The utility of the 1994 versus the revised 2010 Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) Task Force diagnostic criteria for identifying mutation-positive probands with ARVC

Dr Kishal Lukhna
LKHKIS001

SUBMITTED TO THE UNIVERSITY OF CAPE TOWN

Master of Medicine

Department of Medicine
Faculty of Health Sciences
University of Cape Town

Date of submission: 11th October 2019

Supervisors: Professor Ntobeko Ntusi, Dr Sarah Kraus
Department of Medicine, Faculty of Health Sciences, University of Cape Town

Ethics reference number: HREC REF: 454/2016

Declaration

I, Kishal Lukhna, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

Signature: Signed by candidate Date: 11th October 2019

Acknowledgements

First and foremost, I want to take this opportunity to express my sincere thanks and gratitude to my supervisors for their support, guidance and motivation throughout my MMed journey. I would like to acknowledge Dr Sarah Kraus. Her dedication, knowledge and love for cardiomyopathy has played an immense role in steering me throughout the many challenges of academic research. This dissertation would not have been possible without her persistent motivation from beginning to end. I would also like to extend my sincere gratitude to Professor Ntobeko Ntusi. He has been a pillar of strength, providing support, encouragement and enthusiasm throughout my journey. I am indebted to all participants and clinicians who have helped populate the ARVC database; their tireless effort made this project possible.

I am additionally grateful to have been granted this unique opportunity to study a rare clinical condition that has the potential to improve the current understanding of cardiovascular medicine within the African continent. This experience has paved a memorable pathway towards the development of my academic career and a driver for my current interest in medical research. Finally, I would like to give special thanks to my family. It is their love and strict encouragement that has fueled this journey to the end. The voyage of obtaining a master's in medicine has been fantastic and will always remain the root of my academic growth.

Table of Contents

List of Tables

List of Figures

Abbreviations

ARVC	Arrhythmogenic right ventricular cardiomyopathy
CASSA	Cardiac Arrhythmia Society of South Africa
CMR	Cardiovascular magnetic resonance
CRF	Case review form
DP	Diagnostic panel
ECG	Electrocardiogram
EMB	Endomyocardial biopsy
ESC	European Society of Cardiology
GSH	Groote Schuur Hospital
ICD	Implantable cardioverter-defibrillator
IMHOTEP	The Afr**i**can Cardio**m**yopat**h**y and My**o**cardi**t**is R**e**gistry **P**rogramme
ISFC	International Society and Federation of Cardiology
JUP	Junction plakoglobin
LBBB	Left bundle-branch block
LV	Left ventricle/ventricular
MRI	Magnetic resonance imaging
PKP2	Plakophilin-2
PVC	Premature ventricular contraction
RBBB	Right bundle-branch block
RV	Right ventricle/ventricular
RVOT	Right ventricular outflow tract
SAECG	Signal-averaged electrocardiogram
SCD	Sudden cardiac death
SSA	Sub-Saharan Africa
TF	Task Force
TFC	Task Force criteria
UCT	University of Cape Town
VT	Ventricular tachycardia

Abstract

Background: Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited cardiac disorder characterised by structural and functional abnormalities of the right ventricle with or without left ventricular involvement. In 1994, Task Force criteria (TFC) were proposed for the diagnosis of ARVC and were found to be highly specific but lacked sensitivity. In 2010, revised TFC were proposed to increase sensitivity and facilitate diagnosis in those with subtle phenotypes.

Purpose: Many participants of ARVC registries have been enrolled using the 1994 TFC and not re-analysed using the 2010 TFC. We retrospectively compared the utility of both TFC for the diagnosis of mutation-positive probands in the *IMHOTEP (The African Cardiomyopathy and Myocarditis Registry Program)* study with the aim of identifying diagnostic changes that may have clinical impact.

Method: 162 participants with the suspicion of ARVC were referred between May 2003 and May 2018 to our ARVC registry. 150 cases were reviewed using the same ECG and imaging data to fulfil both TFC, and were re-classified by a diagnostic panel at Groote Schuur Hospital, Cape Town.

Results: Sixty-eight participants were diagnosed with ARVC by the diagnostic panel and included into the registry; 14/68 participants with ARVC were found to be mutation-positive. Eighty-two participants were found to have an alternative diagnosis or insufficient criteria and were excluded from the ARVC registry. Mutation-positive probands presented at a significantly younger age compared to the mutation-negative group (29 ± 14 years versus 39 ± 13 years, p=0.009), suggesting an earlier onset of ARVC. Common reasons for presentation in the mutation-positive cohort included palpitations (79%) and presyncope (64%), with

approximately twice the number of participants presenting with sustained ventricular tachycardia (VT) compared to mutation-negative participants (79% versus 47%, p=0.036). The diagnostic yield of the 2010 versus 1994 TFC (n=68) revealed more participants with a definite diagnosis, and less featuring in possible and no criteria categories. A 67% (n=8) change in diagnosis from 1994 borderline to 2010 definite, and an 88% (n=7) change from 1994 possible to 2010 borderline, were observed. Mutation-positive participants had a higher yield for definite ARVC when compared to mutation-negative participants. We subsequently analysed the contribution of each diagnostic modality at fulfilling TFC in our mutation-positive definite participants and found CMR contribution statistically significant, p=0.021.

Conclusion: Our study found that mutation-positive probands were found to be younger, more likely to present with sustained VT, fulfilled a significantly larger number of major 2010 TFC than mutation-negative probands, and that the 2010 TFC for structural and repolarisation abnormalities were more useful in diagnosing ARVC compared to 1994 TFC. We found a significant evolution in classification between both TFC, suggesting that re-classification of participants recruited in traditional ARVC registries according to updated criteria is worthwhile.

Chapter 1 - Literature review

1.1 Introduction

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited myocardial disease characterised by structural and functional abnormalities of the right ventricle (RV) with or without left ventricular (LV) involvement. The pathological hallmark of the disease is defined by progressive myocardial tissue loss with fibro-fatty replacement in an epicardial to an endocardial pattern.[1,2] Affecting 0.02-0.10% of the European population, ARVC is considered autosomal dominant with incomplete penetrance in 30% of cases, with rare autosomal recessive variants described.[3,4] The suspicion of ARVC is often made in otherwise healthy individuals presenting predominantly with lethal tachyarrhythmias, with or without overt structural cardiac abnormalities.[1,3] Diagnosing ARVC can be difficult especially with a normal physical examination reported in at least 50% of patients. There is currently no single diagnostic modality available to confirm ARVC despite advancements in imaging technology, molecular genetics, and rhythm analysis.[5]

In 1994, a Task Force from both sides of the Atlantic Ocean was established to aid in the diagnosis of ARVC. A set of task force criteria was proposed to facilitate the recognition of ARVC and to provide guidance on the interpretation of many frequently used diagnostic modalities. Although highly specific, the 1994 criteria were based mainly on symptomatic cases in the advanced spectrum of disease, and therefore lacked sensitivity for the recognition of early and familial ARVC.[6] In 2010, the revised Task Force criteria (TFC) was proposed to facilitate a clinical diagnosis in those with subtle disease expression. Designed to enhance the sensitivity of disease detection in probands previously classified as *borderline* using the 1994 TFC, the 2010 TFC placed more weight on quantitative criteria, advanced imaging (using cardiovascular magnetic resonance – CMR), and repolarisation abnormalities (such as T-

wave inversion) as early and sensitive markers of disease expression.[6] Genotype analysis was also introduced and referenced as a major criterion in the 2010 TFC.

The ARVC registry of South Africa (SA) was established in 2003. Africa's first confirmed cases of ARVC were reported in 2004.[7,8] Our ARVC registry served as a recruitment model for sub-Saharan Africa (SSA) and was later absorbed into the *African Cardiomyopathy and Myocarditis Registry Program (IMHOTEP)* in 2015 (Kraus S. 2019. PhD Thesis. UCT).

1.2 Data sourced

Using an evidence-based approach, an extensive review of medical literature published in English involving human participants was carefully selected and indexed through PubMed as a primary database. Additional international and local databases relevant to the review were included using a similar search protocol. Key search words using a MeSH search strategy included: 'arrhythmogenic right ventricular cardiomyopathy', and 'ARVC'. Filtered advanced searches included headings such as: 'history', 'genetics', 'diagnostic criteria', '1994 Task Force criteria', '2010 Task Force criteria', and 'diagnostic modalities'. Non-human experimentations and 'paediatrics' were part of the exclusion search criteria. Articles up to May 2019 were included.

1.3 Historical considerations and nomenclature

In 1736, Giovanni Maria Lancisi, the Pope's physician, identified a family with recurrent heart disease across four generations that presented with sudden cardiac death (SCD) and right ventricular failure. Published in a book entitled 'De Motu Cordis et Aneurysmatibus', this represented the first historic case description resembling ARVC.[9] It was only two hundred and fifty years later in 1982 that the first comprehensive case report of ARVC as a clinical entity was published by Dr Frank Marcus. He described a case series of 24 patients with recurrent ventricular tachycardia and right ventricular (RV) enlargement with myocardium replaced by fibro-fatty tissue, and termed the clinical condition 'right ventricular dysplasia', later coined as

'arrhythmogenic right ventricular cardiomyopathy/dysplasia'.[10,11] The term dysplasia was abandoned when ARVC was incorporated into the World Health Organisation (WHO) nomenclature and classification of cardiomyopathies.[12] Approximately 20-years later, ARVC was reported in Africa.[7]

1.4 Epidemiology

The global prevalence of ARVC is estimated between 1 in 2000-5000, with a male predominance of about 70% represented by a 3:1 ratio to females. The higher frequency in males may be linked to specific gender influenced high-intensity sport participation, or a direct influence of sex hormones on phenotypic disease expression.[1] ARVC usually presents in the second to fourth decade of life, with a mean age of presentation at 40 years.[11] Currently, ARVC is responsible for approximately 5-20% of SCD in the young athletic population globally.[3] The annual international mortality rate of ARVC varies between 0.1-3%. A similar finding was represented in our ARVC registry of SA.[13]

1.5 Pathogenesis

The RV myocardial microanatomy can be described by two perpendicularly orientated layers of myocardial fibres. Distortion of these fibres occurs when cell-cell mechanical connections fail, creating an area of weakness prone to shearing forces during cardiac contraction.[14] Replacement of RV myocardium with fibro-fatty scar tissue, unique to ARVC, further leads to free wall thinning and aneurysmal dilatation. Severe or late disease typically manifests in areas of the RV collectively known as the 'triangle of dysplasia', represented by an inflow tract marked by the sub-tricuspid region (RV pulmonary outflow tract) and the RV apex.[1] A pathognomonic site for ARVC related aneurysms commonly occurs in the inferior diaphragmatic wall below the posteroinferior leaflet of the tricuspid valve.[2] The 'triangle of dysplasia', historically described in ARVC since 1982, is predominantly associated with severe cases, and not sensitive enough to apply to early disease manifestations. Over the past

decade, the perception of structural involvement of ARVC has changed to a new biventricular triangle involving the RV basal anteroinferior wall, and LV posterolateral wall (Figure 1). These findings represent early ARVC disease.[15,16]

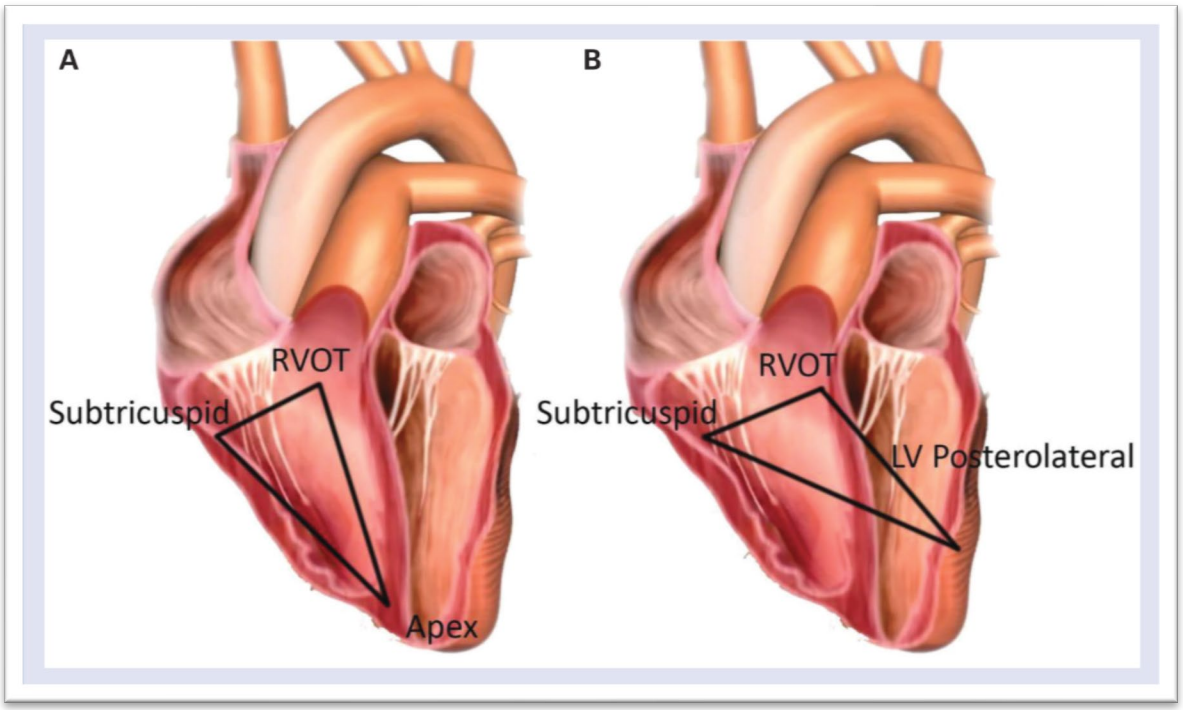


Figure 1. Triangle of dysplasia.
A. Original triangle of dysplasia **B.** Revised bi-ventricular triangle of dysplasia.

From: Mast, T., Teske, A., Doevendans, P., et al. Current and future role of echocardiography in arrhythmogenic right ventricular dysplasia/cardiomyopathy. *Cardiology Journal. 2015;22(4):362-374.*

ARVC is a desmosomal cellular junctional disease. Cellular connections are primarily established by gap junctions, adherens junctions, and desmosomes. Ultrastructural investigation of endomyocardial biopsies reveals intercalated disk remodeling with fewer and shorter desmosomes resulting in intercellular gap widening (Figure 2). Desmosomes are complex multiprotein structures principally responsible for mechanical and electrical attachment of adjacent cells. Mechanical uncoupling in ARVC is strongly associated with cellular death and fibrosis, while electrical uncoupling is associated with significant activation delay; both of these changes clinically manifest as lethal arrhythmias.[15] Desmosomes are

formed from proteins originating from three separate subgroups: desmosomal cadherins, armadillo proteins (plakoglobin and plakophilin), and desmoplakins.

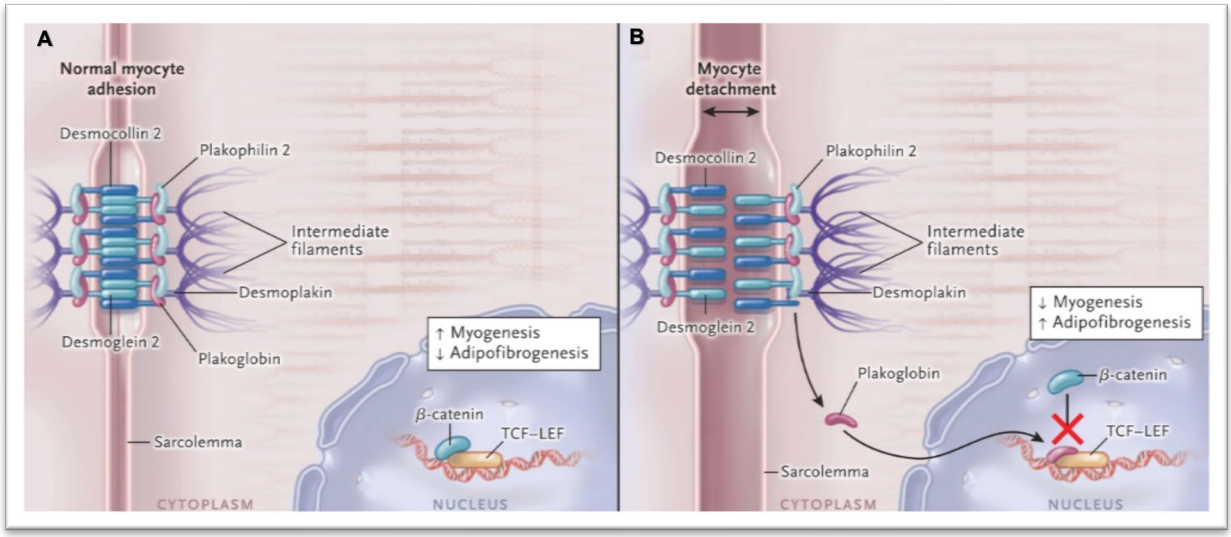


Figure 2. Histopathological features of Arrhythmogenic Right Ventricular Cardiomyopathy. **A.** Normal desmosome. **B.** Abnormal desmosome

From: Corrado D, Link MS CH, Calkins H. Arrhythmogenic Right Ventricular Cardiomyopathy. *New Engl J Med. 2017;376:61–72.*

In the year 2000, a deletion in the plakoglobin gene was found in Naxos disease, an autosomal recessive variant of ARVC.[13] The plakophilin-2 gene was later found to be the most frequently associated pathogenic genetic mutation in ARVC.[13] Adipogenesis is believed to originate from mutated desmosomal plakoglobin proteins that induce intranuclear signaling, suppressing a canonical Wnt–β-catenin signaling pathway.[17] Studies containing mutated plakoglobin have demonstrated increased expression of adipogenic and fibrogenic genes, contributing to the development of fibro-fatty myocardial replacement and scarring.[1,9] Fibro-fatty tissue replacement in ARVC provides an additional substrate for arrhythmogenesis through scar-related macro-reentry phenomena.[1] Remodeling of intercalated discs and the subsequent widening of myocyte gap junctions, significantly contribute to the arrhythmogenicity of ARVC and the potentiate the risk of SCD.[13] Adding to the complexity of diagnosing ARVC

intramyocardial fat deposition in the anterolateral and apical regions of the RV can be seen in normal hearts and is commonly associated with increased body habitus.[13]

1.6 Genetic underpinnings

ARVC is known as a 'disease of the desmosome'.[18] The first gene, *desmoplakin* (*DSP*) encoding the protein desmoplakin, was reported in 2002.[19] Currently, five causative genes responsible for the coding of desmosomal proteins have been identified: namely *junction plakoglobin* (*JUP*), *desmoplakin* (*DSP*), *plakophilin-2* (*PKP2*), *desmoglein-2* (*DSG2*), and *desmocollin-2* (*DSC2*). Other non-desmosomal genes such as *transmembrane protein 43 (TMEM43), cardiac ryanodine receptor* (*RYR2*), and *transforming growth factor-beta 3* (*TGFB3*) have also been implicated in ARVC disease.[20,21,22] The *PKP2* gene is most commonly described and found in 25% of all cases with ARVC.[23] In 2009, under the leadership of Professor Bongani Mayosi, new markers across the *PKP2* locus were found in four unrelated Caucasian families in South Africa. These findings established a genetic founder effect originating from early Dutch settlers.[11] Abnormal desmosomal genes have only been identified in 30-50% of patients with ARVC, highlighting the possibility of many undiscovered genes.[24] By using next-generation genetic sequencing, several non-desmosomal genes frequently found in other forms of cardiomyopathy, such as familial dilated cardiomyopathy, have been found to be associated with ARVC.[15,18] The distinction between these inherited cardiomyopathies, with similar genetic and phenotypical characteristics, have crucial implications in clinical practice, a problem commonly encountered when diagnosing ARVC.[18] In 2017, our group described a pathogenic genetic variant of the *CDH2* gene, that is responsible for the encoding of intercalated disc protein cadherin-2, as a novel genetic cause of ARVC.[22] This unique finding in a three-generation family established new insights into the pathogenesis of ARVC not previously documented in medical literature.[22] Fundamental genetic principles are imperative when interpreting genetic testing as only 16% of healthy individuals carry a mutation variant of ARVC.[19] While positive genotyping is supportive, it is

not confirmatory of ARVC and should not override clinical judgement.[25] A person who has a gene mutation inherits the risk of having the disease, but may not develop the disease.[24] A significant family history changes the probability of developing ARVC from 1:1000-5000 of the general population, to a risk of 1:2.[26] A negative genetic test is non-contributory to the diagnosis of ARVC as only 30-50% of probands carry a known defective gene.[25]

