

## MODERN CRITICAL APPROACH TO THE DIAGNOSIS OF ACUTE VIRAL MYOCARDITIS AND INFLAMMATORY CARDIOMYOPATHIES IN CLINICAL PRACTICE - FOCUS ON THE ROLES OF ECHOCARDIOGRAPHY AND ANTIVIRUS ANTIBODIES

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**Summary: SIGNIFICANCE OF THE PROBLEM:** The diagnosis of acute viral myocarditis is one of the diagnoses most difficult to make in cardiology and medicine in general. Echocardiography and cardiomagnetic resonance play a crucial role in the clinical diagnosis and the serum titer of antiviral antibodies to cardiotropic viruses is still unjustifiably used for the diagnosis of myocarditis in everyday practice. **RESEARCH OBJECTIVES:** To analyze the frequency and significance of echocardiographic parameters in the diagnosis of clinically suspected acute viral myocarditis, to determine the role of antiviral antibody titer (AVA) dynamics for the diagnosis of myocarditis and to compare viral serology and echocardiographic function versus echocardiographic function. **METHODOLOGY:** A retrograde transverse study was performed in the ten-year period from 2006. to 2015, where 126 consecutive patients from the database of the Office of Internal medicine "Dr. Bastać" were analyzed, with a working diagnosis of clinically suspected viral myocarditis. They were clinically, ECG, echocardiographically and serologically monitored for 4 to 8 weeks due to the dynamics of AVA titer. The examined group (A) was divided into subgroups: A1 with elevated AVA class IgM titer in 43 (32%) subjects and subgroup A2 without elevated IgM titer in 83 (68%) patients. The control group of healthy (B) of 103 subjects was comparable. Statistical processing was done in the EXCELL database via descriptive statistics, Student's-T test and Chi<sup>2</sup> test. **RESULTS:** 126 patients had clinically suspected myocarditis ( $\geq 2$  ESC criteria). Diastolic left ventricular dysfunction in 39/126 (31%) patients was the dominant echocardiographic criterion for clinically suspected myocarditis. Reduced ejection fraction (EF < 50%) was measured at 19/126 (15%), followed by left ventricular dilatation. Regional systolic dysfunction was found in 21/126 (17%) and changes in myocardial texture in 17 (13%) subjects. The clinical probability of viral etiology was diagnostically supported by elevated titer of IgM antibodies in 43 (32%) subjects (subgroup A1) where IgM antibodies to Parvo B 19 virus predominate in 36/43 patients (84%). Most were without elevated titer of IgM antibody-subgroup A2 83 (68%). Clear dynamics of IgM antibody titer was observed in 23 persons, a decrease in IgM titer with an increase in IgG titer (seroconversion) in 13 patients. Determination of anti-heart autoantibodies (AHA) was done in 17 severe cases, of which 9 had positive AHA. A comparison of subgroups A1 and A2 did not reveal a statistically significant difference in echocardiographic parameters. The whole group A of clinically suspected myocarditis compared to control group B has statistically highly significantly lower parameters of global systolic (EF=8,7 $\pm$ 4,6 vs. 63 $\pm$ 7,9; p<0,001), longitudinal systolic (S'=6,9 $\pm$ 1,3 vs. 9,9 $\pm$ 2,1) and diastolic function (E/e'=11,9 $\pm$ 4,8 vs. 8,7 $\pm$ 4,6; p<0,001), and a highly statistically significant increase in left ventricular telediastolic dimension, myocardial mass index, and left atrial size. **CONCLUSION:** The diagnosis of acute viral myocarditis in clinical practice is made on the basis of the clinical picture, ECG and echocardiography that indicate myocarditis with the exclusion of cardiac comorbidities, based on the ESC criteria for suspected clinical myocarditis. The whole group A had highly statistically significantly lower parameters of systolic and diastolic function compared to control group B. Normal ECG and echocardiography cannot serve to exclude the diagnosis of myocarditis. Comparison of subgroups A1 and A2 did not reveal a statistically significant difference in echocardiographic parameters.

The sensitivity of IgM titer to cardiotropic viruses is low and should not be used in routine diagnosis of myocarditis.

**key words:** Acute viral myocarditis, inflammatory cardiomyopathy, serum antibodies to cardiotropic viruses, echocardiography, left ventricular systolic dysfunction, left ventricular diastolic dysfunction

## INTRODUCTION

The clinical picture of myocarditis is diverse [1]. Myocarditis (MY) can be the cause of sudden cardiac death in young adults without known heart disease in 20%, idiopathic ventricular tachycardia (VT) in 30%, acute heart failure in 10% [2,3]. MY is one of the leading causes of sudden cardiac death and dilated cardiomyopathy (DCM) in young people [4,5]. In the clinical series of sudden cardiac death, MY is the third leading cause after hypertrophic cardiomyopathy and congenital and atherosclerotic coronary artery disease. [6]. Autopsy studies show that MY is a common cause of DCM in biopsy-proven myocarditis but with large variation from batch to batch: from 0.5% to 67%, the median is 10.3%. Due to the possibility of clinically silent disease and infrequent myocardial biopsy, the exact frequency: incidence and prevalence of MY and inflammatory cardiomyopathy (ICM) is unknown [7,8]. Myocarditis (MY) or myocardial inflammation can be the result of multiple causes, but is commonly associated with infectious agents and more than 20 viruses that damage the myocardium by direct invasion, production of cardiotoxic substances, and inflammation, with or without persistent infection and autoimmune reactions to cardiac epitopes [7,9,10,11]. AVMY is one of the biggest challenges in terms of both diagnosis and therapy [7,12]. Clinical classification of AVMY [7,13]:

