Cardiovascular magnetic resonance and right ventricular angiography in assessment of right ventricular volumes, function and wall motion abnormalities in arrhythmogenic right ventricular cardiomyopathy: a comparative study

Dr. Hsin-Chi Huang MBChB FCP(SA)

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Supervisor: Professor Ntobeko A.B. Ntusi

Cardiologist and Professor of Medicine, Department of Medicine, University of Cape Town and Groote Schuur Hospital

Dedication

I dedicate this work to my parents Teng-Yuan Huang and Hsiu-Lan Huang. Without their love and support, none which I have achieved would be possible.

Acknowledgements

I would like to acknowledge Dr Sarah Kraus for providing the data for the cohort, Mrs Kathryn Manning for the invaluable statistical support and Professor Ntusi for all the guidance and help in making this dissertation possible.

Declaration

I, Hsin-Chi Huang, 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.

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1. Global or regional dysfunction and structural alterations	
Major	Two-dimensional echocardiogram: • 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) • fractional area change ≤33 percent
	Magnetic resonance imaging: • Regional RV akinesia or dyskinesia or dyssynchronous RV contraction and 1 of the following: • Ratio of RV EDV to BSA ≥110 mL/m^2 (male) or ≥100 mL/m^2 (female) • RVEF ≤40 percent
	RV angiography: • Regional RV akinesia, dyskinesia, or aneurysm
Minor	Two-dimensional echocardiogram: • 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) • fractional area change >33 percent to ≤40 percent
	Magnetic resonance imaging: • Regional RV akinesia or dyskinesia or dyssynchronous RV contraction and 1 of the following:

	• Ratio of RV EDV to BSA ≥100 to <110 mL/m^2 (male) or ≥90 to <100 mL/m^2 (female) • RVEF >40 percent to ≤45 percent\
2. Tissue characterization of wall	
Major	• Residual myocytes <60 percent by morphometric analysis (or <50 percent 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 percent to 75 percent by morphometric analysis (or 50 percent to 65 percent if estimated), with fibrous replacement of the RV free wall myocardium in ≥1 sample, with or without fatty replacement of tissue on endomyocardial biopsy
3. Repolarization abnormalities	
Major	• Inverted T waves in right precordial leads (V1, V2, and V3) or beyond in individuals >14 years of age (in the absence of complete right bundle-branch block QRS ≥120 ms)
Minor	• 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 • 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
4. Depolarization/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 the following 3 parameters in the absence of a QRS duration of ≥110 ms on the standard ECG
	  • Filtered QRS duration ≥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 V1, V2, or V3, in the absence of complete right bundle-branch block

5. 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	• 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)
6. 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 categorized as associated or probably associated with ARVC in the patient under evaluation
Minor	• History of ARVC 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 in a first-degree relative • ARVC confirmed pathologically or by current Task Force Criteria in second-degree relative

Table 2 – Demographic and anthropometric measures of cohort

	Sex	Age (years)	Height (cm)	Weight (kg)	BMI (kg/m^2)
1	Male	46	168	98	35
2	Male	49	185	81	24
3	Female	32	163	56	21
4	Male	15	175	71	23
5	Male	43	180	89	27
6	Male	62	170	75	26
7	Male	30	169	67	23
8	Female	31	155	49	20
9	Female	35	163	71	27
10	Female	20	170	51	18
11	Female	41	163	90	34

Table 3 – CMR and RVA RVEDV

Subject	CMR RVEDV (mls)	RVA RVEDV (mls)
1	246.7	130
2	153	170
3	244.9	208
4	230.5	110
5	196.1	184
6	210.1	171
7	158	151
8	554.7	210
9	198	116
10	141.1	121
11	159.9	141

Table 4 - CMR and RVA RVESV

Subject	CMR RVESV (mls)	RVA RVESV (mls)
1	97.4	37
2	95.7	66
3	207.3	76
4	104.6	30
5	108.4	58
6	107.3	64
7	51.6	58
8	514.1	91
9	88.5	32
10	52	48
11	67.9	58

Table 5 – CMR and RVA RVEF

Subject	CMR RVEF (%)	RVA RVEF (%)
1	60.5	71
2	37.5	61
3	15.3	64
4	54.6	73
5	44.7	68
6	48.9	63
7	67.3	61
8	7.3	57
9	55.3	72
10	63.1	60
11	57.5	59

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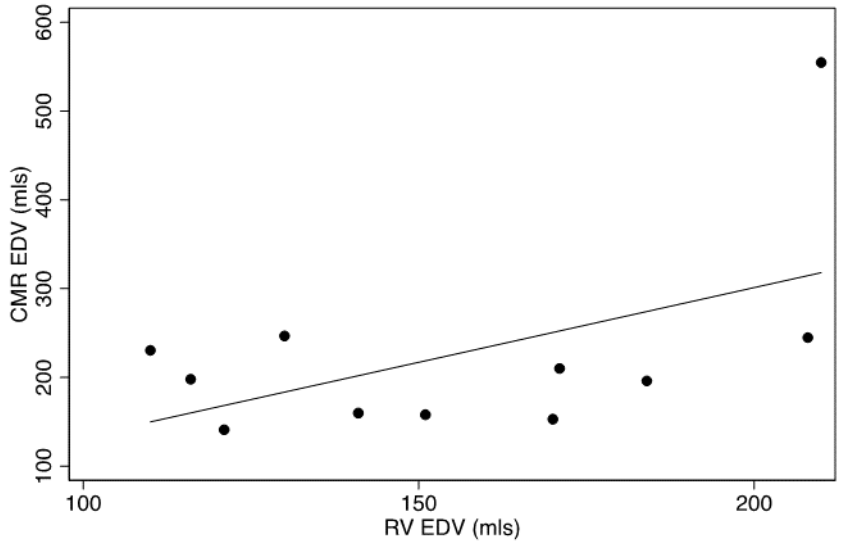


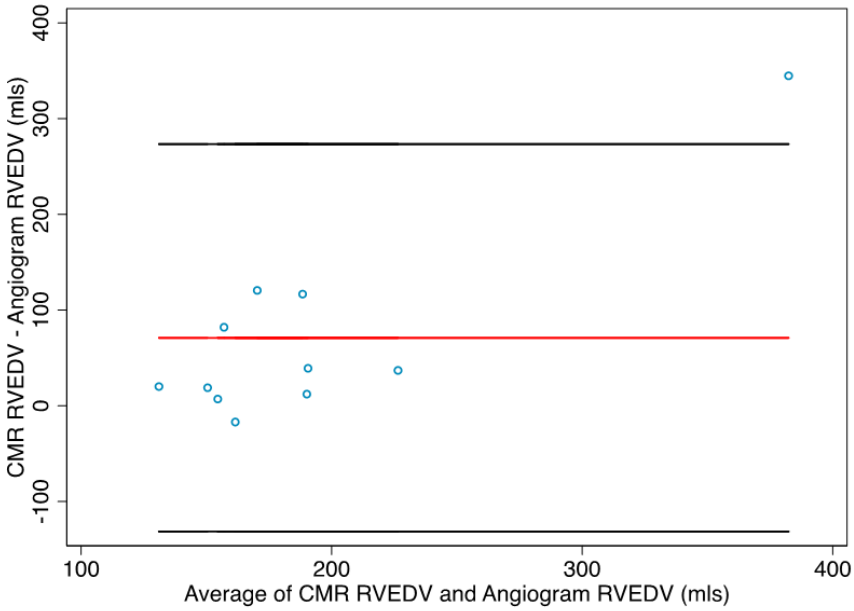
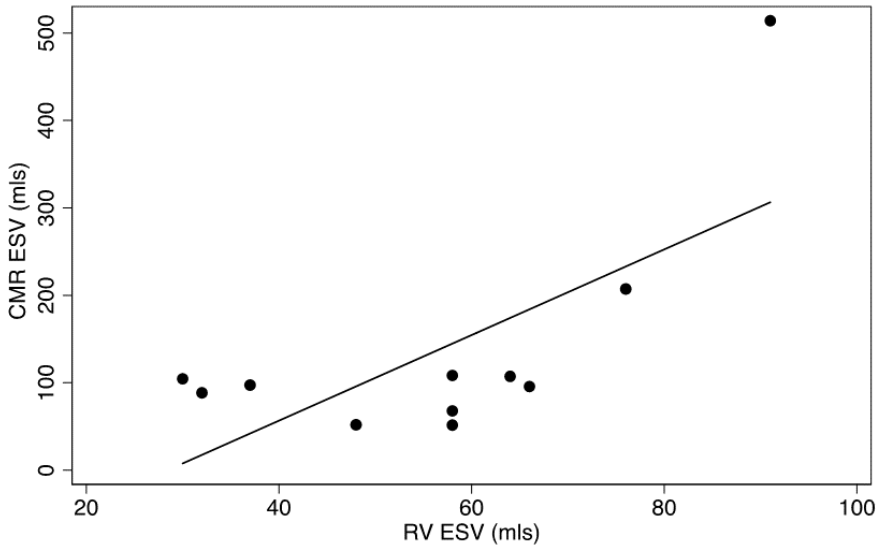


