Assessment of the inflammatory process by endomyocardial biopsy in patients with dilated cardiomyopathy based on pathological and immunohistochemical methods

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Abstract

Introduction: Myocarditis may lead to dilated cardiomyopathy (DCM) in immunogenetically predisposed individuals. The diagnosis of myocardial inflammation is currently based on histopathological and immunohistochemical methods. Previous studies indicate that inflammatory cardiomyopathy occurs in approximately 50% of patients with DCM.

Aim: The goal of the study was to assess the inflammatory process in patients with DCM by endomyocardial biopsy using histopathological and immunohistochemical methods.

Methods: Endomyocardial biopsy specimens was examined using routine histopathological methods and immunochemical staining for T lymphocytes (CD3+, n=84), major histocompatibility complex I (HLA ABC, n=48) and II (HLA DPQR, n=84) antigens and the adhesion molecules ICAM-1 (n=51) and VCAM-1 (n=48) in 84 patients (69 male, 15 female; mean age 35.0±10.5 years) with angiographically-confirmed DCM. Familial disease occurrence was noted in 14 (16.7%) patients. Cardiac samples obtained from 18 patients who died of non-cardiovascular causes were used as a control group.

Results: Myocarditis was diagnosed, according to the Dallas criteria, in 8 (9.5%) patients. The frequency of inflammatory cardiomyopathy, defined as the presence of >2 CD3+ T lymphocytes per high-power field (hpf) in myocardial biopsy, was 14.3%. When broader criteria were applied (presence of >2.0 CD3+ lymphocytes/hpf and/or 1.5 CD3+ lymphocytes/hpf in multiple foci and increased expression of class I/II HLA), inflammatory cardiomyopathy was diagnosed in 32.1% of patients. Inflammatory activation of the endothelium, indicated by increased expression of at least three adhesion molecules (class I and II HLA, ICAM-1, VCAM-1), was present in 22 (45.8%) patients. The expression of HLA DPQR, HLA ABC and ICAM-1 was observed on the endothelium of capillaries and larger vessels, interstitial cells, and the surface of activated lymphocytes; immunohistochemical reactions were diffuse. In patients with markedly elevated expression of the aforementioned adhesion molecules, the expression was also present on cardiomyocyte cell membranes. VCAM-1 was restricted to the endothelium of individual small veins. The control group did not demonstrate any signs of myocarditis, inflammatory cardiomyopathy or inflammatory endothelial activation.

Conclusions: The application of immunohistochemical methods to myocardial biopsy in order to identify the inflammatory cell phenotype and the presence of adhesion molecules permits the diagnosis of inflammatory cardiomyopathy in 14% or 32% of patients, depending on the criteria used, while conventional pathology allows for this diagnosis in 9% of patients. The observed frequency of inflammatory cardiomyopathy, defined as the presence of >2 CD3+ T lymphocytes/hpf in the myocardium, was lower (14%) than in previous studies, while the frequency of inflammatory endothelial activation was similar (45%).

Key words: dilated cardiomyopathy, inflammatory cardiomyopathy, adhesion molecules, immunohistochemistry, myocarditis

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Introduction

Dilated cardiomyopathy (DCM) is the most common type of cardiomyopathy and occurs in relatively young individuals (3rd-5th decade of life). It is the second most frequent indication for heart transplantation [1]. Familial and genetic studies have concluded that familial DCM constitutes 35% of cases. The pathogenesis of sporadic DCM remains unclear.

Experimental and clinical studies indicate that there may be a causative link between viral (entero- and adenoviral) myocarditis and DCM. One of the proposed pathogenetic mechanisms suggests that acute viral myocarditis develops into a chronic inflammatory process in immunogenetically predisposed individuals. This leads to progressive heart failure [2, 3]. An evaluation of endomyocardial biopsy using the Dallas classification, which is based on a routine light-microscope examination, often fails to prove the presence of chronic inflammation in these patients [4]. The main disadvantage of this method is due to the focal character of myocardial inflammatory lesions, which, given the limited size and amount of bioptate, makes it difficult, sometimes impossible, to find inflammatory infiltrates in the myocardium. Furthermore, it is difficult to differentiate between inflammatory cells and, for example, fibroblasts, macrophages, or endothelial cells of small vessels in routine haematoxylin-eosin stains.

