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Histopathologic, Immunohistochemical Features and Profile of Viral Antigens in Patients with Myocarditis

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Abstract. We studied medical records and endomyocardial biopsies of patients with morphological confirmed lymphocytic myocarditis. The patients were divided into two groups: 1 - patients with arrhythmias; group 2 - patients with predominance syndrome heart failure. Morphological verification of myocarditis was based on World Heart Federation Consensus definition of Inflammatory Cardiomyopathy, 1997. Immunohistological study was performed to identify antigens of cardiotrophic viruses. We revealed some features in topic and character of morphological changes in depending on clinical scenario of myocarditis. In patients with chronic heart failure due to myocarditis revealed a high incidence of expression of LMP-antigen Epstein-Barr virus, the lack of expression of adenovirus antigens. Arrhythmic presentation of myocarditis was characterized by a high frequency of expression of enteroviral VP-1 antigen and the type 1 antigen herpes virus. We were not detected expression of the VP-2 antigen parvovirus B19. As a result the most severe inflammatory changes and interstitial fibrosis of intraventricular septum, widespread damage of myocytes the severe myocardial remodeling was found in patients with presentation of myocarditis by chronic heart failure. Interstitial fibrosis of the outflow tracts of the right ventricle, the low activity of inflammation and mild fibrotic changes were feature of arrhythmic scenario of myocarditis.

Introduction

Myocarditis is diagnosed in 34.7% of adult autopsies in cases of sudden death and in 30-40% of biopsy samples of the myocardium of patients with dilated cardiomyopathy [1-2]. Viral infection is leading among the causes of myocarditis [3]. The most frequent clinical scenario of myocarditis is arrhythmias and heart failure. However, it is still unknown what changes of myocardial structure underlie the occurrence of these syndromes. Myocardial biopsies used to confirm or refute the diagnosis, assess the activity of inflammation and fibrosis, the most probability cause of the disease. Therefore, it seems important to study and compare the morphological changes and the expression profile of viral antigens in the myocardium at the most common clinical variants of myocarditis.

Material and Methods

We studied medical records and endomyocardial biopsies of 58 patients, residents of Tomsk Region of Russian Federation, (mean age 41.98±12.75) with morphological suspected lymphocytic myocarditis. Indications for endomyocardial biopsy were unexplained heart failure or arrhythmia. Coronary angiography was performed for exclusion coronary artery disease. Valvular heart diseases were excluded with echocardiography.

Three bioptic samples from each other patient have been taken from the right ventricle site of intraventricular septum, right ventricle apex and out flow tract, via the venous route through

femoral veins (bioptome BiPAL 7, Cordis Corporation, USA). Endomyocardial samples were fixed in 10% buffered formalin at room temperature. Light microscopic examination (microscope Axio Lab A 1, Zeiss) was performed on formalin-fixed and paraffin-embedded serial sections stained by hematoxilin-eosin, picrofuchsine, toluidine blue and immunohistochemical stains. For inflammatory infiltrate assessment CD45, CD3, CD68 antibodies were used. For identify viral antigens we performed immunohistological study with monoclonal antibodies against VP2 protein of parvovirus B19, VP1 protein of enteroviruses, early nuclear protein of cytomegalovirus, LMP antigen of virus Epstain-Barr, adenovirus and polyclonal antibodies against capsid antigens of herpes viruses 1 and 2 types. HRP-DAB detection system (Spring BioScience) was used for antigens visualization. Morphological verification of myocarditis was based on World Heart Federation Consensus definition of Inflammatory Cardiomyopathy, 1997 [4]. Histopathological analysis was based upon a semi-quantitative histological criteria, including inflammatory cell type, semi-quantitative assessment of myocytes damage and inflammation and semi-quantitative assessment of fibrosis (Table 1) [5].

In all cases, for assessment clinical features we used medical records. Patient's complaints, prescription of symptoms, connection with infection diseases, existence and severity of heart failure (NYHA classification), general and biochemical lab values were taken into account. Structural and functional heart condition was evaluated with echocardiography. The existence and character of cardiac arrhythmias and conduction abnormalities is determined by the results of Holter monitoring.

Table 1 - Inflammatory cardiomyopathy classification: grading and staging

	Parameters	0 [score]	1 [score]	2 [score]	3 [score]
Grading [maximum score 5]	Myocytes damage	Absent	Focal	Plurifocal	
	Interstitial inflammation	<7 T [cells/mm²]	7 to ≤ 14 T [cells/mm²]	>14 T [cells/mm²]	
	Endocardial involvement (inflammation, thrombosis)	Absent	Present		
Staging [maximum score 5]	Interstitial/replacement fibrosis	Absent	10 to <20 %	20 to ≤40 %	>40%
	Subendocardial fibrosis	Absent	Present		
	Endocardial fibroelastosis	Absent	Present		

Statistical analyses were performed with Statistica for Windows 10.0. Data were represented as mean ± SD and were compared using *t*-test and LSD-test. A *P* value of less than 0.05 indicated statistical significance.