Desmosomes are present in other parts of the human body besides the cardiac muscle.[24] It has been found in skin and hair follicles in cardio-cutaneous autosomal recessive spectrums of ARVC. Naxos disease, one of the two cardio-cutaneous manifestations of ARVC, presents with palmoplantar keratosis (thickening of palms and soles), woolly hair and features of ARVC.[27] The second cardio-cutaneous variant was discovered in Ecuador and is known as the Carvajal syndrome which has a preference for the left ventricle.[24]

1.7 The effect of exercise on ARVC

Significantly higher pulmonary pressures and RV afterload is attained by athletes during exercise when compared to the LV.[28] ARVC patients participating in competitive sport, such as soccer and basketball, have been shown to have a five-fold increased risk of SCD when compared to non-athletes.[29]. It has been postulated that fatal ventricular arrhythmias are attributed to exercise-induced RV wall stretching following the law of Laplace. Physical exercise further aggravates mechanical cellular uncoupling. Aerobic-exercise has been shown to increase pressure afterload and wall stress disproportionately in the RV compared to the LV (170% versus 23%), triggering malignant ventricular arrhythmias and promoting disease progression.[1,30] Higher wall tension has been found to be an ideal site for mechano-transduction, which is the conversion of mechanical stimuli to intracellular biochemical signals. Mechano-transduction enables cells to adapt to external forces and physical constraints, promoting the RV free wall to act as a potential pathological site in the setting of genetically frail intercellular junctions.[9,31] Competitive sport has been associated with earlier symptom

presentations, larger RV volumes and carries a higher risk of SCD compared to recreational sport.[3] Seventy-one percent of participants in competitive sports at risk for ARVC have inducible VT at electrophysiological studies, compared to 44% of participants involved in recreational sports.[3] At 10-year follow-up, 40% of ARVC patients associated with competitive sport, presented with earlier symptoms compared to 7% in recreational sport.[3] It is currently recommended that ARVC patients avoid competitive and most recreational sports, reducing their risk for ventricular arrhythmias and subsequent death.[3,32] Examples of competitive sport include basketball, soccer, hockey, skiing, running, biking, and tennis; while examples of recreational sport include bowling, golf, weight-lifting and baseball.[33,34]

1.8 Diagnosis

Early disease ARVC is an exceedingly challenging diagnosis to make, especially when there are minimal structural or functional alterations of the RV present. Histological illustrations of transmural fibro-fatty replacement of RV myocardium is a valuable diagnostic indicator, however, not always possible. The segmental nature of ARVC makes endomyocardial biopsies (EMB) of the RV innately difficult, especially with the presence of RV adipose tissue in healthy individuals without ARVC.[35] Misinterpretation of physiologic cardiac fat distribution in the RV apical wall often leads to an overdiagnosis of ARVC.[16] Other conditions commonly misclassified as ARVC include idiopathic right ventricular outflow tract tachycardia (RVOT), cardiac sarcoidosis, congenital heart disease, and dilated cardiomyopathy, often mimic the biventricular variant or end-stage manifestation of ARVC.[1] A diagnosis of ARVC relies heavily on the combination of structural, functional, histological and electrophysiological abnormalities. Scientific communities globally have recognised the difficulties in diagnosing ARVC and thus an international diagnostic task force was assembled. [35]

1.9 1994 Task Force criteria

In 1994, the *Working Group of Myocardial and Pericardial Diseases of the European Society of Cardiology (ESC)* and the *Scientific Council on Cardiomyopathies of the International Society and Federation of Cardiology (ISFC)* established a task force that proposed clinical criteria that would aid in the diagnosis of ARVC. Their aim was to facilitate the recognition and interpretation of non-specific clinical features of ARVC in conjunction with objective data from a combination of diagnostic modalities.[35] The 1994 criteria were structured according to structural imaging, tissue characterisation, and rhythm abnormalities. Each section was categorised into major and minor criteria based on specificity and association with ARVC. A *definite* diagnosis of ARVC was established by fulfilling either two major criteria; or one major plus two minor criteria; or with four minor criteria.[35]

The 1994 TFC concentrated on symptomatic indexes and SCD, representing an advanced spectrum of disease. The criteria focused predominantly on RV disease, reserving the involvement of the LV as a marker of exclusion as of its common association with other cardiomyopathies. The 1994 TFC was deemed highly specific but lacked sensitivity for the recognition of early and familial diseases which often have incomplete expression.[6] Structural and functional imaging criteria were based on a qualitative rather than a quantitative approach that was incorporated in the 2010 TFC. The criteria were also referenced according to literature that lacked our current knowledge and experience with advanced imaging such as cardiovascular magnetic resonance (CMR).[36] Major 1994 TF imaging criteria using either echocardiography, CMR or right ventricular angiography (RVA), required severe dilatation and reduction in RV systolic function without LV impairment, localised RV aneurysms with akinesia or dyskinetic areas, or severe segmental dilatation of the RV. Minor imaging criteria was represented by either mild global RV dilatation with reduced ejection fraction and normal LV, mild segmental dilatation of the RV free wall, or isolated regional RV hypokinesia.[13] The 1994

TF imaging criteria, although helpful, lacked specific quantitative cut-off parameters for grading RV dysfunction according to gender determining body surface area.[36]

In the early 1990s, endomyocardial biopsy (EMB) was heavily weighted and considered the preferred method for diagnosing ARVC. The 1994 TFC incorporated findings from EMB as major criteria, however, these criteria lacked morphometric analysis of myocytes that were replaced by fibro-fatty tissue, an important differentiator from physiological adipogenesis and other cardiac conditions (Figure 3). EMB has a low sensitivity of 67% and higher specificity of 91.5% for diagnosing ARVC.[37] As experience using EMB developed, it became more clearer that EMB held very low diagnostic influence in diagnosing ARVC than it was previously thought. Tissue sampling, predominantly taken at the junction of the ventricular septum and free wall, carries a low risk (0.2%) of perforation, however, due to the segmental nature of ARVC it often yields a low likelihood of active disease.[38,39]

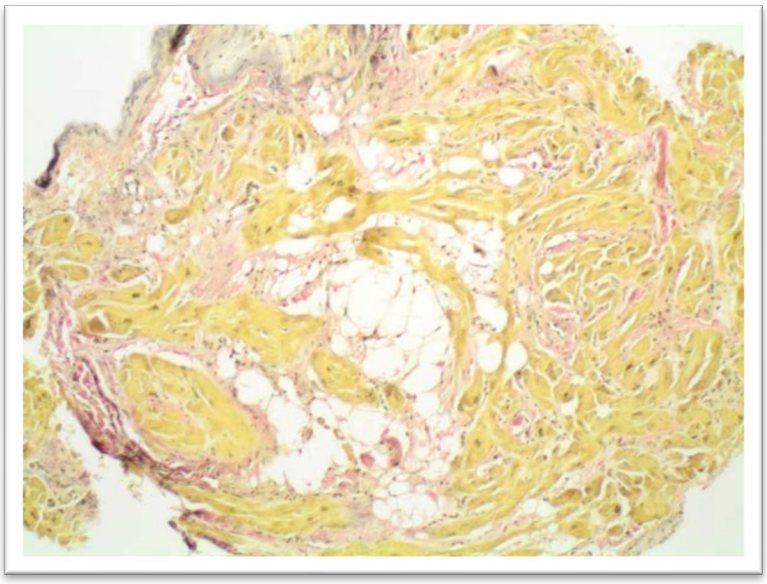


Figure 3. Endomyocardial biopsy sample of an ARVC patient.
Histology specimen of the RV myocardium showing evidence of fibro-fatty infiltration (elastic von Gieson stain, magnification ×100).

From: Mayosi BM, Fish M, Shaboodien G, et al. Identification of Cadherin 2 (CDH2) Mutations in Arrhythmogenic Right Ventricular Cardiomyopathy. *Circ Cardiovasc Genet. 2017;10(2).*

Cardinal diagnostic information obtained by current advancements in medicine were often excluded or designated a minor contributory status in the 1994 TFC. Many diagnostic findings, now prioritised as major criteria in the 2010 TFC, such as right precordial T-wave inversion (TWI) and arrhythmias of RV origin, were weighed less as of their association with other cardiac conditions. With the 1994 TFC directed predominantly at the severe spectrum of ARVC, a new approach facilitating an early clinical diagnosis was needed. The new TFC needed to incorporate advancements in technology and improve diagnostic sensitivity while maintaining the diagnostic specificity of the 1994 TFC.[6]

1.10 2010 Revised Task Force criteria

Fifteen years after their first meeting, the ARVC task force was re-convened to revise the original ARVC diagnostic criteria. Advancements in medical science, imaging and genetics helped design the current 2010 TFC, placing new emphasis on identifying patients in the early phase of ARVC. Although the 2010 TFC was structured to maintain established diagnostic specificity, the sensitivity of probands previously diagnosed as *borderline* using the 1994 TFC was increased.[26,36] An essential prerequisite in the establishment of the 2010 TFC was the maintenance of categories structured in the original 1994 TFC.[18] The new criteria maintained the original sub-divisions of six categories, namely structural abnormalities, tissue characterisation, repolarisation abnormalities, depolarisation abnormalities, arrhythmias, and family history. According to the 2010 criteria, a *definite* diagnosis of ARVC was made by fulfilling either 2 major; 1 major and 2 minor; or 4 minor criteria. A *borderline* diagnosis of ARVC required 1 major and 1 minor; or 3 minor criteria. A *possible* diagnosis of ARVC would require 1 major; or 2 minor criteria.[25] The previous TFC was greatly limited by subjective assessments used to meet imaging criteria, particularly when assessing ventricular structure and function. As a consequence, the revised 2010 criteria focused strictly on quantitative measurements.[40] Separate imaging major and minor criteria defining morphologic RV changes for echocardiography and CMR were included, and no longer limited to previously stipulated

RV regional hypokinesis, microaneurysm and segmental dilatation. To fulfil new imaging criteria, regional wall motion abnormality had to be accompanied by global RV dilatation based on precise gender-specific volumetric measurements and indexed to body surface area or RV systolic dysfunction.[36]

A great shortfall of the 1994 criteria was the frequent misinterpretation of normal RV function as local 'hypokinesis', resulting in large amounts of interobserver variability and overdiagnosis of ARVC. The terminology 'hypokinesis' was subsequently abandoned from the new criteria.[41] Further improvements of the 2010 criteria included the incorporation of both RV functional as well as structural wall motion abnormalities, rather than simple 'hypokinesis' term previously used in establishing a diagnosis.[41,42] The 2010 imaging TFC used terminology such as 'akinesia' which was defined as a lack of motion, 'dyskinesia' defined as an abnormal outward myocardial bulge during systole, and 'dyssynchronous' expressed as regional contractions of surrounding myocardium at different times. These terminologies were standardised and used across all three imaging modalities (echocardiography, CMR and RVA) when assessing regional wall motion abnormalities in ARVC.[15] Fifteen years of medical progression was successfully incorporated into the 2010 TFC with the aim of improving the diagnostic sensitivity of detecting early ARVC while establishing a diagnosis in those previously diagnosed as *borderline* ARVC according to the 1994 TFC.[26]

1.11 Diagnostic imaging modalities

1.11.1 Echocardiography

Revised 2010 TF imaging criteria for two-dimensional echocardiography focused particularly on quantitative RV outflow tract measurements and areas of fractional change for RV systolic function. The 2010 TFC also included qualitative features by commenting on the presence of regional RV akinesia, dyskinesia, or aneurysms.[43] Echocardiography is often limited by technique as the desired quantitative information of RV function is challenging to acquire. A

considerable amount of difficulty arises when attempting to visualise the complex RV anatomy that is shielded by the sternum, requiring special views that are not always easy to acquire in a standardised transthoracic echocardiographic study. Hence to diagnose ARVC by echocardiography is challenging, and requires a high level of expertise.[44] A further limitation when using two-dimensional echocardiography is its weak correlation with CMR-based measurements of RV dilatation and function. Although quantitative measurements have significantly increased TFC sensitivity and specificity when evaluating the RV, technique and probe angulation often reveal variable results even in experienced hands. The segmental nature of ARVC and poor acoustic windows generated by the RVOT makes visualisation of RV dilatation difficult and a reason for reduced fulfilment of echocardiography-based TFC.[45] Discrepancy in fulfilling imaging diagnostic criteria have clinical implications since imaging data is a pivotal diagnostic criterion in a significant number of cases.[45]

1.11.2 Cardiovascular magnetic resonance (CMR)

CMR, with specific quantitative and qualitative measurements, has been a significant addition to the revised TFC. CMR qualitative measurements describe RV wall motion abnormalities as either regional akinesia, dyskinesia, or with dyssynchronous contraction, while its quantitative measurements assess RV end-diastolic volume by body surface area and ejection fraction. The strict quantitative values depicted in the 2010 TFC were standardised using healthy volunteers from the *Multi-Ethnic Study of Atherosclerosis* (*MESA)* as controls when compared to ARVC probands in the North American ARVC registry.[15] CMR, by its high spatial resolution and unlimited orthogonal imaging planes, is currently revered for its precise RV volume and systolic function assessment (Figure 4). Together with high intra-observer and inter-observer agreement and accuracy, CMR has become the preferred non-invasive diagnostic tool for the evaluation of the complex RV anatomy.[15,36]

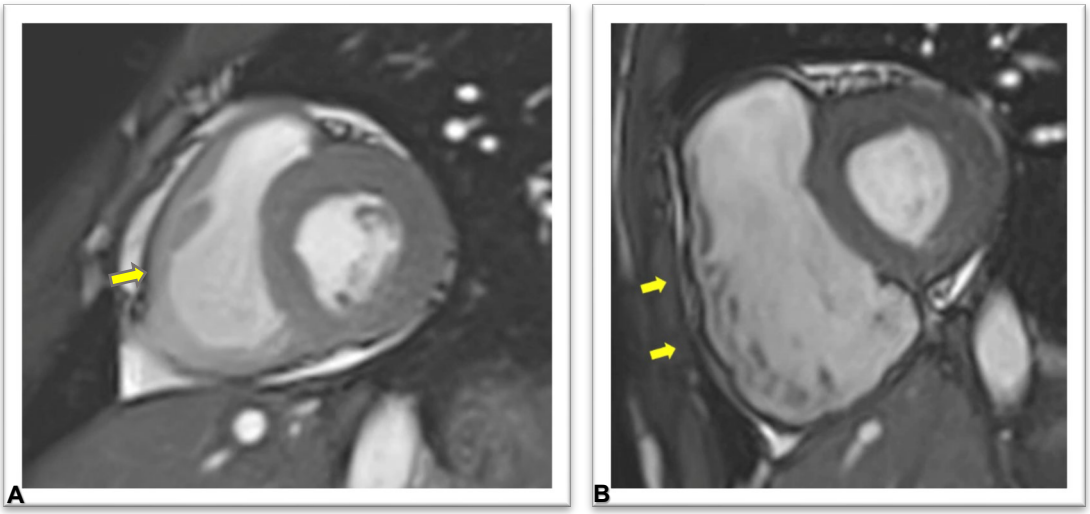


Figure 4: Sample CMR short axis of a normal (A) versus an ARVC patient (B).
A. Normal RV (yellow arrow) **B.** Isolated dilatation and thinning of the RV with segmental RV free wall aneurysm (yellow arrows).

Reproduced from S, Kraus, 2019, PhD Thesis (unpublished) with permission from the *IMHOTEP* registry.

CMR can also characterise tissue and enhance identification of intra-myocardial fat and fibrosis by using late gadolinium enhancement (LGE). CMR, when available, offers a wealth of reliable data and is slowly becoming the chosen imaging modality in ARVC.[44] Although LGE can be used to assess myocardial fibro-fatty infiltration and distinguish ARVC from other cardiomyopathies, there is still some uncertainty on its diagnostic targets and it may carry a risk for the overdiagnosis of ARVC. Additional limiting factors on LGE include the enhancement of RV fat in healthy individuals and the persistent difficulty in differentiating fat from fibrosis in relatively thin RV myocardium. Using LGE highlights a risk of overdiagnosing ARVC, and is not currently part of the current imaging criteria for ARVC.[36]

CMR is seen as an excellent modality when screening asymptomatic relatives at risk of ARVC especially because the sensitivity of echocardiography in these patient populations remain unacceptably low.[45] The major disadvantage of CMR rests in its limited availability and the need for high-level expertise during the assessment and interpretation of data.[44] Unfortunately

despite CMR being an attractive field for training at many academic institutes, clinical experience remains challenging to acquire as of low disease prevalences.[15] Despite CMR emerging as the favoured imaging modality when diagnosing ARVC, electrical abnormalities often precede structural changes in ARVC, emphasising a reason why CMR should not be used in isolation when diagnosing ARVC.[15]

1.11.3 Right ventricular angiography (RVA)

RVA allows for global and regional analysis of the RV anatomy. Fulfilment of quality imaging criteria by the presence of dyskinetic or akinetic myocardium in the infundibular, apical or sub-tricuspid regions of the RV, has a 90% specificity for ARVC.[46] On the contrary, even with good quality angiograms, it is often difficult to assess the complex geometric shape of the RV and thus a negative angiogram does not exclude ARVC.[47] Despite offering detailed functional anatomy, RVA's are limited by its invasive, time-consuming nature that lacks qualitative imaging standardisation that generates a high amount of inter-observer variability.[48] Clinicians have thus moved away from RVA as a preferred diagnostic modality.

1.12 Rhythm Analysis

1.12.1 Electrocardiography (ECG)

The 2010 TFC subdivide ECG findings according to repolarisation and depolarisation abnormalities. The inclusion of repolarisation abnormalities, an early marker for ARVC, in the revised TFC represents an evolution in the TFC.

