Pathophysiology of right ventricular heart disease: the role of structure, apoptosis and inflammation
Campian, M.E.

Citation for published version (APA):
Campian, M. E. (2012). Pathophysiology of right ventricular heart disease: the role of structure, apoptosis and inflammation
CHAPTER VII

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

Maria E. Campian; Hanno L. Tan; Astrid F. van Moerkerken; Raymond Tukkie; Berthe L.F. van Eck-Smit; Hein J. Verberne

“Because things are the way they are, thinks will not stay the way they are.”
Bertolt Brecht
Abstract

Introduction: Arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D) is a myocardial disease that predominantly affects the right ventricle (RV). Its hallmark feature is fibro-fatty replacement of 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 non-invasively in ARVC/D patients.

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 compared to the left ventricular wall ($p = 0.049$) and the inter-ventricular septum ($p = 0.026$). However, significant 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).

Conclusions: 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 non-invasively may aid in the diagnostic course. In addition, the ability to detect apoptosis in vivo with $^{99m}$Tc-annexin V scintigraphy might allow for individual monitoring of disease progression and response to diverse treatments aimed at counteracting ARVC/D progression.
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 cases [1]. ARVC/D is characterized by structural derangements that may cause a broad range of signs and symptoms. Yet, disease expression is highly variable and incomplete in most patients, confounding both the diagnostic process and clinical management, particularly at early disease stages [2].

The histopathological hallmark of ARVC/D is fibro-fatty replacement of 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 fibro-fatty replacement and apoptosis are related in ARVC/D is a matter of speculation. The possibility to detect apoptosis in vivo in ARVC/D may add 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 was 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 experimental models [10, 11]. We aimed to establish whether cardiac apoptosis can be assessed non-invasively in ARVC/D patients.

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. At the time of diagnosis echocardiography (patient 3, 4, 5, and 6) and MRI (patient 1 and 2) were used to describe the RV kinetic abnormalities. Anatomical changes of the RV consist of mild to severe global dilatation, aneurysms, and segmental hypokinesia. Sites of involvement of the RV are found in the triangle of dysplasia, namely the RVOT, the apex and the infundibulum. In all patients, molecular genetic analysis was performed and focused on known mutations related to ARVC/D; this included plakophilin-2 (PKP2), desmoplakin (DSP), desmoglein-2 (DSG2), desmocollin-2 (DSC2), plakoglobin (JUP), and transmembrane protein 43 (TMEM43) [13, 14]. Scintigraphy was performed when they were in clinically stable condition (no ventricular tachyarrhythmias or heart failure symptoms in the 2 months prior to inclusion). No patient had a history of coronary artery disease, diabetes or hypertension.

**Scintigraphy**

Patients were intravenously injected with 600MBq of technetium-99m Hynic-rh-Annexin V (99mTc-annexin V). Four hours after administration, single photon emission computed tomography (SPECT) acquisitions were made using a dual-headed gamma camera equipped with 3/8” NaI(Tl) crystal and combined with a low-dose CT (Infinia, General Electric Medical Systems, Haifa, Israel). SPECT acquisitions were made 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 sec 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 of the Infinia (no intravenous contrast).
**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 (contrast enhanced CT or cardiac MRI performed prior to ICD implantation) performed within 2 months of $^{99m}\text{Tc}$-annexin V scintigraphy, were retrieved for all subjects. To align the anatomical images with the SPECT data, first the matrix size of the anatomical images was adjusted to the SPECT matrix size (128x128) and secondly the images were automatically aligned (*MultiModality, HERMES Medical Solutions, Sweden*). To semi-quantify $^{99m}\text{Tc}$-annexin V myocardial uptake, three regions of interest (ROI) (RV wall, interventricular septum [IVS] and left ventricle [LV] free wall) were drawn on 3 summed mid-myocardial horizontal long axis anatomical images. To correct for background activity (i.e. non-specific uptake) a separate region was drawn in both lungs. As there were no differences in both lung regions these values were aggregated to one value (mean counts per pixel). The ROIs were determined on the anatomical images and subsequently the ROIs were copied to the aligned SPECT images. $^{99m}\text{Tc}$-annexin V uptake in each separate ROI was calculated as the ratio of mean counts per pixel in the specific myocardial region over the mean counts per pixel in the total myocardium (i.e. the sum of all 3 ROIs). Both the regional as the total myocardial activity were corrected for background activity by subtraction of non-specific 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).