Table I. Clinical characteristics of 84 patients with diagnosed dilated cardiomyopathy

Parameter	Value
Age [years]	35.0±10.5
Male	69 (82.1%)
Duration of disease [months]	17.2±27.2
NYHA class	2.6±0.6
Permanent AF	15 (17.8%)
LBBB	11 (13.1%)
LVEDD [mm]	69.9±9.2
%SF	14.4±5.7
Mean PAP [mmHg]	27.1±11.0
Mean PCWP [mmHg]	20.2±8.7
Mean RAP [mmHg]	7.3±5.7
CI [l/min/m^2]	2.7±0.8
LVEF [%]	26.8±10.3

Abbreviations: NYHA class – functional class according to the New York Heart Association; AF – atrial fibrillation; LBBB – left bundle branch block; LVEDD – left ventricular end-diastolic diameter; SF – shortening fraction; PAP – systolic pulmonary artery pressure; PCWP – pulmonary capillary wedge pressure; RAP – right atrial pressure; CI – cardiac index; LVEF – left ventricular ejection fraction

Experimental and human studies indicate that cardiomyocyte necrosis, which is obligatory for the diagnosis of active myocarditis according to the Dallas classification, is only detectable during the first 7-10 days after the onset of symptoms [5]. For this reason, histological criteria are useful in the diagnosis of acute myocarditis, especially when diffuse inflammatory infiltrates and myocytolysis are present, whereas their application in the diagnosis of long-standing cardiac inflammatory disease is limited.

The recently introduced immunohistochemical examinations of the myocardial bioptate can identify the number, phenotype and activation level of inflammatory cells, and demonstrate increased expression of adhesion molecules, indicative of chronic inflammation (class I and II HLA antigens and adhesion molecules ICAM-1, VCAM-1, LFA-3, CD62E, CD62P, CD29, VLA-4). The immunohistological criterion of >2 CD3+/CD2+ T lymphocytes/hpf (high-power field) was used by WHO experts in the definition of inflammatory cardiomyopathy as idiopathic cardiac failure with intramuscular inflammation [6]. Since data concerning the occurrence of inflammation in DCM in a limited amount of publications vary significantly, the goal of the current study was to assess signs of inflammation in endomyocardial biopsy, based on histopathological and immunohistochemical methods.

Methods

Patients

Diagnostic endomyocardial biopsy was performed in 84 patients (69 male, 15 female; mean age: 35.0±10.5 years) with DCM at the National Institute of Cardiology in Warsaw, over the period 1992-2004. The mean disease duration was 17 months. All patients were examined physically and by electrocardiography, echocardiography, cardiac catheterisation and coronary angiography. The diagnosis of DCM was made based on WHO criteria [6], i.e. the presence of a dilated, poorly contracting left ventricle (LVEDD >117% of the age- and body surface area-adjusted expected value) and an ejection fraction of <45%, evaluated by angiography and the exclusion of other known cardiac diseases. Coronary angiography was normal in all the patients. Exclusion criteria included moderate and severe arterial hypertension, coronary artery disease, arrythmogenic right ventricular dysplasia, alcohol abuse, supraventricular tachyarrhythmia, significant valvular defects, systemic disease, pericardial disease, congenital heart defects and cor pulmonale.

The characteristics of DCM patients in whom endomyocardial biopsy was performed are presented in Table I. Familial occurrence (>1 DCM patient in a given

Table II. Type and reactivity of monoclonal antibodies used in immunohistochemical tests

Antigen	Antibody	Dilution	Reactivity
CD3	CD3	1:50	T lymphocytes
HLA-ABC	W6/32	1:50	HLA A, B and C (class I MHC)
HLA-DPQR	CR3/43	1:50	Beta chain of DPDQDR gene subregion products (class II MHC)
VCAM-1/CD106	1.4C3	1:50	VCAM-1, expressed primarily on activated endothelial cells, but also by macrophages and dendritic cells
ICAM-1/CD 54	6.5B5	1:50	ICAM-1 surface glycoprotein, expressed primarily on endothelial cells and monocytes, but also by B and T lymphocytes

family) was noted in 14 (16.7%) patients. Eighteen patients (10 male, 8 female; mean age: 53.61 years) who died of non-cardiovascular causes (neoplasm, 14; pneumonia, 1; stroke, 1; hepatic coma, 1; carotid artery haemorrhage, 1) were used as a control group; cardiac muscle samples were obtained from the right ventricle during autopsy, within 24 hours of death.

