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ORIGINAL ARTICLE

Imaging of programmed cell death in arrhythmogenic right ventricle cardiomyopathy/dysplasia

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Abstract
Background Arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D) is a myocardial disease that predominantly affects the right ventricle (RV). Its hallmark feature is fibrofatty replacement of the RV myocardium. Apoptosis in ARVC/D has been proposed as an important process that mediates the slow, ongoing loss of heart muscle cells which is followed by ventricular dysfunction. We aimed to establish whether cardiac apoptosis can be assessed noninvasively in patients with ARVC/D.
Methods Six patients fulfilling the ARVC/D criteria were studied. Regional myocardial apoptosis was assessed with ^{99m}Tc-annexin V scintigraphy.
Results Overall, the RV wall showed a higher ^{99m}Tc-annexin V signal than the left ventricular wall (p=0.049) and the interventricular septum (p=0.026). However, significantly increased uptake of ^{99m}Tc-annexin V in the RV was present in only three of the six ARVC/D patients (p=0.001, compared to ^{99m}Tc-annexin V uptake in the RV wall of the other three patients).
Conclusion Our results are suggestive of a chamber-specific apoptotic process. Although the role of apoptosis in ARVC/D is unsolved, the ability to assess apoptosis noninvasively may aid in the diagnostic course. In addition, the ability to detect apoptosis in vivo with ^{99m}Tc-annexin V scintigraphy might allow individual monitoring of disease progression and response to diverse treatments aimed at counteracting ARVC/D progression.

Keywords Arrhythmogenic right ventricle cardiomyopathy/dysplasia · Scintigraphy · Apoptosis · ^{99m}Tc-annexin V scintigraphy

Abbreviations

^{99m}Tc	technetium 99m
ARVC/D	arrhythmogenic right ventricular cardiomyopathy/dysplasia
IVS	inter-ventricular septum
LV	left ventricle
PS	phosphatidylserine
ROI	region of interest
RV	right ventricle
VT	ventricular tachycardia

Introduction

Arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D) is a disease that predominantly affects the right ventricle (RV), although biventricular involvement may occur in advanced disease [1]. ARVC/D is characterized by structural derangements that may cause a broad range of

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signs and symptoms. Yet, disease expression is highly variable and incomplete in most patients, confounding both the diagnostic process and clinical management, particularly during early disease stages [2].

The histopathological hallmark of ARVC/D is fibrofatty replacement of the RV myocardium. Apoptosis has been proposed as an important mechanism that mediates the slow, ongoing loss of heart muscle cells which is followed by ventricular dysfunction [3]. How fibrofatty replacement and apoptosis are related in ARVC/D is a matter of speculation. The possibility to detect apoptosis in vivo in ARVC/D may lead to a better understanding of the pathophysiological mechanism underlying disease progression [4]. In vivo imaging of cardiac apoptosis with the use of ^{99m}Tc-annexin V has been proven feasible, as ^{99m}Tc-annexin V binds to exposed phosphatidylserine (PS) on the outer surface of apoptotic cells [5]. Accordingly, ^{99m}Tc-annexin V has been effectively used to noninvasively visualize regions of apoptosis in patients with various pathologies [6–9], as well as in experimental models [10, 11]. We aimed to establish whether cardiac apoptosis can be assessed non-invasively in patients with ARVC/D.

Methods

Patients

The institutional review board approved the study protocol and informed consent was obtained from all study subjects. Six ARVC/D patients were examined. The patients, who fulfilled the ARVC/D Task Force criteria [12], were randomly taken from the cohort of ARVC/D patients at our institution. Patients were evaluated when they were in a clinically stable condition (no ventricular tachyarrhythmias or heart failure symptoms during the 2 months prior to inclusion). In all patients, molecular genetic analysis was performed and focused on known mutations related to ARVC/D; these included plakophilin-2 (PKP2), desmoplakin (DSP), desmoglein-2 (DSG2), desmocollin-2 (DSC2), plakoglobin (JUP), and transmembrane protein 43 (TMEM43) [13, 14]. No patient had a history of coronary artery disease, diabetes or hypertension.