Results

Depending on the clinical scenario of the disease, patients were divided into 2 groups. The groups were matched for age, sex, duration of the disease. The first group comprised 44 patients (mean age 39.72 ± 13.27 years) with a predominance of arrhythmic syndrome. The nine 9 patients (age 45.56 ± 11.92 years) with chronic heart failure mean included into second group (Table 2).

In the first group, the average duration of disease was 6.26 ± 5.24 years. In 6 patients (13.64%) was the definitely link with viral infection. Palpitations (63.64%), disruption of the heart (68.18%) were the most complaints in patients, symptoms of heart failure (HF) in this group were absent or occurred after considerable physical exertion (NYHA 0-1).

In the second group, the average duration of disease was 11.2 ± 10.94 years. Relationship with acute viral infection confirmed in 2 people (22.22%). The main complaints of the patients of the second group were exertional dyspnea (66.67%), cardialgia (44.44%), the edema of low extremities (33.33%). Patients had higher NYHA class of HF. There were inflammatory changes in laboratory parameters. As expected, patients in group 2 had hypertrophy of left ventricle, systolic dysfunction and dilation of the left ventricle. Supraventricular extrasystoles were the most frequently recorded by Holter monitoring. Atrial fibrillation was more often in first group. More than 50% of patients in both groups had ventricular arrhythmias, in the structure of which were also dominated extrasystoles. Unsustained ventricular tachycardia is slightly more common in the first group, and sustained - in the 2nd group. Atria-ventricular blockade and slowing of conduction of bundle-branch block beam also more likely detected in patients with HF (Table 2).

Table 2 - Clinical characteristics

		Group 1 [n=44]	Group 2 [n=9]	p
Age [years]		39.72±13.27	45.56±11.92	NS
Gender	male	26	6	NS
	female	18	3	
NYHA class of HF		1.05±0.99	2.56±0.73	<0,001
Heart Rate		78.79±19.89	88.89±25.22	NS
Systolic blood pressure [mm Hg]		119.49±9.28	111.67±13.23	0.038
Diastolic blood pressure [mm Hg]		77.12±7.43	74.44±11.30	NS
Leukocytes [10^9/l]		11.72±21.60	30.61±43.09	0.049
Laboratory parameters				
Segmented leukocytes [%]		50.83±9.13	65.47±13.84	<0.001
Lymphocytes [%]		38.04±7.38	24.56±11.05	<0.001
Erythrocyte sedimentation rate [mm/h]		11.43±16.55	15.5±11.25	NS
C reactive protein [mg/ml]		4.79±3.29	23.71±36.17	0.014
CPK [U/l]		127.63±109.59	227.75±368.12	NS
CPK MB [U/l]		16.92±14.69	18.83±11.05	NS
Echocardiography results				
Myocardial mass index [g/m^2]		84.17±27.75	110.56±22.6	0.01
EF LV [%]		61.86±11.89	34.33±9.81	<0.001
EDI [ml/m^2]		60.25 ±20.67	101.18±25.69	<0.001
ESI [ml/m^2]		25.79±21.91	74.80±27.67	<0.001
SI l/[min/m^2]		5.69±17.29	28.08±47,18	0.021
Holter monitoring				
Supraventricular arrhythmias		37 (84.09%)	6 (66.67%)	NS
Supraventricular extrasystoles		28 (63.64%)	4 (44.44%)	NS
Atrial fibrillation		19 (43.18%)	3 (33.33%)	NS
Atrial tachycardia		2 (4.55%)	1 (11.11%)	NS
Ventricular arrhythmias		26 (59.09%)	5 (55.56%)	NS
Ventricular extrasystoles		24 (54.55%)	4 (44.44%)	NS
Unsustained tachycardia		5 (11.36%)	1 (1.11%)	NS
Sustained tachycardia		2 (4.55%)	2 (22.22%)	NS
AV blockade		4 (9.3%)	3 (33.33%)	NS

Notes: NS- not significant differences

Enteroviral VP-1 antigen expression, herpes simplex virus 1, 2 type antigens, LMP antigen of the Epstein-Barr virus was detected predominantly in myocardium samples by the immunohistochemical study with antibodies to cardiotropic viruses.

Expression of enteroviral VP-1 antigen, herpes virus type 1 antigen had patients with arrhythmic scenario of myocarditis. There was no expression of VP-2 antigen parvovirus B19 in this group. Patients with HF more often had expression of LMP-antigen Epstein-Barr virus, at the same time there was no expression of adenovirus antigen (Fig. 1).