Endomyocardial biopsy

Three to five samples were obtained in each patient from the interventricular septum, free wall and apex of the right ventricle. Two of the three samples were fixed in 10% buffered formalin and transferred to a paraffin block, using routine methods. Three µm thick sections were stained using haematoxylin-eosin. Histopathological diagnosis of active and borderline myocarditis was made in accordance with the Dallas criteria [4]. According to this classification, active inflammation is characterised by the presence of diffuse or multifocal lymphocyte infiltrates with the cytolysis of adjacent cardiomyocytes. The remaining cardiac muscle samples were embedded in gel (OCT Compound, Miles Inc.) and frozen at -70°C. Serially-cut paraffin and frozen sample slices were placed on silanized slides and stained by the EnVision immunohistochemical method, using the poly- and monoclonal antibodies (DAKO) described in Table II. Positive and negative controls were performed by staining material from reactive lymph nodes and the omission of the first antibody. The quantitative evaluation of CD3+ T lymphocytes was based on the mean value in at least 10 microscopic fields at 400x magnification. The expression of adhesion molecules was assessed semiquantitatively and qualitatively according to the following scale:

0 – lack of staining
1 – weak staining
2 – increased generalised staining
3 – intense generalised staining

For adhesion molecules which are normally present on endothelial and/or interstitial cardiac muscle cells (class I and II HLA, ICAM-1), a value greater than 1 was considered positive. For adhesion molecules which are expressed *de novo* during inflammation (VCAM-1), values greater than 0 were considered positive. The final diagnosis was based on two analyses by two independent pathologists. An inflammatory endothelial activation was considered positive if at least 3 adhesion molecules (class I HLA, HLA DR, VCAM-1, ICAM-1) were expressed on the vascular endothelium.

Statistical analysis

The results are presented as mean ± standard deviation or percentage (%). Fisher's exact test was used to compare the occurrence of given parameters in the study and control groups. Values of $p < 0.05$ were considered statistically significant.

Results

Myocarditis was diagnosed in 8 (9.5%) patients by applying the Dallas criteria to a routine H-E stain (Figure 1). Immunohistochemical tests were performed on both paraffin and frozen sections in 84 patients, and adhesion molecule analyses (class I HLA, ICAM-1, and VCAM-1) were conducted in 48, 51, and 48 patients,

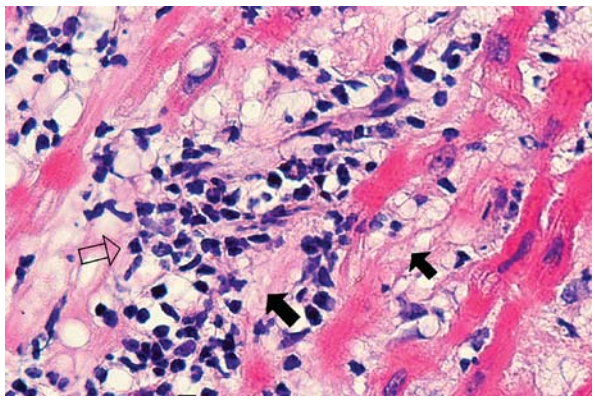

Figure 1. Active myocarditis. Inflammatory interstitial infiltration with lymphoid cells (outlined arrows) and cardiomyocytes demonstrating signs of myocytolysis (solid arrows). H&E stain, 200x mag.

Table III. Expression of adhesion molecules in cardiac biopsy specimens from patients with DCM and control subjects

Parameter	Group I	Group II	Group III	Total	Control group n=8 for HLA DPQR, n=10 for other molecules	p
	DCM (T lymph. >7/mm³) n=12	DCM (T lymph. <7/mm³) n=29	DCM without inflammatory infiltrates n=43	DCM n=84		
HLADPQR	12/12^,& (100%)	15/29 (51.7%)	10/43 (23.3%)	37/84* (44%)	1/18 (5.6%)	*0.005
HLA ABC	7/8$ (87.5%)	10/18 (55.6%)	7/22 (31.8%)	24/48* (50%)	0/10	*0.003
ICAM-1	6/8# (75%)	10/19 (52.6%)	6/24 (25%)	22/51* (43.1%)	0/10	*0.01
VCAM-1	6/8? (75%)	7/18 (38.9%)	4/22 (18.1%)	17/48* (35.4%)	0/10	*0.026

**p vs control group*
HLADPQR: ^ p: Group I vs Group II=0.003; & Group I vs Group II and Group III <0.0001
HLA ABC: $ p: Group I vs Group II and Group III=0.003
ICAM-1: # p vs Group II and Group III=0.048

respectively. Adhesion molecule tests were not performed in some patients due to the lack of frozen material. The results are presented in Table III.