<u>Repolarisation abnormalities</u>

Repolarisation abnormalities, a primary diagnostic feature in ARVC, are early and sensitive markers of disease expression.[6] These abnormalities, found in individuals older than 14 years in the absence of a right bundle branch block (RBBB), are defined as major criteria in the 2010

TFC when T-wave inversion (TWI) in right precordial leads extend from lead V1 to V3.[49] Anteroseptal TWI (V1-V3) are uncommon in patients with a RBBB who do not have ARVC.[40] Characteristically, this particular pattern of TWI is seen in 87% of patients with ARVC and only present in <3% of healthy individuals without ARVC.[50] Interestingly, anteroseptal TWI is commonly found in 12.7% of black athletes and can be differentiated from ARVC by the presence of preceding convex ST-segment elevation, a finding rarely found in ARVC.[41,51]

Pre-participation athletic screening programs introduced in Italy in 1982 have substantially decreased SCD in young competitive athletes.[52] Cardinal ECG observations including right precordial TWI, QRS widening and epsilon waves have led to athletic disqualification and has resulted in a sharp decline in ARVC-related SCD during sporting activities globally.[9]

Depolarisation abnormalities

Depolarisation abnormalities represent markers of intraventricular conduction delay. Electrophysiologically, these abnormalities, a common feature found in the severe spectrum of ARVC, denote surviving myocardium interspersed between fibro-fatty tissue that fragments electrical current.[13] Ventricular depolarisation abnormalities in ARVC are confined to conduction delay in right precordial leads (without a RBBB) and are represented by coined abnormalities referred to as epsilon waves, late potentials (typically described using signal-averaged ECG), and terminal activation delay of the QRS complex. An epsilon wave (Figure 5), first described in 1977 by Dr Guy Fontaine, is a post-excitation ventricular wave occurring in 30% of cases with ARVC.[47,53] Commonly small in amplitude; the epsilon wave mirrors a pre-excitation delta wave by occurring after the QRS complex at the start of the ST-segment. Epsilon waves are a histopathological representation of delayed activation in RV myocardial fibres and are considered an electrophysiological substrate for ventricular tachyarrhythmias.[14,47]

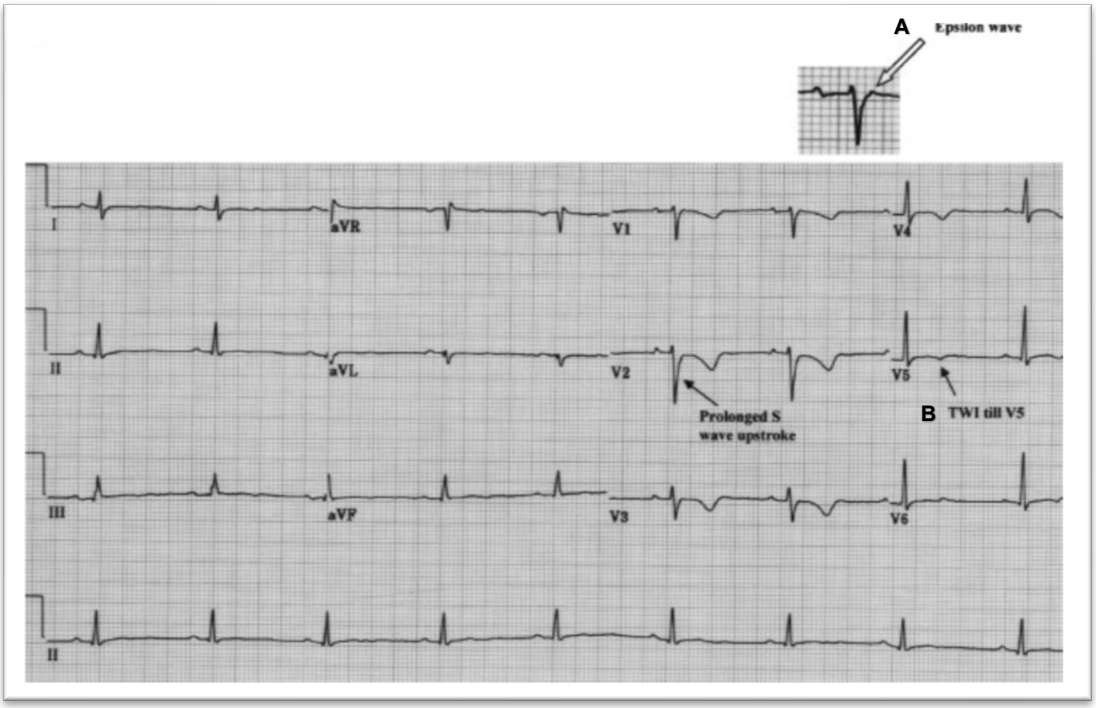


Figure 5: Sample ECG of an ARVC patient.

B. Depolarisation changes in the form of an Epilson wave in V1. **B.** Repolarisation changes with TWI from V1-V5.

From: Nasir, K., Bomma, C., Tandri, H., et al. Electrocardiographic features of arrhythmogenic right ventricular dysplasia/cardiomyopathy according to disease severity: A need to broaden diagnostic criteria. *Circulation.2004;110(12):1527-1534*.

1.12.2 Signal-averaged electrocardiography (SAECG)

SAECG is a diagnostic modality used to assess for late potentials and populates minor depolarisation TFC. It averages multiple consecutive QRS complexes (approximately 250) and filters random noise. Late potentials are defined as prolongation of right precordial QRS complexes and assessed by having either:

1) a filtered QRS duration (using a 40-250Hz filter) ≥ 114ms,
2) duration of low terminal signal QRS (<40 mV) ≥ 38ms, and/or
3) root-mean-square voltage of a QRS terminal 40ms ≤ 20 mV.

If ≥ 1 of these abnormal parameters are found, a minor depolarisation criterion for ARVC is fulfilled. Analysis of each parameter has a sensitivity ranging from 58 to 60% with a specificity

of 94 to 96%.[40] Abnormal SAECG is not specific for ARVC, and is seen in other conditions with abnormal myocardial tissue.[44] Despite its weak contribution to TFC, it remains a useful non-invasive tool for screening ARVC in family members.[47]

1.12.3 Terminal activation duration (TAD)

TAD is demonstrated in QRS complexes found in anteroseptal precordial leads (V1-V3) with delayed S-waves exceeding 55ms. It is a manifestation of conduction delay and contributes one minor depolarisation criterion to the 2010 TFC. TAD is measured from the nadir of the S-wave to the end of depolarisation. Although epsilon waves are defined as separate entities found at the end of QRS complexes; ambiguity often arises when its end is not clearly demarcated. TAD avoids this dilemma by including the last depolarisation deflection in its measurement (i.e. including epsilon waves if present).[44] Duplication of TFC is always a concern when working with overlapping modalities, however, duplicated TFC is fundamentally prevented as one can only fulfil either major or minor criteria, and never both. The limitation of TAD is seen in its contra-indication in complexes with a RBBB, an uncommon finding in patients with ARVC and TWI in V1-V3.[6]

1.12.4 Arrhythmias

Sustained or non-sustained (<30 seconds) ventricular arrhythmias originating from the RV inferior wall or apex are considered major criteria for ARVC. The VT's QRS morphology and axis help reflect its site of origin. A superior axis is defined as a predominantly negative or indeterminate QRS complex in leads II, III, and aVF and a positive QRS complex in lead aVL. An inferior axis is defined as a predominantly positive QRS complex in leads II, III, and aVF and a negative QRS complex in lead aVL. A VT with a left bundle branch block (LBBB) morphology and inferior axis suggests an origin from the RV outflow tract (RVOT), fulfilling a minor criterion according to the 2010 TFC. Importantly, a VT with a LBBB morphology and superior axis suggests origins in the RV inferior wall and fulfils a major criterion.[44]

Characteristically, patients with ARVC and a near-normal LV function, tolerate a sustained RV VT ranging between 200–250 beats/minute for many hours before presentation.[41]

1.12.5 24-hour Holter monitoring

Although the presence of ventricular ectopy increases with age, >200 premature ventricular complexes (PVC's) in 24-hours in an adult <50 years is suggestive of an underlying myocardial disease, not explicitly ARVC.[40] A Holter measuring >500 PVCs in 24-hours satisfies a minor (arrhythmia) criterion in the 2010 TFC. Due to the limited number of leads used in the Holter, no restricted QRS PVC morphology is stipulated in the 2010 TFC.[44] Although represented as a tool for investigating ARVC, 24-hour Holter devices are particularly useful in monitoring patients with ARVC.

1.13 Family History

The 2010 TFC incorporated the evolving genetic background of ARVC by including genotyping as a diagnostic modality that contributes a major criterion towards diagnosing ARVC.

1.13.1 Genotyping

The variability of the genotypic–phenotypic presentations of ARVC, especially with incomplete penetrance, creates an unclear risk of inheritance in family members.[51] Asymptomatic relatives with desmosomal mutations have a six-fold increased risk of developing ARVC compared to family members of probands without a pathogenic mutation.[54] Although genotyping is integrated as a tool for screening early disease, in light of its flaws at predicting disease, genetic counselling is emphasised.[15]

1.14 The rationale for this study

ARVC manifests with life-threatening arrhythmias in the young, economically active population. Structural abnormalities in ARVC are easily overlooked, especially in the early

stages of disease when the diagnosis is challenging. With non-specific clinical findings and no gold standard investigation to diagnose ARVC, the best strategy rests in the combination of highly sensitive and specific criteria from selected evidence-based diagnostic modalities to improve diagnosis.[55] Importantly, ARVC needs to be distinguished from other inherited cardiomyopathies with similar genetic and phenotypical characteristics.[18]

The ARVC registry of South Africa was established in 2003 (HREC: 047/2003) under the auspices of the Cardiac Arrhythmia Society of South Africa (CASSA) at GSH.[8,23] The objective of this registry was to determine the clinical characteristics, survival, and genetics of ARVC in South Africa. Published previously, fifty unrelated cases who met the 1994 TFC were enrolled between January 2004 and April 2009. The mean age of symptom onset was 32.6 years with a male predominance of 66%.[23] Professional endurance athletes represented 28% of the cohort.[56] The mean age at death was 36.9 years, an early mortality no different to the general population of South Africa whose lifespan was shortened mostly to the epidemics of HIV/AIDS at that time.[56] The cohort revealed an annual mortality rate of 2.8% in 2010, with a five-year cumulative mortality of 10%. Emphasis must be placed on the fact that participants were in the advanced phase of ARVC.[56] The ARVC registry of South Africa was later absorbed into the *African Cardiomyopathy and Myocarditis Registry Programme (IMHOTEP)* in 2015 (HREC:767/2014), and approved by the Human Research Ethics Committee of UCT. The *IMHOTEP* registry is designed to improve the diagnostic classification of cardiomyopathy, determine the role of genetics and myocarditis in the pathogenesis of the disease, and establish long-term outcomes. For the first time, participants suspected to have ARVC in *IMHOTEP* have been classified using both Task Force criteria. Re-analysis would help identify a diagnostic change in cases previously categorised using the 1994 TFC. This study will help discuss the clinical impact TFC have on predicting genotype status in a resource-restricted environment.

Chapter 2 - Methods

2.1 Aims and Objectives

Our study aimed at comparing the utility of the original 1994 TFC to the revised 2010 TFC (Table 2.1) in diagnosing mutation-positive unrelated probands with ARVC in the *IMHOTEP* study. Probands were defined as the first affected individual in a family seeking medical attention for ARVC (i.e. index case in a family). The following objectives were considered:

Primary objectives

1. All index cases (probands) referred with the suspicion of ARVC to the ARVC registry of South Africa and/or *IMHOTEP* registry were reassessed according to the 1994 TFC.
2. All index cases (probands) referred with the suspicion of ARVC to the ARVC registry of South Africa and/or *IMHOTEP* registry were reassessed according to the revised 2010 TFC.
3. A comparison of the diagnostic yield using both TFC was made, identifying a diagnostic change in a sub-Saharan African setting.

Secondary objective

1. A comparison of the diagnostic yield of the TFC and of the diagnostic modalities used when diagnosing ARVC in the South African cohort, were made against groups described internationally.

Table 2.1 Comparison of original 1994 and revised 2010 TFC

Original Task Force Criteria	Revised Task Force Criteria
I. Global or regional dysfunction and structural alterations*	
Major	
• Severe dilatation and reduction of RV ejection fraction with no (or only mild) LV impairment • Localized RV aneurysms (akinetic or dyskinetic areas with diastolic bulging) • Severe segmental dilatation of the RV	**By 2D echo:** • Regional RV akinesia, dyskinesia, or aneurysm • *and* 1 of the following (end diastole): — PLAX RVOT ≥32 mm (corrected for body size [PLAX/BSA] ≥19 mm/m^2) — PSAX RVOT ≥36 mm (corrected for body size [PSAX/BSA] ≥21 mm/m^2) — *or* fractional area change ≤33% **By MRI:** • Regional RV akinesia or dyskinesia or dyssynchronous RV contraction • *and* 1 of the following: — Ratio of RV end-diastolic volume to BSA ≥110 mL/m^2 (male) or ≥100 mL/m^2 (female) — *or* RV ejection fraction ≤40% **By RV angiography:** • Regional RV akinesia, dyskinesia, or aneurysm
Minor	
• Mild global RV dilatation and/or ejection fraction reduction with normal LV • Mild segmental dilatation of the RV • Regional RV hypokinesia	**By 2D echo:** • Regional RV akinesia or dyskinesia • *and* 1 of the following (end diastole): — PLAX RVOT ≥29 to <32 mm (corrected for body size [PLAX/BSA] ≥16 to <19 mm/m^2) — PSAX RVOT ≥32 to <36 mm (corrected for body size [PSAX/BSA] ≥18 to <21 mm/m^2) — *or* fractional area change >33% to ≤40% **By MRI:** • Regional RV akinesia or dyskinesia or dyssynchronous RV contraction • *and* 1 of the following: — Ratio of RV end-diastolic volume to BSA ≥100 to <110 mL/m^2 (male) or ≥90 to <100 mL/m^2 (female) — *or* RV ejection fraction >40% to ≤45%
II. Tissue characterization of wall	
Major	
• Fibrofatty replacement of myocardium on endomyocardial biopsy	• 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% to 75% by morphometric analysis (or 50% to 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
III. Repolarization abnormalities	
Major	
	• Inverted T waves in right precordial leads (V_1, V_2, and V_3) or beyond in individuals >14 years of age (in the absence of complete right bundle-branch block QRS ≥120 ms)
Minor	
• Inverted T waves in right precordial leads (V_2 and V_3) (people age >12 years, in absence of right bundle-branch block)	• Inverted T waves in leads V_1 and V_2 in individuals >14 years of age (in the absence of complete right bundle-branch block) or in V_4, V_5, or V_6 • Inverted T waves in leads V_1, V_2, V_3, and V_4 in individuals >14 years of age in the presence of complete right bundle-branch block

(*Continued*)

From: Marcus FI, et al. Diagnosis of Arrhythmogenic Right Ventricular Cardiomyopathy/Dysplasia: Prosed Modification of the Task Force Criteria. Circulation 2010;121:1533-1541.

Table 2.1 continued

Original Task Force Criteria	Revised Task Force Criteria
IV. Depolarization/conduction abnormalities	
Major	
• Epsilon waves or localized prolongation (>110 ms) of the QRS complex in right precordial leads (V_1 to V_3)	• Epsilon wave (reproducible low-amplitude signals between end of QRS complex to onset of the T wave) in the right precordial leads (V_1 to V_3)
Minor	
• Late potentials (SAECG)	• Late potentials by SAECG in ≥1 of 3 parameters in the absence of a QRS duration of ≥110 ms on the standard ECG • Filtered QRS duration (fQRS) ≥114 ms • Duration of terminal QRS <40 μV (low-amplitude signal duration) ≥38 ms • Root-mean-square voltage of terminal 40 ms ≤20 μV • Terminal activation duration of QRS ≥55 ms measured from the nadir of the S wave to the end of the QRS, including R′, in V_1, V_2, or V_3, in the absence of complete right bundle-branch block
V. Arrhythmias	
Major	
	• Nonsustained or sustained ventricular tachycardia of left bundle-branch morphology with superior axis (negative or indeterminate QRS in leads II, III, and aVF and positive in lead aVL)
Minor	
• Left bundle-branch block–type ventricular tachycardia (sustained and nonsustained) (ECG, Holter, exercise) • Frequent ventricular extrasystoles (>1000 per 24 hours) (Holter)	• Nonsustained or sustained ventricular tachycardia of RV outflow configuration, left bundle-branch block morphology with inferior axis (positive QRS in leads II, III, and aVF and negative in lead aVL) or of unknown axis • >500 ventricular extrasystoles per 24 hours (Holter)
VI. Family history	
Major	
• Familial disease confirmed at necropsy or surgery	• ARVC/D confirmed in a first-degree relative who meets current Task Force criteria • ARVC/D confirmed pathologically at autopsy or surgery in a first-degree relative • Identification of a pathogenic mutation† categorized as associated or probably associated with ARVC/D in the patient under evaluation
Minor	
• Family history of premature sudden death (<35 years of age) due to suspected ARVC/D • Familial history (clinical diagnosis based on present criteria)	• History of ARVC/D in a first-degree relative in whom it is not possible or practical to determine whether the family member meets current Task Force criteria • Premature sudden death (<35 years of age) due to suspected ARVC/D in a first-degree relative • ARVC/D confirmed pathologically or by current Task Force Criteria in second-degree relative

PLAX indicates parasternal long-axis view; RVOT, RV outflow tract; BSA, body surface area; PSAX, parasternal short-axis view; aVF, augmented voltage unipolar left foot lead; and aVL, augmented voltage unipolar left arm lead.

Diagnostic terminology for original criteria: This diagnosis is fulfilled by the presence of 2 major, or 1 major plus 2 minor criteria or 4 minor criteria from different groups. Diagnostic terminology for revised criteria: definite diagnosis: 2 major or 1 major and 2 minor criteria or 4 minor from different categories; borderline: 1 major and 1 minor or 3 minor criteria from different categories; possible: 1 major or 2 minor criteria from different categories.

*Hypokinesis is not included in this or subsequent definitions of RV regional wall motion abnormalities for the proposed modified criteria.

†A pathogenic mutation is a DNA alteration associated with ARVC/D that alters or is expected to alter the encoded protein, is unobserved or rare in a large non–ARVC/D control population, and either alters or is predicted to alter the structure or function of the protein or has demonstrated linkage to the disease phenotype in a conclusive pedigree.

From: Marcus FI, et al. Diagnosis of Arrhythmogenic Right Ventricular Cardiomyopathy/Dysplasia: Prosed Modification of the Task Force Criteria. Circulation 2010;121:1533-1541.

2.2 Methodology

2.2.1 Study population and study design

We performed a retrospective analysis of all participants referred with the suspicion of ARVC to the established ARVC Registry of South Africa (HREC: 049/2003) and *IMHOTEP* registry (HREC: 766/2014) between May 2003 and May 2018. Groote Schuur hospital is a multicentre referral point for ARVC in sub-Saharan Africa. Over the study period, 162 participants were referred to the registry from both private and public healthcare institutions in South Africa, with a majority of referrals originating from the Cardiac Clinic at Groote Schuur hospital, Cape Town. Participants of all ages were eligible for inclusion; however, as the diagnostic criteria do not apply to young children, individuals <14 years at the time of diagnostic evaluation were excluded. Participants were also excluded from the analysis if they had insufficient clinical data available for review. Informed consent for partaking in research was taken from participants by the ARVC registry of South Africa and/or *IMHOTEP* investigators at the time of participant recruitment.

2.2.2 Diagnostic evaluation

Original clinical data were gathered for all referred participants and reviewed by the *IMHOTEP* diagnostic panel (DP), comprising of clinicians specialised in clinical cardiology, electrophysiology, imaging (including CMR), pathology, and clinical and molecular genetics at the Cardiac Clinic, Groote Schuur hospital. The DP's primary role was to confirm a diagnosis of ARVC by reviewing the medical history and investigations performed for each participant. To generate consistent data for diagnostic comparison using both the 1994 and 2010 TFC across the cohort, outcomes from imaging modalities such as echocardiograms, CMR and RVA, together with EMBs, and electrophysiological rhythm analysis using ECGs, SAECGs and 24-hour Holter monitoring were analysed. All quantitative studies were performed in a single academic institute by the DP. Due to cost and resource limitations, tissue re-characterisation using histomorphometric tissue analyses, as defined in 2010 TFC, was only

performed on participants where a positive result would affect diagnosis and subsequent management. The DP classified participants according to the original 1994 TFC and revised 2010 TFC, placing each participant into either a *definite* (2 major, or 1 major plus 2 minor, or 4 minor criteria), *borderline* (1 major plus 1 minor, or 3 minor criteria), *possible* (1 major, or 2 minor criteria) or *no criteria* (0 major, or 0 minor criteria) category. A confirmed diagnosis of ARVC for inclusion into *IMHOTEP* by the DP was based on the following criteria:

1. A classification of either *definite* or *borderline* ARVC according to the revised 2010 TFC (current standard of care); **and**
2. The exclusion of alternative pathologies/diagnoses that may mimic ARVC.

