Pathophysiology of right ventricular heart disease: the role of structure, apoptosis and inflammation
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CHAPTER VI

Assessment of inflammation in patients with arrhythmogenic right ventricular cardiomyopathy/dysplasia

Maria E. Campian; Hein J. Verberne; Maxim Hardziyenka; Elisabeth A.A. de Groot; Astrid F. van Moerkerken; Berthe L.F. van Eck-Smit; Hanno L. Tan


“*The teacher who is indeed wise does not bid you to enter the house of his wisdom but rather leads you to the threshold of your mind.*”

Kahlil Gibran
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. However, patchy inflammatory infiltrates in RV are also consistently reported using autopsy and myocardial biopsy. Although the role of inflammation in ARVC/D is unresolved, the ability to assess inflammation noninvasively may aid in the diagnostic process. We aimed to establish whether cardiac inflammation can be assessed noninvasively in ARVC/D patients.

Methods: In 8 ARVC/D patients and 9 control patients from the hematology/oncology department, the level of inflammatory activation was assessed by measuring plasma levels of inflammatory cytokines. Regional myocardial inflammation was assessed with $^{67}$Gallium ($^{67}$Ga)-scintigraphy.

Results: ARVC/D patients had higher plasma levels than controls of the pro-inflammatory cytokines IL-1β (1.22±0.07 vs. 0.08±0.01pg/ml, p<0.0001), IL-6 (3.86±0.44 vs. 0.38±0.04pg/ml, p<0.0001), and TNF-α (9.16±0.90 vs. 0.40±0.06pg/ml, p<0.0001), while levels of the anti-inflammatory cytokine IL-10 were not significantly different (1.28±0.15 vs. 1.56±0.30pg/ml, p=0.74). $^{67}$Ga uptake in RV was higher in ARVC/D patients than in controls. In ARVC/D patients, $^{67}$Ga uptake in the RV wall was higher than in the inter-ventricular septum or left ventricular wall.

Conclusion: Inflammation in the RV wall of ARVC/D patients can be detected noninvasively with the combined analysis of plasma levels of inflammatory cytokines and cardiac $^{67}$Ga-scintigraphy.
Introduction

Arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D) is a myocardial 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 histopathologic hallmark of ARVC/D is fibro-fatty replacement of RV myocardium [3]. Nevertheless, patchy inflammatory infiltrates in RV are also consistently reported [4,5]. How fibro-fatty replacement and inflammatory infiltrates are related is a matter of speculation.

It has been proposed that the progressive loss of RV myocardium is caused by an inflammatory injury, and that subsequent fibro-fatty replacement is part of a healing process [4-6].

At present, detection of myocardial inflammation requires the use of endomyocardial biopsies or analysis of autopsy material. Although sampling of endomyocardial biopsies is relatively safe, particularly when guided by electroanatomic mapping [7], the availability of noninvasive diagnostic tools would provide obvious advantages for clinical practice. Thus far, the pro-inflammatory cytokines ((interleukin-1β [IL-1β], interleukin-6 [IL-6], tumor necrosis factor-α [TNF-α])) have been shown to be activated and to play a role in chamber dysfunction in patients with left ventricular heart failure [8,9]. Yet, in ARVC/D, there are no data on possible activation and detection of the pro-inflammatory cytokines. Although the pro-inflammatory cytokines are possibly sensitive markers for state of disease, the interpretation is hampered by the lack of organ-specific identification. In
addition to systemic evaluation, localization of disease to a specific organ, i.e. within the myocardium, is essential.

Previously, with the use of $^{67}$Ga-scintigraphy, we have shown that activation of inflammatory infiltrates can be noninvasively detected in the ventricular myocardium [10].

Therefore, the aim of the present study was to test whether inflammation in ARVC/D may be monitored noninvasively with the complementary use of plasma inflammatory cytokine analysis and cardiac $^{67}$Gallium ($^{67}$Ga)-scintigraphy.