Myocarditis (defined as >7 CD3+ lymphocytes per mm³ [which corresponds to >2 CD3+ T lymphocytes/hpf]) was diagnosed by myocardial biopsy in 14.3% of patients (12/84). When expanded criteria were applied (>2 CD3+ T lymphocytes/hpf and/or >1.5 CD3+ T lymphocytes/hpf in multiple foci and increased expression of class I/II HLA), inflammatory cardiomyopathy was diagnosed in 32.1% of patients (27/84).

Inflammatory activation of the endothelium, defined as increased expression of at least three different adhesion molecules (HLA ABC, HLA DPQR, ICAM-1, VCAM-1), was observed in 22 (45.8%) patients. The frequency of expression of individual adhesion molecules was higher than in the control group (HLA DPQR, 44% *vs* 5.6% in the control group, p=0.005; HLA ABC, 50% *vs* 0%, p=0.003; ICAM-1, 43.1% *vs* 0%, p=0.01; VCAM-1, 35.4% *vs* 0%, p=0.026). Adhesion molecules were expressed most often in samples containing intense inflammatory infiltrates – bioptates fulfilling the Dallas criteria and found to contain >7 CD3+ T lymphocytes per mm³ in immunohistochemical tests. Adhesion molecule expression was less evident in bioptates containing limited inflammatory infiltrates (<7 lymphocytes per mm³). Expression was also observed in bioptates from patients with DCM, in which no immunohistochemical signs of inflammatory infiltration were demonstrated. Endothelial activation was present in 16/26 bioptates (61.5%), which fulfilled the expanded criteria of inflammatory cardiomyopathy, but only 6/22 bioptates (27.3%) from patients with DCM without signs of inflammation demonstrated increased expression of three adhesion molecules (p <0.037).

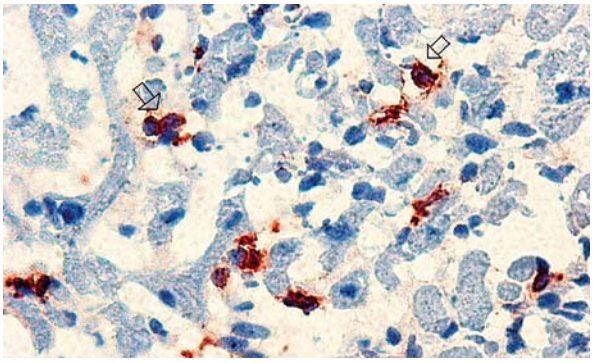

Figure 2. CD3-expressing inflammatory cells in the myocardium (arrows). EnVision stain, 200x mag.

Routine (H-E) and immunohistochemical stains were applied to myocardial samples obtained from the control subjects; individual CD3+ T lymphocytes (<1/hpf) and increased expression of HLA DPQR were present in 1 of 18 patients (5.6%). This patient concerned had died of severe bacterial pneumonia. Inflammatory lymphocyte infiltrates and signs of inflammatory endothelial activation (increased expression of ICAM-1, VCAM-1, and HLA ABC) were not observed in the control group.

The lymphocyte infiltrates observed in cardiobioptates from DCM patients using immunohistochemical methods were both focal and diffuse (Figure 2). Positive immunohistochemical reactions for HLA DPQR, HLA ABC, and ICAM-1 were mainly demonstrated on the endothelium of capillaries and larger vessels and the surface of activated T lymphocytes; in patients with more intense inflammatory processes these molecules were also located on interstitial cells and the surface of cardiomyocytes (Figures 3-7). These reactions were diffuse in character, i.e. they affected the entire cardiac muscle sample, independently of focal inflammatory

infiltrates. The adhesion molecule expression on cardiomyocyte membranes was both focal and diffuse. In contrast to the other adhesion molecules, VCAM-1 was restricted to the endothelial surface of small venous vessels (Figure 8).