For this study, participants were divided into 2 arms, 'DP included' (i.e. confirmed clinical diagnosis of ARVC) and 'DP excluded' (i.e. insufficient criteria to diagnose ARVC - *possible early ARVC,* or alternative diagnosis). Further subdivision of the 'DP included' cohort was made according to genotype status. These were referred to as mutation-positive, mutation-negative and mutation-unknown in the study. Genotype status was provided by the *IMHOTEP* investigators were mutation analysis was done for desmosomal genes encoding plakophilin-2 (*PKP2*), desmoplakin (*DSP*), desmoglein-2 (*DSG2*), desmocollin-2 (*DSC2*), plakoglobin (*JUP*), and non-desmosomal genes encoding phospholamban (*PLN*) and cadherin 2 (*CDH2*) for index patients, and reported at the cardiovascular molecular genetics laboratory, Hatter Institute, UCT (Kraus,S. 2019. Ph.D. Thesis, UCT; Mbele, M. 2014. Ph.D. Thesis, UCT; Fish, M. 2016. Ph.D. Thesis, UCT; Machipisa, T. 2016. M.Phil. Thesis, UCT; Kamuli, S. 2016. M.Phil. Thesis, UCT).[23] The definitions of genotype status are described in Table 2.2.

Table 2.2 Mutation status	
Mutation status	**Definition**
Mutation-positive	The presence of a known pathogenic genetic variant (mutation) defined by the following criteria: 1. associated with ARVC, 2. unobserved or rare in large control populations, and 3. alters or is predicted to alter the structure or function of protein, or linkage to a disease phenotype that has been demonstrated in a conclusive pedigree.[6]
Mutation-negative	The absence of a known pathogenic variant in participants who have undergone molecular genetic analysis.
Mutation-unknown	Participants were the genotype status is unknown as molecular genetic analysis has not yet been performed.

As in our primary objectives, all index cases referred for the diagnosis of ARVC were re-assessed according to both the original 1994 and the revised 2010 TFC (Figure 6 and 7) to compare the yield of the two diagnostic criteria in a sub-Saharan African setting. Our rationale for these objectives is that participants referred to the ARVC Registry prior to 2010 would have been included or excluded based exclusively on the fulfilment of the 1994 TFC. In order to accurately compare the performance of the 1994 versus the 2010 TFC, and minimise clinician interpretation bias, the original diagnostic classifications done by the ARVC registry investigators were not used in this study.

2010 Task Force criteria classification

Definite	Borderline	Possible	No Criteria
2 major, or	1 major plus 1 minor, or	1 major, or	0 major, or
1 major plus 2 minor, or	3 minor criteria	2 minor criteria	0 minor criteria
4 minor criteria			

1994 Task Force criteria classification

Definite	Borderline	Possible	No Criteria
2 major, or	1 major plus 1 minor, or	1 major, or	0 major, or
1 major plus 2 minor, or	3 minor criteria	2 minor criteria	0 minor criteria
4 minor criteria			

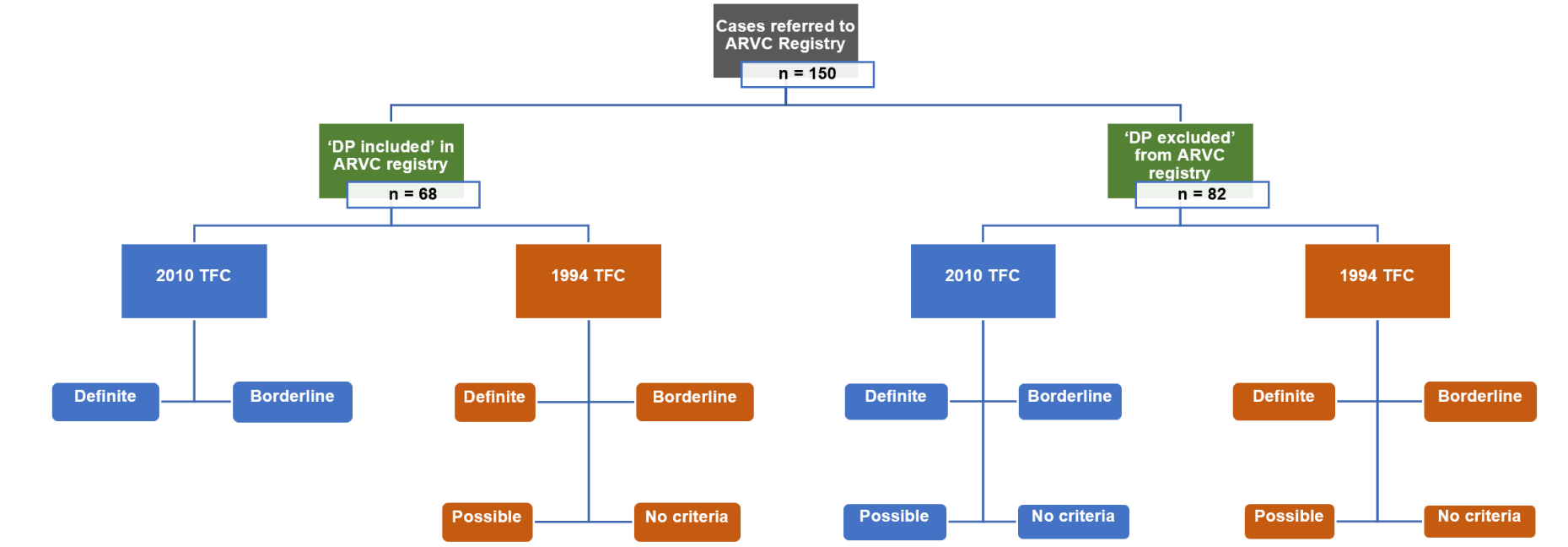


Figure 6. Schematic representation of primary objectives in the ARVC registry

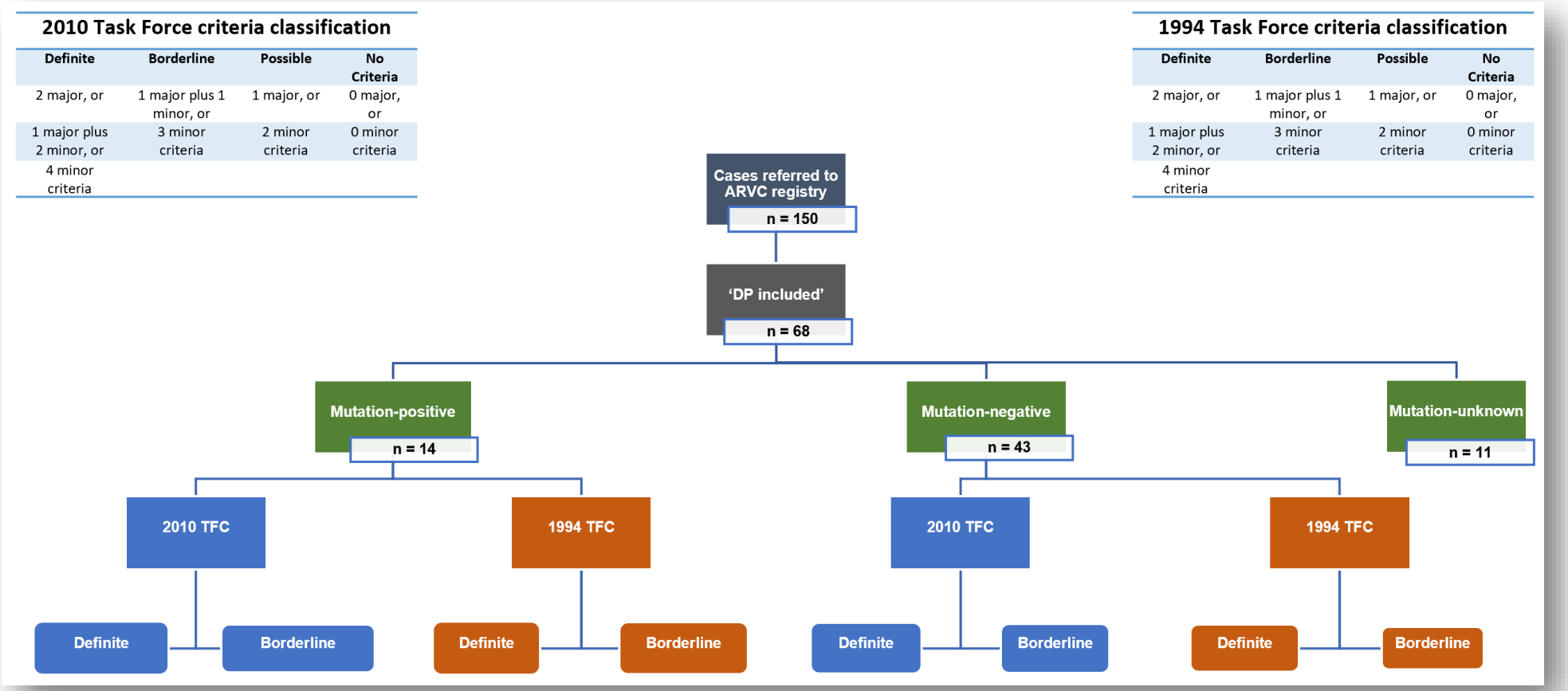


Figure 7. Schematic representation of primary objectives according to mutation status

2.2.3 Data collection

Diagnostic classification of data according to the 1994 and 2010 TFC were captured using prepared case report forms (appendix 1 and 2, respectively) by the DP and subsequently transferred into an electronic database. In addition to diagnostic data, baseline demographics (age at presentation, gender and ethnicity) were collected for each participant at the time of recruitment into the registry or collected from hospital records. Symptoms at presentation, including those who presented after surviving a sudden cardiac arrest and ventricular tachycardia, were also included in the analysis. Data was recorded on an online database, OpenClinica®. Unique study numbers previously assigned by the ARVC registry and/or *IMHOTEP* were used, respecting participant confidentiality.

2.2.4 Ethical considerations

Our study was conducted under the ethics approval (HREC REF: 454/2016) given by the Human Research Ethics Committee (HREC) at our institution (appendix 4) and was registered as a sub-study under the *African Cardiomyopathy and Myocarditis Registry Program: IMHOTEP* (HREC 766/2014) (appendix 3). The ARVC registry of South Africa (HREC 047/2003) was incorporated into *IMHOTEP* at the time of its inception and approved by HREC in October 2014. Our research was grounded by ethics, holding participant safety, rights, and well-being as a priority in keeping with the South African principles of Good Clinical Practice (GCP). Informed consent for partaking in research (including genetics analysis) was provided by participants (or their legal guardian in the case of minors) at the time of recruitment into the ARVC Registry of South Africa and/or *IMHOTEP.*

2.2.5 Safety

Structured as a low-risk retrospective observational study, collected data offered no potential harm to each participant's standard of care. All obtained information complied with the Data Protection Act, 1998. To maintain a chain of confidentiality, participant records were

requested, analysed and returned based on strict security precautions outlined in the study protocol. A unique identifier was assigned to each participant, avoiding capture of identifiable personal information. Results from the TFC classification of participants evaluated by the DP were made available to attending clinicians of participants referred, especially if data held future management implications.

2.2.6 Statistical methods

Statistical analysis was performed using International Business Machines (IBM) Statistical Package for Social Sciences (SPSS) 2017 (Version 25.0, Chicago, USA). Descriptive statistics were used to describe study population data. Categorical data were presented as a number of cases and percentage, and the Chi-squared test was used to determine statistical differences between groups. Continuous data were tested for distribution using histogram for visualisation and Shapiro-Wilks for test of normality. Continuous variables were presented as either mean ± standard deviation (normally distributed data), or median and ± interquartile range (non-normally distributed data). Statistically significant differences between groups were determined using T-test (2 samples) or ANOVA table (more than 2 samples) for normally distributed data, and Wilcoxon sum rank (2 samples) and Kruskal-Wallis (more than 2 samples) for non-normally distributed data. Levene's test was used to verify the equality of variances. The McNemar test was used to determine differences in classification using the 1994 versus the 2010 TFC. A *p*-value of <0.05 was considered to be statistically significant.

Chapter 3 - Results

One hundred and sixty-two participants with suspected ARVC were referred to the ARVC registry of South Africa and/or *IMHOTEP* registry between May 2003 and May 2018. Of those referred, 12 participants were excluded at the outset; 5 were incorrectly classified on the consent form and were not suspected of having ARVC, and a further 7 had insufficient clinical information available to complete either TFC (clinical data unavailable, n=4; histological diagnosis made at postmortem or post-transplant, n=2; infant, n=1). One hundred and fifty participants were included in this study and evaluated by the DP (Figure 8).

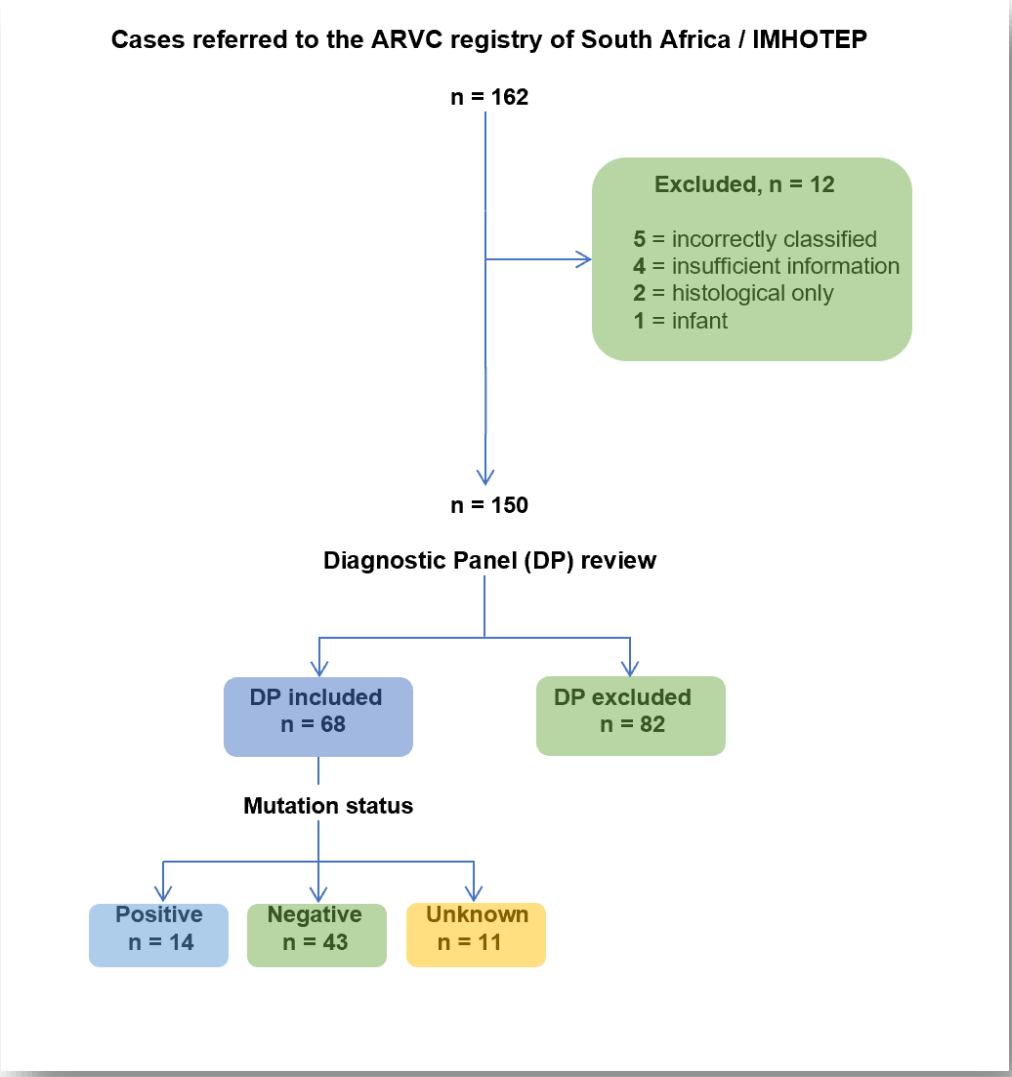


Figure 8. Schematic representation of the ARVC Registry and/or *IMHOTEP*

Of the cohort of 150 unrelated participants, 68 were diagnosed with ARVC by the DP and included into the ARVC registry (i.e. 'DP included'). Of those diagnosed with ARVC, 14 participants were mutation-positive (*PKP2* gene, n=12, *CDH2* gene, n=2), 43 were mutation-negative, and 11 were mutation-unknown. The remaining 82 participants were found to have an alternative diagnose or *possible early ARVC* (i.e. insufficient criteria to confirm ARVC) and were therefore excluded from the ARVC registry by the DP (i.e. 'DP excluded'). Twenty-four percent of those excluded were thought to have *possible early ARVC* with insufficient criteria at baseline and follow-up to confirm a diagnosis. Seventeen percent were found to have different types of cardiomyopathy (dilated cardiomyopathy, n=5; hypertrophic cardiomyopathy, n=5; endomyocardial fibrosis, n=1; left ventricular noncompaction, n=1; and myocarditis, n=2), 14.6% had idiopathic right ventricular outflow tract ventricular tachycardia (RVOT VT), 6.1% had a supraventricular tachycardia, 3.7% had athletes heart, 2.4% had cardiac sarcoidosis, and 14.6% had a non-cardiac diagnosis (Figure 9).

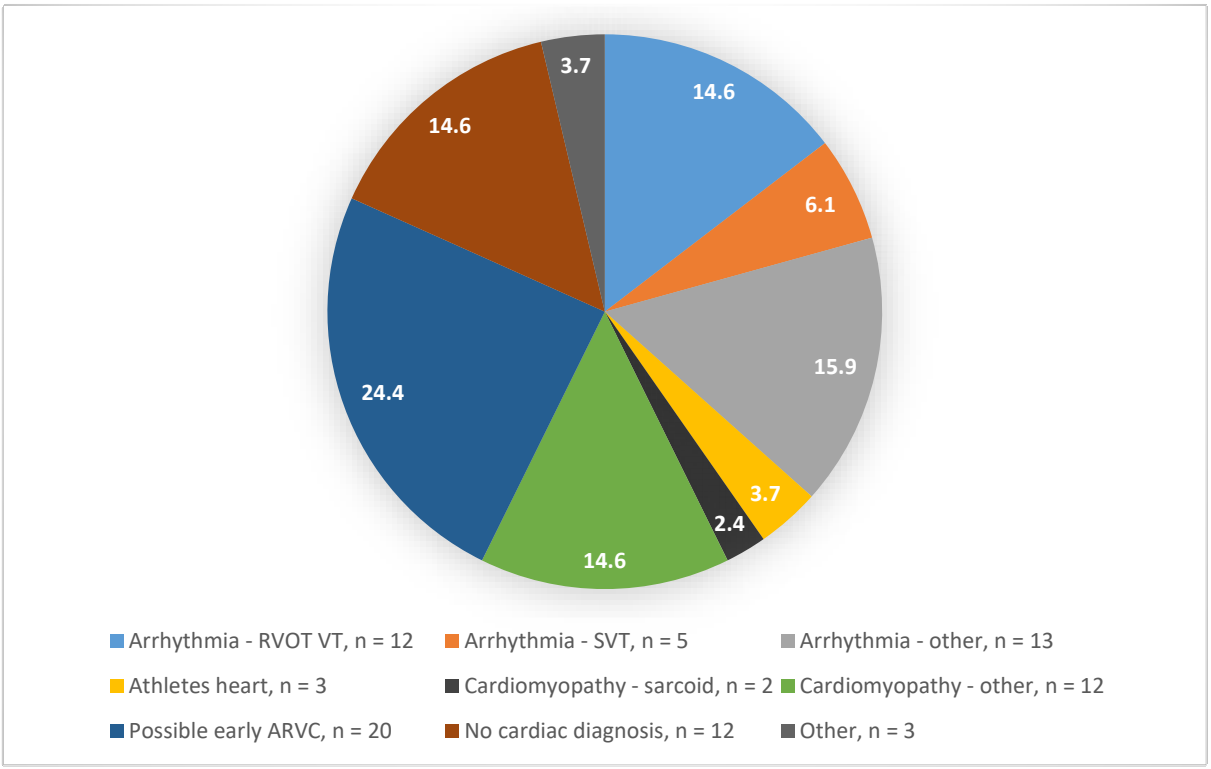


Figure 9. Percentage of participants excluded by the diagnostic panel ('DP excluded').