Figure 3 – Spearman's coefficient of CMR RVESV and RVA RVESV



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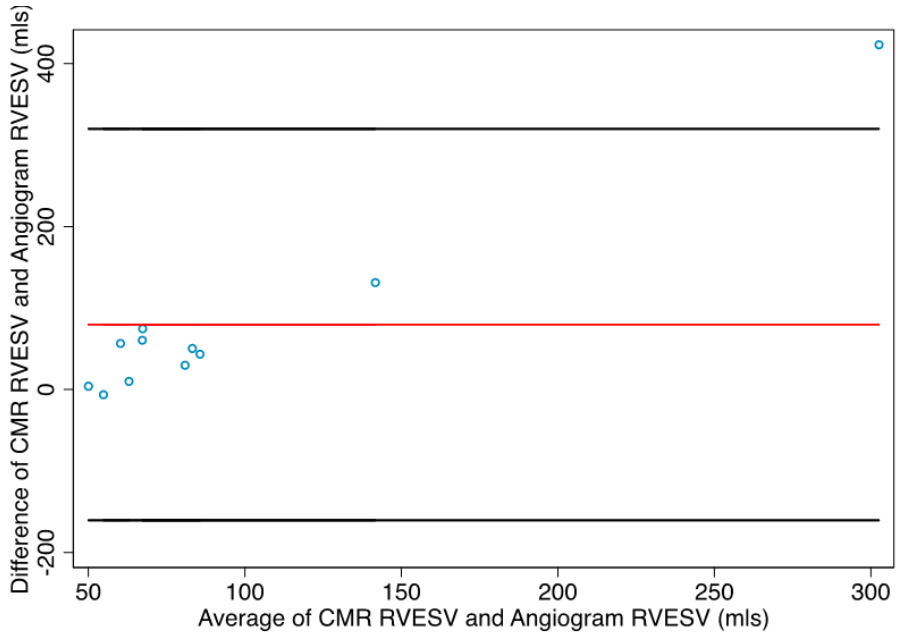


Figure 5– Spearman's coefficient of CMR RVEF and RVA RVEF

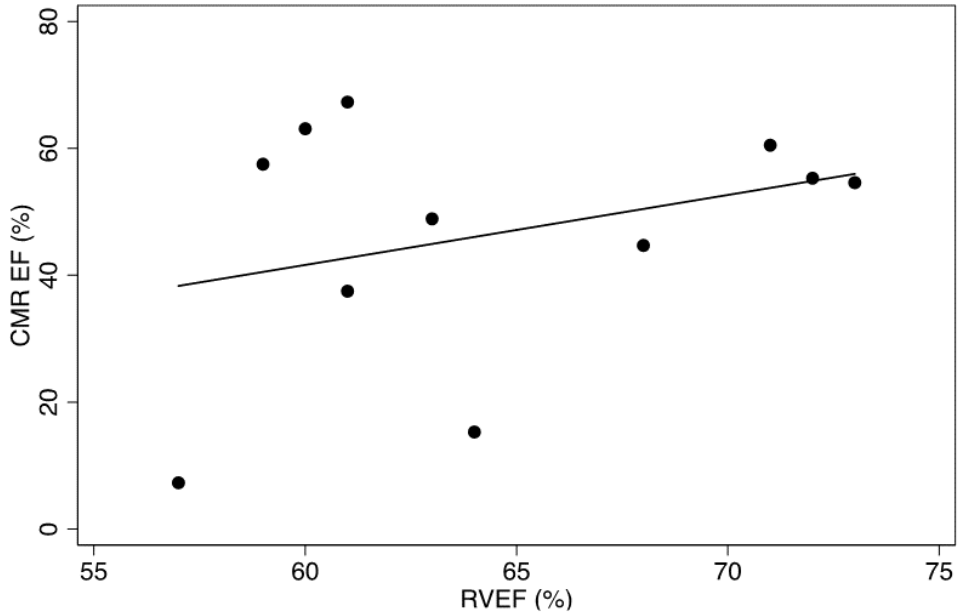
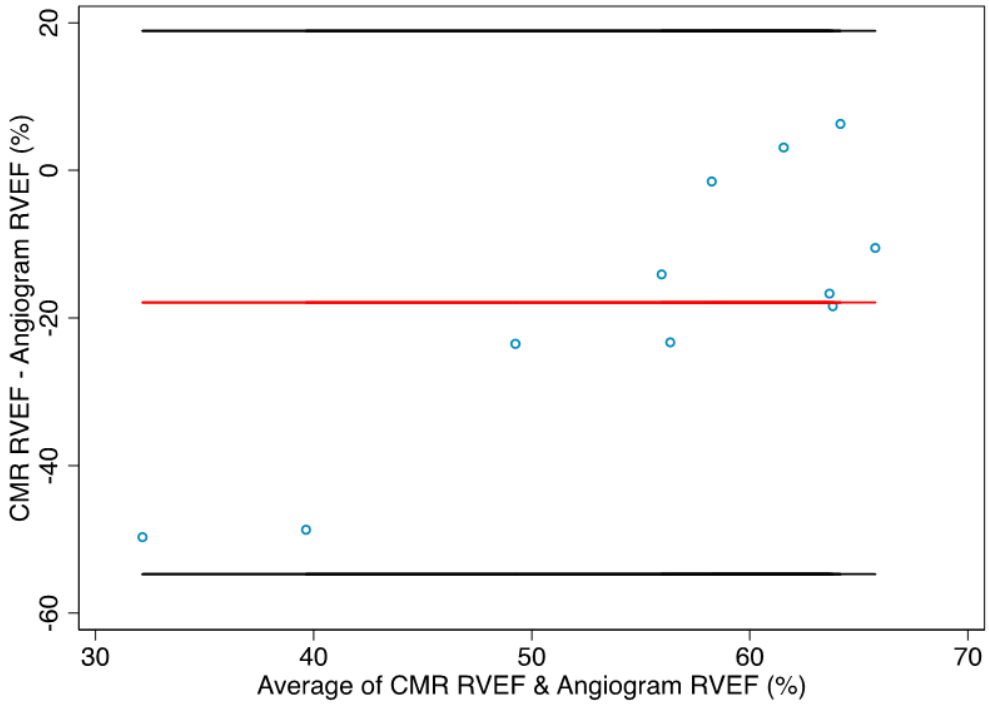


Figure 6– Bland-Altman of CMR RVEF and RVA RVEF



Abbreviations

ACM	Arrhythmogenic cardiomyopathy
ARVC	Arrhythmogenic right ventricular cardiomyopathy
BSA	Body surface area
CDH2	Cadherin-2
CMR	Cardiovascular magnetic resonance
CPVT	Catecholaminergic polymorphic ventricular tachycardia
CTNNαT	α-T-catenin
DES	Desmin
ECG	Electrocardiogram/electrocardiography
EDV	End-diastole volume
EMB	Endomyocardial biopsy/biopsies
ESV	End-systolic volume
IMHOTEP	African Cardiomyopathy and Myocarditis Registry Programme
LAO	Left anterior oblique
LGE	Late gadolinium enhancement
LMNA	Lamin A/C
LV	Left ventricle/ventricular
PLAX	Parasternal long-axis view
PLN	Phospholamban
PSAX	Parasternal short-axis view
RAO	Right anterior oblique
RV	Right ventricle/ventricular
RVA	Right ventricular angiogram/angiography
RVEF	Right ventricular ejection fraction
RVOT	Right ventricular outflow tract

RyR2	Ryanodine-2 receptor
SAECG	Signal averaged electrocardiogram
SCD	Sudden cardiac death
SV	Stroke volume
TFC	Task Force Criteria
TGF-β3	Transforming growth factor beta 3
TMEM43	Transmembrane protein 43
TTN	Titin
VT	Ventricular tachycardia

Chapter 1: Introduction and study rationale

Introduction

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited cardiomyopathy characterised by structural and functional changes classically to the right ventricle (RV) – but may also involve the left ventricle (LV) – and predisposes to malignant ventricular arrhythmias, heart failure and sudden cardiac death (SCD).[1] In ARVC, normal myocardium is replaced by fibro-fatty tissue. Although RV involvement is the hallmark of ARVC, there can be predominant LV involvement or biventricular involvement, with some studies demonstrating up to 52% of probands having involvement of the LV.[2, 3] Therefore, ARVC may be more appropriately considered as simply an arrhythmogenic cardiomyopathy (ACM).

The cardiocutaneous syndromes, Naxos and Carvajal syndromes, are described cutaneous disorders associated with ventricular structural abnormalities and ventricular tachycardia (VT). In 2000, the discovery that Naxos and Carvajal syndrome were caused by genetic mutations in the desmosomal genes, plakoglobin and desmoplakin, respectively, led to the association between ARVC and desmosomal gene mutations.[1,4] Understanding of the genetic basis of ARVC represented a turning point in our understanding of the pathophysiology of ARVC.

ARVC is an autosomal dominant disease with incomplete penetrance and variable expression with cardiac desmosomal genes largely thought to be the culprit genes.[1,4] ARVC-causing mutations are found in genes encoding plakophilin-2, desmoglein-2, desmocollin-2, desmoplakin and plakoglobin.[4] Desmosomes are critical for the structural integrity of the heart. The cadherins (desmoglein and desmocollin) link individual cells, while armadillo proteins (plakoglobin and plakophilin) in turn link the cadherin tails to desmoplakin, which anchors the

entire desmosomal structure to the intermediate filaments of the cell.[5] More recently, our department has discovered that mutations in cadherin-2 (CDH-2) can cause ARVC.[6,7]

The current hypothesis for the pathogenesis of ARVC is that genetic mutations in these desmosomal genes lead to abnormal cell-to-cell adhesions, which in turn, cause cell uncoupling, inflammation, myocardial cell death and, finally, fibrosis. This hypothesis is supported by experimental mouse models with plakophilin, plakoglobin and desmoplakin knock-out genes. Mice deficient in these desmosomal genes had myocyte death with resultant fibrosis that subsequently provided a substrate for arrhythmias.[8] The desmosome hypothesis is especially attractive as it explains why strenuous activity would increase cell uncoupling and thus accelerate disease progression. It also explains why the disease has a predilection for the RV, which is thinner than the LV and thus more susceptible to pathology. [4]

The most common mutation worldwide is reported in the plakophilin-2 gene, which is found in up to 40% of ARVC probands.[9] The South African ARVC registry, coordinated from our centre, found similar results in South Africa, with the plakophilin-2 gene mutation being found in 25% of cases.[10,11]