Discussion

Myocardial biopsy is an important element in the diagnosis of heart failure of unknown origin in DCM; a specific diagnosis is, however, rarely made. Application of the Dallas criteria to routine histopathological studies reveals inflammation as the cause of DCM in approximately 10% of patients [7]. We observed active myocarditis, defined according to these criteria as the presence of diffuse or multifocal inflammatory infiltrates with damage to the neighbouring cardiomyocytes, in 9.5% of patients. Conventional histopathological

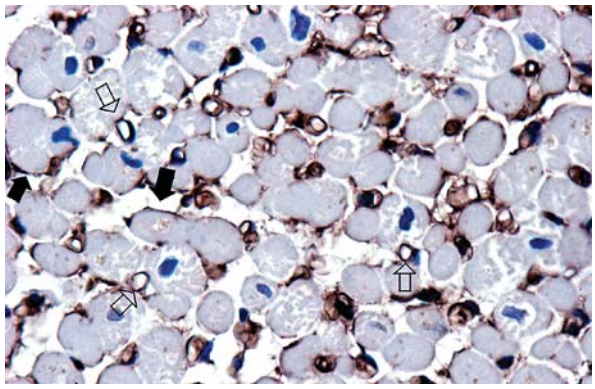

Figure 3. Positive test for HLA DPQR on capillary endothelial (outlined arrows) and cardiomyocyte (solid arrows) surfaces in a patient with inflammatory cardiomyopathy. EnVision stain, 200x mag.

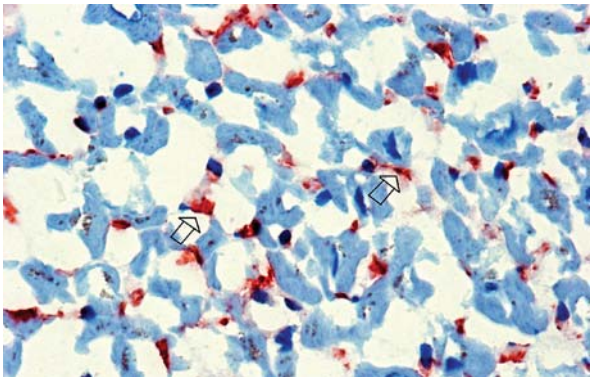

Figure 4. Normal expression of HLA ABC in myocardial bioptate from a patient without inflammatory cardiomyopathy. A positive reaction is observed in a small number of cells (arrows). EnVision stain, 200x mag.

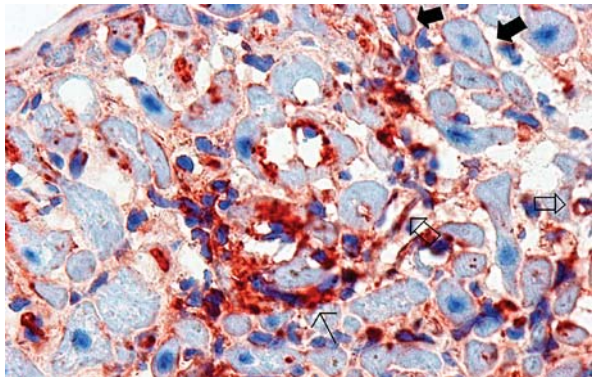

Figure 5. Increased expression of ICAM-1 on small vessel endothelium (outlined arrows), interstitial cells (thin arrows) and, focally, on cardiomyocyte surfaces (solid arrows) in myocardial bioptate from a patient with inflammatory cardiomyopathy. EnVision stain, 200x mag.

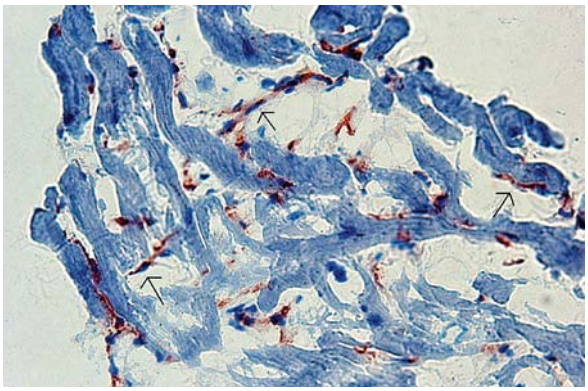

Figure 6. Normal expression of ICAM-1 (on a small number of interstitial cells; arrows) in a patient with DCM without signs of inflammatory cardiomyopathy. EnVision stain, 200x mag.