**Methods**

*Patients and control subjects*

The institutional review board approved the study protocol and informed consent was obtained from all study subjects. Eight ARVC/D patients and nine controls were examined. The patients, who fulfilled the ARVC/D Task Force criteria [11], were randomly taken from the cohort of ARVC/D patients at our institution, a tertiary referral center. Patients were included if they were in clinically stable condition (no ventricular tachyarrhythmias or heart failure symptoms in the 2 months before inclusion). Genetic analysis for plakophilin-2 (PKP2), desmoplakin (DSP), desmoglein-2 (DSG2), desmocollin-2 (DSC2), plakoglobin (JUP), and transmembrane protein 43 (TMEM43) were conducted in all patients or the probands in their family, [12,13].

Controls were retrospectively taken from our institution's hematological/oncological database, and were individuals in whom $^{67}$Ga-scintigraphy was routinely performed in their diagnostic workup. Their primary disease locus was extra-thoracic, and the acquisition was made
before any form of chemotherapy or radiotherapy. Neither ARVC/D patients nor controls had a history of coronary artery disease, diabetes or hypertension.

**Plasma level of inflammatory cytokines**

We analyzed plasma levels of pro-inflammatory cytokines (IL-1β, IL-6, TNF-α), and the anti-inflammatory cytokine interleukin-10 (IL-10) using commercially available ultrasensitive enzyme-linked immunosorbent assay (ELISA) kits for human IL-1β, IL-6, TNF-α, and IL-10 according to the manufacturer's recommendations (Bio-Rad Laboratories, USA). The 95%-confidence interval of the in-hospital reference values obtained in 62 healthy volunteers were for IL-1β: 0.0018-0.2118pg/ml; for IL-6: 0.0895-1.2238pg/ml; for TNF-α: 0.0212-2.2556pg/ml; and for IL-10: 0.0483-16.7000pg/ml.

**67Ga-scintigraphy**

SPECT of the thorax was performed 48h after an intravenous injection of 200MBq of 67Ga-citrate by use of a γ-camera (Infinia, General Electric, USA) with a medium-energy all-purpose collimator and a 128x128 matrix. Fifteen percent windows were set for the 3 main energy peaks of 67Ga (93, 184, and 296keV). SPECT images were iteratively reconstructed (OSEM) and corrected for attenuation using the low-dose CT of the Infinia (no intravenous contrast). 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 (within 6 months of 67Ga-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 $^{67}$Ga myocardial uptake, 3 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. These ROIs on the anatomical images were copied to the aligned SPECT images. $^{67}$Ga uptake in each separate ROI was calculated as the ratio of mean counts per pixel in the specific myocardial region over mean counts per pixel in the total myocardium (the sum of all 3 ROIs).

**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 (two-way ANOVA) using a post-hoc Bonferroni correction. The correlation coefficient was used to study possible associations between plasma levels of inflammatory cytokines and myocardial $^{67}$Ga-uptake (SPSS for Windows 15.1, SPSS Inc., USA). A $p<0.05$ was considered to indicate statistical significance.

**Results**

**Patient characteristics**

Table 1 summarizes the demographic/clinical data and the presence/absence of the ARVC/D criteria in the ARVC/D patients. All ARVC/D patients fulfilled the ARVC/D Task Force criteria [14]. Their mean age was 36.8±13.3 years (range 20-55 years) and 62.5% (n=5) were female. One patient had the C796R mutation in PKP2, while one had the V158G mutation in DSG2. Both variants were previously reported as ARVC/D-
causing mutations (12,15). There were no clinical signs of heart failure and
echocardiography revealed normal left ventricular function (not show). The
mean age of controls was 55.4±11.9 and 22.2% (n=2) were female.
**Table 1.**


<table>
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<th>Patient</th>
<th>Age/Gen</th>
<th>Symptoms</th>
<th>Age at Diagnosis</th>
<th>IDC Mutation</th>
<th>Drugs</th>
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<th>ARVC/D</th>
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<th>LBBB</th>
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**Clinical characteristics of subjects with arrhythmogenic right ventricular cardiomyopathy/dysplasia.** +: present; -: absent; ARVC/D: arrhythmogenic right ventricular cardiomyopathy/dysplasia; DSG2-V158G: V158G missense mutation in desmoglein-2; ICD, implantable cardioverter/defibrillator; LBBB, left bundle branch block; na, not analyzed; PKP2-C796R: C796R missense mutation in plakophilin-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.
**Plasma levels of inflammatory cytokines**

The ARVC/D patients had higher plasma levels than controls of the pro-inflammatory cytokines IL-1β (1.22±0.07 vs. 0.08±0.01pg/ml, p<0.0001), IL-6 (3.86±0.44 vs. 0.38±0.04pg/ml, p<0.0001), and TNF-α (9.16±0.90 vs. 0.40±0.06pg/ml, p<0.0001). The levels of the anti-inflammatory cytokine IL-10 were statistically not significantly different between both groups (1.28±0.15 vs. 1.56±0.30pg/ml, p=0.72) (Figure 1).