Scintigraphy

Patients were intravenously injected with 600 MBq of ^{99m}Tc HYNIC-rh-annexin V (^{99m}Tc-annexin V), and 4 h after administration, single photon emission computed tomography (SPECT) scans were acquired using a dual-headed gamma camera equipped with a 3/8” NaI(Tl) crystal and combined with a low-dose CT scanner (Infinia; General Electric Medical Systems, Haifa, Israel). SPECT scans were acquired with low-energy, high-resolution collimators, a 15% energy window on the 140 keV photopeak, according to a step-and-shoot protocol with a total of 90 frames and 30 s per frame in a 128×128 matrix and a zoom of 1.28. SPECT images were iteratively reconstructed (OSEM) and corrected for attenuation using the low-dose CT scans from the Infinia scanner (no intravenous contrast material).

Analysis of scintigraphic data

To define the anatomical borders of the myocardium within the thorax, anatomical tomographic images are essential and the low-dose CT images of the Infinia could not be used for this purpose. Therefore, tomographic anatomical images from contrast-enhanced CT or cardiac MR imaging performed prior to implantable cardioverter defibrillator (ICD) implantation and within 2 months of ^{99m}Tc-annexin V scintigraphy were reviewed for all subjects. To align the anatomical images with the SPECT data, first the matrix size of the anatomical images was adjusted to the matrix size of the SPECT data (128×128) and second the images were automatically aligned (MultiModality; HERMES Medical Solutions, Sweden). To semiquantify ^{99m}Tc-annexin V myocardial uptake, three regions of interest (ROI) including the RV wall, interventricular septum (IVS) and left ventricle (LV) free wall were drawn on three summed mid-myocardial horizontal long axis anatomical images. To correct for background activity (i.e. nonspecific uptake), a separate region was drawn on both lungs. As there were no differences between the two lung regions, these values were aggregated to one value (mean counts per pixel). The ROIs were determined on the anatomical images and were subsequently copied to the aligned SPECT images. ^{99m}Tc-annexin V uptake in each separate ROI was calculated as the ratio of the mean counts per pixel in the specific myocardial region to the mean counts per pixel in the total myocardium (i.e. the sum of all three ROIs). Both the regional and the total myocardial activity were corrected for background activity by subtraction of nonspecific uptake. The attenuation-corrected SPECT data were used for analysis. The reader was blinded to the clinical information.

Follow-up

Long-term follow-up data were obtained from at least one of three sources: visit to the outpatient clinic; review of the patient’s hospital records; personal communication with the patient’s physician. This analysis focused on the occurrence of ventricular arrhythmias, appropriate ICD discharge, and sudden cardiac death. One patient was lost to follow-up. The mean follow-up was 27±8 months (range 18–57 months).

Table 1 Clinical characteristics of patients with ARVC/D

Patient no.	Gender	Age at scintigraphy (years)	Symptoms at diagnosis	Age at diagnosis (years)	Mutation	Medication	ARVC/D Task Force criteria									
							Family history			ECG depolarization/ conduction		ECG repolarization	Arrhythmias		RV dysfunction	
							Disease confirmed at necropsy (major)	Sudden cardiac death (minor)[a]	ARVC/D (minor)	Epsilon wave (major)	Late potential (minor)	Negative T wave (minor)	LBBB-VT (minor)	>1,000 PVC/24 h (minor)	Severe (major)	Mild (minor)
1	M	24	Syncope	21	PKP2: C796R[b]	Sotalol	−	+	+	−	+	+	+	−	−	+
2	F	55	VT	49	No	–	−	+	+	−	NA	+	+	−	−	+
3	F	48	VT	38	No	Sotalol	−	−	−	−	NA	+	+	NA	−	+
4	M	33	VT	30	No	Sotalol	−	−	+	−	−	+	+	NA	−	+
5	M	19	VT	16	No	Sotalol	−	−	−	−	NA	+	-	NA	−	+
6	M	41	VT	27	DSG2: T335A[c]	–	−	−	−	−	+	+	+	NA	+	−

LBBB left bundle branch block, *NA* not analysed, *PVC* premature ventricular complex.