3.1 Baseline characteristics of all cases referred to the ARVC Registry

The mean age of participants referred was 35.9 ±14.9 years with a male predominance of 64.7%. Participant ethnicity comprised predominantly of Caucasian (55.3%), followed by mixed ancestry (29.3%), black African (10.7%) and Indian (4.7%). Symptoms at presentation, in descending order of frequency, were palpitations (71.3%), presyncope (46%), syncope (34%), chest pain (32.7%) and dyspnoea (20.7%). Very few cases (3.3%) were asymptomatic at the time of referral. There were no significant differences in mean age, gender, ethnicity or symptoms at presentation between the 'DP included' and 'DP excluded' cohort, with the exception of presyncope (55.9% versus 37.8%, p=0.039). There was, however, a significant difference in sustained VT at presentation between these groups; with VT occurring in 54.4% of those in the 'DP included' cohort and only in 31.7% of those in the 'DP excluded' group (p=0.008). There was no difference in the frequency of survived SCD between the groups (8.8% versus 7.3%, p=0.735).

Table 3.1.1. Baseline demographic characteristics of all cases referred to the ARVC Registry				
Participant characteristics	**Cases referred to ARVC registry, n=150 (%)**	**Comparison between**		
		'DP included', n=68 (%)	**'DP excluded', n=82 (%)**	***p*-value***
☺**Age of onset in years**				
Mean (±SD)	35.9 (14.9)	35.6 (13.8)	36.2 (15.8)	0.814
Median (±IQR)	37 (23.8 - 46.0)	38 (25.3 - 44.8)	35 (22.8 - 48.3)	
Male	97 (64.7)	45 (66.2)	52 (63.4)	0.725
Ethnicity				
Caucasian	83 (55.3)	41 (60.3)	42 (51.2)	0.208
Black African	16 (10.7)	5 (7.4)	11 (13.4)	
Mixed ancestry (Coloured)	44 (29.3)	17 (25)	27 (32.9)	
Indian	7 (4.7)	5 (7.4)	2 (2.4)	
Symptoms at presentation				
Palpitation	107 (71.3)	53 (77.9)	54 (65.9)	0.110
Presyncope	69 (46)	38 (55.9)	31 (37.8)	0.039
Syncope	51 (34)	22 (32.4)	29 (35.4)	0.517
Chest pain	49 (32.7)	24 (35.3)	25 (30.5)	0.430
Dyspnoea	31 (20.7)	17 (25.0)	14 (17.1)	0.252
Asymptomatic	5 (3.3)	0	5 (6.1)	0.066
Symptoms unknown	2 (1.3)	1 (1.5)	1 (1.2)	0.894
#VT at presentation	63 (42)	37 (54.4)	26 (31.7)	0.008
Survived SCD	12 (8)	6 (8.8)	6 (7.3)	0.735

SD = standard deviation; IQR = interquartile range.
#VT = sustained ventricular tachyarrhythmias lasting greater than 30 seconds.
**p-value represents the comparison between demographic characteristics in the 'DP included' (n=68) and 'DP excluded' (n=82) cohort.*

☺Age of onset = testing for normality varied between categories; therefore, both mean (±SD), and median (±IQR) are represented. Multiple tests applied (including Skewness, Kurtosis, Shapiro-Wilk, and visualisation with histogram, Q-Q plot and box plots) with varying results. Shapiro-Wilk test for all cases, p= 0.011; 'DP included' p=0.141; 'DP excluded' p=0.025.

When comparing the baseline characteristics of those with ARVC (n=68) according to mutation status (mutation-positive versus mutation-negative), there were no significant differences found in gender, ethnicity or symptoms at presentation between the groups (Table 3.1.2). There was, however, a significant difference in the age of onset between mutation-positive and mutation-negative participants (mean age in years, 28.5 ± 13.96 versus 39.4 ± 12.91, p=0.009). Mutation-positive participants were also more likely to present with VT than those who were mutation-negative (78.6% versus 46.5%, p=0.036).

Table 3.1.2. Baseline demographic characteristics according to mutation status

Participant characteristics	Mutation-positive, n=14 (%)	Mutation-negative, n=43 (%)	Mutation-unknown, n=11 (%)	*p*-value*
Age of onset in years				
Mean (±SD)	28.5 (13.96)	39.4 (12.91)	29.8 (12.64)	0.009
Median (±IQR)	28 (14.5 - 44)	39.0 (32 - 47)	28 (16 - 41)	-
Male	9 (64.3)	28 (65.1)	8 (72.7)	0.955
Ethnicity				
Caucasian	10 (71.4)	23 (53.5)	8 (72.7)	0.536
Black African	1 (7.1)	3 (7)	1 (9.1)	
Mixed ancestry (Coloured)	3 (21.4)	13 (30.2)	1 (9.1)	
Indian	0	4 (9.3)	1 (9.1)	
Symptoms				
Palpitation	11 (78.6)	35 (81.4)	7 (63.6)	0.780
Presyncope	9 (64.3)	21 (48.8)	8 (72.7)	0.548
Syncope	5 (35.7)	14 (52.6)	3 (27.3)	0.836
Chest pain	3 (21.4)	17 (39.5)	4 (36.4)	0.366
Dyspnoea	6 (42.9)	10 (23.3)	1 (9.1)	0.330
Asymptomatic	0	0	0	
Symptoms unknown	0	1 (2.3)	0	
#VT at presentation	11 (78.6)	20 (46.5)	6 (54.5)	0.036
Survived SCD	2 (14.3)	3 (7)	1 (9.1)	0.401

SD = standard deviation; IQR = interquartile range.
**p-value represents the comparison between demographic characteristics in the mutation-positive (n=14) and mutation-negative (n=43) cohort.*
#VT = sustained ventricular tachyarrhythmias lasting greater than 30 seconds.

3.2 Diagnostic classification of all cases referred to the ARVC Registry according to the 2010 and 1994 TFC

When comparing the 2010 and 1994 TFC in the 'DP included' group (n=68); 52 (76.5%) versus 47 (69.1%), [McNemar test, p=0.267] were found to have a *definite* diagnosis of ARVC, and 16 (23.5%) versus 12 (17.6%) [McNemar test, p=0.503] fulfilled *borderline* criteria. Nine participants classified as *definite* or *borderline* ARVC using the 2010 TFC were classified as *possible* (n=8, 11.8%) or *no criteria* (n=1, 1.5%) when using the 1994 TFC, respectively (Table 3.2.1 and 3.2.2; or Figure 10). Comparatively, when using the 2010 versus 1994 TFC in the 'DP excluded' cohort, 4 (4.9%) versus 9 (11%) [McNemar test, p=0.180] participants fulfilled criteria for a *definite* diagnosis, and 3 (3.7%) versus 16 (19.5%) [McNemar test, p=0.002] were classified as *borderline* (Table 3.2.1). Importantly, 7 of the 'DP excluded' cases fulfilling 2010 *definite* and *borderline* TFC were considered 'mimics' with confirmed alternative diagnosis, including hypertrophic cardiomyopathy (n=2), endomyocardial fibrosis (n=1), sarcoidosis (n=2), Brugada syndrome (n=1) and asymptomatic athlete's heart (n=1). Eight cases fulfilling 1994 *definite* and *borderline* TFC were considered to have *possible early ARVC*; however, none of these cases had sufficient 2010 TFC to confirm a diagnosis at baseline or follow-up and were therefore excluded from the registry by the DP.

Table 3.2.1. Summary of both TFC in all cases referred to the ARVC Registry

Task Force Criteria	Cases referred to ARVC registry, n=150 (%)	'DP included', n=68 (%)	'DP excluded', n=82 (%)
1994			
Definite	56 (37.3)	47 (69.1)	9 (11)
Borderline	28 (18.7)	12 (17.6)	16 (19.5)
Possible	40 (26.7)	8 (11.8)	32 (39)
No criteria	26 (17.3)	1 (1.5)	25 (30.5)
2010			
Definite	56 (37.3)	52 (76.5)	4 (4.9)
Borderline	19 (12.7)	16 (23.5)	3 (3.7)
Possible	25 (16.7)	0	25 (30.5)
No criteria	50 (33.3)	0	50 (61)

Table 3.2.2. Comparing the diagnostic yields of the 2010 versus 1994 TFC in the 'DP included' cohort				
	2010 Definite, n=52 (%)	**2010 Borderline, n=16 (%)**	**2010 Possible, n=0 (%)**	**2010 No criteria, n=0 (%)**
1994 Definite, n=47 (%)	43 (82.7)	4 (25)	0	0
1994 Borderline, n=12 (%)	8 (15.4)	4 (25)	0	0
1994 Possible, n=8 (%)	1 (1.9)	7 (43.8)	0	0
1994 No criteria, n=1 (%)	0	1 (6.3)	0	0

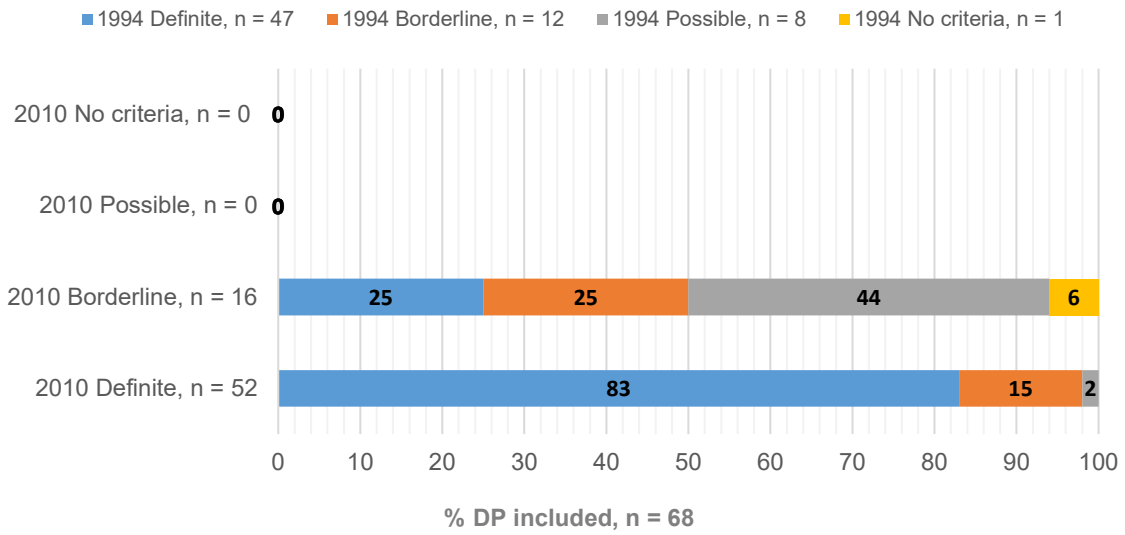


Figure 10. Comparing the diagnostic yields of the 2010 versus 1994 TFC in the 'DP included' cohort in percentage.

We further analysed the utility of the 2010 versus 1994 TFC according to mutation-status. A *definite* diagnosis confirmed by 2010 TFC was seen in all (100%) mutation-positive participants, and only in 72.1% of mutation-negative and 63.6% of mutation-unknown participants. The remaining mutation-negative and mutation-unknown participants fulfilled *borderline* criteria (Table 3.2.4). Comparatively, a *definite* diagnosis according to 1994 TFC was seen in 85.7% (12/14) of mutation-positive participants, with 14.3% (2/14) of mutation-positive participants fulling only *borderline* criteria (Table 3.2.4 or Figure 11). No mutation-positive participants were considered to have a *possible* or a *no criteria* diagnosis by either 2010 or 1994 TFC.

Table 3.2.3. Comparing the diagnostic yields of the 2010 versus 1994 TFC in the mutation-positive cohort				
	2010 Definite, n=14 (%)	**2010 Borderline, n=0 (%)**	**2010 Possible, n=0 (%)**	**2010 No criteria, n=0 (%)**
1994 Definite, n=12 (%)	12 (85.7)	0	0	0
1994 Borderline, n=2 (%)	2 (14.3)	0	0	0
1994 Possible, n=0 (%)	0	0	0	0
1994 No criteria, n=0 (%)	0	0	0	0

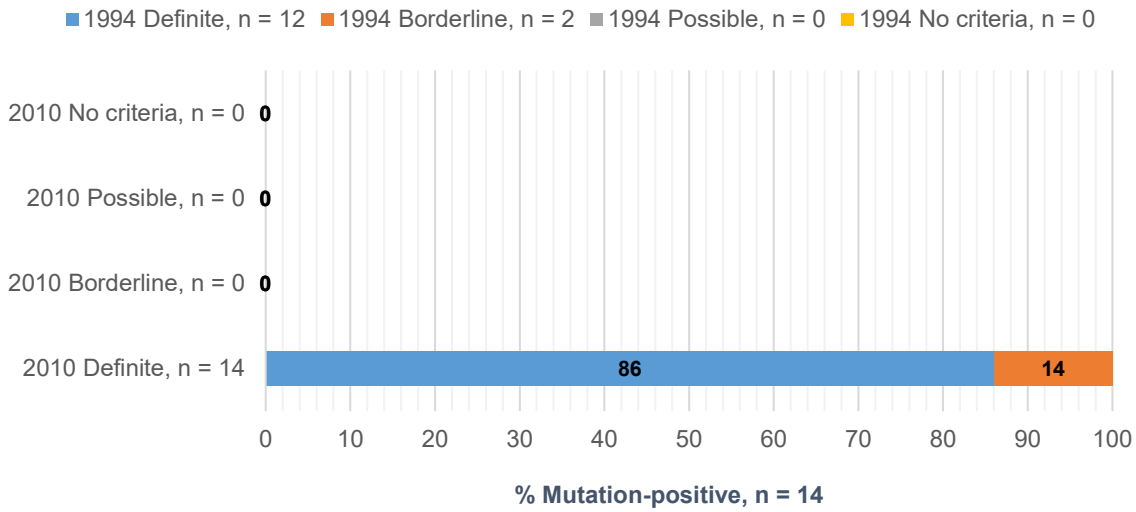


Figure 11. Comparing the diagnostic yields of the 2010 versus 1994 TFC in the mutation-positive cohort in percentage.

When comparing the mean number of major and minor criteria according to mutation status (Table 3.2.4), we found a significant difference in the number of 2010 major criteria between mutation-positive and mutation-negative groups. A statistically significant difference was noted even with the exclusion of gene mutation as a criterion (2.50 ±0.855 versus 1.74 ±0.848, p=0.005). A summary of the specific criteria according to mutation status is summarised in Table 3.2.7.

Table 3.2.4. Summary of the diagnostic yield from each TFC according to mutation status					
Task Force Criteria	**'DP included', n=68 (%)**	**Mutation-positive, n=14 (%)**	**Mutation-negative, n=43 (%)**	**Mutation unknown, n=11 (%)**	***p*-value***
1994					
Mean major criteria (±SD)	1.00 (±0.810)	1.29 (±0.825)	0.88 (±0.762)	1.09 (±0.944)	0.092
Mean minor criteria (±SD)	2.22 (±0.770)	2.57 (±0.938)	2.16 (±0.721)	2.0 (±0.632)	0.092
Definite	47 (69.1)	12 (85.7)	27 (62.8)	8 (72.7)	
Borderline	12 (17.6)	2 (14.3)	9 (20.9)	1 (9.1)	
Possible	8 (11.8)	0	6 (14)	2 (18.2)	
No criteria	1 (1.5)	0	1 (2.3)	0	
2010					
Mean major criteria (±SD) (mutation included)	2.10 (±1.161)	3.50 (±0.855)	1.74 (±0.848)	1.73 (±1.272)	<0.001
Mean major criteria (±SD) (mutation excluded)	1.90 (±0.964)	2.50 (±0.855)	1.74 (±0.848)	1.73 (±1.272)	0.005
Mean minor criteria (±SD)	1.16 (±0.784)	1.07 (±0.829)	1.19 (±0.699)	1.18 (±1.079)	0.596
Definite	52 (76.5)	14 (100)	31 (72.1)	7 (63.6)	
Borderline	16 (23.5)	0	12 (27.9)	4 (36.4)	
Possible	0	0	0	0	
No criteria	0	0	0	0	

SD = standard deviation
**p-value represents the comparison between the diagnostic yield of each TFC in the mutation-positive (n=14) and mutation-negative (n=43) cohort.*

Tables 3.2.5 and 3.2.6 describe the specific criteria fulfilled by participants in both 'DP included' (n=68) and 'DP excluded' (n=82) groups. When comparing specific criteria of the 2010 versus 1994 TFC; there was notable variability in the yield of various major and minor criteria (illustrated in Figure 12 and 13). The variations illustrated were attributed to:

1) the inclusion of quantifiable criteria in the 2010 TFC for structural abnormalities (in both echocardiography and CMR) and tissue characterisation (EMB);
2) the distinction between major and minor repolarisation and arrhythmia criteria; **and**
3) the inclusion of molecular genetic results in the 2010 TFC.

Table 3.2.5. 2010 Task Force criteria in all cases referred to the ARVC registry

	'DP included', n=68					'DP excluded', n=82				
	All, n=68 (%)	**Definite, n=52 (%)**	**Borderline, n=16 (%)**	**Possible, n=0 (%)**	**No criteria, n=0 (%)**	**All, n=82 (%)**	**Definite, n=4 (%)**	**Borderline, n=3 (%)**	**Possible, n=25 (%)**	**No criteria, n=50 (%)**
Structural abnormalities										
Structural major	35 (51.5)	34 (65.4)	1 (6.3)	-	-	5 (6.1)	2 (50)	0	3 (12)	0
Structural minor	1 (1.5)	1 (1.9)	0	-	-	1 (1.2)	0	0	1 (4)	0
Unknown - No imaging	6 (8.8)	3 (3.8)	3 (18.8)	-	-	1 (1.2)	0	0	0	1 (2)
Tissue characteristics										
Major	7 (10.3)	7 (13.5)	0	-	-	1 (1.2)	1 (25)	0	0	0
Minor	5 (7.4)	4 (7.4)	1 (6.3)	-	-	3 (3.7)	0	0	2 (8)	1 (2)
FFR[#] not quantified	8 (11.8)	7 (13.5)	1 (6.3)	-	-	4 (4.9)	0	0	0	4 (8)
Unknown - EMB not done	32 (47.1)	24 (46.2)	8 (50)	-	-	26 (31.7)	1 (25)	1 (33.3)	7 (28)	17 (34)
Repolarisation abnormalities										
Repolarisation major	50 (73.5)	41 (78.8)	9 (56.3)	-	-	9 (11)	2 (50)	3 (100)	4 (16)	0
Repolarisation minor	9 (13.2)	3 (5.8)	6 (37.5)	-	-	11 (13.4)	1 (25)	0	5 (20)	5 (10)
Unknown	0	0	0	-	-	1 (1.2)	0	0	0	1 (2)
Depolarisation abnormalities										
Depolarisation major	4 (5.9)	4 (7.7)	0	-	-	0	0	0	0	0
Depolarisation minor	36 (52.9)	31 (59.6)	5 (31.3)	-	-	26 (31.7)	2 (50)	3 (100)	10 (40)	11 (22)
Unknown	0	0	0	-	-	1 (1.2)	0	0	0	1 (2)
Arrhythmia										
Arrhythmia major	32 (47.1)	31 (59.6)	1 (6.3)	-	-	6 (7.3)	3 (75)	0	3 (12)	0
Arrhythmia minor	28 (41.2)	14 (26.9)	14 (87.5)	-	-	30 (36.6)	1 (25)	0	10 (40)	19 (38)
Family history										
Family history major - excl. mutation	2 (2.9)	2 (3.8)	0	-	-	1 (1.2)	0	0	1 (4)	0
Family history major - incl. mutation	15 (22.1)	15 (28.8)	0	-	-	0	0	0	0	0
Family history minor	2 (2.9)	2 (3.8)	0	-	-	0	0	0	0	0

