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Original Article

Ventricular myocarditis coincides with atrial myocarditis in patients

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ABSTRACT

Introduction: Atrial fibrillation (AF) is a common complication in myocarditis. Atrial inflammation has been suggested to play an important role in the pathophysiology of AF. However, little is known about the occurrence of atrial inflammation in myocarditis patients. Here, we analyzed inflammatory cell numbers in the atria of myocarditis patients without symptomatic AF.
Methods: Cardiac tissue was obtained postmortem from lymphocytic myocarditis patients ($n=6$), catecholamine-induced myocarditis patients ($n=5$), and control patients without pathological evidence of heart disease ($n=5$). Tissue sections of left and right ventricle and left and right atrium were stained for myeloperoxidase (neutrophilic granulocytes), CD45 (lymphocytes), and CD68 (macrophages). These cells were subsequently quantified in atrial and ventricular myocardium and atrial adipose tissue.
Results: In lymphocytic myocarditis patients, a significant increase was observed for lymphocytes in the left atrial adipose tissue. In catecholamine-induced myocarditis patients, significant increases were found in the atria for all three inflammatory cell types. Infiltrating inflammatory cell numbers in the atrial myocardium correlated positively with those in the ventricles, especially in catecholamine-induced myocarditis patients.
Conclusions: To a varying extent, atrial myocarditis occurs concurrently with ventricular myocarditis in patients diagnosed with myocarditis of different etiology. This provides a substrate that potentially predisposes myocarditis patients to the development of AF and subsequent complications such as sudden cardiac death and heart failure.

1. Introduction

Myocarditis is a cardiac disease which is heterogeneous in etiology and pathophysiology. Most commonly myocarditis is caused by viral infection, resulting in a distinct lymphocytic infiltration of the myocardium (lymphocytic myocarditis) [1–3]. A noninfectious form of myocarditis, which over the past 20 years has become a clinical topic of increasing interest, is catecholamine-induced myocarditis, which is found in 62% of patients diagnosed with stress-induced cardiomyopathy [4,5]. Although the mechanisms underlying catecholamine-induced myocarditis are still not well understood, an increased infiltration of different inflammatory cells was described in human ventricular heart tissue. Indeed, Yoshida et al. [6] observed mononuclear cell infiltration in endomyocardial biopsy samples taken from the left ventricle of three stress-induced cardiomyopathy patients, while Nef et al. [7] described increased macrophages and lymphocytes in the right ventricle in stress-induced cardiomyopathy. However, both the diagnosis and research into myocarditis are focused almost entirely on the ventricles of the heart (ventricular myocarditis), whereas very little is known about how myocarditis affects the atria.

Nonetheless, myocarditis is a known precursor of atrial fibrillation (AF) [8], and AF is found in 14% of patients with suspected myocarditis

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[9]. AF can result in sudden cardiac death, and indeed myocarditis is a commonly found underlying cause of sudden cardiac death, especially in younger patients [10,11]. In addition, AF can result in the development and enhancement of heart failure [12]. There is strong evidence linking both systemic and local inflammation to the initiation and perpetuation of AF in general [13,14]. For instance, increased infiltration of macrophages [15] and lymphocytes [16] has been described in the left and right atria of patients with symptomatic AF, compared to control patients. These studies suggest that (cardiac) inflammation may predispose patients into developing AF. However, it is not known whether ventricular myocarditis coincides with infiltration of inflammatory cells in the atria (referred to as atrial myocarditis) in myocarditis patients. The presence of atrial myocarditis in myocarditis patients may predispose these patients to the development of AF. In this study, we quantitatively analyzed neutrophilic granulocytes, lymphocytes, and macrophages in the atria and ventricles of lymphocytic and catecholamine-induced myocarditis patients.

2. Materials and methods

2.1. Patients

The use of patient material as described in this article is approved by the medical ethics committee of the VU Medical Center (VUmc), Amsterdam, the Netherlands, in concordance with the guidelines established by the World Medical Association (Declaration of Helsinki). Human heart tissue was obtained at autopsy at the Pathology department of the VUmc and used in this study retrospectively. In the VUmc, as part of the patient contract, or in case relatives have given explicit prior written consent, tissue taken at autopsy can be used for research after completion of the diagnostic process. From each patient, tissue from the left ventricular anterior wall, the right ventricular lateral wall, and the left and right atrial appendages (auricles) of the heart was obtained for analysis.