[a] Death before 35 years of age due to suspected ARVC/D.

[b] C796R missense mutation in plakophilin-2.

[c] T335A missense mutation in desmoglein-2.

Statistical analysis

Data are presented as means ± SD. Mean values were compared for differences using the (un)paired Student's *t*-test when appropriate. For multiple comparisons, means were compared for differences using analysis of variance (ANOVA) with a post-hoc Bonferroni correction (SPSS for Windows 16.0.2.1; SPSS, Chicago, IL). A p value <0.05 was considered to indicate statistical significance.

Results

Clinical spectrum

Table 1 summarizes the demographic/clinical data. All patients fulfilled the ARVC/D Task Force criteria [12]. Their mean age at clinical presentation was 36.7±13.9 years (range 19–55 years) and 33% (two) were women. In five patients, ventricular tachycardia (VT) with left bundle branch block morphology was the first expression of ARVC/D. One patient presented with syncope. Two patients had a positive family history of premature sudden cardiac death. All patients had normal LV function by echocardiography and all patients had an ICD. All patients had a history of haemodynamically unstable VT. Four patients were on antiarrhythmic agents. One patient had the C796R mutation in PKP2, while one had the T335A mutation in DSG2. In the remaining patients, no DNA mutations were found. One patient (patient 6) had severe segmental dilatation of the RV on echocardiography (major ARVC/D Task Force criterion [12]). The other five patients had regional RV hypokinesia (patients 2, 3 and 5), mild segmental dilatation of the RV (patient 4) and mild global RV dilatation with normal LV function (patient 1) on echocardiography (minor ARVC/D Task Force criteria [12]).

Myocardial ^{99m}Tc-annexin V uptake

Figure 1 shows a typical example of a patient who exhibited increased ^{99m}Tc-annexin V uptake in the RV wall (patient 2). Overall, the RV wall showed a higher ^{99m}Tc-annexin V uptake (1.328±0.437) than the LV wall (0.936±0.175, p=0.049) or the IVS (0.902±0.222, p=0.026). There was no difference in ^{99m}Tc-annexin V uptake between the LV wall and the IVS (p=0.986). However, the overall higher uptake of ^{99m}Tc-annexin V in the RV wall could be explained by the fact that 50% of patients (patients 3, 5 and 6) showed increased ^{99m}Tc-annexin V uptake in the RV compared to the other three patients (patients 1, 2 and 4; 1.788±0.133 vs 0.983±0.034 respectively, p=0.001; Fig. 2).

Within 2 months of ^{99m}Tc-annexin V scintigraphy, cardiac MR images were available in three patients (patients 2, 3 and 4). Only patient 3 showed increased uptake of ^{99m}Tc-annexin V. The increased uptake of ^{99m}Tc-annexin V was located in the lateral wall of the RV, while the MR images showed an overall dilated RV with regional dyskinesia of the apex. It is therefore not possible to draw any conclusions as to a potential correlation between cardiac MRI RV abnormalities and the location of increased uptake of ^{99m}Tc-annexin V.

Follow-up

The extent of ^{99m}Tc-annexin V uptake in the RV wall did not distinguish patients with arrhythmias within 2 years after ^{99m}Tc-annexin V scintigraphy from those without, nor did it distinguish patients in whom a gene mutation was found from those in whom it was not (Table 2).