1. Possible subclinical acute myocarditis (typical viral syndrome without cardiac symptoms and with ECG changes, positive biomarkers of CK-MB and troponin, with echocardiographic findings: decreased EF and regional anomalies of left ventricular wall mobility and changes in myocardial texture)
2. Probable clinical acute myocarditis (all previous + symptoms: pain, shortness of breath, palpitations, etc.)
3. Definitive myocarditis (confirmed pathohistological, immunohistochemical and PCR viral genome via EMB)

This classification has not yet been revised by cardiomagnetic resonance imaging (CMR), which would be necessary. The term ICM was introduced in 1995 by the World Health Organization [14] and involves myocarditis with systolic dysfunction and/or left ventricular dilatation, but it does not describe the phenotype and does not define the cause [15]. By their course, viral myocardites are divided into subacute and chronic, they are often talked about but rarely proven [15].

There is a change in the most common types of viral myocarditis, previously Coxsackie B viruses and adenoviruses, and in the last two decades Parvo B19, herpes virus type 6, hepatitis C virus, and now less commonly Coxsackie B viruses, adenoviruses, Epstein-Barr virus and Cytomegalovirus [7,11,12]. Myocarditis can also develop in patients with HIV infection, hepatitis C or Lyme disease. [7,11,12]. Proven cases of myocarditis caused by the SARS CoV-2 virus have been occurring since 2019 during the COVID 19 epidemic, but not enough is known about it [16-20].

Most patients with acute viral myocarditis recover without sequelae, but some patients progress to chronic inflammatory and dilated cardiomyopathy, heart failure, and become candidates for heart transplantation [1,5,12,13,15].

To this day, there has not existed the so-called non-invasive gold standard for AVMY diagnosis due to the low specificity and sensitivity of traditional diagnostic tests, but the development of cardiomagnetic resonance imaging is promising [12,21,22]. Endomyocardial biopsy with pathohistological examination and the presence of viral genome is the most reliable method, if representative myocardial samples are obtained [7,9] and it allows the application of a therapeutic algorithm, but this invasive diagnosis is mostly reserved for more severe and unclear cases of inflammatory cardiomyopathies. Therefore, the clinical picture, ECG, biomarkers and imaging methods, primarily in practice the easiest echocardiography and increasingly magnetic resonance imaging, can, in the form of a mosaic, complement the diagnosis of myocarditis based

on the clinical picture and various diagnostic categories with an ESC score of 2 or more points [11,12].

The main symptoms of AVMY are common: fatigue, palpitations, chest pain, shortness of breath on exertion; physical examination reveals tachycardia, weakened first S1 tone and often S3 gallop rhythm and de novo mesosystolic murmur [13,15,21]. The usual ECG nonspecific finding in clinically suspected AVMY is most commonly sinus tachycardia and various dysrhythmias: ventricular and supraventricular extrasystoles, rarely ventricular tachycardia and atrial fibrillation, and less frequently bradycardia and heart blocks; ECG changes in the ST segment and T wave are quite specific for myocardial lesions: transient changes in the ST segment and T wave, depression or elevation of the ST segment, deep negative T waves, block of the left branch of the His bundle and sometimes images of myocardial infarction [13,15,21].

Elevated cardiac troponins are detected in the laboratory and there are also newer markers. In children with fulminant myocarditis, higher levels of creatinine, lactate and aspartate transaminase (AST) are associated with increased hospital mortality [23]. Natriuretic peptide (NT-pro-BNP) is elevated in children with acute ICM and generally declines rapidly in recovery of left ventricular function [24]. In adults, higher concentrations of interleukin-10 are associated with an increased risk of death. Myocardial antibodies (AHAs) have been reported to predict an increased risk of death or the need for transplantation. [25]. Circulating viral antibody titers do not correlate well with tissue viral genomes and are rarely useful for diagnostic use in practice due to their low sensitivity [11,12,26].

**NON-INVASIVE IMAGING TECHNIQUES.** The concept of imaging has evolved from a monomodality to a multimodality imaging strategy where each test adds information that increases the specificity of the diagnostic marker for the diagnosis of myocarditis. Transthorax Echocardiography (TTE) is the most available method at the patient's bed, which can be used to suspect myocarditis. Echocardiographic signs of clinically suspected AVMY are variable and heterogeneous: most often left ventricular dysfunction with regional segmental kinetic disorders, left ventricular dilatation or pericardial effusion, rarely intracardiac