Nondesmosomal genes have also been associated with ARVC, though many of these associations have not stood up to epidemiological scrutiny. Nondesmosomal genes include transmembrane protein 43 (TMEM43), transforming growth factor beta 3 (TGF-β3), cardiac ryanodine-2 receptor (RyR2), desmin (DES), titin (TTN), lamin A/C (LMNA), phospholamban (PLN) and α-T-catenin (CTNNαT).[4] In fact, the mutations in the calcium-dependent RyR2 gene have been shown to be a cause of catecholaminergic polymorphic ventricular tachycardia (CPVT).[12] Why nondesmosomal genes should contribute to ARVC is poorly understood. PLN seems to be the most common nondesmosomal gene, with a Dutch study finding that this mutation accounted for 32% of patients who had nondesmosomal genetic mutations.[13]

In addition to the mechanics of cell-to-cell uncoupling, desmosomal mutations have also been implicated in interfering with the canonical/Wnt/β-catenin/Tcf/Lef pathway which regulates apoptosis, adipogensis and fibrogenesis.[9,14,15] Experiments involving desmoplakin knock-out mice have shown that plakoglobin translocates into the nucleus to interfere with this pathway.[15-17] Since arrhythmias can occur in the concealed phase of ARVC where there is insignificant myocardial fibrosis and structural abnormalities, it has been postulated that desmosomes, gap junctions and voltage-gated sodium channels are intricately linked and dysfunction in one domain impacts on the function of the all the other units.[17] This hypothesis is supported by the observation that mice with decreased expression of plakophilin-2 exhibit a decrease in intercalated disk connexion 43, which plays an important role in sodium channel physiology.[16] Despite these insights into the genetic basis and disease mechanisms of ARVC, what is clear is that our understanding of the pathogenesis of ARVC is far from complete. Only 58% of patients with ARVC have identifiable genetic mutations, while 16% of healthy control individuals also have the same gene abnormalities.[1]

Currently, ARVC is diagnosed using the 2010 Task Force Criteria (TFC). The 2010 TFC has major and minor criteria in 6 categories (Table 1). These categories are: (1) global or regional dysfunction and structural alterations, (2) tissue characterisation of the myocardium, (3) repolarisation abnormalities, (4) depolarisation/conduction abnormalities, (5) arrhythmias and (6) a family history of the disease. A definite diagnosis of ARVC is made with the fulfilment of 2 major or 1 major and 2 minor criteria. Borderline ARVC is diagnosed with 1 major + 1 minor or 3 minor criteria. Possible ARVC is present if 1 major or 2 minor criteria are met.[18]

The diagnostic criteria have evolved as technology and our understanding of the disease has improved. One of the categories in the 2010 TFC is the demonstration of global or regional dysfunction and structural alterations. The modalities that can be used to demonstrate this are two-dimensional echocardiography, cardiovascular magnetic resonance (CMR) and right ventricular angiography (RVA). Two-dimensional echocardiography has the benefit of being

noninvasive, but as the RV lies behind the sternum and has a curved shape, image acquisition is often problematic and suboptimal. RVA provides better image acquisition than two-dimensional echocardiography but is invasive, involves ionising radiation and has the potential for complications. Although endomyocardial biopsies (EMB) can be performed at the time of RVA, its use is limited by the fact that sampling the thin dyskinetic RV free wall, which is the most likely area to provide a specimen with the highest yield, carries a much higher risk of complications than if another area was sampled. Major criteria for RVA findings are regional RV akinesia, dyskinesia or aneurysms.[18]

CMR has emerged as the imaging modality of choice as it can achieve better imaging of the RV compared to two-dimensional echocardiography and is noninvasive compared to RVA. CMR can detect fat infiltration, wall motion abnormalities, myocardial fibrosis as well as measure ventricular size accurately.[19] Major criteria for CMR are regional RV akinesia, dyskinesia or dyssynchronous contraction plus one sign of functional impairment. Functional impairment is defined as the ratio of RV end-diastolic volume (RVEDV) to body surface ratio being >110 mL/m^2 for males and >100 mL/m^2 for females or RV ejection fraction (RVEF) of <40%. It should however be noted that the 2010 TFC do not make provision for CMR detection of fat infiltration and fibrosis in the RV as diagnostic criteria.[18]

Rationale for Study

CMR and RVA both investigate functional and structural abnormalities of the RV. The RVA is the older of the two modalities. To date, there have been limited data comparing CMR and RVA, and no such studies from the African continent. This study compares CMR and RVA in the assessment of ARVC in the South African ARVC registry.

Aims and objectives

The aim of this study was to compare CMR and RVA in the assessment of ARVC in the South African ARVC registry.

Primary objective:

1. Comparison of CMR and RVA in detecting RV structural abnormalities.

Secondary objectives:

1. Comparison of CMR and RVA end-systolic volumes (ESV) and EDV
2. Comparison of CMR and RV angiography RVEF
3. Sensitivity of CMR and RVA in ARVC diagnosis

Hypotheses

We hypothesise that CMR and RV angiography will be comparable in the detection of structural abnormalities, ventricular volumes and ejection fractions. We also hypothesise that CMR will be superior in its sensitivity in the diagnosis of ARVC.

References

1. Basso C, Bauce B, Corrado D, Thiene G. Pathophysiology of arrhythmogenic cardiomyopathy. *Nat Rev Cardiol* 2012;9:223-233.
2. Saguner AM, Brunckhorst C, Duru F. Arrhythmogenic ventricular cardiomyopathy: A paradigm shift from right to biventricular disease. *World J Cardiol* 2014;6(4):154-174.
3. López-Ayala JM, Gómez-Milanés I, Sánchez Muñoz JJ, Ruiz-Espejo F, Ortíz M, González-Carrillo J, López-Cuenca D, Oliva-Sandoval MJ, Monserrat L, Valdés M, Gimeno JR. Desmoplakin truncations and arrhythmogenic left ventricular cardiomyopathy: characterizing a phenotype. *Europace* 2014;16(12):1838-1846.
3. Elmaghawry M, Alhashemi M, Zorzi A, Yacoub MH. A global perspective of arrhythmogenic right ventricular cardiomyopathy. *Global Cardiol Sci Pract* 2013;2012(2):81-92.
5. Calkins H. Arrhythmogenic Right Ventricular Dysplasia. *Curr Probl Cardiol* 2013;38:103-123.
6. Mayosi BM, Fish M, Shaboodien G, Mastantuono E, Kraus S, Wieland T, Kotta MC, Chin A, Laing N, Ntusi NAB, Chong M, Horsfall C, Pimstone SN, Gentilini D, Parati G, Strom TM, Meitinger T, Pare G, Schwartz PJ, Crotti L. Identification of Cadherin 2 (*CDH2*) Mutations in Arrhythmogenic Right Ventricular Cardiomyopathy. *Circ Cardiovasc Genet* 2017;10(2):e001605.
7. Turkowski KL, Tester DJ, Bos JM, Haugaa KH, Ackerman MJ. Whole exome sequencing with genomic triangulation implicates CDH2-encoded N-cadherin as a novel pathogenic substrate for arrhythmogenic cardiomyopathy. *Congenit Heart Dis* 2017;12(2):226-235.
8. Lodder EM, Rizzo S. Mouse models in arrhythmogenic right ventricular cardiomyopathy. *Front Physiol* 2012;3:221.

9. Campuzano O, Alcalde M, Allegue C, Allegue C, Iglesias A, Garcia-Pavia, Partemi S, Oliva A, Pascali VL, Berne P, Sarquella-Brugada G, Brugada J, Brugada P, Brugarda R. Genetics of arrhythmogenic right ventricular cardiomyopathy. *J Med Genet* 2013;50:280-289.

10. Watkins DA, Hendricks N, Shaboodien G, Mbele M, Parker M, Vezi BZ, Latib A, Chin A, Little F, Badri M, Moolman-Smook JC, Okreglicki A, Mayosi BM; ARVC Registry of the Cardiac Arrhythmia Society of Southern Africa (CASSA). Clinical features, survival experience, and profile of plakophylin-2 gene mutations in participants of the arrhythmogenic right ventricular cardiomyopathy registry of South Africa. *Heart Rhythm* 2009;6(11 Suppl):S10-S17.

11. Hendricks N, Watkins DA, Mayosi BM. Lessons from the first report of the Arrhythmogenic Right Ventricular Cardiomyopathy Registry of South Africa. *Cardiovasc J Afr* 2010;21(3):129-130.

13. Postma AV, Denjoy I, Kamblock J, Alders M, Lupoglazoff J-M, Vaksmann G, Dubosq-Bidot L, Sebillon P, Mannens MMAM, Guicheney P, Wilde AAM. Catecholaminergic polymorphic ventricular tachycardia: RYR2 mutations, bradycardia, and follow up of the patients. *J Med Genet* 2005;42:863-870.

14. Groeneweg JA, van der Zwaag PA, Olde Nordkamp LRA, Bikker H, Jongbloed JDH, Jongbloed R, Wiesfeld ACP, Cox M, van der Heijden JF, Atsma DE, de Boer K, Doevendans PA, Vink A, van Veen T, Dooijes D, van den Berg M, Wilde A, van Tintelen JP, Hauer RN. Arrhythmogenic right ventricular dysplasia/cardiomyopathy according to revised 2010 Task Force Criteria with inclusion of non-desmosomal phospholamban mutation carriers. *Am J Cardiol* 2013;112(8):1197-1206.

15. Saffitza JE, Asimaki A, Huang H. Arrhythmogenic right ventricular cardiomyopathy: new insights into mechanisms of disease. *Cardiovasc Pathol* 2010;19:166-170

16. Calore M, Lorenzon A, De Bortoli M, Poloni G, Rampazzo A. Arrhythmogenic cardiomyopathy: a disease of intercalated discs. *Cell Tissue Res* 2015;360(3):491-500.

17. Delmar M. Desmosome–Ion Channel Interactions and Their Possible Role in Arrhythmogenic Cardiomyopathy. *Pediatr Cardiol* 2012;33:975-979.

18. Marcus FI, McKenna WJ, Sherrill D, Basso C, Bauce B, Bluemke DA, Calkins H, Corrado D, Cox MG, Daubert JP, Fontaine G, Gear K, Hauer R, Nava A, Picard MH, Protonotarios N, Saffitz JE, Sanborn DM, Steinberg JS, Tandri H, Thiene G, Towbin JA, Tsatsopoulou A, Wichter T, Zareba W. Diagnosis of arrhythmogenic right ventricular cardiomyopathy/dysplasia: proposed modification of the task force criteria. *Circulation* 2010;121(13):1533-41.