Discussion

Apoptosis is a significant pathophysiological feature of ARVC/D and is a consistent post-mortem finding in both the RV and LV [1, 15]. In this study, ^{99m}Tc-annexin V scintigraphy was performed with the purpose of establishing whether apoptosis can be visualized in vivo in patients with ARVC/D. Our results demonstrated increased ^{99m}Tc-annexin V uptake in the RV free wall of three ARVC/D patients, suggestive of RV-specific apoptotic activity in

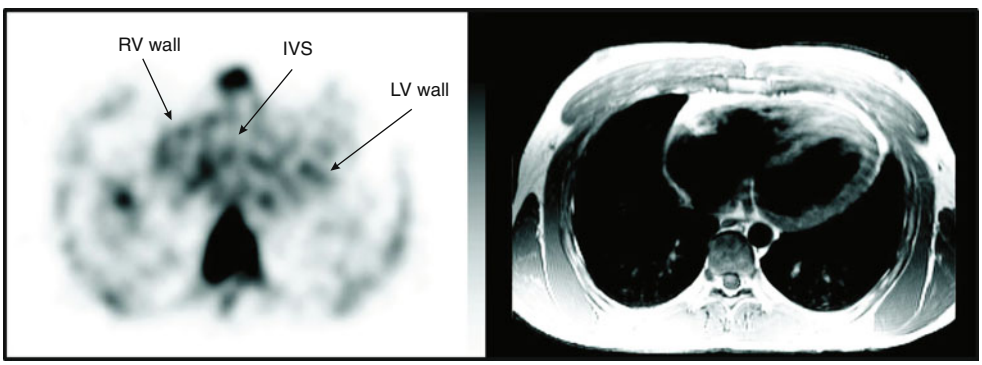

Fig. 1 Coregistered transaxial images of patient 3 (*left* cardiac MR image, *right* ^{99m}Tc-annexin SPECT scintigraphy image). There is increased ^{99m}Tc-annexin V uptake in the RV wall (*IVS* interventricular septum, *LV* left ventricular free wall)

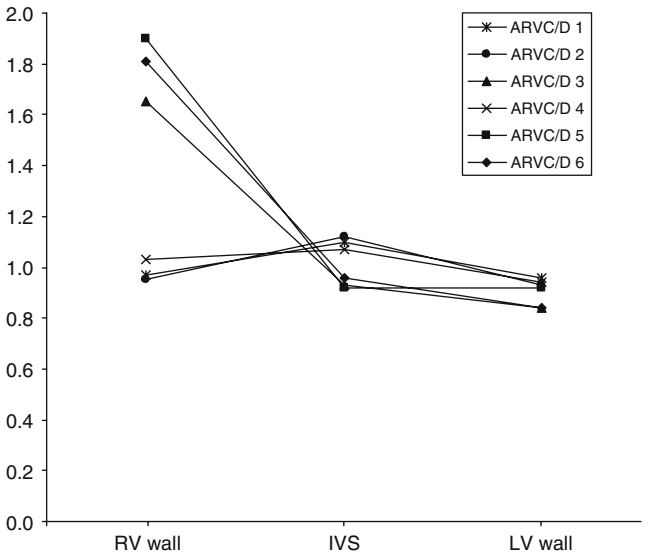

Fig. 2 ^{99m}Tc-annexin uptake in the RV wall, IVS and LV wall calculated as the ratio of the uptake (mean counts per pixel) in the vvparticular myocardial region to the uptake in the total myocardium (i.e. the sum of all three ROIs)

these patients. The variation in myocardial uptake of ^{99m}Tc-annexin V between patients is not surprising and might partly be explained by the random distribution and the episodic nature of the apoptotic process [16]. Furthermore, patients differed with respect to the time since diagnosis and severity of morphological abnormalities. These variations were probably reflected by differences in myocardial uptake of ^{99m}Tc-annexin V.