thrombus, but the finding can be normal, too [11,12,27]. When the echocardiographic window is inadequate, an important step in diagnostics is transesophageal echocardiography [28]. Imaging of deformation by speckle tracking echocardiography (speckle tracking strain) usually shows a reduced longitudinal pattern of myocardial deformation but it is also a non-specific sign of myocardial disease. The advantage of the method is that it can recognize early changes in myocardial function before we see them using "ordinary" or conventional methods based on measuring the ejection fraction of the left ventricle (EF) [29,30,31,32,33]. Reduction of global longitudinal deformation (GLS) has a diagnostic value and affects the prognosis of the disease in inflammatory cardiomyopathy and heart failure. Cardiac magnetic resonance imaging (CMR) is useful in diagnosing AVMY and for monitoring disease progression, and the presence of late gadolinium accumulation (LGE) is the best independent predictor of cardiac mortality [21,34,35]. CMR shows a gadolinium binding in the medial part of the left ventricular myocardium and subepicardially, which is completely different from the findings in ischemic cardiomyopathy [9,11,12,35].

**Endomyocardial biopsy (EMB)** with pathohistological examination and the presence of viral genome by means of PCR and immunohistochemical evidence of inflammation is the most reliable method and allows the application of a therapeutic algorithm, but this invasive diagnosis is mostly reserved for severe cases and cardiomyopathies [7,9]. If myocardial samples are not representative, false-negative EMB findings are possible. Yet most authorities support the concept that EMB should be the gold standard for the diagnosis of definitive myocarditis [7,9,11,12].

The basis of AVMY treatment is the treatment of heart failure and arrhythmias. Specific treatment for fulminant and acute AVMY is antiviral therapy and for post-viral chronic autoreactive myocarditis the treatment is immunosuppressive therapy with corticosteroids and cyclosporine [36] and more recently with CD3 muromonab [22].

**RESEARCH OBJECTIVES:** To analyze the type and significance of echocardiographic parameters and characteristics in the diagnosis of clinically suspected acute viral myocarditis in everyday practice. To determine the role of

antiviral antibody titer dynamics for the diagnosis of clinically suspected acute viral myocarditis and to compare viral serology in relation to echocardiographic parameters of diastolic and systolic function of the left ventricle.

#### MATERIAL AND METHODS

A retrograde transverse study was performed in the ten-year period from 2006 to 2015, where 126 consecutive patients clinically suspected of acute viral myocarditis, were isolated from the database of the Office of Internal medicine "Dr. Bastać", having been clinically, echocardiographically and serologically monitored due to the dynamics of antibody titers to cardiotropic antibodies. The examined group had an average age of  $43.3 \pm 8.9$  years, body mass index BMI  $27.8 \pm 5.9$ , dominated by female gender-78 (62%). Mean values of systolic and diastolic pressure on arrival were  $127 \pm 14/78 \pm 11$  mmHg. The control group had

comparable characteristics: 103 persons with average age  $46 \pm 12$  years, body mass index BMI  $29.3 \pm 6.4$ , 53 persons (52%) female. Mean systolic and diastolic pressure on arrival were  $136 \pm 14/71 \pm 11$  mmHg

**Exclusion criteria:** Absence of hypertension, known coronary heart disease, valvular defects of other relevant diseases and with low pre-test probability (PTP)  $<15\%$  on ischemic heart disease. **Inclusion criteria:** the criteria of Dennert et al. from 2007 were used first [7] and later were re-evaluated through the criteria of the Working Group on Myocardial and Pericardial Diseases of the European Society of Cardiology from 2013 for clinically suspected myocarditis [11]. 2 criteria at least were required: one at least from the group of clinical presentations and one at least from the group of diagnostic categories as shown in the **TABLE 1** [11]

**TABLE 1.** The criteria of the Working Group on Myocardial and Pericardial Diseases of the European Society of Cardiology from 2013 for clinically suspected myocarditis [11]

ESC CRITERIA FOR CLINICALLY SUSPECT MYOCARDITIS:	
ONE OR MORE $\geq 1$ -CRITERIA OF CLINICAL PRESENTATIONS (1-5)	$\geq 1$ DG CRITERIA FROM DIFFERENT CATEGORIES (I-IV),
1. SIMILAR TO ACUTE CORONARY SYNDROME	I. ECG: ECG / HOLTER / STRESS TEST - new abnormalities, any of the following: 1. block I-III degree or branch block, 2. ST / T changes-ST elevation / depression or without, T wave inversion 3. sinus arrest, VT or ventricular flutter and asystole, 4. AF, 5. reduced amplitude of R tooth, 6.intraventricular block 7. Q tooth 8. low voltage 9. frequent VES 10. PSVT
2. De novo OR EXCESSIVE HEART FAILURE in the absence of coronary heart disease and other known causes of heart failure	II. MYOCARDIOCYTOLYSIS MARKERS (troponins I, T)
3. CHRONIC HEART FAILURE	III FUNCTIONAL AND STRUCTURAL ANOMALIES OF THE MYOCARDIUM - ECHOCARDIOGRAPHY, CMR, PET, PET CT SCAN New, different, unexplained LV and/or RV structural and functional abnormalities: 1. regional disorders of segment kinetics or 2. global systolic or diastolic anomalies with or without: 3.ventricular dilatation, with or without 4. increased wall thickness, with or without 5.pericardial effusion and with or without 6.endocavitary thrombus
4. PALPITATIONS AND/OR UNEXPLAINED SYMPTOMS OF ARRHYTHMIA AND/OR PRESINCOPE AND SYNCOPE AND/OR RESUSCITATED PATIENTS	
5. UNEXPLAINED CARDIOGENIC SHOCK	IV. TISSUE CHARACTERIZATION on Cardiomagnetic Resonance (CMR) -edema, late gadolinium accumulation mesomicardially or subepicardially (LGE) classic myocardial pattern
If the patient is asymptomatic - 2 or more criteria from different Dg. categories	