19. Moosa S, Ntusi NAB. Role of cardiovascular magnetic resonance in the evaluation of cardiomyopathy. *South Afr J Radiol* 2016;20(2):a1055.

Chapter 2: Literature review

Search strategy

Pubmed was searched using combinations of the following terms: “arrhythmogenic right ventricular cardiomyopathy’’, “Southern Africa”, “South Africa”, “cardiovascular magnetic resonance”, “magnetic resonance imaging”, “angiography” and “angiogram”.

Summary of literature

ARVC is a rare, inherited cardiomyopathy characterised by fibro-fatty infiltration of mostly the RV that predisposes to ventricular arrhythmias and SCD. [1] It is an autosomal dominant disease with incomplete penetrance and variable expression.[1, 2] The cardiac desmosomal genes, plakophilin-2, desmoglein-2, desmocollin-2, desmoplakin and plakoglobin are the main causative genes in ARVC.[2] The current hypothesis for the pathogenesis of ARVC is genetic mutations in desmosomal genes leading to abnormal cell-to-cell adhesions, which in turn, cause cell uncoupling, inflammation, myocardial cell death and, finally, fibrosis. The most common mutation worldwide is the plakophilin-2 gene, which is found in up to 40% of cases.[3]

Nondesmosomal genes have also been associated with ARVC, though many of these associations have not stood up to epidemiological scrutiny. Nondesmosomal genes implicated in ARVC development include TMEM43, TGF-β3, RyR2, DES, TTN, LMNA, PLN and CTNNαT.[4] Mutations in the calcium-dependent RyR2 gene have been shown to be a cause of CPVT. What is clear is that our understanding of the pathogenesis of ARVC is far from complete. Whilst only 58% of patients with ARVC have identifiable genetic mutations, 16% of healthy control individuals also have these same gene abnormalities.[1]

ARVC is a complex disease with no single gold standard test. The diagnosis is established using the 2010 TFC. The 2010 TFC has major and minor criteria in 6 categories (Table 1).[5] These categories are:

(1) global or regional dysfunction and structural alterations

(2) tissue characterisation of the myocardium

(3) repolarisation abnormalities

(4) depolarisation/conduction abnormalities

(5) arrhythmias

(6) family history of the disease.

A definite diagnosis of ARVC is made with the fulfilment of 2 major or 1 major and 2 minor criteria. Borderline ARVC is diagnosed with 1 major and 1 minor or 3 minor criteria. Possible ARVC is present if 1 major or 2 minor criteria are met.[5]

ARVC in South Africa

The first case of ARVC in Africa was diagnosed in 2000, with the South African ARVC registry also established in 2000.[6,7] A study of the profile of South African ARVC patients showed that 80% of affected individuals were white, while the most common gene mutation was the plakophilin-2 gene, found in 25% of probands.[7] Mortality for the South African cohort was 2.8%, with the mean age of death being 20 years younger than a French series.[7] Our group concluded that ARVC in Southern Africa, with the exception of age of death, was similar to that reported in the international literature. In the South African ARVC registry, the predominance of white probands was postulated to be due to racial healthcare inequalities and differential healthcare access, although a founder effect could not be excluded.[7] More recently, our group postulated new gene mutation in CDH-2 was responsible for ARVC.[8] The newly reported ARVC-causing mutation was found in 2.7% probands in the South African

ARVC registry.[8] ARVC caused by CDH-2 mutations has also been recently confirmed by a group from the Mayo Clinic.[9] From the literature, and based on our experience, it is thus reasonable to infer that ARVC in South Africa is similar to international trends, although the patients and age of death is younger. These observations suggest that a more aggressive approach to treatment in our setting is warranted.

Utility of RV angiography in ARVC

RVA allows for assessment of the structure and function of the RV, making it useful in the diagnosis of ARVC. The sensitivity and specificity of angiography is considered high. A small study of 17 probands by White*, et al* found a 100% specificity and sensitivity for RVA.[10] A positive predictive value of 85% and a negative predictive value of 95% has been reported in the literature for RVA.[10,11] It should, however, be noted that acquiring angiography images can be technically difficult with incorrect catheter position, for example, giving sub-optimal images by inducing ectopic beats. Failing to distribute the contrast throughout the RV correctly can also impact on volume calculations. Ahmed*, et al* also noted poor inter-observer variations in qualitative measurements which is of concern as the 2010 TFC major criteria for angiography are limited to qualitative measurements rather than quantitative measurements.[12]

Angiography has the advantage of allowing histology to be attained via EMB at the time of the procedure. The sensitivity of histology, however, is considered poor as the low yield septal area, rather than the RV free wall, is most often sampled due to fear of complications.[13,14] From the literature, RVA is considered as an excellent test in the diagnosis of ARVC, provided correct procedures are followed.

Utility of CMR in ARVC

CMR is an attractive imaging modality, as it is safe, reproducible and can achieve excellent images of the RV in any orthogonal plane. Importantly, CMR is the gold-standard technique for assessment of RV volumes. CMR has excellent spatial and temporal resolution and does not involve any ionising radiation, compared to RVA. [15] CMR can assess anatomical abnormalities such as wall motion abnormalities, ventricular size, RVEF and detect fatty infiltration and myocardial fibrosis.[15] The importance of CMR was underscored by a study conducted in a paediatric population which concluded that CMR was needed in half of the study patients in order to secure the diagnosis of definite ARVC.[16] In this study, the authors concluded that, in decreasing order of importance, the contribution of individual investigations to the diagnosis of ARVC was an abnormal CMR, positive family history, EMB findings, abnormal depolarisation on electrocardiography (ECG), abnormal echocardiogram, abnormal repolarisation on ECG, and finally, the presence of arrhythmias.[16] Similarly, in the South African ARVC registry, CMR was useful for the confirmation of the diagnosis of ARVC in the majority of probands.[17]

The original 1994 TFC required the demonstration of RV dilation, aneurysms and a reduction in RVEF.[18] These criteria were criticised as being too subjective and were subsequently revised. The revised 2010 Task Force CMR criteria required the demonstration of both structural RV abnormalities and functional abnormalities in the form of quantitative cut-offs in RVEF and RVEDV.[5] These cut-off values in RVEF and EDV were derived after comparing 462 normal patients in the Multi-Ethnic Study of Atherosclerosis study to 44 North American Multidisciplinary ARVC study probands and was targeted to achieve a specificity of 95% once a major criteria was fulfilled.[19] There have been many studies to assess the impact of the new criteria on the diagnosis of ARVC. Most of these studies have noted a reduction in patients meeting major and minor criteria after the introduction of the 2010 TFC, which is not surprising considering the more stringent criteria.[20-22]

Major CMR criteria have been reported in the literature to have a sensitivity of between 68% and 100%, with specificity of up to 94%.[21-23] Femia, *et al* showed that the positive predictive value of CMR is 55%, while negative predictive value is 100%.[20] CMR has been shown to have excellent inter-observer correlation, with correlation coefficients between observers for RVEDV, RVESV and RVEF being in excess of 90%.[23]

The detection of intra-myocardial fat is a very attractive diagnostic parameter in the diagnosis of ARVC. However, studies have shown that the sensitivity and specificity of intra-myocardial fat is quite variable, ranging from 22% to 100%.[23-25] This has been attributed to the RV being so thin walled that, practically, it is difficult to separate normal epicardial from pathological myocardial fat.[24,25] Fat infiltration is also not specific for ARVC as it can also be present in the elderly, obese, steroid users and in right ventricular outflow tract tachycardias.[24,25]

Late gadolinium enhancement (LGE), which detects myocardial fibrosis, is another technique which may improve CMR diagnostics, although Marra, *et al* found that invasive endocardial voltage mapping was significantly more sensitive than contrast-enhanced CMR in the identification of RV scars.[26] It follows that CMR, as a diagnostic and prognostic tool, should be used synergistically rather than alone in the diagnosis of ARVC. Indeed, Aquaro, *et al* showed that pre- and postcontrast signal abnormalities markedly increased the sensitivity of CMR, and that by combining the traditional functional parameters with signal abnormalities yielded a 96% sensitivity and 100% specificity.[27] In addition to the diagnosis of ARVC, CMR can be useful in prognosticating probands with ARVC, with an abnormal CMR having a 96.9% positive predictive value for adverse cardiac events, and with involvement of the LV being an especially strong predictor.[28]

In summary, CMR is an important imaging tool in the diagnosis of ARVC. The variable sensitivity in the literature may be attributable to the evolving accuracy of CMR analytic tools

relative to when the studies were done. Although sensitivity is variable, specificity is consistently high in the literature. Other than the traditional structural and functional parameters, new techniques have been developed that may enhance CMR capabilities, including presence of LGE. It is highly likely that an updated TFC will incorporate these emerging CMR techniques in the future.

Comparison of CMR and RVA

There have been few studies in which compared RVA to CMR directly.

In 2004, White, *et al* examined 17 patients who had presented with arrhythmias and were being evaluated for ARVC. Out of this cohort, 7 were subsequently diagnosed with definite ARVC, according to 1994 TFC. CMR sensitivity and specificity for this cohort was 86% and 60%, respectively, while angiography had a sensitivity and specificity of 100%.[10] In 2012, Indik, *et al* studied 17 probands from the North American ARVC registry who had undergone both CMR and RVA. This study noted a correlation coefficient of 72% for RVEDV, 68% for RVEF, with RVA volumes being generally greater than CMR volumes.[29]

RVA is an established and tested technique. CMR on the other hand is newer and has advantages of being noninvasive, providing better viewing planes of the RV and allows for fat and fibrosis detection. Since the publication of the comparative study in 2004,[10] CMR protocols have become more refined, machines more sophisticated and interpreters much more experienced. It is likely that CMR will provide greater reproducibility than RVA, as has been recently reported.[29] Indeed, the revised 2010 TFC no longer includes quantitative criteria for RVA, suggesting that CMR has largely superseded RVA in diagnostic imaging.