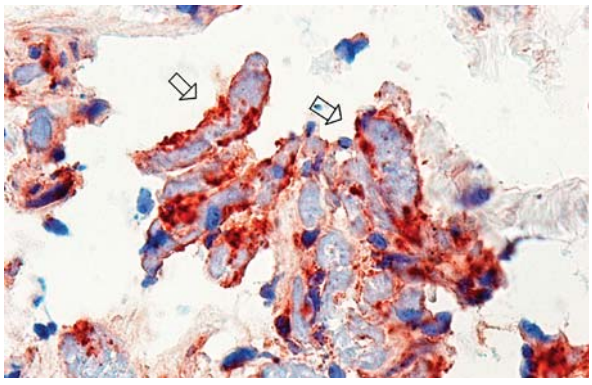

Figure 7. Increased expression of HLA ABC on cardiomyocytes (arrows) in inflammatory cardiomyopathy. EnVision stain, 200x mag.

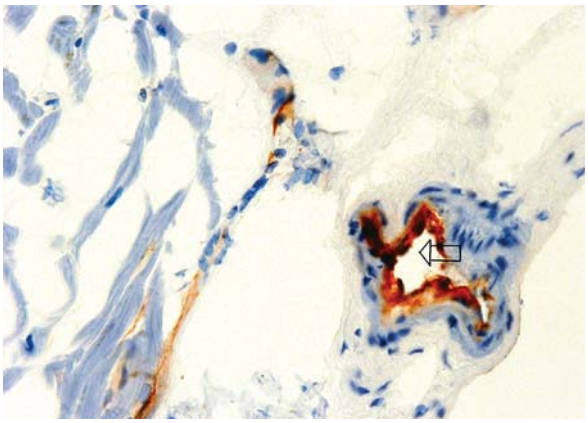

Figure 8. Expression of VCAM-1 in a single small vein (arrows) in inflammatory cardiomyopathy. EnVision stain, 200x mag.

examinations of the remaining cardiac muscle bioptates revealed, both in our material and in previous studies, nonspecific changes, such as cardiomyocyte hypertrophy or damage and varying degrees of interstitial fibrosis [8].

When one considers the pathogenetic relationship between myocarditis and DCM, it seems that the number of inflammatory processes diagnosed is surprisingly low. This may be due to the fact that the inflammatory infiltrates observed in myocarditis are often focal, which, given the limited size and number of bioptates, as well as the fact that biopsies are often taken from patients with long-standing DCM, renders it difficult to observe an active inflammatory process in the cardiac muscle [9]. The development of immunohistochemical methods for the assessment of myocardial bioptates has made it possible to diagnose inflammatory cardiomyopathy based on the phenotype of inflammatory cells, which limits the number of errors made in the histopathological analysis (due to misinterpretations of the inflammatory cells, e.g. mistaking a fibroblast for a lymphocyte) [10, 11].

The most important criterion in the diagnosis of inflammatory cardiomyopathy is the evaluation of CD3+ T lymphocyte infiltrates. Immunohistochemical tests performed on frozen material increase the sensitivity of the quantitative analysis. The criterion most frequently applied is the presence of >7 CD3+ T lymphocytes per mm^3 of endomyocardial tissue. In our material, this amount was observed in 12 (14.3%) patients. A comparison with previous studies indicates that this percentage is similar to that obtained by Wojnicz et al. [11] (12%), and lower than the result presented by Schultheiss et al. [1] (approx. 50%). The fact that we studied subjects with familial DCM, who did not fulfil these criteria, may be another factor which decreases the frequency of inflammatory cardiomyopathy in our material.

Inflammatory infiltrates observed in healthy cardiac muscle samples taken during autopsies, pre-transplant samples from heart donors, and samples from patients with coronary artery disease and most patients with heart failure of unknown origin, without DCM, do not exceed 1.5 T lymphocytes/hpf [12, 13]. It is worth noting that, besides naive CD3+ T lymphocytes, activated and cytotoxic T lymphocytes, macrophages, NK (natural killer) cells, and B lymphocytes also play a role in myocardial inflammation. An analysis of the frequency with which various inflammatory cell phenotypes occurred in the myocardium demonstrated a significant correlation between the presence of CD3+ T lymphocytes and activated (CD45RO+ and CD69+) T lymphocytes, as well as the presence of cell adhesion molecule VLA-4 receptor on inflammatory cells [13].