**METHODOLOGY.** In addition to routine clinical methods: anamnesis and physical examination, ECG, anthropometry, basic blood biochemistry, echocardiography and serology of IgM and IgG antiviral antibodies were performed

on all of them. In individual cases, radiography of the thorax was performed, as well as troponin T, pro BNP and D dimer. Very rarely, the proposed examination on cardio-magnetic resonance imaging was completed, while

endomyocardial biopsy was performed in only 2 patients.

**Echocardiography.** Echocardiographic examinations were performed using Toshiba Power Vision 6000, Toshiba Xario CV and GE Vivid 7 multifrequency sector probes from 2.0 to 4.5 MHz with harmonic imaging. All subjects underwent standard protocols, according to the then valid recommendations [37,38] and they were interpreted in the light of the latest recommendations for standards in performing echocardiography [39,40]. Echocardiographic examinations were performed by conventional M-mode and two-dimensional (B-mode) echocardiography, and Doppler analysis of transmitral flow during diastole was performed, as well as pulse tissue Doppler examination. Of the

structural parameters, left ventricular diameter (LA), left ventricular telediastolic diameter (LVEDD), left ventricular telesystolic diameter (LVESD), posterior left ventricular wall thickness (PWTd), and interventricular septum IV were measured. The criterion for left ventricular dilatation was the telediastolic dimension of the left ventricle  $\geq 54$  mm for women and  $\geq 59$  mm for men [37]. Left ventricular volumes and left ventricular ejection fractions (EF) were automatically calculated using the Teichholz method and biplane Simpson method [37] and then the left ventricular mass (LVM) was calculated by the Devereux formula and the left ventricular mass index (LVMI).

$$(LVMI (g/m^2) = [(TDD + ZZd + IVSd)^3 - TDD^3] \times 1.05 - 13.4 / BSA(m^2) [37]$$

Normal myocardial mass is up to 224 g for males and up to 162 g for females. Myocardial mass index is less than 95 g/m<sup>2</sup> for females and less than 115 g/m<sup>2</sup> for males. Diastolic function was assessed by measuring the maximum velocity of the early (E) and late (A) phases of ventricular filling, the deceleration time of the E velocity (DTE, normally 160-200 ms), and by calculating the E/A ratio (normal E/A  $\geq 0.8$ ). Using the tissue Doppler technique, measurements of tissue diastolic (e') and systolic velocities (S') of the myocardium on the septal and lateral sides of the mitral annulus were performed and the mean value (e') was taken, and then the ratio E/e' was calculated [38], as a surrogate for left ventricular filling pressure. Diastolic function is categorized as:

- (a) normal (E/A  $\geq 0.8$  -  $< 1.5$ , E-DTE deceleration time  $> 160$  ms, mean E/e'  $\leq 8$ );
- (b) Grade 1, impaired relaxation (E/A  $< 0.8$ , DTE  $> 200$  ms, mean E/e'  $\leq 8$ );
- (c) Grade 2, Pseudonormalization (E/A  $\geq 0.8$  and  $< 1.5$ , DTE 160–200 ms, mean E/e' = 9–12);
- (d) Grade 3, Restrictive pattern (E/A  $\geq 1.5$ , DTE  $< 160$  ms, mean E/e'  $\geq 13$ ).

Regional disorders in left ventricular contractility are segmental hypokinesia, akinesia, dyskinesia. Changes in myocardial texture; hyperechoic extensive subendocardial or transmural zones are a clear finding while oval hyperechoic zones of the myocardium- most often in the intraventricular septum are a controversial parameter. Only extensive zones or 3 smaller zones with a diameter of  $\geq 3$  mm or transmural

involvement (signs of fibrosis and cicatrix) with hypokinesia are significant. Based on the above criteria, clinically suspected myocarditis was established - until 2015, these patients were routinely tested for serum IgM and IgG antibodies to Parvo B19, Coxsackie and Adenovirus, and exceptionally to less potential agents (Ebstein Bar virus, cytomegalovirus, influenza virus, hepatitis C) it was determined from 2 samples of paired sera at 2 to 8 weeks. Antiviral antibodies and anti-heart antibodies were determined by enzyme-linked immunosorbent assay (ELISA). **Based on the positivity of IgM antiviral antibodies, the examined group (A) was divided into subgroups:** A1 with elevated IgM antibody titer in 43 (32%) subjects (SUBGROUP A1) and A2 without elevated IgM antibody titer (Group A2) - 83 (68%) patients (SUBGROUP A2). Statistical processing was done in the EXCEL database using the methods of descriptive statistics, Student's-T test and Chi<sup>2</sup> test.