Existing gaps in the literature

The only 2 studies that have been performed, comparing CMR and RVA, are dated and have involved small numbers. Further, there have also been no studies to document the African experience. The University of Cape Town and Groote Schuur Hospital host the South African ARVC registry and the African Cardiomyopathy and Myocarditis Registry Program (IMHOTEP) and are in an opportune position to have the capacity to undertake such a contemporaneous comparative analysis.

References

1. Basso C, Corrado D, Bauce B, Thiene G. Arrhythmogenic right ventricular cardiomyopathy. *Circ Arrhythmia Electrophysiol* 2012;5(6):1233-1246.
2. Elmaghawry M, Alhashemi M, Zorzi A, Yacoub MH. A global perspective of arrhythmogenic right ventricular cardiomyopathy. *Global Cardiol Sci Pract* 2013;2012(2):81-92.
3. Campuzano O, Alcalde M, Allegue C, Allegue C, Iglesias A, Garcia-Pavia, Partemi S, Oliva A, Pascali VL, Berne P, Sarquella-Brugada G, Brugada J, Brugada P, Brugarda R. Genetics of arrhythmogenic right ventricular cardiomyopathy. *J Med Genet* 2013;50:280-289.
4. Calkins H. Arrhythmogenic Right Ventricular Dysplasia. *Curr Probl Cardiol* 2013;38:103-123.
5. Marcus FI, McKenna WJ, Sherrill D, Basso C, Bauce B, Bluemke DA, Calkins H, Corrado D, Cox MG, Daubert JP, Fontaine G, Gear K, Hauer R, Nava A, Picard MH, Protonotarios N, Saffitz JE, Sanborn DM, Steinberg JS, Tandri H, Thiene G, Towbin JA, Tsatsopoulou A, Wichter T, Zareba W. Diagnosis of arrhythmogenic right ventricular cardiomyopathy/dysplasia: proposed modification of the task force criteria. *Circulation* 2010;121(13):1533-41.
6. Sliwa K, Mayosi BM. Recent advances in the epidemiology, pathogenesis and prognosis of acute heart failure and cardiomyopathy in Africa. *Heart* 2013;99(18):1317-1322.
7. Watkins DA, Hendricks N, Shaboodien G, Mbele M, Parker M, Vezi BZ, Latib A, Chin A, Little F, Badri M, Moolman-Smook JC, Okreglicki A, Mayosi BM; ARVC Registry of the Cardiac Arrhythmia Society of Southern Africa (CASSA). Clinical features, survival experience, and profile of plakophylin-2 gene mutations in participants of the arrhythmogenic right ventricular cardiomyopathy registry of South Africa. *Heart Rhythm* 2009;6(11 Suppl):S10-S17.

8. Mayosi BM, Fish M, Shaboodien G, Mastantuono E, Kraus S, Wieland T, Kotta MC, Chin A, Laing N, Ntusi NAB, Chong M, Horsfall C, Pimstone SN, Gentilini D, Parati G, Strom TM, Meitinger T, Pare G, Schwartz PJ, Crotti L. Identification of Cadherin 2 (CDH2) Mutations in Arrhythmogenic Right Ventricular Cardiomyopathy. *Circ Cardiovasc Genet* 2017;10(2):e001605.

9. Turkowski KL, Tester DJ, Bos JM, Haugaa KH, Ackerman MJ. Whole exome sequencing with genomic triangulation implicates CDH2-encoded N-cadherin as a novel pathogenic substrate for arrhythmogenic cardiomyopathy. *Congenit Heart Dis* 2017;12(2):226-235.

10. White JB, Razmi R, Nath H, Kay GN, Plumb VJ, Epstein AE. Relative utility of magnetic resonance imaging and right ventricular angiography to diagnose arrhythmogenic right ventricular cardiomyopathy. *J Interv Card Electrophysiol* 2004;10(1):19-26.

11. Francés RJ. Arrhythmogenic right ventricular dysplasia/cardiomyopathy. A review and update. *Int J Cardiol* 2006;110(3):279-287.

12. Ahmed J, Smith WM, Ruygrok PN, Occleshaw C. Right ventricular morphology on catheter angiography: variations and its implications for the diagnosis of arrhythmogenic right ventricular cardiomyopathy. *Heart Lung Circ* 2012;21(11):700-705.

13. Sreetharan S, MacIntyre CJ, Fatah M, Warren AE, Wilson GJ, Hamilton RM. Clinical utility of endomyocardial biopsy in the diagnosis of arrhythmogenic right ventricular cardiomyopathy in children. *Pediatr Res* 2018;84(4):552-557.

14. Kayser HWM, van der Wall EE, Sivananthan MU, Plein S, Bloomer TN, de Roos A. Diagnosis of arrhythmogenic right ventricular dysplasia: a review. *Radiographics* 2002;22(3):639-648.

15. Moosa S, Ntusi NAB. Role of cardiovascular magnetic resonance in the evaluation of cardiomyopathy. *South Afr J Radiol* 2016;20(2):a1055.

16. Etoom Y, Govindapillai S, Hamilton R, Manlhiot C, Yoo SJ, Farhan M, Sarikouch S, Peters B, McCrindle BW, Grosse-Wortmann L. Importance of CMR within the task

force criteria for the diagnosis of ARVC in children and adolescents. *J Am Coll Cardiol* 2015;65(10):987-995.

17. Kraus S. The rationale, design and implementation of the African Cardiomyopathy and Myocarditis (IMHOTEP) registry. University of Cape Town PhD thesis 2019. Unpublished.
18. McKenna WJ, Thiene G, Nava A, Fontaliron F, Blomstrom-Lundquist G, Fontaine G, Camerini F, on behalf of the 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 and Federation of Cardiology. Diagnosis of arrhythmogenic right ventricular dysplasia cardiomyopathy. *Br Heart J* 1994;71:215-218.
19. Vermes E, Strohm O, Otmani A, Childs H, Duff H, Friedrich MG. Impact of the revision of arrhythmogenic right ventricular cardiomyopathy/dysplasia task force criteria on its prevalence by CMR criteria. *JACC Cardiovasc Imaging* 2011;4(3):282-287.
20. Femia G, Hsu C, Singarayar S, Sy RW, Kilborn M, Parker G, McGuire M, Semsarian C, Puranik R. Impact of new task force criteria in the diagnosis of arrhythmogenic right ventricular cardiomyopathy. *Int J Cardiol* 2014;171(2):179-183.
21. Liu T, Pursnani A, Sharma UC, Vorasettakarnkij Y, Verdini D, Deeprasertkul P, Lee AM, Lumish H, Sidhu MS, Medina H, Danik S, Abbara S, Holmvang G, Hoffmann U, Ghoshhajra BB. Effect of the 2010 task force criteria on reclassification of cardiovascular magnetic resonance criteria for arrhythmogenic right ventricular cardiomyopathy. *J Cardiovasc Magn Reson* 2014;16(1):47.
22. te Riele A, Tandri H, Bluemke DA. Arrhythmogenic right ventricular cardiomyopathy (ARVC): cardiovascular magnetic resonance update. *J Cardiovasc Magn Reson* 2014;16(1):50.
23. Tandri H, Castillo E, Ferrari VA, Nasir K, Dalal D, Bomma C, Calkins H, Bluemke DA. Magnetic resonance imaging of arrhythmogenic right ventricular dysplasia: sensitivity,

specificity, and observer variability of fat detection versus functional analysis of the right ventricle. *J Am Coll Cardiol* 2006;48(11):2277-2284.

24. Jain A, Tandri H, Calkins H, Bluemke DA. Role of cardiovascular magnetic resonance imaging in arrhythmogenic right ventricular dysplasia. *J Cardiovasc Magn Reason* 2008;10(1):32.

25. Tandri H, Macedo R, Calkins H, Marcus F, Cannom D, Scheinman M, Daubert J, Estes M 3rd, Wilber D, Talajic M, Duff H, Krahn A, Sweeney M, Garan H, Bluemke DA; Multidisciplinary Study of Right Ventricular Dysplasia Investigators. Role of magnetic resonance imaging in arrhythmogenic right ventricular dysplasia: Insights from the North American arrhythmogenic right ventricular dysplasia (ARVD/C) study. *Am Heart J* 2008;155(1):147-153.

26. Marra MP, Leoni L, Bauce B, Corbetti F, Zorzi A, Migliore F, Silvano M, Rigato I, Tona F, Tarantini G, Cacciavillani L, Basso C, Buja G, Thiene G, Iliceto S, Corrado D. Imaging study of ventricular ccar in arrhythmogenic right ventricular cardiomyopathy. Comparison of 3D Standard electroanatomical voltage mapping and contrast-enhanced cardiac magnetic resonance. *Circ Arrhythm Electrophysiol* 2012;5(1):91-100.

27. Aquaro GD, Barison A, Todiere G, Grigoratos C, Ait Ali L, Di Bella G, Emdin M, Festa P. Usefulness of Combined Functional Assessment by Cardiac Magnetic Resonance and Tissue Characterization Versus Task Force Criteria for Diagnosis of Arrhythmogenic Right Ventricular Cardiomyopathy. Am J Cardiol 2016; 118(11):1730-1736.

28. Aquaro GD, Pingitore A, Bella GD, Piaggi P,MS, Gaeta R, Grigoratos C, Altinier A, Pantano A, Strata E, De Caterina R, Sinagra G, Emdin M. Prognostic Role of Cardiac Magnetic Resonance in Arrhythmogenic Right Ventricular Cardiomyopathy. Am J Cardiol 2018;122(10):1745-1753.

29. Indik JH, Dallas WJ, Gear K, Tandri H, Bluemke DA, Moukabary T, Marcus FI. Right ventricular volume analysis by angiography in right ventricular cardiomyopathy. *Int J Cardiovasc Imaging* 2012;28(5):995–1001.