**Figure 1**

![Graph showing plasma concentrations of cytokines](image)

**Plasma concentrations of cytokines in ARVC/D patients and controls.** IL-1β, interleukin-1 beta; IL-6, interleukin-6; IL-10, interleukin-10; TNF-α, tumor necrosis factor alpha.

**Myocardial $^{67}$Ga uptake**

Figure 2 shows a typical example of an ARVC/D patient with increased $^{67}$Ga uptake in the RV wall. ARVC/D patients had significantly higher uptake of $^{67}$Ga in the RV wall and IVS than in the LV wall (1.11±0.08, 1.04±0.06, and 0.89±0.06, respectively, Figure 3A). In controls, no differences between these regions were observed (0.97±0.11, 1.04±0.05, and
0.99±0.04, respectively, Figure 3B). Moreover, $^{67}$Ga uptake in the RV wall was significantly higher in ARVC/D patients than in controls (1.11±0.08 vs. 0.97±0.11, $p=0.01$), while $^{67}$Ga uptake was not different between both groups in IVS (1.04±0.06 vs. 1.04±0.05, $p=0.90$) and LV wall (0.89±0.06 vs. 0.99±0.04, $p=0.06$).

**Figure 2.**

ARVC/D patient with increased $^{67}$Ga uptake in right ventricular wall. Coregistered transaxial images of cardiac magnetic resonance imaging (left) and $^{67}$Ga SPECT scintigraphy (right). There is increased $^{67}$Ga uptake in the right ventricular (RV) wall. IVS, interventricular septum; LV, left ventricular free wall.

**Correlations between plasma levels of inflammatory cytokines and myocardial $^{67}$Ga uptake**

Plasma levels of IL-1β and $^{67}$Ga uptake in the RV appeared to correlate positively, but this correlation did not reach statistical significance ($r=0.62$; $p=0.1$). No correlations were found between plasma levels of IL-6, TNF-α, or IL-10 and the $^{67}$Ga uptake in the RV wall. Similarly, no correlations were found between plasma levels of IL-6, TNF-α, or IL-10 and $^{67}$Ga uptake in the LV wall or IVS.
Myocardial $^{67}$Ga uptake in ARVC/D patients and controls. Semi-quantitative myocardial $^{67}$Ga uptake in ARVC/D subjects (A) and controls (B). $^{67}$Ga 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).
Discussion

With a combined analysis of plasma level of inflammatory cytokines and cardiac $^{67}$Ga-scintigraphy, we demonstrate that myocardial inflammation can be noninvasively detected in ARVC/D patients.

The pathophysiology behind the loss of RV myocardium in ARVC/D is unresolved. A common finding is patchy cell death with inflammatory infiltration [16]. This has spawned the proposal that ARVC/D has an infectious/inflammatory etiology, involving a primary chronic myocarditis that evokes an inflammatory injury, and culminates in fibro-fatty repair and progressive loss of RV myocardium. Different types of cardiotropic viruses may play a role in the pathophysiology of ARVC/D. A genetic predisposition may lead to increased viral susceptibility and myocarditis [17].

One explanation for the presence of pro-inflammatory cytokines in ARVC/D is the “cytokines hypothesis” of heart failure, which proposes that a precipitating event triggers innate stress response [18]. These cytokines are believed to be produced by nucleated cells in the heart and subsequently released into the blood stream [19]. In patients with left ventricular heart failure, pro-inflammatory cytokines contribute, by various mechanisms, to the deterioration of cardiovascular function [18, 20, 21]. Furthermore, various cytokines, including TNF-α, IL-2, interferon-γ, and IL-1β-converting enzyme are increased in viral myocarditis [22]. In ARVC/D, no data on a possible association between pro-inflammatory cytokines and disease are available.