## RESULTS

126 patients (GROUP A) had clinically suspected myocarditis (KSVMY with  $\geq 2$  ESC criteria). The most common symptoms were palpitations 107/126 (85%), chest pain 83/126 (66%) and fatigue, feeling tired, shortness of breath and dyspnea on exertion 62/126 (49%) in various combinations (TABLE 2)

**TABLE 2. Symptoms, physical signs, and ECG changes in 126 patients with suspected myocarditis and/or inflammatory cardiomyopathy**

Symptoms and physical signs in clinically suspected myocarditis - clinical presentations	Group A N=126	%
<b>SYMPTOMS - CLINICAL PRESENTATIONS</b>		
I. Palpitations	106	84%
II. Chest pain: anginal, pericardial or pseudischemic	83	66%
III. Fatigue, feeling tired, Dyspnea - lack of air on exertion	62	49%
IV. Symptoms and signs of chronic heart failure	21	17%
V. Life-threatening conditions: Acute heart failure	3	2%
<b>PHYSICAL SIGNS</b>		
Tachycardia > 90 / min at rest	106	84%
Bradycardia < 50 / min at rest	3	2,4%
Irregular heart rhythm-dysrhythmias	102	81%
Muffled tones / gallop rhythm	3	2,4%
De novo systolic murmur	2	1,6%
Pericardial friction	2	1,6%
<b>ECG CHANGES</b>		
ANY	112	89%
TACHYCARDIA SINUALIS	106	84%
ARRHYTHMIA EXTRASYSTOLICA VENTRICULARIS VES	78	62 %
ARRHYTHMIA EXTRASYSTOLICA SUPRAVENTRICULARIS	34	27%
DIFFUSE ST-SEGMENT DEPRESSION	33	26%
NEGATIVE T WAVES	30	24%
HISS BUNDLE LEFT BRANCH BLOCK	9	7%
SINUS BRADICARDIA < 50 WITH AV BLOCK GRADUS I	6	5%
SECOND II AND THIRD III DEGREE AV BLOCKS	3	2,5%
NORMAL ECG	14	11%

The physical finding in KSVMY (TABLE 2) was dominated by tachycardia 106/126 (84%), irregular heart rhythm 102/126 (81%) and much less frequent were more severe clinical forms: signs of cardiac decompensation 21/126 (17%), (late inspiratory crackles in the lungs, tachypnea, dyspnea at rest, swollen veins in the neck, late inspiratory crackles in the lungs, hepatomegaly, peripheral edema). Objective, physical findings were normal in 14/126 (11%) subjects

Of the 126 cases of clinically suspected myocarditis, most had some ECG changes-112/126 (89%), and with a normal ECG there were only 14/126 (11%) but echocardiographic changes were found in them. ECG analysis (TABLE 2) registers a high frequency of nonspecific disorders-dysrhythmias: sinus tachycardia in 112/126 (89%), ventricular extrasystoles 78/126 (62%), supraventricular extrasystoles 24/126 (19%) and electropathological changes for clinically suspected myocarditis: diffuse ST segment depression 33/126 (26%), diffuse negative T waves 30/126 (24%) and His bundle left branch block in 9 (7%) patients.

**The analysis of parameters measured by transthoracic echocardiography (TTE), in**

the presence of echocardiographic criteria for KSVMY (TABLE 3) was dominated by left ventricular diastolic dysfunction in 39/126 (31%), of which 17 (14%) had severe diastolic dysfunction grade III.

Global left ventricular systolic dysfunction quantified by left ventricular ejection fraction (EF) less than 50% (EF < 50%) was found in 19/126 (15%) and all had mild to moderate left ventricular dilatation and criteria for inflammatory cardiomyopathy (ICM). Increased left ventricular myocardial mass and left ventricular myocardial mass index (LVMI) due to possible myocardial edema were registered in 16 (13%) of these 19 patients. Regional systolic dysfunction (hypokinesia of 2 or more left ventricular myocardial segments), which, most commonly by distribution are not coronary artery perfusion, was found in 21/126 (17%), with cicatrix present in 11 patients, most commonly infero-postero-lateral. Myocardial akinesia was not present in the study group and septal dyskinesia was present in the left branch block (not taken into account) in 9 patients (7%). Changes in the texture of the myocardium - extensive hyperechoic zone of the myocardium and fibrosis-cicatrix were found in 17 (13%) subjects. However, 24/126 (19%) patients had a completely normal echocardiographic finding,

but with clinical and ECG criteria for myocarditis..