Statistical analysis
Data are presented as mean ± SD. Mean values were compared for differences using the (un)-paired Student’s T-test when appropriate. In case of multiple comparisons, means were compared for differences with analysis of variance (ANOVA) using a post-hoc Bonferroni correction (SPSS for Windows 16.0.2.1, SPSS Inc., USA). 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% (n=2) were women. In five patients, ventricular tachycardia (VT) with left bundle branch block (LBBB) 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 implantable cardioverter defibrillator (ICD). All patients had a history of haemodynamically unstable VT. Four patients were on anti-arrhythmic 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 dilation 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 dilation 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]).
### Table 1.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Gender</th>
<th>Age at scintigraphy (yrs)</th>
<th>Symptoms at diagnosis</th>
<th>Age at diagnosis</th>
<th>Mutation</th>
<th>Medication</th>
<th>ARVC/D Task Force criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Family history</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ECG depolarization/conduction</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ECG repolarization</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Arrhythmias</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>RV dysfunction</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Disease confirmed at necropsy (major)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sudden cardiac death (minor)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ARVC/ D wave (minor)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Late potential (minor)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Negative T wave (minor)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>LBBB- &gt;1,000 PVC/ VT 24h (minor)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Severe (major)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mild (minor)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>M</td>
<td>24</td>
<td>Symcope</td>
<td>21</td>
<td>PKP2: C796R&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Sotalol</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>55</td>
<td>VT</td>
<td>49</td>
<td>No</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>48</td>
<td>VT</td>
<td>38</td>
<td>Sotalol</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>33</td>
<td>VT</td>
<td>30</td>
<td>Sotalol</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>19</td>
<td>VT</td>
<td>16</td>
<td>Sotalol</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>41</td>
<td>VT</td>
<td>27</td>
<td>DSG2: T335A&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>LBBB</sup> left bundle branch block, <sup>NA</sup> not analyzed, <sup>PVC</sup> premature ventricular complex.

<sup>a</sup>Death before 35 years of age due to suspected ARVC/D.

<sup>b</sup>C796R missense mutation in plakophilin -2.

<sup>c</sup>T335A missense mutation in desmoglein -2.

**Clinical characteristics of subjects with arrhythmogenic right ventricular cardiomyopathy/dysplasia.** +: present; -: absent; ARVC/D: arrhythmogenic right ventricular cardiomyopathy/dysplasia; LBBB: left bundle branch block; NA: not analyzed; PKP2-C796R: C796R missense mutation in plakophilin-2; DSG2-T335A: T335A missense mutation in desmoglein-2; Pt, patient; PVC/24 h: premature ventricular complex per 24 hours; SCD: family history of sudden cardiac death (<35 years of age) due to suspected ARVC/D; VT: ventricular tachycardia.
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 ($1.328 \pm 0.437$) than the LV wall ($0.936 \pm 0.175$, $p = 0.049$) or the IVS ($0.902 \pm 0.222$, $p = 0.026$). 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 (patient 3, 5 and 6) showed increased $^{99m}$Tc-annexin V uptake in the RV compared to the other 3 patients (patient 1, 2 and 4) ($1.788 \pm 0.133$ vs $0.983 \pm 0.034$ respectively, $p = 0.001$, Figure 2).

**Figure 1**

ARVC/D patient with increased $^{99m}$Tc-annexin V uptake in right ventricular wall. Coregistered transaxial images of patient 3 with cardiac magnetic resonance imaging (left) and $^{99m}$Tc-annexin SPECT scintigraphy (right). There is increased $^{99m}$Tc-annexin V uptake in the right ventricular (RV) wall. IVS: interventricular septum; LV: left ventricular free wall
Myocardial $^{99m}$Tc-annexin uptake in ARVC/D patients. $^{99m}$Tc-annexin uptake in the right ventricular (RV) wall, interventricular septum (IVS), or left ventricular (LV) wall was calculated as the ratio of uptake (mean counts per pixel) in this myocardial region over the uptake in the total myocardium (i.e., the sum of all 3 regions of interest).

**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).
Table 2.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ICD shok</td>
<td>Sudden cardiac death</td>
<td>ICD shok</td>
<td>Sudden cardiac death</td>
<td>ICD shok</td>
<td>Sudden cardiac death</td>
<td>ICD shok</td>
<td>Sudden cardiac death</td>
</tr>
<tr>
<td>1</td>
<td>Normal</td>
<td>2001</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Increased</td>
<td>1998</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Increased</td>
<td>1994</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Normal</td>
<td>2001</td>
<td>Lost to follow-up</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Increased</td>
<td>2001</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Normal</td>
<td>1990</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Follow-up in ARVC/D patients on the occurrence of ventricular arrhythmias, appropriate ICD discharge and sudden cardiac death. Patient 4 was lost to follow-up. +: present; -: absent. Abbreviations as in Table 1
Discussion

Apoptosis is a significant pathophysiological feature of ARVC/D and is a consistent post-mortem finding of both 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 demonstrate increased $^{99m}$Tc-annexin V uptake in the RV free wall of three ARVC/D patients, suggestive of RV-specific apoptotic activity in 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 morphologic abnormalities. These variations are 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 found frequently in the regions of myocardium which are not subjected to the invasion of 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. Non-apoptotic PS externalization is induced by several activation stimuli, including engagement of immunoreceptors. Externalized
PS is observed in apoptotic, injured, infected, senescent, or necrotic cells and becomes a target for recognition by phagocytes [18-20]. Thus, in addition to acting as a marker for 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 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-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**

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

Recently a proposal for modification of the in 1994 published highly specific ARVC/D Task Force criteria has become available [25]. The revision has incorporated 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 in 1994 published ARVC/D Task Force criteria were used [12]. As expected, because of the relatively unchanged specificity, re-evaluation of the patients included in our study according these new criteria did not change the clinical diagnosis in any of the patients.

**Conclusion**

Apoptosis may be detected noninvasively in ARVC/D. This possibility may provide us with a tool that may be used to obtain a better understanding of the role of apoptosis in the pathophysiology of ARVC/D. Whether it allows for monitoring of the disease course or the response to various treatments aimed at counteracting disease progression remains to be studied.
References