Details of the included patients are listed in Supplementary Table 1. The patient groups did not differ between each other in gender or age. Patients were diagnosed postmortem with lymphocytic myocarditis based on the presence of aggregates of lymphocytes adherent to cardiomyocytes, in combination with myocytolysis (objectified as complement factor 3d-positivity of cardiomyocytes) in the ventricular myocardium. Instead, patients were diagnosed with catecholamine-induced myocarditis when myocytolysis coincided with a diffuse infiltration of neutrophilic granulocytes, macrophages, and lymphocytes in the ventricular myocardium rather than aggregates of lymphocytes. In addition, control patients were included without pathological evidence of heart disease. Of each patient, a lactate dehydrogenase staining was performed on a ventricular cross-sectional heart slice to detect putative infarctions. As this study aims to investigate atrial myocarditis as a possible predisposing factor for AF, only patients were selected who did not have symptomatic AF. Patients with infarctions were also excluded from this study.

2.2. Immunohistochemistry

For immunohistochemical analysis, formalin-fixed, paraffin-embedded tissue was cut into 4-μm sections, deparaffinized, rehydrated, and incubated in methanol/H_2O_2 (0.3%) for 30 min to block endogenous peroxidases. Antigen retrieval was performed by heat inactivation in sodium-citrate buffer (10 mM, pH 6.0) for slides to be stained for myeloperoxidase (MPO; neutrophilic granulocytes) and CD68 (macrophages). Slides stained for CD45 (lymphocytes) did not require antigen retrieval. The slides were incubated with either rabbit antihuman MPO (1:700; Dako, Eindhoven, the Netherlands), mouse antihuman CD68 (1:400; Dako), or mouse antihuman CD45 (1:50; Dako) for 1 h at room temperature. The sections were then washed with phosphate-buffered saline and incubated with Real EnVision HRP α-mouse/rabbit (undiluted; Dako) for 30 min at room temperature. The staining was visualized using 3,3′-diaminobenzidine (0.1 mg/ml, 0.02% H_2O_2). The sections were then counterstained with hematoxylin, dehydrated, and covered. With each staining, a phosphate-buffered saline control and an isotype control were included. All these controls yielded negative results (not shown).

2.3. Quantitative immunohistochemical analysis

The extravascular MPO-positive (neutrophilic granulocytes), CD68-positive (macrophages), and CD45-positive cells (lymphocytes) were quantified in the myocardium of the left and right ventricle and the left and right atria of the heart. CD45 (leukocyte common antigen) is also present on nonlymphocytic cells, but can be used as general lymphocyte marker based on morphology of positive-staining cells [17]. Only compact cells with a high staining intensity were counted as lymphocytes. Recently, we observed in left atrial appendage tissue of AF patients that inflammatory cells also infiltrated the adipose tissue [18]. Therefore, in this study, we analyzed the atrial adipose tissue separately. In the atria, we identified areas of cardiomyocytes as "myocardium" and areas of adipose tissue as "adipose tissue," regardless of whether this adipose tissue was part of the epicardium or embedded in the myocardium. In one lymphocytic myocarditis patient, the adipose tissue was not analyzed as the atrial tissue slides contained an insufficient amount of adipose tissue (<0.3 mm^2). The total surface area of each sample was measured using QPRODIT, and the numbers of cells were calculated as the total score for each specimen.

2.4. Collagen staining and quantification

Atrial collagen was visualized histochemically using a picrosirius red staining. Formalin-fixed paraffin-embedded tissue was cut into 4-μm sections, deparaffinized, and rehydrated. The sections were incubated in a 0.2% Sirius Red solution (VWR International, Radnor, PA, USA; solution made in 1.2% picric acid) for 60 min. Subsequently, the staining was differentiated in 0.01 N HCl for 2 min. Following this, the sections were washed, dehydrated, and covered. For quantitative analysis, six photos were taken randomly throughout the myocardium of each atrial tissue section, using a Leica DM/RB microscope equipped with a polarization filter. NIH Image software 1.63 was used to calculate the percentage of collagen on each photo, and the mean percentage was taken as data value of an individual patient. The quantitative analysis was performed blinded.

2.5. Statistical analysis

Statistical analysis was performed using Prism 6.0 (GraphPad Software, La Jolla, CA, USA). Differences in cell numbers per mm^2 tissue and collagen deposition were calculated with the Mann–Whitney *U* test, while correlations were determined using Spearman's rank correlation coefficient. A correlation coefficient ranging from −0.29 to 0.29 constituted absence of correlation, from 0.30 to 0.49 or −0.30 to −0.49 constituted a medium correlation, and from 0.50 to 1.00 or −0.50 to −1.00 constituted a strong correlation. Correlations were calculated separately for each myocarditis group. For the correlations, we also calculated the coefficient of determination. Age and gender differences between groups were determined using the Kruskal–Wallis test. *P* values <.05 were considered statistically significant.

3. Results

3.1. Inflammatory cells infiltrating the atria

In general, a diffuse pattern of infiltrating neutrophilic granulocytes (Fig. 1A), lymphocytes (Fig. 1B), and macrophages (Fig. 1C) was observed in the atria of myocarditis patients. All these inflammatory cells