Chapter 3: Methods

Study design

The study was a retrospective analysis of ARVC cases from the South African ARVC and IMHOTEP registries. RVESV, RVEDV, RVEF for both CMR and RVA were measured. The presence of structural abnormalities was also noted. All RVA and CMR data were interpreted by experienced observers, independently.

Study population

The study population consisted of definite, possible and borderline ARVC cases from these afore-mentioned registries, who had both CMR and RVA data.

Data collection and management

The collected data was entered onto an electronic database that was password protected to ensure privacy. Each proband had the following data entered: CMR RVEDV; CMR RVESV; RVA RVEDV; RVA RVESV; presence of aneurysms/regional wall abnormalities on CMR; presence of aneurysms/regional wall abnormalities on RVA. No personal identifiable data were recorded.

Study assessments

CMR: CMR studies were performed using a 1.5 T MR system (Avanto and Aera, Siemens Healthcare, Germany). A 32-channel phased-array chest coil was used for all data acquisition, except for short-Tau inversion recovery (STIR) imaging, for which the body coil was used. A

complete stack of short axis images was obtained during breath hold and cardiac gating for cine and LGE imaging. LGE imaging was performed using a T1-weighted phase-sensitive inversion recovery sequence about 8 minutes after intravenous administration of contrast agent (0.15 mmol/kg body weight).

Analysis of LV and RV ejection fraction was performed using Argus software (Version VB17, 2011, Siemens Medical Solutions). LV and RV short axis epicardial and endocardial borders were manually contoured at end-diastole and end-systole. LV end-systolic (LVESV) and end-diastolic (LVEDV) volumes were used to calculate stroke volume (SV) and ejection fraction (EF) – (EF = SV/EDV). Myocardial mass was also calculated by subtracting the endocardial volume from the epicardial volume, based on prior knowledge of myocardial specific gravity (1.05 g/cm^3). Left atrial diameter was measured in the LV outflow tract (3-chamber) view. The RVEDV and RVESV were calculated in a similar fashion, as above.

Images were evaluated qualitatively for the presence or absence, pattern (subendocardial, midwall, subepicardial, transmural) and regional distribution of LGE areas by three observers, each with at least 4 years of CMR experience. The detection of LGE was made by consensus of all 3 observers. In addition, endocardial and epicardial regions of interest (ROI) were manually contoured in the LGE images, together with a reference ROI in the anterior LV wall without visual LGE, and focal areas of LGE were defined quantitatively as those with SI ≥ 2.0 standard deviations above the mean SI of normal myocardium.

RVA: For calculation of RV angiographic volumes, the 30° right anterior oblique (RAO) and 60° left anterior oblique (LAO) views were utilised. The RV angiographic volumes were analysed blindly by two observers, independently. A cardiac cycle was analysed that had good contrast opacification of the RV and not immediately proceeded by a premature ventricular contraction. The diastolic and systolic images were selected, and a contour drawn in each phase. The area within the contour was then calculated. The length-scale

was calibrated by measuring the projected size of the magnified angio-catheter shaft within the RV, which was usually a pigtail 5Fr catheter, to allow an accurate and reproducibly consistent measurement. The RV volume was then assessed by using both the RAO and LAO projections ($V_{TWO\text{-}VIEW}$) or by using the RAO projection alone (V_{RAO}).

$V_{TWO\text{-}VIEW}$ was calculated as:

$$\mathbf{V_{TWO\text{-}VIEW} = 0.6 * A_{RAO} * A_{LAO} / L_{RAO} + 3.9(ml)} \quad (1)$$

where A_{RAO} was the projected area within the drawn contour in the RAO view, A_{LAO} was the projected area in the LAO view, and L_{RAO} was the projected distance in the RAO view from the pulmonic valve to the point that bisected the inferior wall.

The ejection fraction was then computed as follows:

$$\mathbf{RVEF_{TWO\text{-}VIEW} = (V_{TWO\text{-}VIEW\text{-}DIA} - V_{TWO\text{-}VIEW\text{-}SYS}) / V_{TWO\text{-}VIEW\text{-}DIA}} \quad (2)$$

where $V_{TWO\text{-}VIEW\text{-}DIA}$ was the volume computed at end-diastole and $V_{TWO\text{-}VIEW\text{-}SYS}$ was the volume computed at end-systole of the chosen cardiac cycle by Equation 1.

Using the RAO view alone, the volume was calculated as:

$$\mathbf{V_{RAO} = (0.4 * A_{RAO} * A_{RAO} / L_{RAO} + 3.9) * 0.88 + 7.71\ ml} \quad (3)$$

The RVEF was computed using the RAO computed volumes at end-diastole and end-systole as follows:

$$\mathbf{RVEF_{RAO} = (V_{RAO\text{-}DIA} - V_{RAO\text{-}SYS}) / V_{RAO\text{-}DIA}} \quad (4)$$

where $V_{RAO\text{-}DIA}$ was the volume computed at end-diastole and $V_{RAO\text{-}SYS}$ was the volume computed at end-systole of the chosen cardiac cycle, by the RAO volume formula (Equation 3). Body surface area (BSA) was computed and all volumes were adjusted for BSA.

Ethical considerations and obtaining informed consent

This proposal was submitted to the University of Cape Town Human Research Ethics Committee. This study was a substudy of a study which has already been given ethics approval for the ARVC Registry of South Africa (HREC Reference number: 047/2003) and IMHOTEP study (HREC Reference number: 766/2014). Informed consent has therefore already been obtained from participants and was not obtained again as this was a retrospective assessment of data that already had been collected.

Statistical analysis

Descriptive statistics were used to summarise patient characteristics. Continuous variables were summarised as mean ± standard deviation, and minimum and maximum range where applicable. Categorical variables were presented as frequencies and percentages. Spearman's rank correlation was used to analyse the strength and direction of the linear relationship between RVA and CMR measures. Bland-Altman plots were used to assess the agreement between angiogram and MRI measurements. Limits of agreement (95% lower and upper bounds) were calculated as the mean difference ± 2 standard deviations. All analysis was performed using Stata v.14.1 software (StataCorp, College Station, TX, USA).

Risks and benefits

Risks: This study was a retrospective cross-sectional study of data already collected. There was therefore no risk to participants.

Benefits: CMR is far less invasive with less complications than RVA. If the findings of the study confirm that CMR is similar or superior to RVA in the assessment of the RV in ARVC, this could improve patient safety as CMR is not an invasive technique. There was no obvious

benefit for the participants of this study, but such work may benefit future patients suspected of ARVC.

Publication and dissemination

The results of the study will be made available through the University of Cape Town Masters of Medicine catalogue. The study findings shall also be made available through published conference proceedings and publication in peer-reviewed scientific journals.

Chapter 4: Submissible manuscript

Abstract

Background: Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited cardiomyopathy characterised by structural changes to mostly the right ventricle (RV) that predisposes to ventricular arrhythmias heart failure and sudden cardiac death. ARVC is diagnosed using the 2010 Task Force Criteria which include RV angiography (RVA) and cardiovascular magnetic resonance (CMR). There has been a dearth of studies to document the comparison of the performance of CMR and RVA, and none undertaken in Africa. The aim of this study was to compare CMR and RVA in the assessment of ARVC in the South African ARVC registry.

Methods: The study is a retrospective analysis of definite, possible and borderline ARVC cases from the South African ARVC registry and the African Cardiomyopathy and Myocarditis Registry Program (IMHOTEP) that have both CMR and RVA data. RV end-systolic and diastolic volumes, RV ejection fractions and the presence of absence of structural abnormalities derived from RVA and CMR are compared. Sensitivity of CMR and RVA for the diagnosis of definite, possible and borderline ARVC was also calculated.

Results: A total of 11 patients out of 62 from the registry met the inclusion criteria. The Spearman's coefficient for RV end-systolic volume was 0.48 (p=0.12). The Spearman's coefficient for RV end-diastolic volume was 0.28 (p=0.4). The Spearman's coefficient for RV ejection fraction was 0.06 (p=0.85). CMR detected regional wall abnormalities in 4 out of 11 patients while RVA did not detect any regional wall abnormalities. Sensitivity of CMR and RVA for the diagnosis of definite, possible and borderline ARVC was 48% and 55%, respectively.

Conclusions: We show that South African ARVC patients had poor correlation between CMR and RVA parameters, and CMR was also more likely to reveal RV free wall regional wall motion abnormalities.

Keywords

Arrhythmogenic right ventricular cardiomyopathy, right ventricular angiography, cardiovascular magnetic resonance, right ventricular volumes, South Africa.