All our ARVC/D patients had a history of documented VT episodes. Mallat et al. speculated that apoptosis in ARVC/D might result from repetitive ventricular tachyarrhythmia episodes [15]. Furthermore, apoptotic myocytes are frequently found in regions of the myocardium which are not subjected to invasion by adipocytes and fibrosis, suggesting that the loss of myocytes through apoptosis occurs as a primary process before adipocytes and fibrous tissues fill the vacant cellular space. Also, Valente et al. have reported that apoptosis is present in endomyocardial biopsy samples of patients with ARVC/D, especially in the early symptomatic phase of the disease [17].

The exposure of PS on the cell surface is a general marker of apoptotic cells. Externalization of nonapoptotic PS is induced by several activation stimuli, including engagement of immunoreceptors. Externalized PS is observed in apoptotic, injured, infected, senescent and necrotic cells, and becomes a target for recognition by phagocytes [18–20]. Thus, in addition to acting as a marker of apoptosis, annexin V may be a marker of inflammation and cell stress. Accordingly, the myocardial uptake of ^{99m}Tc-annexin V is most likely not only a marker of

Table 2 Follow-up in ARVC/D patients on the occurrence of ventricular arrhythmias, appropriate ICD discharge and sudden cardiac death

Patient no.	^{99m}Tc-annexin V uptake	Year of diagnosis	VT	2005			2006			2007			2008			2009			2010		
				ICD shock	Sudden cardiac death		VT	ICD shock	Sudden cardiac death	VT	ICD shock	Sudden cardiac death	VT	ICD shock	Sudden cardiac death	VT	ICD shock	Sudden cardiac death	VT	ICD shock	Sudden cardiac death
1	Normal	2001	–	–	–		–	–	–	–	–	–	+	–	–	–	–	–	–	–	–
2	Increased	1998	–	–	–		–	–	–	–	–	–	–	–	–	–	–	–	–	-	–
3	Increased	1994	–	–	–		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4	Normal	2001	Lost to follow-up																		
5	Increased	2001	+	+	–		+	+	–	+	–	–	–	–	–	–	–	–	–	–	-
6	Normal	1990	–	–	–		–	–	–	–	–	+									

apoptosis, but may also partly reflect local inflammation. Patchy inflammatory infiltrates in RV are consistently reported in ARVC/D, both in in vitro and in in vivo examinations [3, 21, 22].

Patchy cell death combined with inflammatory infiltration is a common histological finding in ARVC/D [23]. The inflammation might be a reaction to proinflammatory cytokines induced by cell death and/or apoptosis or caused by an infectious myocarditis (e.g. viral infection) [21, 24]. Although it is most likely that these factors are, at least to some extent, interrelated, it is not known whether there is a causal relationship between inflammation and cell death in ARVC/D. However, it remains unclear whether myocarditis in ARVC/D is disease-initiating (a primary event) or a reaction to processes initiated by ARVC/D.

Study limitations and clinical usefulness

The first limitation of our study is the small number of patients. The observation of ^{99m}Tc-annexin V myocardial uptake in three out of six patients might be explained by the random distribution and the episodic nature of the apoptotic process. Second, no cardiac biopsies were obtained. Therefore, a validation of the ^{99m}Tc-annexin V myocardial uptake with histology was not possible.

Recently modification of the highly specific ARVC/D Task Force criteria (published in 1994) has been proposed [25]. The revision incorporates new knowledge and technology to improve especially the sensitivity of the Task Force criteria without changing the high specificity. However, at the time of patient inclusion the 1994 criteria were used [12]. As expected, because of the relatively unchanged specificity, reevaluation of the patients included in our study according to the new criteria did not change the clinical diagnosis in any patient.

Conclusion

Apoptosis may be detected noninvasively in ARVC/D, and this may lead to a better understanding of the role of apoptosis in the pathophysiology of ARVC/D. Whether it will allow monitoring of the disease course or the response to various treatments aimed at counteracting disease progression remains to be studied.

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Conflicts of interest None.

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