[#]*FFR = Fibrofatty replacement; EMB = Endomyocardial biopsy*

Table 3.2.6. 1994 Task Force criteria in all cases referred to the ARVC registry										
	‘DP included’, n=68					‘DP excluded’, n=82				
	All, n=68 (%)	**Definite, n=47 (%)**	**Borderline, n=12 (%)**	**Possible, n=8 (%)**	**No criteria, n=1 (%)**	**All, n=82 (%)**	**Definite, n=9 (%)**	**Borderline, n= 16 (%)**	**Possible, n=32 (%)**	**No criteria, n=25 (%)**
Structural abnormalities										
Structural major	37 (54.4)	32 (68.1)	5 (41.7)	0	0	7 (8.5)	3 (33.3)	2 (12.5)	2 (6.3)	0
Structural minor	18 (26.5)	11 (23.4)	6 (50)	1 (12.5)	0	49 (59.8)	6 (66.7)	11 (68.8)	24 (75)	8 (32)
Unknown - No Imaging	6 (8.8)	3 (6.4)	0	2 (25.0)	1 (100)	1 (1.2)	0	0	0	1 (4)
Tissue characterisation										
Major	18 (26.5)	18 (38.3)	0	0	0	17 (20.7)	5 (55.6)	9 (56.3)	3 (9.4)	0
Unknown - EMB not done	32 (47.1)	21 (44.7)	6 (50)	4 (50)	1 (100)	26 (31.7)	1 (11.1)	3 (18.8)	12 (37.5)	10 (40)
Repolarisation abnormalities										
Repolarisation minor	50 (73.5)	37 (78.7)	7 (58.3)	6 (75)	0	11 (13.4)	4 (44.4)	2 (12.5)	2 (6.3)	3 (12)
Unknown	0	0	0	0	0	1 (1.2)	0	0	0	1 (4)
Depolarisation abnormalities										
Depolarisation major	10 (14.7)	10 (21.3)	0	0	0	5 (6.1)	2 (22.2)	1 (6.3)	2 (6.3)	0
Depolarisation minor	19 (27.9)	15 (31.9)	2 (16.7)	2 (25)	0	19 (23.2)	4 (44.4)	5 (31.3)	7 (21.9)	3 (12)
Unknown	0	0	0	0	0	1 (1.2)	0	0	0	1 (4)
Arrhythmia minor	60 (88.2)	41 (87.2)	11 (91.7)	7 (87.5)	1 (100)	34 (41.5)	6 (66.7)	6 (37.5)	15 (46.9)	7 (28)
Family history										
Family history major	3 (4.4)	3 (6.4)	0	0	0	1 (1.2)	0	0	1 (3.1)	0
Family history minor	2 (2.9)	2 (4.3)	0	0	0	0	0	0	0	0

EMB = endomyocardial biopsy

Table 3.2.7. Summary of both Task Force criteria according to mutation status				
Task Force criteria	**'DP included', n=68 (%)**	**Mutation-positive, n=14 (%)**	**Mutation-negative, n=43 (%)**	**Mutation unknown, n=11 (%)**
2010				
Structural abnormalities				
Major criteria	35 (51.5)	11 (78.6)	19 (44.2)	5 (45.5)
Minor criteria	1 (1.5)	0	1 (2.3)	0
Imaging not done	6 (8.8)	0	5 (11.6)	1 (9.1)
Tissue characteristics				
Major criteria	7 (10.3)	2 (14.3)	4 (9.3)	1 (9.1)
Minor criteria	5 (7.4)	0	4 (9.30	1 (9.1)
FFR* not quantified	8 (11.8)	3 (21.4)	5 (11.6)	0
EMB not done	32 (47.1)	5 (35.7)	22 (51.2)	5 (45.5)
Repolarisation abnormalities				
Major criteria	50 (73.5)	12 (85.7)	32 (74.4)	6 (54.5)
Minor criteria	9 (13.2)	1 (7.1)	5 (11.6)	3 (27.3)
Depolarisation abnormalities				
Major criteria	4 (5.9)	1 (7.1)	2 (4.7)	1 (9.1)
Minor criteria	36 (52.9)	9 (64.3)	22 (51.2)	5 (45.5)
Arrhythmia				
Major criteria	32 (47.1)	9 (64.3)	17 (39.5)	6 (54.5)
Minor criteria	28 (41.2)	5 (35.7)	19 (44.2)	4 (36.4)
1994				
Structural abnormalities				
Major criteria	37 (54.4)	11 (78.6)	21 (48.8)	5 (45.5)
Minor criteria	18 (26.5)	2 (14.3)	12 (27.9)	4 (36.4)
Imaging not done	6 (8.8)	0	5 (11.6)	1 (9.1)
Tissue characteristics				
Major criteria	18 (26.5)	4 (28.6)	12 (27.9)	2 (18.2)
EMB not done	32 (47.1)	5 (35.7)	22 (51.2)	5 (45.5)
Repolarisation abnormalities				
Minor criteria	50 (73.5)	12 (85.7)	32 (74.4)	6 (54.5)
Depolarisation abnormalities				
Major criteria	10 (14.7)	1 (7.1)	4 (9.3)	5 (45.5)
Minor criteria	19 (27.9)	5 (35.7)	12 (27.9)	2 (18.2)
Arrhythmia				
Minor criteria	60 (88.2)	14 (100)	36 (83.7)	10 (90.9)
Family History				
Major criteria	3 (4.4)	2 (14.3)	1 (2.3)	0
Minor criteria	2 (2.9)	1 (7.1)	1 (2.3)	0

**FFR = fibrofatty replacement; EMB = endomyocardial biopsy*

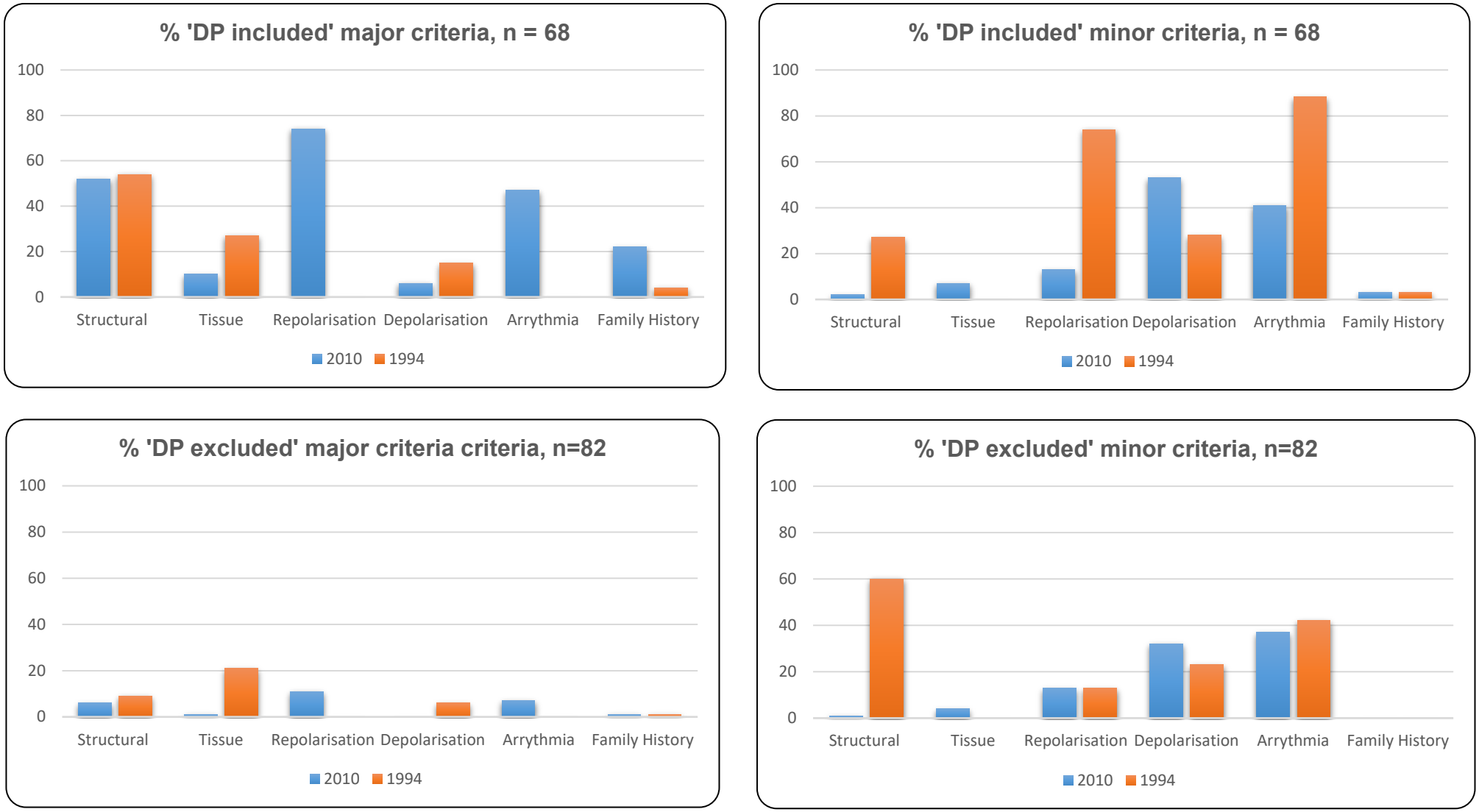
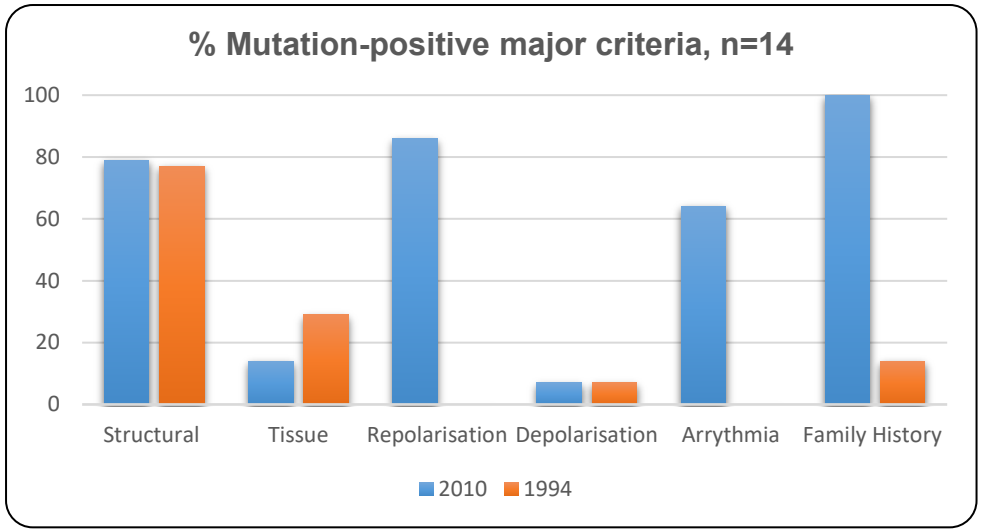
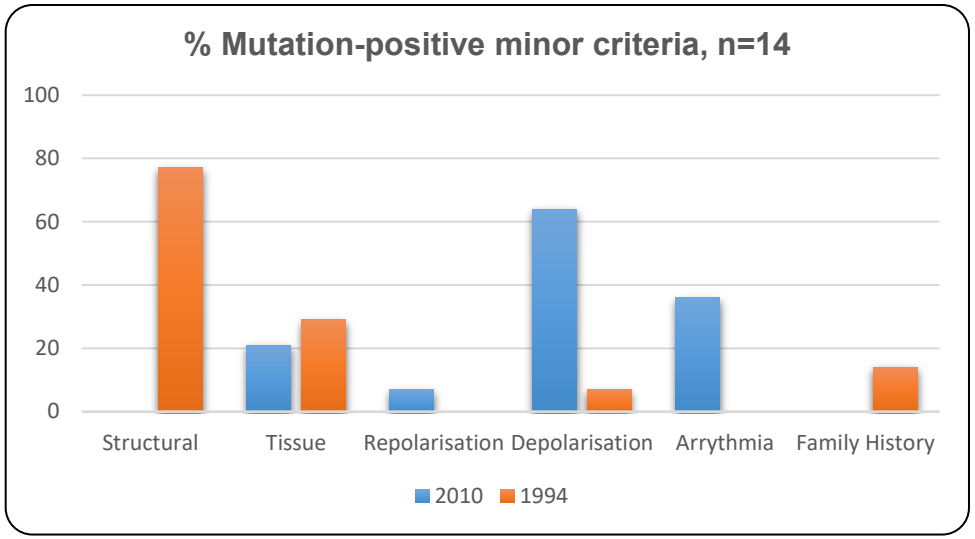
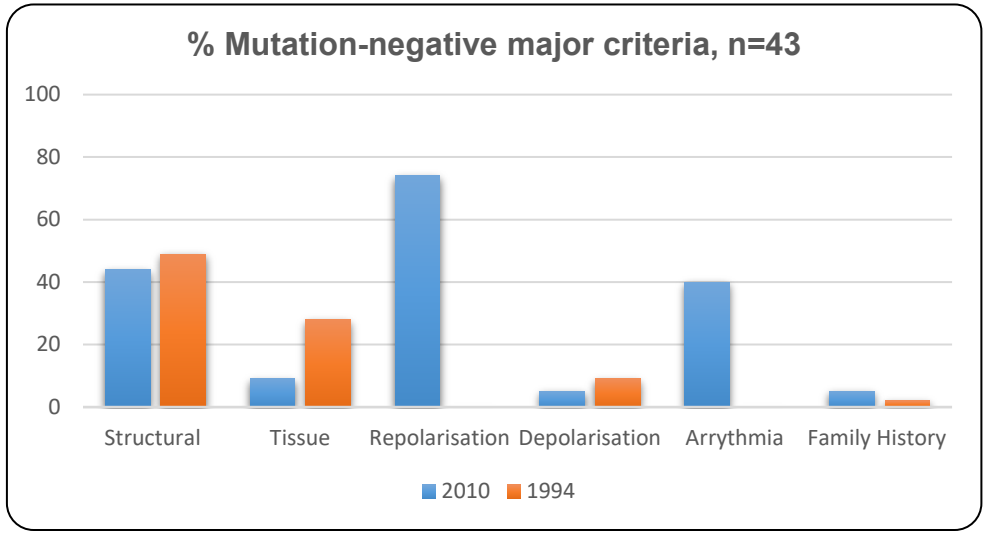


Figure 12. Comparing the diagnostic yields of the 2010 versus 1994 TFC major and minor criteria in cases referred to the ARVC registry.







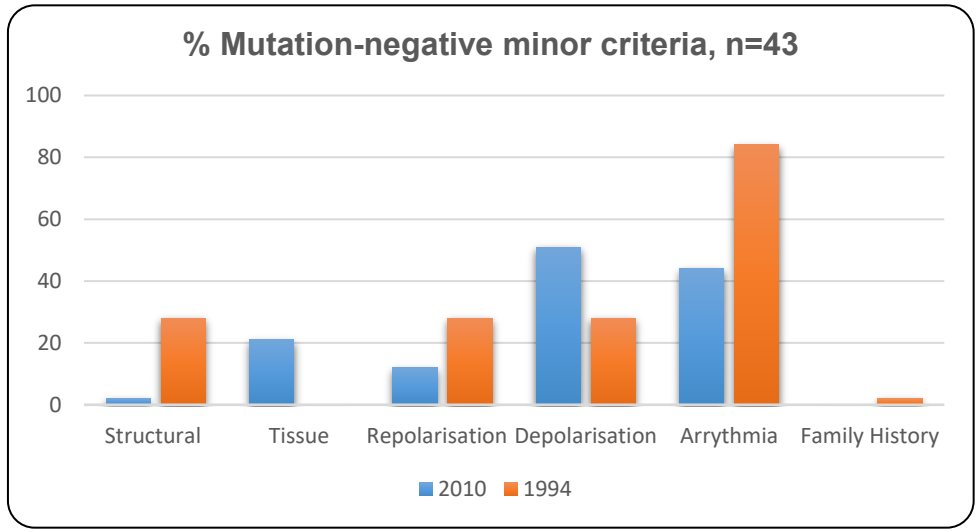


Figure 13. Comparing diagnostic yields of the 2010 versus 1994 major and minor criteria according to mutation-positive and mutation-negative status.

3.3 The outcome of diagnostic modalities using the 2010 TFC in cases referred to the ARVC registry

The TFC utilise information obtained from a spectrum of at least seven diagnostic modalities, excluding DNA analysis, to help make a diagnosis of ARVC. In the referred cohort (n=150) and using the 2010 TFC, there was no significant difference in the number of diagnostic modalities utilised in the workup of participants in either the 'DP included' or 'DP excluded' groups, with the exception for CMR (42.6% versus 63.4%, p=0.011) and echocardiography (86.8% versus 96.3%, p=0.031) (Table 3.3.1 and Figure 14).

Table 3.3.1. Summary of diagnostic modalities performed using the 2010 TFC in cases referred to the ARVC Registry

	Cases referred to ARVC registry, n=150 (%)	'DP included', n=68 (%)	'DP excluded', n=82 (%)	*p*-value*
Echocardiogram	138 (92)	59 (86.8)	79 (96.3)	0.031
CMR	81 (54)	29 (42.6)	52 (63.4)	0.011
RVA	95 (63.3)	44 (64.7)	51 (62.2)	0.751
ECG	150 (100)	68 (100)	82 (100)	-
SAECG	109 (72.7)	48 (70.6)	61 (74.4)	0.603
24-hour Holter	62 (41.3)	28 (41.2)	34 (41.5)	0.972
EMB	92 (61.3)	36 (52.9)	56 (68.3)	0.055

CMR = cardiovascular magnetic resonance; RVA = right ventricular angiography; SAECG= Signal-averaged electrocardiogram; EMB = endomyocardial biopsy.
**p-value represents the comparison between each diagnostic modality in 'DP included' (n=68) and 'DP excluded' (n=82) cohort.*

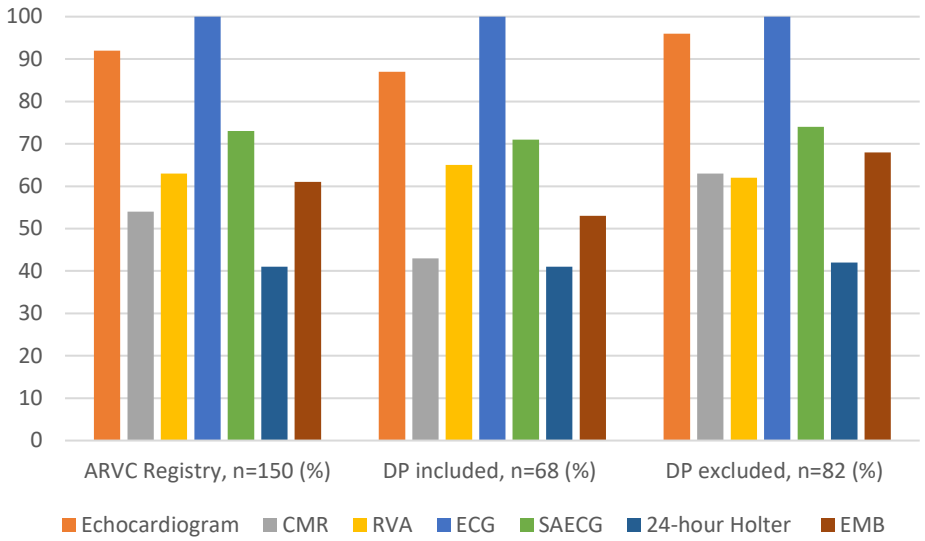


Figure 14. Percentage of diagnostic modalities performed using the 2010 TFC in cases referred to the ARVC Registry.

We also analysed the number of diagnostic modalities performed according to mutation status and found no statistically significant differences between both the mutation-positive and mutation-negative groups (Table 3.3.2 and Figure 15).