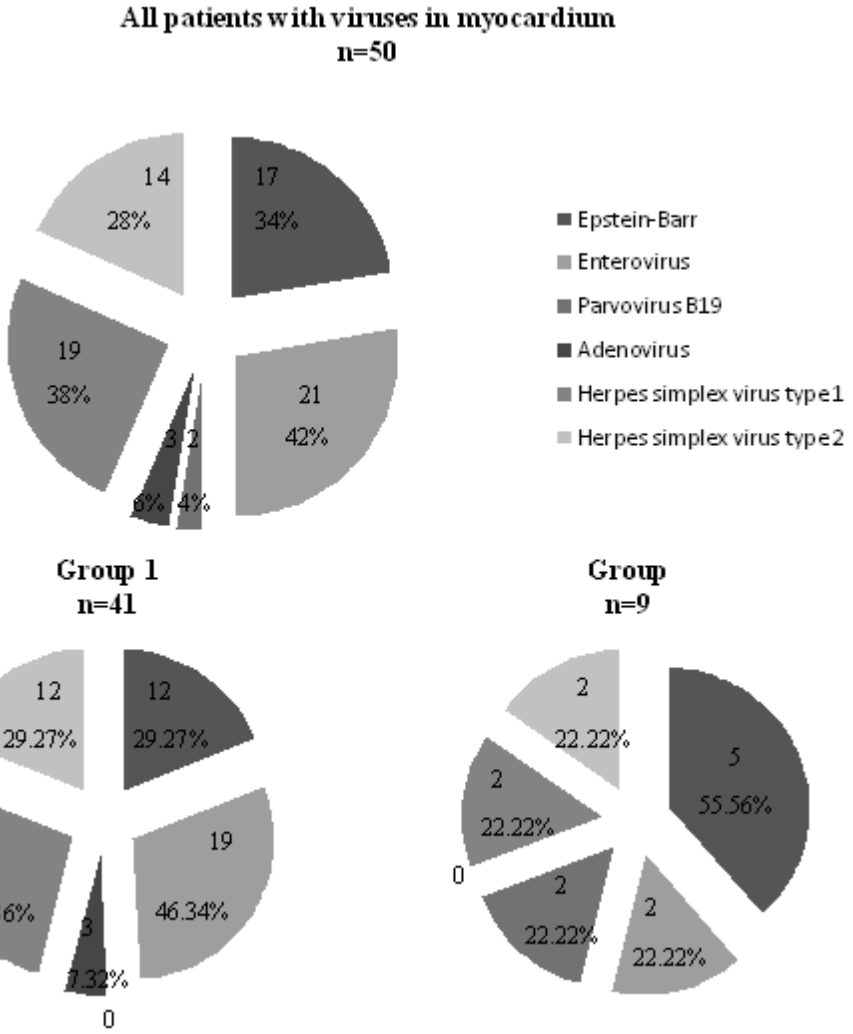

Fig. 1. Profile of viral antigens in the myocardium of patients with lymphocytic myocarditis. *p_{1-2}=0.006.

Morphological changes in patients with arrhythmic scenario of myocarditis were characterized by the absence or any focal lesions myocytes, a small number of T-lymphocytes in the infiltrate, reflecting the low activity of inflammation. Fibrotic changes were mild, the total score on the indicators characterizing the stage of myocarditis, did not exceed 2. A morphological feature of this clinical variant of myocarditis is the presence of interstitial fibrosis in the outflow tracts of the right ventricle (Table 3).

In patients with HF, myocardial lesions had more widespread, noted focal and multifocal myocytes damage, greater number of T-lymphocytes in the infiltrate, reflecting the high activity of inflammation. Fibrotic changes in the myocardium of patients in this group were also more pronounced (Table 3).

Table 3 - Results of histology

Parameters		Group 1 [n=44]	Group 2 [n=9]	p
Apex of right ventricle [score]	Myocytes damage	0.63±0,49	1.0±0	NS
	Interstitial inflammation	0.57±0.50	0.33±0.58	NS
	Endocardial involvement (inflammation, thrombosis)	0.09±0.28	0±0	NS
	Grading	1.26±0.92	1.33±0.58	NS
	Interstitial/replacement fibrosis	0.92±0.76	0.67±0.58	NS
	Subendocardial fibrosis	0.33±0.48	0.33±0.58	NS
	Endocardial fibroelastosis	0.39±0.49	1.0±0	0.04
	Staging	1.62±0.98	2.0±1.0	NS
Interventricular septum [score]	Myocytes damage	0.76±0.5	1.6±0.55	0.001
	Interstitial inflammation	0.79±0.55	1.2±0.45	NS
	Endocardial involvement (inflammation, thrombosis)	0.15±0.36	0.2±0.45	NS
	Grading	1.69±0.92	3.0±1.22	0.008
	Interstitial/replacement fibrosis	0.89±0.58	1.6±0.89	0.038
	Subendocardial fibrosis	0.34±0.48	0,2±0.45	NS
	Endocardial fibroelastosis	0.49±0.51	0.4±0.55	NS
	Staging	1.71±0.96	2.2±1.79	NS
Right ventricular outflow [score]	Myocytes damage	0.77±0.57	1.0±0	NS
	Interstitial inflammation	0.77±0.50	-	NS
	Endocardial involvement (inflammation, thrombosis)	0.17±0.38	-	NS
	Grading	1.70±1.18	1.0±0	NS
	Interstitial/replacement fibrosis	0.97±0.56	-	0.022
	Subendocardial fibrosis	0.48±0.57	1.0±0	NS
	Endocardial fibroelastosis	0.48±0.51	1.0±0	NS
	Staging	1.84±0.97	2.0±0	NS