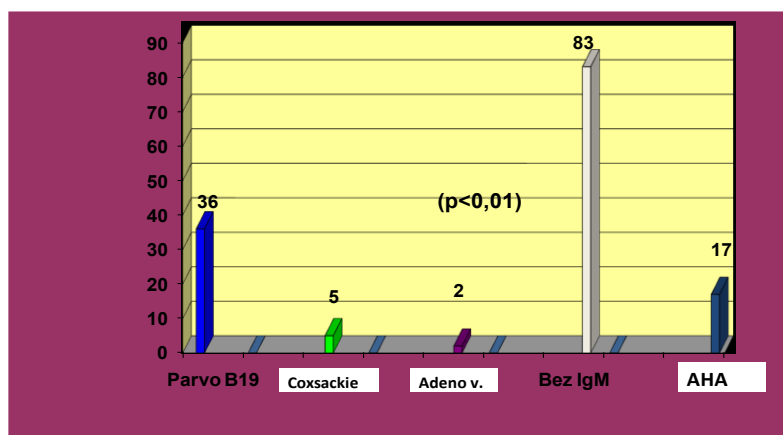
TABLE 3. ECHOCARDIOGRAPHIC PARAMETERS IN INDIVIDUAL DISTRIBUTION in clinically suspected myocarditis

ECHOCARDIOGRAPHIC PARAMETERS	GROUP A, N=126 patients	PERCENTAGE
<b>Pathological findings on echocardiography</b>	102	81%
Normal echocardiographic findings	24	(19%)
Diastolic dysfunction represented by the ratio $-E / e' \geq 9$	39	(31%)
Severe diastolic dysfunction grade III ( $E / e'_{\text{prim}} \geq 14$ )	17	13,5%
Regional systolic dysfunction with normal EF (hypokinesia of myocardial segments)	21	(17%)
Changes in the texture of the myocardium-significant hyperechogenic zone (fibrosis-cicatrix)	17	13,5%)
Systolic dysfunction - EF <50% and Left ventricular dilatation EDD > 54 or 58mm,)	19	(15%)
Increased myocardial mass	16	(13%)
Pericardial effusion-Myopericarditis	4	(3%)
Mitral regurgitation due to papillary muscle dysfunction	3	3%

**In patients with clinically suspected myocarditis, the clinical probability of viral etiology was diagnostically supported by elevated IgM antibody titer in 43 (32%)**

subjects- (subgroup A1) (CHART 1) while most were without elevated IgM antibody titer (Group A2) - 83 (68%) patients.

CHART 1. Distribution of IgM serological positivity in 43 (34%) of 126 patients examined for suspected recent virus infection and evidence of autoimmune response via elevated AHA antibodies serum titer

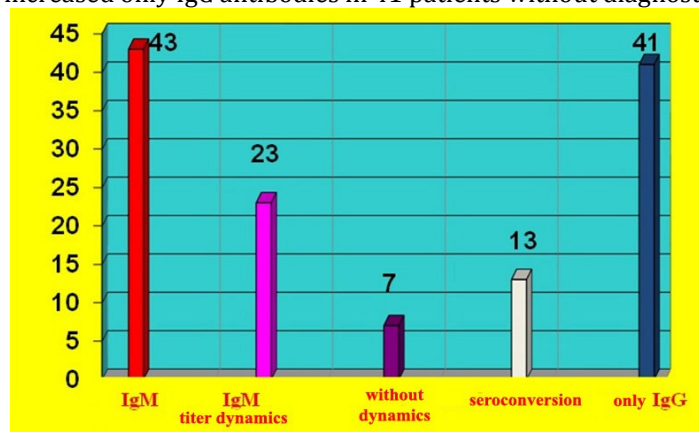


There is a predominance of IgM antibodies to Parvo B 19 virus in 36/43 (84%) patients ( $p < 0.01$ ) and only in 5/43 (12%) cases to Coxsackie B and in 2/43 (5%) patients to Adenovirus. The majority of patients were without elevated IgM antibody titer - subgroup A2 of 83 (68%) patients and about half of them - 41/126 (32%) have only elevated serum titer of IgG antibodies to cardiotropic which has no

diagnostic significance on its own, without IgM antibody titer dynamics.

Clear IgM titer dynamics was recorded in 23/126 (18%) subjects and a decrease in titer, with an increase in IgG titer (seroconversion) in 13/126 (10%) patients, while there were 7 patients without captured titer dynamics (CHART 2)

CHART 2. Dynamics of IgM antibody titer to cardiotropic viruses and IgM seroconversion to IgG in 43 patients and increased only IgG antibodies in 41 patients without diagnostic significance



Elevated IgG antibody titer has no diagnostic significance on its own, without IgM antibody titer dynamics. In group A2 without IgM, 41/126 (32%) had elevated serum IgG antibodies to cardiotropic viruses, most often to parvo B19, adenovirus and coxsackie B. As many as 42/126 patients (33%) did not have elevated IgM or IgG titers. antiviral antibodies, but had clear criteria (2 and more) for clinical myocarditis and 8 of them had elevated anti-heart antibodies and signs of inflammatory CMP.

Determination of anti-heart autoantibodies (aha) was performed more recently in severe cases of 17 inflammatory cardiomyopathy (CHART 1) of which 8 had antimyocardial autoantibodies, but their role has not yet been defined.

Quantitative echocardiographic parameters in patients with clinically suspected myocarditis are shown in TABLE 4 and CHARTS 3 and 4.