Table 3.3.2. Summary of diagnostic modalities performed using the 2010 TFC according to mutation status

	Mutation-positive, n=14 (%)	Mutation-negative, n=43 (%)	Mutation-unknown, n=11 (%)	*p*-value*
Echocardiogram	13 (92.9)	36 (83.7)	10 (90.9)	0.393
CMR	5 (35.7)	19 (44.2)	5 (45.5)	0.577
RVA	10 (71.4)	26 (60.5)	8 (72.7)	0.460
ECG	14 (100)	43 (100)	11 (100)	-
SAECG	11 (78.6)	30 (69.8)	7 (63.6)	0.524
24-hour Holter	7 (50)	16 (37.2)	5 (45.5)	0.397
EMB	9 (64.3)	21 (48.8)	6 (54.5)	0.315

CMR = cardiovascular magnetic resonance; RVA = right ventricular angiography; SAECG= Signal-averaged electrocardiogram; EMB = endomyocardial biopsy.
**p-value represents the comparison between each diagnostic modality in mutation-positive (n=14), and mutation-negative (n=43) cohort.*

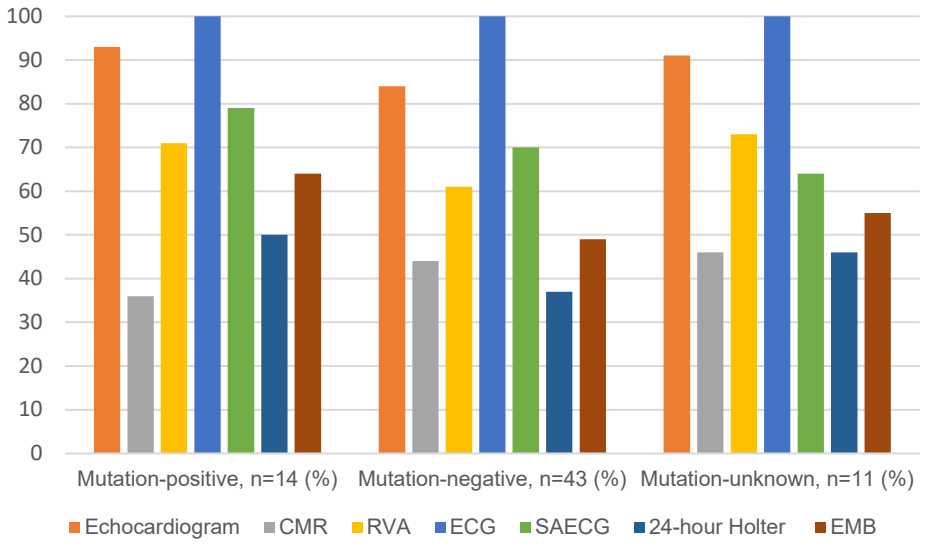


Figure 15. Percentage of diagnostic modalities performed using the 2010 TFC according to mutation status.

3.4 Performance of diagnostic modalities using the 2010 versus 1994 TFC at diagnosing mutation-positive *definite* probands

We reported on the independent performance of frequently utilised diagnostic modalities in mutation-positive *definite* participants using the 2010 and 1994 TFC (Figure 16 and 17). Modalities fulfilling structural imaging and tissue characterisation were seen less frequently when using the stricter quantified 2010 TFC. Echocardiography, when comparing the 2010 versus 1994 TFC, satisfied only half the number of positive criteria (23.1% versus 46.2%). A similar finding was appreciated when endomyocardial biopsy was compared (22.2% versus 44.4%). Remarkably, only CMR provided a complete set of positive criteria (100%) using both TFC (p=0.021). Repolarisation abnormalities contributed positive diagnostic criteria in 85.7% of cases using the 2010 TFC, while depolarisation abnormalities including epsilon waves only provided positive criteria in 7.1% of cases when using either TFC.

Table 3.3.3. Summary of positive criteria fulfilled by specific diagnostic modalities using both TFC according to mutation status												
	Echocardiography		**CMR**		**RVA**		**EMB**		**ECG Repolarisation**		**ECG Depolarisation**	
Major criteria	**2010 n (%)**	**1994 n (%)**	**2010 n (%)**	**1994 n (%)**	**2010 n (%)**	**1994 n (%)**	**2010 n (%)**	**1994 n (%)**	**2010 n (%)**	**1994**	**2010 n (%)**	**1994 n (%)**
Mutation-positive, n=14	3/13 (23.1)	6/13 (46.2)	5/5 (100)	5/5 (100)	6/10 (60)	7/10 (70)	2/9 (22.2)	4/9 (44.4)	12/14 (85.7)	-	1 (7.1)	1 (7.1)
Mutation-negative, n=43	4/36 (11.1)	7/36 (19.4)	6/19 (31.6)	8/19 (42.1)	14/26 (53.8)	15/26 (57.7)	4/21 (19)	12/21 (57.1)	31/43 (74.4)	-	2 (4.7)	4 (9.3)
Mutation-unknown, n=11	1/10 (10)	2/10 (20)	3/5 (60)	3/5 (60)	3/8 (37.5)	4/8 (50)	1/6 (16.7)	2/6 (33.3)	6/11 (54.5)	-	1 (9.1)	5 (45.5)
p*-value	0.523	0.151	0.021	0.066	0.617	0.675	0.963	0.547	0.210	-	0.834	0.007

CMR = cardiovascular magnetic resonance; RVA = right ventricular angiography; EMB = endomyocardial biopsy.
**p-value represents the comparison of positive criteria contributed by each diagnostic modality in the mutation-positive cohort (n=14) using the 2010 and 1994 TFC.*

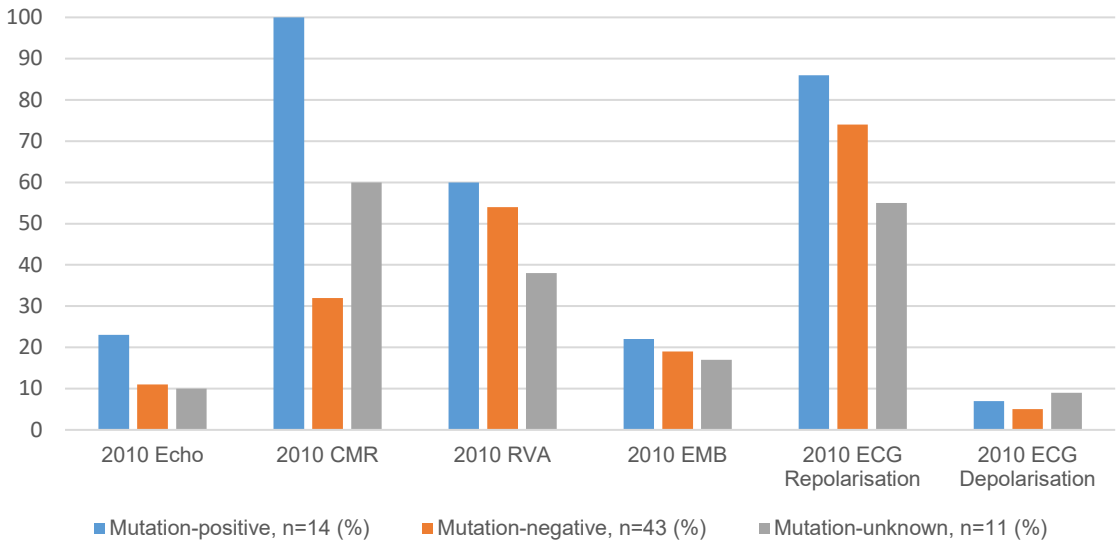


Figure 16. Percentage of positive criteria fulfilled by specific diagnostic modalities using the 2010 TFC according to mutation status.

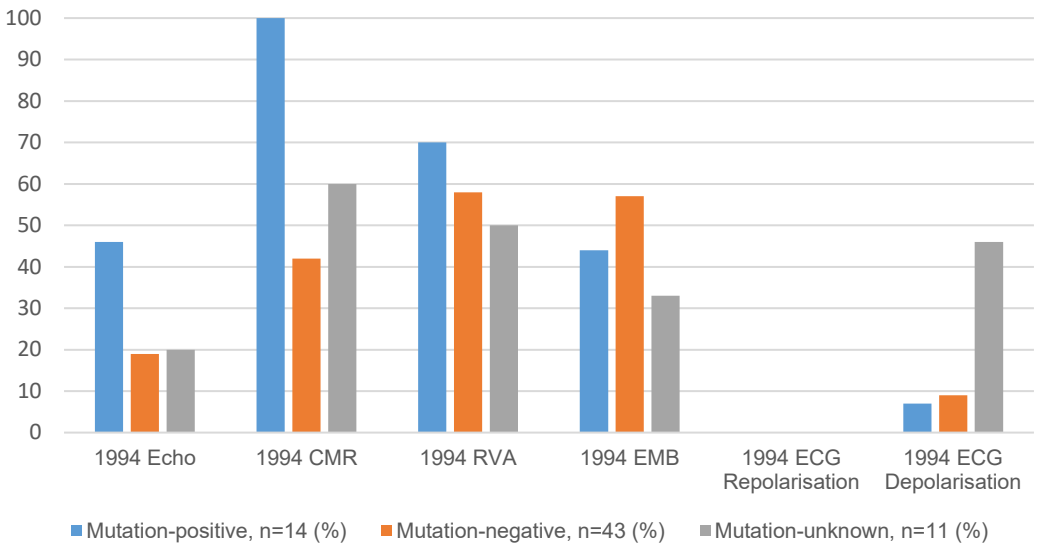


Figure 17. Percentage of positive criteria fulfilled by specific diagnostic modalities using the 1994 TFC according to mutation status.

Chapter 4 - Discussion

In this study, we report a comparison between the original 1994 and revised 2010 TFC in diagnosing ARVC, and the utility of the criteria in identifying mutation-positive probands. One hundred and fifty participants were evaluated by the DP; 68 were diagnosed with ARVC and included into the ARVC registry ('DP included') within the *IMHOTEP* study, and 82 participants were excluded ('DP excluded') with alternative diagnosis or insufficient criteria. There were no differences in gender, ethnicity or symptoms at presentation between the 'DP included' and 'DP excluded' groups with the exception of presyncope. Importantly, both groups were investigated similarly with one exception; imaging modalities such as CMR and echocardiography were more frequently performed in the 'DP excluded' group compared to the 'DP included' group. These observations infer that all cases in the 'DP excluded' group were adequately investigated and were not excluded due to incomplete clinical work-up. Furthermore, an alternative diagnosis was made in the majority of excluded cases, and no pathogenic genetic variants were found in the participants screened. The mean age of participants referred (n=150) was 35.9 ± 14.9 years, with no substantial differences found between the 'DP included' and 'DP excluded' groups. A statistically significant difference was noted in the mean age of onset between mutation-positive and mutation-negative probands, suggesting the earlier onset of ARVC in those with a pathogenic genetic variant. Sustained VT at presentation occurred in more than half of those with ARVC ('DP included') and less than a third of those in the 'DP excluded' cohort. Mutation-positive participants were more frequently found to present with VT compared to those in the mutation-negative group. Our study found young Caucasian males presenting with sustained VT were more likely to have a pathogenic genetic variant of ARVC.

International comparison

Large trans-Atlantic studies have described mutation-positive participants as earlier presenters of ARVC (mean age of onset, 34 ±14 years) when compared to mutation-negative individuals.[57] In our study, representing cases from SSA, mutation-positive participants were found to present significantly younger (mean age of onset, 28.5 ±13.96 years) than those described internationally. Our study also identified a greater predominance of sustained VT at presentation in our mutation-positive group compared to the global north (78.6% versus 52.0%).[57] Participants in our study contributed a similar number of positive 2010 TFC as described in international cohorts.[57] The overall number of diagnostic modalities responsible for fulfilling imaging criteria were also found to be similar, however, our cohort had a higher trend towards invasive modalities.[57] These findings are in keeping with investigative strategies prior to the arrival of CMR in SSA. After analysing each diagnostic modality's ability at contributing major 2010 TFC in mutation-positive *definite* participants, we found the addition of CMR statistically significant and a preferred imaging tool when screening for ARVC.

Improvement of diagnosis

Our study emphasised the importance of re-classifying all participants previously referred and classified using outdated criteria (i.e. 1994), with updated (i.e. 2010) TFC in a traditional ARVC registry. Of the 52 participants classified as 2010 TFC *definite* in our 'DP included' cohort, only 83% comprised of 1994 *definite* cases, while 15% represented previously classified 1994 *borderline* cases. Of the 16 participants classified with a 2010 TFC *borderline* diagnosis ('DP included' cohort), a quarter originated from cases previously diagnosed as 1994 *definite*, while only 25% remained unchanged with a 1994 *borderline* diagnosis. A significant majority of the 2010 *borderline* cases originated from cases previously classified as 1994 *possible*, and interestingly a further 6% arose from cases that occupied *no criteria* according to the 1994 TFC. The most striking reasons for diagnostic change were found in 3 main categories: 1) the appreciation of precordial T-wave inversion as major criteria; 2) morphometric analysis of

myocardial biopsies quantifying fibro-fatty involvement in keeping with ARVC pathology, and; 3) the recognition of VT with a superior axis as a major criterion of ARVC. The overall diagnostic yield of the 2010 versus 1994 TFC revealed more participants with a *definite* diagnosis, and less featuring in *possible* and *no criteria* categories. A considerable upgrade (66.7%) in diagnosis from 1994 *borderline* to 2010 *definite* was observed, with an 8.5% downgrade from 1994 *definite* to 2010 *borderline*. One of the main reasons for a downgrade in diagnosis was seen after the removal of wide QRS complexes as a 1994 TF major depolarisation criterion. A further 12.5% of cases previously classified as 1994 *possible* were re-classified as having a 2010 *definite* diagnosis. Changes in diagnostic classification have significant implications when risk stratifying patients for future management and were mainly seen in individuals recruited prior to 2010. Our 'DP excluded' cohort revealed fewer participants as *definite* (4.9% versus 11%), and *borderline* ARVC (3.75% versus 19.5%), and more cases as *no criteria* (61% versus 30.5%), when using the 2010 versus 1994 TFC. The utility of the 2010 and 1994 TFC strictly according to mutation-positive status found a higher yield for *definite* ARVC, and led to a complete change in diagnosis from 1994 *borderline* to 2010 *definite*.

Application of Task Force criteria

Our study found the total number of major clinical criteria using the 2010 TFC as a predictive marker in identifying mutation-positive individuals with ARVC even with the exclusion of genotype as a criterion (2.50 ±0.855 versus 1.74 ±0.848, p=0.005). We identified similar patterns in the distribution of major criteria across the 'DP included' and mutation-positive cohorts when using both the 2010 and 1994 TFC. In keeping with international literature, we found a greater fulfilment of 2010 TF repolarisation criteria, with fewer fulfilment of depolarisation and tissue characterisation criteria. These findings beautifully demonstrate the shift in diagnostic weight between each TFC with the 2010 TFC placing more emphasis on repolarisation abnormalities over previously favoured depolarisation and histological

abnormalities. The quantification driven 2010 TFC was further highlighted when fewer tissue characterisation and structural imaging criteria were fulfilled in the 'DP excluded' group, emphasising the improved diagnostic accuracy of 2010 TFC at identifying and excluding mimics. The 1994 TFC contributed an overall greater amount of structural imaging criteria, a common late finding in ARVC. Comparatively, the 2010 TFC contributed a higher amount of repolarisation abnormalities, an established sensitive marker for early disease ARVC. Our study successfully compared the utility of the original 1994 TFC to the revised 2010 TFC at diagnosing mutation-positive probands with ARVC and demonstrated a new shift in clinical focus towards diagnosing early ARVC.

Conclusion

Our study found that mutation-positive probands were found to be younger, more likely to present with sustained VT, fulfilled a significantly larger number of major 2010 TFC than mutation-negative probands, and that the 2010 TFC for structural and repolarisation abnormalities were more useful in diagnosing ARVC compared to 1994 TFC. We found a significant evolution in classification between both TFC, suggesting that re-classification of participants recruited in traditional ARVC registries according to updated criteria is worthwhile.

Study Limitations

Our study was limited by its retrospective design. In addition, ARVC is a rare disease requiring sophisticated investigations and clinical expertise that are not readily available at all levels of care in sub-Saharan Africa. As a consequence, the number of participants with ARVC in this study were relatively small, particularly in the mutation-positive group. Due to the small number of mutation-positive participants and the fact that mutation-negative individuals may habour a undiscovered pathogenic variant, the statistical analysis should be interpreted with some

caution. Furthermore, the majority of participants were recruited at a tertiary centre with notable historical referral bias; therefore, it is likely that this cohort represents a more severe spectrum of disease and not truly representative of the population. As this data was collected for the purposes of a registry, each participant was not investigated with all diagnostic modalities, which is a reflection of choices made in daily clinical practise in a resource-restricted environment. As ARVC cannot be diagnosed by a single diagnostic modality and disease expression varies between individual patients, there was a large variation in clinical criteria used to establish a diagnosis of ARVC in this cohort. These factors have limited our ability to make more precise deductions about the sensitivity and specificity both the TFC and individual diagnostic modalities have, therefore, have not been included in the analysis. Despite these limitations, this study has highlighted important insights into the utility of the diagnostic criteria in our local context. Our study has generated opportunities for future research in the clinical outcomes of family members of probands with ARVC in sub-Saharan Africa.

Source of Funding

There was no designated funding for this sub-study; however, the parent study, *IMHOTEP*, is funded by the Newton Fund (Medical Research Council of South Africa, MRCSA; and GlaxoSmithKline, GSK).

Disclosures

None.

References

1. Corrado D, Link MS CH. Arrhythmogenic Right Ventricular Cardiomyopathy. New Engl J Med. 2017;376:61–72.
2. Thiene G, Marcus F. Arrhythmogenic cardiomyopathy: A biventricular disease in search of a cure. Hear Rhythm. 2013;10(2):290–1.
3. Ruwald AC, Marcus F, Estes 3rd NA, Link M, McNitt S, Polonsky B, et al. Association of competitive and recreational sport participation with cardiac events in patients with arrhythmogenic right ventricular cardiomyopathy: results from the North American multidisciplinary study of arrhythmogenic right ventricular cardiomyopath. Eur Hear J. 2015;36(27):1735–43.
4. Thiene G, Basso C, Danieli G, Rampazzo A, Corrado D, Nava A. Arrhythmogenic right ventricular cardiomyopathy: A still underrecognized clinic entity. Trends Cardiovasc Med. 1997;7(3):84–90.
5. Rossi PA. Arrhythmogenic right ventricular dysplasia--clinical features. Eur Heart J. 1989 Sep 2;10(suppl D):7–9.
6. Marcus FI, McKenna WJ, Sherrill D, Basso C, Bauce B, Bluemke D a., et al. Diagnosis of Arrhythmogenic Right Ventricular Cardiomyopathy/Dysplasia: Proposed Modification of the Task Force Criteria. Circulation. 2010 Apr 6;121(13):1533–41.
7. Munclinger MJ, Patel JJ, Mitha AS. Follow-up of patients with arrhythmogenic right ventricular cardiomyopathy dysplasia. S Afr Med J. 2000 Jan;90(1):61–8.
8. Latib MA, Michaels K MB. Initial report of the Arrhythmogenic Right Ventricular Cardiomyopathy Registry of South Africa: 2004. Cardiovasc J South Afr. 2004;15:237–238.
9. Thiene G. The research venture in arrhythmogenic right ventricular cardiomyopathy: A paradigm of translational medicine. Eur Heart J. 2015;36(14):837–46.
10. Marcus FI, Fontaine GH, Guiraudon G, Frank R, Laurenceau JL, Malergue C, et al.

Right ventricular dysplasia: a report of 24 adult cases. Circulation. 1982 Feb 1;65(2):384–98.