NS- non significant differences

Myocarditis presents in many different ways, ranging from completely asymptomatic, through flu-like symptoms, mild symptoms of chest pain suggestive of myocardial infarction and palpitations associated with transient ECG changes to life-threatening cardiogenic shock and ventricular arrhythmia [6]. It is still unknown what changes of myocardial structure underlie the occurrence of these syndromes. Although the aetiology of human myocarditis often remains undetermined, there is evidence for viral and autoimmune mechanisms, acting in individuals with or without a genetic predisposition. Molecular techniques, mainly (reverse transcriptase) (RT)-PCR amplification [6] suggest that viral infections are the most important cause of myocarditis in North America and Europe with genomes of enterovirus, adenovirus, influenza viruses, human herpes virus-6 (HHV-6), Epstein-Barr-virus, cytomegalovirus, hepatitis C virus, and parvovirus B19 reported in the myocardium of patients with myocarditis. In our small study found histopathologic, immunohistochemical features and profile of viral antigens in patients with myocarditis of Tomsk region.

Conclusion

In patients with chronic heart failure due to myocarditis revealed a high incidence of expression of LMP-antigen Epstein-Barr virus, the lack of expression of adenovirus antigens.

Arrhythmic presentation of myocarditis was characterized by a high frequency of expression of enteroviral antigen VP-1 antigen and the type 1 antigen herpes virus, the absent of expression of the VP-2 antigen parvovirus B19.

As a result widespread damage of myocytes the severe myocardial remodeling was found in patients with presentation of myocarditis by chronic heart failure.

Interstitial fibrosis of the outflow tracts of the right ventricle, the low activity of inflammation and mild fibrotic changes were feature of arrhythmic scenario of myocarditis.

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References

[1] H. Wang , Q. Yao , S. Zhu , G. Zhang , Z. Wang , Z. Li , R. Sun , C. Lu , C. Li , J. Pu , The autopsy study of 553 cases of sudden cardiac death in Chinese adults, Heart and Vessels. 29, 4 (2014) 486-495.

[2] Alida L.P. Caforio, R. Marcolongo, R. Jahns, M. Fu, S. B. Felix, S. Iliceto, Immune-mediated and autoimmune myocarditis: clinical presentation, diagnosis and management, Heart Fail. Rev. 18, 60 (2013) 715-732.

[3] A. Shauer , I. Gotsman , A. Keren, D. R Zwas., Y. Hellman , R. Durst , D. Admon, Acute Viral Myocarditis: Current Concepts in Diagnosis and Treatmen, Israel Med. Association J. 15, 3 (2013) 180-185.

[4] B. Maisch, B. Bultman, S. Factor, et al, World Heart Federation consensus conferences' definition of inflammatory cardiomyopathy (myocarditis): report from two expert committees on histology and viral cardiomyopathy, Heart Beat. 4 (1999) 3-4.

[5] C. Basso, F. Calabrese, A. Angelini, E. Carturan, G. Thiene, Classification and histological, immunohistochemical, and molecular diagnosis of inflammatory myocardial disease, Heart Fail. Rev. 18, 6 (2013) 673-681.

[6] A. L. P. Caforio, S. Pankuweit, E. Arbustini, C. Basso, J. Gimeno-Blanes, S. B. Felix, M. Fu, T. Heliö, S. Heymans, R. Jahns, K. Klingel, A. Linhart, B. Maisch,W. McKenna, J. Mogensen, Y. M. Pinto, A. Ristic, H. P. Schultheiss, H. Seggewiss, L. Tavazzi, G. Thiene, A. Yilmaz, Ph. Charron, P. M. Elliott, Current state of knowledge on aetiology, diagnosis, management, and therapy of myocarditis: a position statement of the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases, Eur. Heart J. 34, 33 (2013) 2636-2648.