Inflammatory mediators such as TNF-α and IL-1β, known to be elevated in viral myocarditis, can stimulate the expression of inducible nitric oxide synthase (iNOS) [23]. Induction of iNOS leads to strand breaks, p53
accumulation, and apoptosis, and these processes were prevented by iNOS inhibition [24]. Apoptosis, in turn, evokes an inflammatory response.

The inflammatory response in ARVC/D may not only be an initial response to a noxious trigger (e.g., viral infection), but it may also be enhanced because of apoptosis induced by pro-inflammatory cytokines. Our findings, (increased plasma levels of pro-inflammatory cytokines and ⁶⁷Ga uptake in the RV wall), did not allow us to distinguish whether they indicated an initial response to infection or a secondary inflammatory response induced by apoptosis. Moreover, to determine whether inflammation is a viral-related inflammation or a post-apoptosis inflammatory reaction is essential to perform endo-myocardial biopsies.

Although pro-inflammatory cytokines may be sensitive disease markers, they cannot localize the disease to a specific organ. Previously, we reported that ⁶⁷Ga-scintigraphy can be used to map inflammation noninvasively in the ventricular myocardium [10]. Although ⁶⁷Ga-scintigraphy has a high sensitivity, a major disadvantage of ⁶⁷Ga-scintigraphy is its limited specificity [29/25]. In recent years, ¹⁸F-fluorodeoxyglucose positron emission tomography (¹⁸F-FDG PET) has emerged as an alternative and superior method to assess inflammation. However, one of the preferred substrates of myocardial metabolism is glucose. This limits the use of ¹⁸F-FDG for the detection of localized myocardial inflammation. Therefore, ⁶⁷Ga-scintigraphy is most likely to be superior to ¹⁸F-FDG for the detection of myocardial muscle inflammation, despite its limited specificity. ⁶⁷Ga thus marks areas of inflammation (e.g., an infection site) and rapid cell division, allowing sites with tumor, inflammation, and both acute and chronic infection to be visualized with
scintigraphic techniques [26-28]. Controversial data were reported in case of inflammatory foci in myocarditis.

**Study limitations and clinical usefulness**

Firstly, a limitation of our study is the small patient number. The finding that the correlations between plasma levels of pro-inflammatory cytokines and myocardial uptake of $^{67}$Ga in the RV wall failed to reach statistical significance probably relates to this limited number. Still, the results were highly consistent, as both plasma levels of pro-inflammatory cytokines and myocardial $^{67}$Ga uptake in the RV wall were increased in ARVC/D patients. Secondly, it was recently suggested that ARVC/D and myocarditis cannot be distinguished with the use of currently established clinical criteria, and that a distinction requires analysis of biopsy specimens [7]. Although we conducted no cardiac biopsies, we obtained support for the notion that we studied patients with ARVC/D, rather than myocarditis, by including 6 patients (patients 1, 2, 3, 4, 6, 7) who had first-degree relatives that also fulfilled established criteria for ARVC/D, and one other patient (patient 5) with a mutation that was previously reported to be associated with ARVC/D.

The ability to detect inflammation noninvasively provides us with a tool that may be used to obtain a better understanding of the role of inflammation in the pathophysiology of ARVC/D as it does in other diseases [29,30].

**Conclusion**

Inflammation may contribute to disease progression in ARVC/D and can be assessed non-invasively with the combined analysis of plasma inflammatory cytokine levels and cardiac $^{67}$Ga-scintigraphy. The ability to detect inflammation non-invasively provides us with a tool that may be used to
obtain a better understanding of the role of inflammation in the pathophysiology of ARVC/D as it does in other diseases [29, 30]. The clinical implications of these findings remain to be assessed in future studies.

Acknowledgments

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11. Corrado D, Fontaine G, Marcus FI et al. Arrhythmogenic right ventricular dysplasia/cardiomyopathy: need for an international registry. Study group on arrhythmogenic right ventricular dysplasia/cardiomyopathy of the working group on myocardial and pericardial disease and arrhythmias of the European Society of Cardiology and of the Scientific Council on