11. Elmaghawry M, Alhashemi M, Zorzi A, Yacoub MH. A global perspective of arrhythmogenic right ventricular cardiomyopathy. Glob Cardiol Sci Pract. 2012 Dec;2012(2):26.
12. Report of the 1995 World Health Organization/International Society and Federation of Cardiology Task Force on the Definition and Classification of Cardiomyopathies. Circulation. 1996 Mar 1;93(5):841 LP – 842.
13. Thiene G, Corrado D, Basso C. Arrhythmogenic right ventricular cardiomyopathy/dysplasia. Orphanet J Rare Dis. 2007;2(1):45.
14. Fontaine G, Chen HV. Arrhythmogenic Right Ventricular Dysplasia Back in Force. Am J Cardiol. 2014 May;113(10):1735–9.
15. te Riele AS, Tandri H, Bluemke DA. Arrhythmogenic right ventricular cardiomyopathy (ARVC): cardiovascular magnetic resonance update. J Cardiovasc Magn Reson. 2014 Dec 20;16(1):50.
16. te Riele AS, James C, Philips B, Rastegar N, Bhonsale A, Groeneweg J, et al. Mutation-Positive Arrhythmogenic Right Ventricular Dysplasia/Cardiomyopathy: The Triangle of Dysplasia Displaced. J Cardiovasc Electrophysiol. 2013 Dec;24(12):1311–20.
17. Garcia-Gras E. Suppression of canonical Wnt/ -catenin signaling by nuclear plakoglobin recapitulates phenotype of arrhythmogenic right ventricular cardiomyopathy. J Clin Invest. 2006 Jul 3;116(7):2012–21.
18. te Riele ASJM, Hauer RN. Arrhythmogenic right ventricular dysplasia/cardiomyopathy: Clinical challenges in a changing disease spectrum. Trends Cardiovasc Med. 2014;25(3):191–8.
19. Dunn KE, Ashley EA. Arrhythmogenic Right Ventricular Cardiomyopathy: Toward a Modern Clinical and Genomic Understanding. Circ Cardiovasc Genet. 2015;8(3):421–4.

20. McKoy G, Protonotarios N, Crosby A, Tsatsopoulou A, Anastasakis A, Coonar A, et al. Identification of a deletion in plakoglobin in arrhythmogenic right ventricular cardiomyopathy with palmoplantar keratoderma and woolly hair (Naxos disease). Lancet (London, England). 2000;355(9221):2119–24.
21. Asimaki A, Tandri H, Huang H, Halushka MK, Gautam S, Basso C, et al. A new diagnostic test for arrhythmogenic right ventricular cardiomyopathy. N Engl J Med. 2009;360(11):1075–84.
22. Mayosi BM, Fish M, Shaboodien G, Mastantuono E, Kraus S, Wieland T, et al. Identification of Cadherin 2 (CDH2) Mutations in Arrhythmogenic Right Ventricular Cardiomyopathy. Circ Cardiovasc Genet. 2017;10(2).
23. Watkins D a., Hendricks N, Shaboodien G, Mbele M, Parker M, Vezi BZ, et al. Clinical features, survival experience, and profile of plakophylin-2 gene mutations in participants of the Arrhythmogenic Right Ventricular Cardiomyopathy Registry of South Africa. Hear Rhythm. 2009 Nov;6(11 suppl.):S10-7.
24. Marcus FI, Edson S, Towbin J a. Genetics of arrhythmogenic right ventricular cardiomyopathy: A practical guide for physicians. J Am Coll Cardiol. 2013;61(19):1945–8.
25. Basso C, Corrado D, Bauce B, Thiene G. Arrhythmogenic right ventricular cardiomyopathy. Circ Arrhythmia Electrophysiol. 2012;5(6):1233–46.
26. Marcus FI, McKenna WJ, Sherrill D, Basso C, Bauce B, Bluemke D a., et al. Diagnosis of arrhythmogenic right ventricular cardiomyopathy/dysplasia. Eur Heart J. 2010 Apr;31(7):806–14.
27. Mejia-Lopez E, Romero J, Mejia-Lopez E, Manrique C, Lucariello R. Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC/D): A Systematic Literature Review. Clin Med Insights Cardiol. 2013 May;7:97.
28. Heidbuchel H, Prior DL, Gerche a. L. Ventricular arrhythmias associated with long-term endurance sports: what is the evidence? Br J Sports Med. 2012;46(Suppl_1):i44–50.

29. Corrado D, Basso C, Rizzoli G, Schiavon M, Thiene G. Does Sports Activity Enhance the Risk of Sudden Death in Adolescents and Young Adults? J Am Coll Cardiol. 2003;42(11):1959–63.

30. Rojas A, Calkins H. Present understanding of the relationship between exercise and arrhythmogenic right ventricular dysplasia/cardiomyopathy. Trends Cardiovasc Med. 2015;25(3):181–8.

31. Dupont S, Morsut L, Aragona M, Enzo E, Giulitti S, Cordenonsi M, et al. Role of YAP/TAZ in mechanotransduction. Nature. 2011;474(7350):179–83.

32. James CA, Bhonsale A, Tichnell C, Murray B, Russell SD, Tandri H, et al. Exercise Increases Age-Related Penetrance and Arrhythmic Risk in Arrhythmogenic Right Ventricular Dysplasia/Cardiomyopathy–Associated Desmosomal Mutation Carriers. J Am Coll Cardiol. 2013 Oct;62(14):1290–7.

33. Ruwald A, Marcus F, Iii NAME, Link M, Mcnitt S, Polonsky B, et al. Association of competitive and recreational sport participation with cardiac events in patients with arrhythmogenic right ventricular cardiomyopathy : results from the North American multidisciplinary study of arrhythmogenic right ventricular cardiomyopathy. 2015;1735–43.

34. Pelliccia A, Fagard R, Bjørnstad HH, Anastassakis A, Arbustini E, Corrado D, et al. Recommendations for competitive sports participation in athletes with cardiovascular disease A consensus document from the Study Group of Sports Cardiology of the Working Group of Cardiac Rehabilitation and Exercise Physiology and the Working Group of Myocardial and Pericardial Diseases of the European Society of Cardiology. 2005;1422–45.

35. McKenna WJ, Thiene G, Nava A, Fontaliran F, Blomstrom-Lundqvist C, Fontaine G, et al. Diagnosis of arrhythmogenic right ventricular dysplasia/cardiomyopathy. Task Force of the Working Group Myocardial and Pericardial Disease of the European Society of Cardiology and of the Scientific Council on Cardiomyopathies of the International Society. Heart. 1994 Mar 1;71(3):215–8.

36. Femia G, Hsu C, Singarayar S, Sy RW, Kilborn M, Parker G, et al. Impact of new task force criteria in the diagnosis of arrhythmogenic right ventricular cardiomyopathy. Int J Cardiol. 2014;171(2):179–83.

37. Angelini A, Thiene G, Boffa GM, Calliaris I, Daliento L, Valente M, et al. Endomyocardial biopsy in right ventricular cardiomyopathy. Int J Cardiol. 1993 Jul 18;40(3):273–82.

38. Basso C, Ronco F, Marcus F, Abudureheman A, Rizzo S, Frigo AC, et al. Quantitative assessment of endomyocardial biopsy in arrhythmogenic right ventricular cardiomyopathy/dysplasia: An in vitro validation of diagnostic criteria. Eur Heart J. 2008;29(22):2760–71.

39. Iesaka Y, Hiroe M, Aonuma K, Nitta J, Nogami A, Tokunaga T, et al. Usefulness of electrophysiologic study and endomyocardial biopsy in differentiating arrhythmogenic right ventricular dysplasia from idiopathic right ventricular tachycardia. Heart Vessels Suppl. 1990;5:65–9.

40. Marcus FI, McKenna WJ, Sherrill D, Basso C, Bauce B, Bluemke DA, et al. Diagnosis of Arrhythmogenic Right Ventricular Cardiomyopathy/Dysplasia: Proposed Modification of the Task Force Criteria. Circulation. 2010 Apr 6;121(13):1533–41.

41. Marcus FI, Abidov A. Arrhythmogenic right ventricular cardiomyopathy 2012: Diagnostic challenges and treatment. J Cardiovasc Electrophysiol. 2012 Oct;23(10):1149–53.

42. Basso C, Bauce B, Corrado D, Thiene G. Pathophysiology of arrhythmogenic cardiomyopathy. Nat Rev Cardiol. 2012;9(4):223–33.

43. Smith W. Guidelines for the Diagnosis and Management of Arrhythmogenic Right Ventricular Cardiomyopathy. Hear Lung Circ. 2011 Dec;20(12):757–60.

44. Haugaa KH, Haland TF, Leren IS, Saberniak J, Edvardsen T. Arrhythmogenic right ventricular cardiomyopathy, clinical manifestations, and diagnosis. Europace. 2016;18(7):965–72.

45. Borgquist R, Haugaa KH, Gilljam T, Bundgaard H, Hansen J, Eschen O, et al. The

diagnostic performance of imaging methods in ARVC using the 2010 Task Force criteria. Eur Hear J - Cardiovasc Imaging. 2014 Nov 1;15(11):1219–25.

46. Daliento L, Rizzoli G, Thiene G, Nava A, Rinuncini M, Chioin R, et al. Diagnostic accuracy of right ventriculography in arrhythmogenic right ventricular cardiomyopathy. Am J Cardiol. 1990 Sep;66(7):741–5.

47. Francés RJ. Arrhythmogenic right ventricular dysplasia/cardiomyopathy. A review and update. Int J Cardiol. 2006 Jun;110(3):279–87.

48. Bilchick KC, Dimarco JP. Coming full circle: Contouring the right ventricle in arrhythmogenic right ventricular dysplasia/cardiomyopathy. J Cardiovasc Electrophysiol. 2008;19(1):46–7.

49. Fontaine G, Fontaliran F, Hébert JL, Chemla D, Zenati O, Lecarpentier Y, et al. Arrhythmogenic Right Ventricular Dysplasia. Annu Rev Med. 1999 Feb;50(1):17–35.

50. Marcus FI. Prevalence of T-wave inversion beyond V1 in young normal individuals and usefulness for the diagnosis of arrhythmogenic right ventricular cardiomyopathy/dysplasia. Am J Cardiol. 2005;95(9):1070–1.

51. Marcus FI. Arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C): We′ve come a long way, baby. Trends Cardiovasc Med. 2015 Apr;25(3):199–200.

52. Corrado D, Basso C, Pavei A, Michieli P, Schiavon M TG. Trends in Sudden Cardiovascular Death in Young Competitive Athletes. Jama. 2006;296(13):1593–601.

53. Wu S, Wang P, Hou Y, Yang P, Xiao Y, Zhan X. Epsilon wave in arrhythmogenic right ventricular dysplasia/cardiomyopathy. PACE - Pacing Clin Electrophysiol. 2009;32(1):59–63.

54. Cox MGPJ, Van Der Zwaag PA, Van Der Werf C, Van Der Smagt JJ, Noorman M, Bhuiyan ZA, et al. Arrhythmogenic right ventricular dysplasia/cardiomyopathy: Pathogenic desmosome mutations in index-patients predict outcome of family screening: Dutch arrhythmogenic right ventricular dysplasia/cardiomyopathy genotype-phenotype follow-up study. Circulation. 2011;123(23):2690–700.

55. Basso C, Corrado D, Marcus FI, Nava A, Thiene G. Arrhythmogenic right ventricular

cardiomyopathy. Lancet. 2009;373(9671):1289–300.

56. Hendricks N, Watkins D a, Mayosi BM. Lessons from the first report of the Arrhythmogenic Right Ventricular Cardiomyopathy Registry of South Africa. Cardiovasc J Afr. 2010;21(3):129–30.

57. Groeneweg JA, Bhonsale A, James CA, Te Riele AS, Dooijes D, Tichnell C, et al. Clinical Presentation, Long-Term Follow-Up, and Outcomes of 1001 Arrhythmogenic Right Ventricular Dysplasia/Cardiomyopathy Patients and Family Members. Circ Cardiovasc Genet. 2015;8(3):437–46.

Appendix

Appendix 1: 1994 ARVC Task Force criteria - Case Report Form

Unique ID: _____ SEX: _

KEY
0= absent
1= present

GLOBAL AND / OR REGIONAL DYSFUNCTION AND STRUCTURAL ALTERATIONS	
MAJOR	
o Severe dilatation and reduction of right ventricular ejection fraction with no (or only mild) LV impairment o Localised right ventricular aneurysms (akinetic or dyskinetic areas with diastolic bulging) o Severe segmental dilatation of the right ventricle	
MINOR	
o Mild global right ventricular dilatation and/or ejection fraction reduction with normal left ventricle o Mild segmental dilatation of the right ventricle o Regional right ventricular hypokinesia	
TISSUE CHARACTERISATION OF WALLS	
MAJOR	
o Fibrofatty replacement of myocardium on endomyocardial biopsy	
REPOLARISATION ABNORMALITIES	
MINOR	
o Inverted T waves in right precordial leads (V2 and V3) (people aged more than 12 years; in absence of right bundle branch block)	
DEPOLARISATION / CONDUCTION ABNORMALITIES	
MAJOR	
o Epsilon waves or localised prolongation (> 110ms) of the QRS complex in right precordial leads (V1-V3)	
MINOR	
o Late potentials (signal averaged ECG)	
ARRHYTHMIAS	
MINOR	
o Left bundle branch block type ventricular tachycardia (sustained and non-sustained) (ECG, Holter, exercise testing). o Frequent ventricular extra systoles (more than 1000/24 h) (Holter)	
FAMILY HISTORY	
MAJOR	
o Familial disease confirmed at necropsy or surgery	
MINOR	
o Familial history of premature sudden death (<35 year) due to suspected right ventricular dysplasia. o Familial history (clinical diagnosis based on present criteria	

Diagnosis	Major	Minor

Definite	Borderline	Possible	No Criteria
2 major; or	1 major plus 1 minor; or	1 major; or	0 major; or
1 major plus 2 minor; or	3 minor criteria	2 minor criteria	0 minor criteria
4 minor criteria			

Appendix 2: 2010 ARVC Task Force Criteria - Case Report Form

Unique ID: _____ SEX: ____

KEY
0= absent
1= present

GLOBAL OR REGIONAL DYSFUNCTION AND STRUCTURAL ALTERATIONS	
MAJOR	
By 2D echo**:** Regional RV akinesia, dyskinesia, or aneurysm *and* 1 of the following: o PLAX RVOT ≥ 32 mm (corrected for body size [PLAX/BSA] ≥ 19 mm/m^2) o PSAX RVOT ≥ 36 mm (corrected for body size [PSAX/BSA] ≥ 21 mm/m^2) o Fractional area change ≤ 33%	
By MRI: Regional RV akinesia or dyskinesia or dyssynchronous RV contraction *and* 1 of the following: o Ratio of RV end-diastolic volume to BSA ≥ 110 mL/m^2(male) or ≥ 100 mL/m^2(female) o RV ejection fraction ≤ 40%	
By RV angiography**:** o Regional RV akinesia, dyskinesia, or aneurysm	
MINOR	
By 2D echo**:** Regional RV akinesia or dyskinesia *and* 1 of the following: o PLAX RVOT ≥ 29 to < 32 mm (corrected for body size [PLAX/BSA] ≥ 16 to < 19 mm/m^2) o PSAX RVOT ≥ 32 to < 36 mm (corrected for body size [PSAX/BSA] ≥ 18 to < 21 mm/m^2) o Fractional area change > 33% to ≤ 40%	
By MRI: Regional RV akinesia or dyskinesia or dyssynchronous RV contraction *and* 1 of the following: o Ratio of RV end-diastolic volume to BSA ≥ 100 to < 110 mL/m^2 (male) or ≥ 90 to < 100 mL/m^2 (female) o RV ejection fraction > 40% to ≤ 45%	
TISSUE CHARACTERISATION OF WALL	
MAJOR	
o 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	
o Residual myocytes 60% to 75% by morphometric analysis (or 50% to 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	
REPOLARISATION ABNORMALITIES	
MAJOR	
o Inverted T waves in right precordial leads (V1, V2, and V3) or beyond in individuals > 14 years of age (in the absence of complete right bundle-branch block QRS ≥ 120 ms)	
MINOR	
o Inverted T waves in leads V1 and V2 in individuals > 14 years of age (in the absence of complete right bundle-branch block) or in V4, V5, or V6	
o Inverted T waves in leads V1, V2, V3, and V4 in individuals > 14 years of age in the presence of complete right bundle-branch block	

DEPOLARISATION / CONDUCTION ABNORMALITIES	
MAJOR	
○ Epsilon wave (reproducible low-amplitude signals between end of QRS complex to onset of the T wave) in the right precordial leads (V1 to V3)	
MINOR	
○ Late potentials by SAECG in ≥ 1 of 3 parameters in the absence of a QRS duration of ≥ 110 ms on the standard ECG • Filtered QRS duration (fQRS) ≥ 114 ms • Duration of terminal QRS < 40 μV (low-amplitude signal duration) ≥ 38 ms • Root-mean-square voltage of terminal 40 ms ≤ 20 μV ○ *or* Terminal activation duration of QRS ≥ 55 ms measured from the nadir of the S wave to the end of the QRS, including R', in V1, V2, or V3, in the absence of complete right bundle-branch block	
ARRHYTHMIAS	
MAJOR	
○ Non-sustained or sustained ventricular tachycardia of left bundle-branch morphology with superior axis (negative or indeterminate QRS in leads II, III, and aVF and positive in lead aVL)	
MINOR	
○ Non-sustained or sustained ventricular tachycardia of RV outflow configuration, left bundle-branch block morphology with inferior axis (positive QRS in leads II, III, and aVF and negative in lead aVL) or of unknown axis ○ > 500 ventricular extra systoles per 24 hours (Holter)	
FAMILY HISTORY	
MAJOR	
○ ARVC confirmed in a first-degree relative who meets current Task Force criteria ○ ARVC confirmed pathologically at autopsy or surgery in a first-degree relative ○ Identification of a pathogenic mutation† categorised as associated or probably associated with ARVC/D in the patient under evaluation	
MINOR	
○ History of ARVC/D in a first-degree relative in whom it is not possible or practical to determine whether the family member meets current Task Force criteria ○ Premature sudden death (< 35 years of age) due to suspected ARVC/D in a first-degree relative ○ ARVC confirmed pathologically or by current Task Force Criteria in second-degree relative	

Diagnosis	Major	Minor

Definite	Borderline	Possible	No Criteria
2 major; or	1 major plus 1 minor; or	1 major; or	0 major; or
1 major plus 2 minor; or	3 minor criteria	2 minor criteria	0 minor criteria
4 minor criteria			

Appendix 3: Human Research Ethics Approval - HREC REF 766/2014

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E52-24 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 404 7682 • **Facsimile** [021] 406 6411
Email: nosi.tsama@uct.ac.za
Website: www.health.uct.ac.za/research/humanethics/forms

21 October 2014

HREC REF: 766/2014

Prof B Mayosi
Department of Medicine
Old Main Building

Dear Prof Mayosi

PROJECT TITLE: RATIONALE, DESIGN AND IMPLEMENTATION OF THE AFRICAN CARDIOMYOPATHY AND MYOCARDITIS RESGISTRY (ACMR) (incorporating the following studies: A CLINICAL AND GENETIC STUDY OF FAMILIAL DILATED CARDIOMYOPATHY IN SOUTH AFRICA HREC REF 197/96, THE ARRHYTHMOGENIC RIGHT VENTRICULAR CARDIOMYOPATHY REGISTRY OF SOUTH AFRICA HREC REF 047/2003)(PhD candidate Dr Sarah Kraus)

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30th October 2015.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/research/humanethics/forms)

Please add the PI contact details and the HREC contact details to all the informed consent documents.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

We acknowledge that the PhD student, Dr Sarah Kraus is also involved in this study.

Please quote the HREC reference no in all your correspondence.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN ETHICS
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical

HREC 303/2014

Appendix 4: Human Research Ethics Approval - HREC REF 454/2016

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E53-46 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6626
Email: shuretta.thomas@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

28 June 2016

HREC REF: 454/2016

Dr N Ntusi
Division of Cardiology
E22, NGSH

Dear Dr Ntusi

PROJECT TITLE: THE UTILITY OF THE 1994 VERSUS THE REVISED 2010 ARRHYTHMOGENIC RIGHT VENTRICULAR CARDIOMYOPATHY (ARVC) TASK FORCE DIAGNOSTIC CRITERIA FOR IDENTIFYING MUTATION-POSITIVE PROBANDS WITH ARVC (MMed-candidate-Dr K Lukhna) Sub-study linked to 766/2014

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30th June 2017.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval before the research may occur.

The HREC acknowledge that the student, Dr Kishal Lukhna will also be involved in this study.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

HREC 454/2016