Since the presence of inflammatory infiltrates is related to activation of cell adhesion molecules, an evaluation of the expression of these molecules is another component of immunohistochemical diagnostics in myocardial inflammation. Inflammatory activation of the endothelium by cytokines leads to increased or *de novo* expression of adhesion molecules. These molecules act as a signal for circulating immunocompetent cells which adhere to the activated endothelium and subsequently migrate via the endothelial cells into the damaged tissue [14]. Class I and II HLA molecules and ICAM-1 may be a constitutional component of vessel endothelium and interstitial cell membranes. Under normal conditions, there is no (or limited) expression of these molecules; during inflammation, this expression increases significantly. VCAM-1, however, is observed exclusively on activated endothelial cells. The fact that adhesion molecules (with the exception of VCAM-1) are expressed throughout the myocardium in inflammatory cardiomyopathy is especially valuable in immunohistochemical diagnosis, as it makes it possible to diagnose inflammation despite the focal distribution of inflammatory infiltrates. A semiquantitative evaluation of adhesion molecule expression in myocardial bioptates may, however, be an imprecise method, as it depends on the subjective interpretation of expression as normal or increased.

The presence of various adhesion molecules in cardiac bioptates in DCM has been confirmed in previous studies [11, 15-17]. Digital imaging analysis has also demonstrated a correlation between the endothelial expression of adhesion molecules and the presence of inflammatory

cells expressing counter-receptors for these molecules [18]. We studied the expression of four adhesion molecules. The expression of HLA DPQR was observed in 44%, HLA ABC in 50%, ICAM-1 in 43.1%, and VCAM-1 in 35.4% of patients with DCM. The expression of individual adhesion molecules may be increased in a few percent of patients without heart failure; this has been proven in autopsy studies. In our control group, one of 18 patients demonstrated increased expression of HLA DPQR.

Noutsias et al. [10], who studied a broad range of adhesion molecules (the immunoglobulin, selectin, and β_1- and β_2-integrin families) in myocardial bioptates in DCM, observed simultaneous increased expression of at least three adhesion molecules in 67% of patients. Since simultaneous expression of three adhesion molecules did not occur in the control group, the authors proposed this as a marker of inflammatory endothelial activation. We observed simultaneous expression of at least 3 of the 4 studied adhesion molecules in 45.8% of our patients, and none in the control subjects. The induction of adhesion molecules precedes the formation of inflammatory infiltrates, which means that increased expression of adhesion molecules on endothelial and interstitial cells in endomyocardial bioptates may be interpreted as inflammatory activation of the myocardium, even if no inflammatory cells are observed [19]. Based on this observation, Noutsias et al. expanded the diagnostic criteria of inflammatory cardiomyopathy, including patients with a number of CD3+ T lymphocytes below 2/hpf, but increased expression of adhesion molecules on endothelial and myocardial interstitial cells [13]. These criteria were fulfilled by 67% of patients in Noutsias's material, and 32.1% of our patients.

We observed the expression of adhesion molecules on endothelial and interstitial cells in most of our patients. In patients with especially intense inflammatory processes, the induction of adhesion molecules was also demonstrated on the surface of cardiomyocytes, in either a focal or a diffuse distribution. This observation has previously been made by other authors [11, 16, 20, 21]. The expression of adhesion molecules on the surface of cardiomyocytes, which, unlike the expression on endothelial and interstitial cells, does not occur under normal conditions, may represent an autoimmune cardiomyocyte reaction in response to injury and/or cytokine stimulation by inflammatory cells. Certain researchers deny the presence of positive immunohistochemical reactions on the surface of cardiomyocytes, attributing it to a diagnostic error related to the methods used to generate immunohistochemical reactions [10, 15, 18].

Experimental studies have demonstrated that cytokine-induced expression of adhesion molecules may be attenuated by antioxidants and certain drugs, such as cyclosporine and statins. Node et al. [22] observed lower levels of adhesion molecule-inducing cytokines (TNF-α, IL-6) in patients with DCM treated with statins, compared to placebo. Wojnicz et al. [23] demonstrated a beneficial effect of immunosuppressive treatment in patients with heart failure due to DCM and increased expression of HLA in myocardial bioptates. These studies indicate that demonstrating increased activation of endothelial and interstitial cells in cardiac bioptates identifies a group of patients who may benefit from treatment with drugs inhibiting this process, such as statins.

Conclusions

The application of immunohistochemical methods to myocardial biopsy allows for the diagnosis of inflammatory cardiomyopathy in 14% or 32% of patients, depending on the criteria used, and demonstrates inflammatory activation of the endothelium in 45% of patients. The frequency of inflammatory cardiomyopathy defined as the presence of >2.0 CD3+ T lymphocytes/hpf in the myocardium was lower than in other publications, while inflammatory endothelial activation occurred in a similar number of patients.