TABLE 4. Quantitative echocardiographic parameters in relation to viral serology in clinically suspected myocarditis

QUANTITATIVE ECHO-CARDIOGRAPHIC Xsr±SD	The whole group (A) N=126	Subgroup A1 N=43/126 (34,1%) POSITIVE IgM	Subgroup A2 N=83/126 (66%) NEGATIVE IgM	Control group B N=103	Statistically Significant difference student's T-test p NS=UNSIGNIFICANT
DIASTOLIC DYSFUNCTION REPRESENTED BY RELATIONSHIP E/e'	11,9± 4,8	12,3±5,3	11,6±4,7	8,7±4,6	A vs B, <0,001 A1 VS A2 0,400, NS A1 VS B <0,001 A2 vs B, 0,00019
LONGITUDINAL SYSTOLIC FUNCTION (TISSUE DOPPLER) - SYSTOLIC RATE OF THE LATERAL ANULUS S'	6,9± 1,3	7,2 ± 1,4	6,9± 1,2	9,9± 2,1	A vs B <0,001 A1 VS A2 0,300, NS A1 Vs B- <0,0001 A2 vs B- <0,0001
LEFT ATRIAL SIZE (mm)	42,87±4,60	43,39 ±4,43	42,35 ±4,74	37,92± 3,72	A vs B <0,001 A1 VS A2. 0,113, NS A1 VS B <0,001 A2 vs B, <0,001
LEFT VENTRICULAR EJECTION FRACTION-EF (%)	59,1±7,6	59,7±6,9	58,7±8,2	63±7,9	A VS B <0,001 A1 vs A2- 0,554 NS A1 Vs B- 0,0004 A2 vs B- 0,0001
LEFT VENTRICULAR DIMENSION TDD (mm)	52,84± 5,85	53,58± 6,05	52,10 ±5,57	49,56±4,26	A vs B <0,001 A1 VS A2 0,076 NS A1 VS B <0,001 A2 vs B, 0,0004
LEFT VENTRICULAR MYOCARDIAL MASS INDEX g/m <sup>2</sup>	121,8±28,5	123,3±29,6	119,5±30,9	98,1± 20.2	A vs B <0,001 A1 vs A2 0,425 NS A1 VS B <0,001 A2 vs B <0,001



CHART 3. Quantitative echocardiographic parameters of tissue Doppler: diastolic function and longitudinal systolic function in relation to viral serology in clinically suspected myocarditis

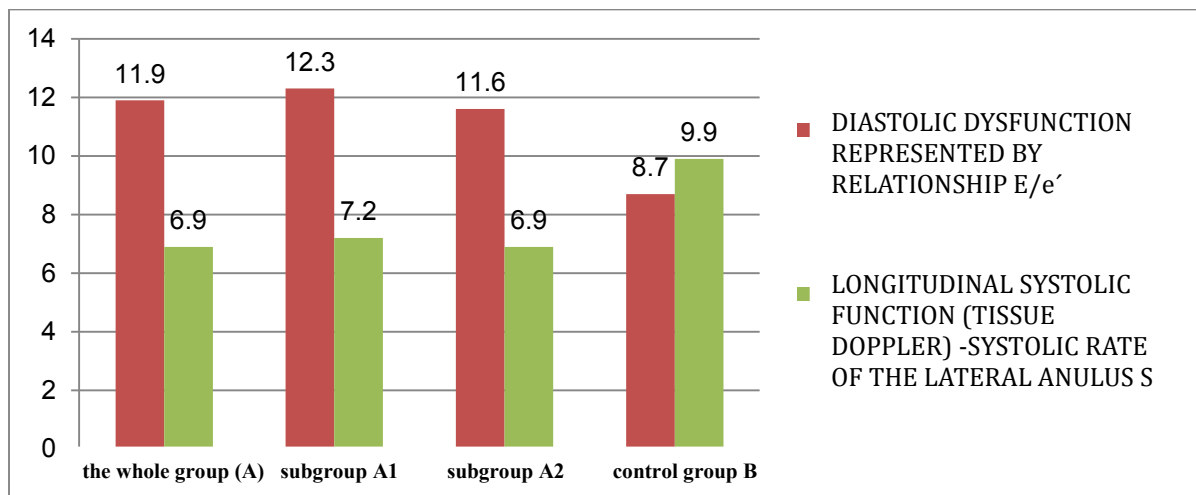
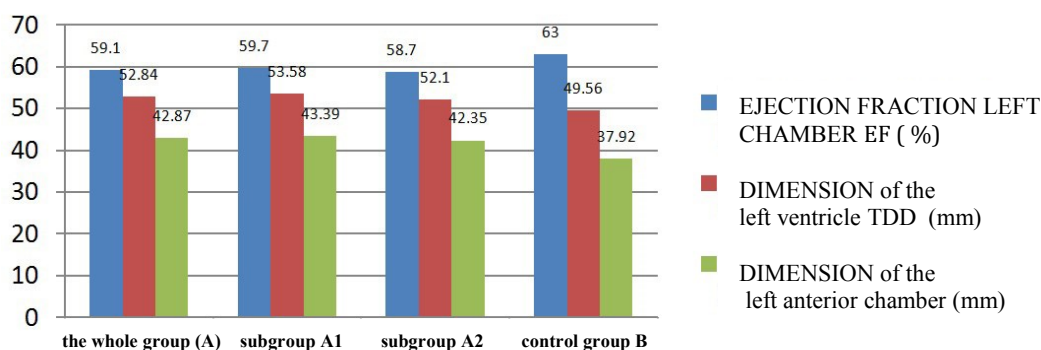


CHART 4. Echocardiographic parameters of left ventricular and atrial systolic function and dimensions in relation to viral serology in clinically suspected myocarditis



The whole group A of clinically suspected myocarditis compared to control group B had statistically highly significantly reduced parameters of systolic function ( $EF = 59.1 \pm 7.6\%$  vs.  $63 \pm 7.9\%$ ;  $p < 0.001$ ) (Table 4 and Chart 3) including longitudinal systolic function  $S'$  via tissue Doppler  $6.9 \pm 1.3$  cm / s vs.  $9.9 \pm 2.1$ ;  $p < 0.001$  (Table 4 and Chart 4).