Introduction

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited cardiomyopathy characterised by structural changes to mostly the right ventricle (RV) that predispose to ventricular arrhythmias, heart failure and sudden cardiac death (SCD).[1] ARVC is diagnosed using the 2010 Task Force Criteria (TFC) (Table 1).[2] The 2010 TFC have major and minor criteria in 6 categories (Table 1). These categories are: (1) global or regional dysfunction and structural alterations, (2) tissue characterisation of the myocardium, (3) repolarisation abnormalities, (4) depolarisation/conduction abnormalities, (5) arrhythmias and (6) a family history of the disease. A definite diagnosis of ARVC is made with the fulfilment of 2 major or 1 major and 2 minor criteria. Borderline ARVC is diagnosed with 1 major + 1 minor or 3 minor criteria. Possible ARVC is present if 1 major or 2 minor criteria are met.[2] The diagnostic criteria have evolved as technology and our understanding of the disease has improved. One of the categories in the 2010 TFC is the demonstration of global or regional dysfunction and structural alterations. The modalities that can be used to demonstrate this are two dimensional echocardiography, cardiovascular magnetic resonance (CMR) and right ventricular angiography (RVA).[2]

RVA is invasive and has the potential for complications. Although endomyocardial biopsies (EMB) can be performed at the time of RVA, its use is limited by the observation that sampling the thin dyskinetic RV free wall, which is the most likely area to produce the highest yield, carries the highest risk of complications. CMR is the imaging modality of choice as it can achieve more accurate quantification of RV volumes and imaging of the RV structure compared to 2-dimensional echocardiography, is noninvasive and without ionising radiation when compared to RVA.[3,4] CMR can detect fat infiltration, wall motion abnormalities, ventricular sizes and myocardial fibrosis in the RV.[3] Major criteria for CMR are regional RV akinesia, dyskinesia or dyssynchronous contraction plus one sign of functional impairment.[2]

There have been few studies which have compared RVA to CMR directly. In 2004, White, *et al* examined 17 patients who had presented with arrhythmias and were being evaluated for ARVC.[5] Out of this cohort, 7 were subsequently diagnosed with definite ARVC according to 1994 TFC. CMR sensitivity and specificity for this cohort was 86% and 60%, respectively, while RVA had a sensitivity and specificity of 100%.[5] In the other comparative study, published in 2012, Indik, *et al* studied 17 probands from the North American ARVC registry who had undergone both CMR and RVA, and noted a correlation coefficient of 0.72 for RV end-diastolic volume (RVEDV), 0.68 for RV ejection fraction (RVEF) with RVA volumes generally bigger than CMR.[6]

There have been no studies to document comparisons of CMR and ARVC on the African continent. The University of Cape Town hosts the African Cardiomyopathy and Myocarditis Registry Program (IMHOTEP) and the South African ARVC registry. Therefore, we aimed to investigate the performance of CMR and RVA in the assessment of ARVC in the South African population.

Methods

The study was a retrospective analysis of definite, possible and borderline ARVC cases from the IMHOTEP and South African ARVC registries that had both CMR and RVA data. RV end-systolic volume (RVESV), RVEDV, RVEF and the presence of structural abnormalities were noted in CMR and RVA. Data were entered onto an electronic database that was password protected to ensure privacy.

CMR studies were performed using a 1.5 T MR system (Avanto and Aera, Siemens Healthcare, Germany). A 32-channel phased-array chest coil was used for all data acquisition. A complete stack of short axis images was obtained during breath hold and cardiac gating for cine and LGE imaging. LGE imaging was performed using a T1-weighted phase-sensitive inversion recovery sequence about 8-15 minutes after intravenous administration of contrast agent (0.15 mmol/kg body weight).

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For calculation of RV angiographic volumes, the 30° right anterior oblique (RAO) and 60° left anterior oblique (LAO) views were utilised. The RV angiographic volumes were analysed blindly by two observers, independently. A cardiac cycle was analysed that had good

contrast opacification of the RV and not immediately proceeded by a premature ventricular contraction. The diastolic and systolic images were selected, and a contour drawn in each phase. The area within the contour was then calculated. The length-scale was calibrated by measuring the projected size of the magnified angio-catheter shaft within the RV, which was usually a pigtail 5Fr catheter, to allow an accurate and reproducibly consistent measurement. The RV volume was then assessed by using both the RAO and LAO projections ($V_{TWO\text{-}VIEW}$) or by using the RAO projection alone (V_{RAO}).

$V_{TWO\text{-}VIEW}$ was calculated as:

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where A_{RAO} was the projected area within the drawn contour in the RAO view, A_{LAO} was the projected area in the LAO view, and L_{RAO} was the projected distance in the RAO view from the pulmonic valve to the point that bisected the inferior wall.

The ejection fraction was then computed as follows:

$$RVEF_{TWO\text{-}VIEW} = (V_{TWO\text{-}VIEW\text{-}DIA} - V_{TWO\text{-}VIEW\text{-}SYS}) / V_{TWO\text{-}VIEW\text{-}DIA} \quad (2)$$

where $V_{TWO\text{-}VIEW\text{-}DIA}$ was the volume computed at end-diastole and $V_{TWO\text{-}VIEW\text{-}SYS}$ was the volume computed at end-systole of the chosen cardiac cycle by Equation 1.

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The RVEF was computed using the RAO computed volumes at end-diastole and end-systole as follows:

$$RVEF_{RAO} = (V_{RAO\text{-}DIA} - V_{RAO\text{-}SYS}) / V_{RAO\text{-}DIA} \quad (4)$$

where $V_{RAO\text{-}DIA}$ was the volume computed at end-diastole and $V_{RAO\text{-}SYS}$ was the volume computed at end-systole of the chosen cardiac cycle, by the RAO volume formula (Equation 3). Body surface area (BSA) was computed and all volumes were adjusted for BSA.

The study was approved by the University of Cape Town Human Research Ethics Committee. This study was a substudy of the ARVC Registry of South Africa (HREC Reference number: 047/2003) and IMHOTEP study (HREC Reference number: 766/2014). Informed consent had therefore already been obtained from participants and was not obtained again as this was a retrospective assessment of data that already had been collected.

Descriptive statistics were used to summarise patient characteristics. Continuous variables were summarised as mean ± standard deviation and minimum and maximum range. Categorical variables were presented as frequencies and percentages. Spearman's rank correlation was used to analyse the strength and direction of the linear relationship between RVA and CMR measures. Absolute error was calculated for every subject for each measure. Mean difference was calculated as the difference between RVA and CMR measures. Bland-Altman plots were used to assess the agreement between angiogram and MRI measurements. Limits of agreement (95% lower and upper bounds) were calculated as the mean difference ± 2 standard deviations. Plots were observed to assess whether data values fall within ± 2s of the mean difference. All analysis was performed using Stata v.14.1 software (StataCorp, College Station, TX, USA).

Results

Demographic and anthropometric measures

11 patients out of 62 from the IMHOTEP and South African ARVC registries met the inclusion criteria of the study. Of these 11 ARVC patients, 6 were male; the mean age was 36 ± 14 years (range of 15 to 62 years) (Table 2). The mean height was 1.69 ± 8.48 m and mean weight 72.5 ± 16.21 kg.

Comparative analysis of RVEDV

CMR derived mean RVEDV was 226 ± 155 ml, with a range of between 141 and 554 ml (Table 3). RVA derived mean RVEDV was 155 ± 35 ml, with a range of between 110 and 210 ml. The Spearman's coefficient for RVEDV was 0.28 (p=0.4) (Figure 1). Bland-Altman analysis showed that the bias (mean difference in scores ± standard deviation) between MRI and angiogram RVEDV was 71 ± 101 ml (Figure 2). The upper limit of agreement is 273 ml and the lower limit of agreement is -131 ml. CMR derived RVEDV were greater than those derived from RVA.

Comparative analysis of RVESV

CMR derived mean RVESV was 135 ± 132 ml, with a range of between 51 and 514 ml (Table 4). RVA derived mean RVESV was 56 ± 18 ml with range of between 30 and 91 ml. The Spearman's coefficient for RVESV was 0.48 (p=0.12) (Figure 3). Bland-Altman analysis showed that the bias between CMR and RVA value for RVESV was 79 ± 36 ml (Figure 4). The upper limit of agreement was 319 ml and lower limit of agreement of -160 ml. Similar to above, we found that RVA underestimated RVESV compared to CMR.

Comparative analysis of RVEF

CMR derived RVEF was 46 ± 19 %, with a range of between 7.3 and 67% (Table 5). RVA derived RVEF was 64 ± 5 %, a range of between 57 and 73%. The Spearman's coefficient for RVEF was 0.06 (p=0.85) (Figure 5). Bland-Altman analysis showed that mean difference between CMR and RVA value is 18 ± 5.5 % (Figure 6). The upper limit of agreement is 19% and lower limit of agreement is -55%.

Comparative analysis of RV wall motion abnormalities

CMR detected regional wall abnormalities in 4 of 11 (36%) ARVC patients, while RVA did not detect any regional wall abnormalities, suggesting that CMR may be ideally placed for this assessment.

Diagnostic sensitivity

Out of the 62 definite, possible and borderline borderline cases, 27 had CMR data. 13 of these 27 ARVC patients with CMR fulfilled major criteria for ARVC based on CMR imaging criteria. Sensitivity for a major CMR criterion was therefore 48%. There were 38 RVA available for analysis from the 62 patients in the registries. Of these 38 patients with RVA, 21 fulfilled major criteria for ARVC based on RVA imaging criteria. Sensitivity for RVA major criteria was therefore 55%. Specificity could not be calculated as we did not have the overall denominator of patients referred for assessment of ARVC who were found not to have the diagnosis.

Discussion

We compared the diagnostic performance of CMR and RVA in 11 patients with ARVC. We found that (1) the patients were young, with a mean age of 37 years, (2) CMR derived end-diastolic and end-systolic volumes were greater, (3) CMR derived RVEF was lower, (4) there was great variability between CMR and RVA measures, with poor correlation on Bland-Altman analysis, (5) only CMR revealed focal dyskinesia of the RV, and (6) that diagnostic sensitivity was poor for both modalities in this study. We believe these data have significant implications for clinical practice, and suggest that CMR may be a preferred diagnostic tool for imaging in ARVC. First, CMR is noninvasive and does not involve ionising radiation. Second, CMR was better able to detect focal dyskinesia and dyssynchrony in the RV free wall.

Our hypothesis in this study was that there would be a high degree of correlation between CMR and RVA, which was not supported by the results. In fact, our study revealed poor correlation between CMR and RVA with worsening Spearman's correlation for RVESV, RVEDV and RVEF, respectively. Several possibilities exist to explain the discrepant results, including the small sample size, variable RVA technique, and temporal differences in the acquisition of the data from the 2 imaging modalities.

Of interest, our observations of the low sensitivity of CMR (48%) and RVA (55%) were not in keeping with previously published data. RVA has been reported to have a sensitivity of 100%.[5] Similarly, prior studies have reported a CMR sensitivity of 68%-100%.[7-9] The diagnostic accuracy of CMR was assessed in a series of 232 patients undergoing evaluation for suspected ARVC using the 1994 TFC.[9] In this series, 64 patients fulfilled 1994 TFC for the diagnosis of ARVC, 63 fulfilled diagnostic criteria modified for familial ARVC, and another 7 were obligate gene carriers. The following were noted: (1) 183 of CMR studies were interpreted as diagnostic or strongly suspicious for ARVC; and (2) all patients who fulfilled 1994 TFC modified for familial ARVC, or those who were obligate gene carriers had abnormal CMR results (diagnostic or strongly suspicious), thus giving a sensitivity and specificity of CMR for clinical ARVC of 100% and 50%, respectively.[9] Possible reasons for the low sensitivity observed in our study are indicated in the study limitations described below.