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Ocena procesu zapalnego w biopsji endomiokardialnej u chorych z kardiomiopatią rozstrzeniową na podstawie badań histopatologicznych i immunohistochemicznych

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Streszczenie

Wstęp: Zapalenie mięśnia sercowego może prowadzić do kardiomiopatii rozstrzeniowej (DCM) u osób z predyspozycją immunogenetyczną. Rozpoznanie procesu zapalnego w mięśniu sercowym stawia się obecnie na podstawie badania histopatologicznego i badań immunohistochemicznych. Z danych z literatury wynika, że kardiomiopatia zapalna występuje u ok. 50% chorych z DCM.

Cel: Celem pracy była ocena procesu zapalnego w biopsji endomiokardialnej u chorych z DCM na podstawie badań histopatologicznych i immunohistochemicznych.

Metodyka: Przebadano biopsje endomiokardialne rutynową metodą histopatologiczną i wykonując barwienia immunohistochemiczne na obecność limfocytów T (CD3+, n=84), antygenów zgodności tkankowej klasy I (HLA ABC, n=48) i klasy II (HLA DPQR, n=84) oraz cząsteczek adhezyjnych ICAM-1 (n=51) i VCAM-1 (n=48) u 84 chorych (69 mężczyzn i 15 kobiet, średni wiek 35,0±10,5 lat) z angiograficznie potwierdzoną DCM. Rodzinne występowanie choroby stwierdzono u 14 (16,7%) chorych. Kontrolę stanowiły wycinki z serca od 18 chorych zmarłych z przyczyn pozasercowych.

Wyniki: Na podstawie histopatologicznych kryteriów z Dallas u 8 (9,5%) chorych rozpoznano zapalenie mięśnia sercowego. Częstość kardiomiopatii zapalnej rozpoznanej na podstawie obecności >2 limfocytów T CD3+ w dużym polu widzenia (hpf) w biopsji miokardialnej wynosiła 14,3%. Przy zastosowaniu rozszerzonych kryteriów rozpoznania (stwierdzenie obecności >2,0 limfocytów T CD3+/hpf i/lub >1,5 limfocytów T CD3+/hpf w licznych ogniskach oraz wzmożonej ekspresji antygenów HLA klasy I/II) częstość kardiomiopatii zapalnej wynosiła 32,1%. Aktywację zapalną śródbłonka wyrażającą się wzmożoną ekspresją co najmniej 3 cząsteczek adhezyjnych (HLA klasy I i II, ICAM-1, VCAM-1) stwierdzono u 22 (45,8%) chorych. Ekspresję antygenów HLA DPQR, HLA ABC i ICAM-1 obserwowano na śródbłonkach włośniczek i większych naczyń, na komórkach tkanki śródmiąższowej oraz na powierzchni zaktywowanych limfocytów, a reakcje immunohistochemiczne miały charakter rozlany. W przypadkach ze szczególnie nasiloną ekspresją ww. molekuł odczyny dodatnie były również na powierzchni błony komórkowej kardiomiocytów. Cząsteczka adhezyjna VCAM-1 obecna była jedynie na śródbłonkach pojedynczych, drobnych naczyń żylnych. W grupie kontrolnej nie było cech zapalenia mięśnia serca, kardiomiopatii zapalnej ani aktywacji zapalnej śródbłonka.

Wnioski: Zastosowanie metod immunohistochemicznych w biopsji mięśnia serca w celu określenia fenotypu komórek zapalnych i obecności cząsteczek adhezyjnych pozwala na rozpoznanie kardiomiopatii zapalnej w zależności od przyjętych kryteriów u 14% lub 32% chorych, a konwencjonalne badanie histopatologiczne u 9% chorych. Częstość kardiomiopatii zapalnej rozpoznanej na podstawie obecności >2,0 limfocytów T CD3+/hpf w mięśniu serca w naszym materiale jest niższa (14%) niż w publikacjach innych autorów, natomiast aktywacja zapalna śródbłonka występuje u podobnej liczby chorych (45%).

Słowa kluczowe: kardiomiopatia rozstrzeniowa, kardiomiopatia zapalna, cząsteczki adhezyjne, immunohistochemia, zapalenie mięśnia sercowego

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