Nowadays, in most centres, RVA is rarely performed in patients suspected of having ARVC, reflecting the fact that CMR provides high-quality quantitative information on RV size and function, as well as tissue characteristics through use of LGE imaging. However, in centers where CMR is not available, or in patients in whom EMB is planned, RVA is still performed. CMR enables identification of global and regional ventricular dilation, global and regional ventricular dysfunction, intramyocardial fat, LGE and focal wall thinning.[8-13] Importantly, neither wall thinning nor intramyocardial fat is included in the diagnostic criteria for ARVC, and

they should not be relied on for diagnosis. Further, CMR abnormalities are unlikely in the absence of ECG, echocardiographic, and/or arrhythmic manifestations of ARVC, though the possible role of LGE as an isolated, early marker of disease expression requires additional evaluation.[14]

While not assessed in this study, CMR may play an important role in the risk stratification of ARVC patients. Among a cohort of 175 ARVC patients (52 definite, 50 borderline, and 73 possible by 2010 revised TFC) who underwent CMR and were followed for a median of 4.3 years, 35 (20%) patients experienced a hard cardiac event (SCD, resuscitated cardiac arrest, or appropriate ICD shock).[15] Of the 35 patients with an event, 34 had CMR abnormalities (defined as RV or LV wall motion abnormality, RV or LV dilation, RV or LV systolic dysfunction, fat infiltration, or LGE), suggesting that patients with a normal CMR are at low risk for cardiac arrhythmic events. Measurements of myocardial strain by CMR correlate well with scar (as detected by LGE or by electroanatomic mapping during invasive electrophysiology studies) and may be helpful in identifying patients at risk for VT with arrhythmogenic substrate for ablation.[16] Indeed, in our study, CMR was able to detect wall motion abnormalities in 36%, when these were not detected on RVA.

Our data suggest that CMR may be a more reliable tool for the assessment of RV volumes and function in ARVC. However, concerns with CMR have been noted in the published literature. First, although some CMR parameters are highly specific for gene-carrier status (e.g., specificity of 100% for each of these three parameters: RV dilation and/or systolic impairment, RV LGE, and severe RV segmental dilation/regional wall motion abnormalities and/or aneurysms), others demonstrated low specificity (e.g., specificity of 56% for abnormal trabeculations and 44% for mild RV localized dilation and/or regional wall motion abnormalities).[9] Second, CMR interobserver variability in identifying features of ARVC has been noted, and substantial interobserver variability may be seen identified between readers, likely related to lack of experience with CMR in the diagnosis of ARVC.[8,12,17] Consequently,

CMR should ideally be performed in a center with expertise in the evaluation of ARVC with CMR.

This study has several limitations. First, it has all the flaws of a retrospective design. Second, the sample size of 11 ARVC patients included is small. Third, the RVA technique was noted to be quite variable and the imaging planes used for RVA were not consistent. Fourth, the readers for CMR and RVA were not all blinded to the results. Fifth, the CMR and RVA were not always performed at the same time point and may explain some of the discrepancies noted between the two, due to disease progression between imaging time points. Sixth, our study was unable to assess specificity of CMR and RVA, as we did not have data on total numbers of patients who underwent imaging and subsequently found not have the disease. Nonetheless, despite these limitations, we think that this study provides useful insights on the diagnostic performance of CMR and RVA in the evaluation of patients with ARVC.

Conclusion

In conclusion, we compared the diagnostic performance of CMR and RVA in patients with ARVC and found that CMR reported greater volumes, lower global systolic function, and was more likely to reveal RV dyskinesia or dyssynchrony. There was great variability between CMR and RVA measures of cardiac structure and function and poor correlation on Bland-Altman analysis. Finally, in our study, the diagnostic sensitivity of both CMR and RVA was low compared to existing literature. It would be ideal for these observations to be confirmed in larger studies, but such confirmation is unlikely as few centres performed RVA in ARV nowadays.

References

1. Basso C, Corrado D, Bauce B, Thiene G. Arrhythmogenic right ventricular cardiomyopathy. *Circ Arrhythmia Electrophysiol* 2012;5(6):1233-1246.
2. Marcus FI, McKenna WJ, Sherrill D, Basso C, Bauce B, Bluemke DA, Calkins H, Corrado D, Cox MG, Daubert JP, Fontaine G, Gear K, Hauer R, Nava A, Picard MH, Protonotarios N, Saffitz JE, Sanborn DM, Steinberg JS, Tandri H, Thiene G, Towbin JA, Tsatsopoulou A, Wichter T, Zareba W. Diagnosis of arrhythmogenic right ventricular cardiomyopathy/dysplasia: proposed modification of the task force criteria. *Circulation* 2010;121(13):1533-41.
3. Moosa S, Ntusi NAB. Role of cardiovascular magnetic resonance in the evaluation of cardiomyopathy. *South Afr J Radiol* 2016;20(2):a1055.
4. Ntusi NAB, Samuels P, Moosa S, Mocumbi AO. Diagnosing cardiac disease during pregnancy: imaging modalities. *Cardiovasc J Afr* 2016;27(2):95-103.
5. White JB, Razmi R, Nath H, Kay GN, Plumb VJ, Epstein AE. Relative utility of magnetic resonance imaging and right ventricular angiography to diagnose arrhythmogenic right ventricular cardiomyopathy. *J Interv Card Electrophysiol* 2004;10(1):19-26.
6. Indik JH, Dallas WJ, Gear K, Tandri H, Bluemke DA, Moukabary T, Marcus FI. Right ventricular volume analysis by angiography in right ventricular cardiomyopathy. *Int J Cardiovasc Imaging* 2012;28(5):995–1001.
7. Liu T, Pursnani A, Sharma UC, Vorasettakarnkij Y, Verdini D, Deeprasertkul P, Lee AM, Lumish H, Sidhu MS, Medina H, Danik S, Abbara S, Holmvang G, Hoffmann U, Ghoshhajra BB. Effect of the 2010 task force criteria on reclassification of cardiovascular magnetic resonance criteria for arrhythmogenic right ventricular cardiomyopathy. *J Cardiovasc Magn Reson* 2014;16(1):47.
8. Tandri H, Castillo E, Ferrari VA, Nasir K, Dalal D, Bomma C, Calkins H, Bluemke DA. Magnetic resonance imaging of arrhythmogenic right ventricular dysplasia: sensitivity,

specificity, and observer variability of fat detection versus functional analysis of the right ventricle. *J Am Coll Cardiol* 2006;48(11):2277-2284.

9. Sen-Chowdhry S, Prasad SK, Syrris P, Wage R, Ward D, Merrifield R, Smith GC, Firmin DN, Pennell DJ, McKenna WJ. Cardiovascular magnetic resonance in arrhythmogenic right ventricular cardiomyopathy revisited: comparison with task force criteria and genotype. *J Am Coll Cardiol* 2006;48(10):2132-140.
10. Boxt LM, Rozenshtein A. MR imaging of arrhythmogenic right ventricular dysplasia. *Magn Reson Imaging Clin N Am* 2003;11(1):163-171.
11. Midiri M, Finazzo M, Brancato M, Hoffmann E, Indovina G, Maria MD, Lagalla R. Arrhythmogenic right ventricular dysplasia: MR features. *Eur Radiol* 1997;7(3):307-312.
12. Bluemke DA, Krupinski EA, Ovitt T, Gear K, Unger E, Axel L, Boxt LM, Casolo G, Ferrari VA, Funaki B, Globits S, Higgins CB, Julsrud P, Lipton M, Mawson J, Nygren A, Pennell DJ, Stillman A, White RD, Wichter T, Marcus F. MR Imaging of arrhythmogenic right ventricular cardiomyopathy: morphologic findings and interobserver reliability. *Cardiology* 2003;99(3):153-162.
13. Tandri H, Saranathan M, Rodriguez ER, Martinez C, Bomma C, Nasir K, Rosen B, Lima JA, Calkins H, Bluemke DA. Noninvasive detection of myocardial fibrosis in arrhythmogenic right ventricular cardiomyopathy using delayed-enhancement magnetic resonance imaging. *J Am Coll Cardiol* 2005;45(1):98-103.
14. Norman M, Simpson M, Mogensen J, Shaw A, Hughes S, Syrris P, Sen-Chowdhry S, Rowland E, Crosby A, McKenna WJ. Novel mutation in desmoplakin causes arrhythmogenic left ventricular cardiomyopathy. *Circulation* 2005;112(5):636-42.
15. Aquaro GD, Pingitore A, Bella GD, Piaggi P,MS, Gaeta R, Grigoratos C, Altinier A, Pantano A, Strata E, De Caterina R, Sinagra G, Emdin M. Prognostic Role of Cardiac Magnetic Resonance in Arrhythmogenic Right Ventricular Cardiomyopathy. *Am J Cardiol* 2018;122(10):1745-1753.

16. Zghaib T, Ghasabeh MA, Assis FR, Chrispin J, Keramati A, Misra S, Berger R, Calkins H, Kamel I, Nazarian S, Zimmerman S, Tandri H. Regional strain by cardiac magnetic resonance imaging improves detection of right ventricular scar compared with late gadolinium enhancement on a multimodality scar evaluation in patients with arrhythmogenic right ventricular cardiomyopathy. *Circ Cardiovasc Imaging* 2018;11(9):e007546.

17. Tandri H, Calkins H, Marcus FI. Controversial role of magnetic resonance imaging in the diagnosis of arrhythmogenic right ventricular dysplasia. *Am J Cardiol* 2003;92(5):649.