Diastolic dysfunction ( $E/e' 11.9 \pm 4.8$  vs.  $8.7 \pm 4.6$ ;  $p < 0.001$ ) shown in Table 4 and Graph 3, was highly significant in the study group vs. control group. The increase in left ventricular telediastolic dimension (TDD, EDD), myocardial mass index (LVMI) and left atrial size (TABLE 4 and CHART 4) was statistically significantly increased in the group of clinically suspected myocarditis. The whole group A of clinically

suspected myocarditis has a myocardial mass index statistically significantly higher, which is explained by myocardial edema and not hypertrophy as in hypertension.

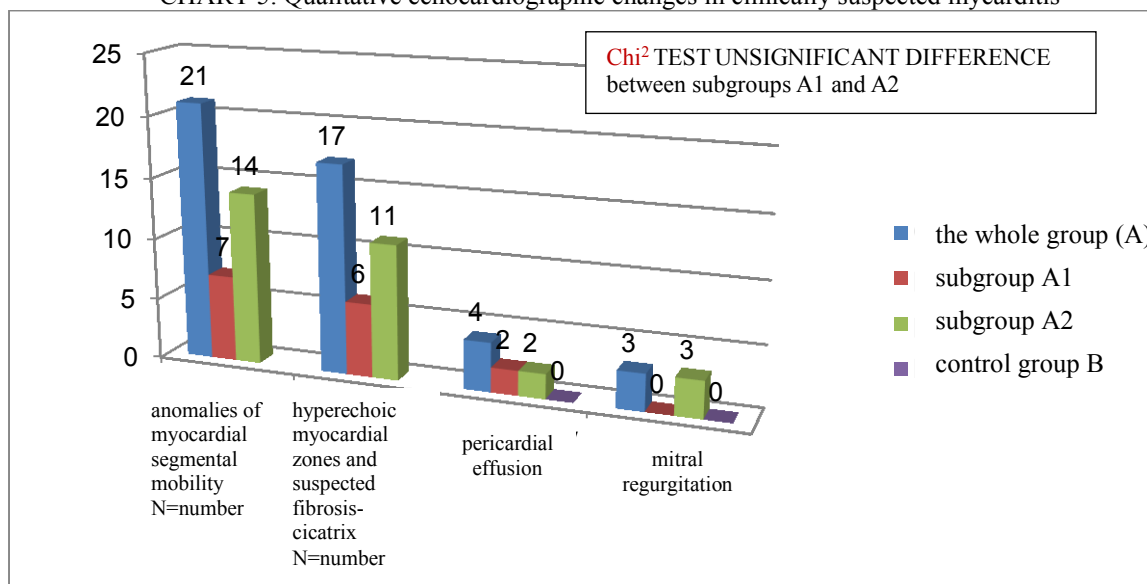
Comparison of subgroups A1 and A2 did not find a statistically significant difference between IgM positive and IgM negative patients in relation to quantitative echocardiographic changes (TABLE 4 AND CHARTS 3 AND 4), which means that elevated IgM antibody titer and seroconversion do not indicate the degree of myocardial damage and thus to a more severe form of myocarditis.

Qualitative echocardiographic changes are shown in CHART 5. These changes do not occur in the control group, which indicates their good specificity. As for quantitative

echocardiographic parameters, there is no statistically significant difference between

subgroups A1 and A2 (Chi<sup>2</sup> test of insignificant difference).

CHART 5. Qualitative echocardiographic changes in clinically suspected myocarditis



## DISCUSSION

To this day, there has not existed the so-called gold standard for the diagnosis of acute myocarditis due to the low specificity and sensitivity of traditional diagnostic tests. Endomyocardial biopsy with pathohistological examination, immunohistochemistry and the presence of viral genome is the most reliable method and allows the application of a therapeutic algorithm, but this invasive diagnosis is mainly reserved for more severe and unclear cases of dilated and / or inflammatory cardiomyopathies. Acute viral myocarditis is generally a mild and self-limiting consequence of systemic infection with cardiotropic viruses [41]. However, patients may develop temporary or permanent impairment of cardiac function, including acute cardiomyopathy with hemodynamic compromise or severe arrhythmia. Acute fulminate myocarditis is rare, it occurs primarily in children as cardiogenic shock or pulmonary edema, and recognizing it in time saves lives. EF usually returns almost to normal but residual diastolic dysfunction may limit greater exertion in some who have experienced fulminant myocarditis [13]. The proportion of dilated cardiomyopathies (DCM) due to viral infection remains controversial [42]. In the largest series of 1426 children, myocarditis was the cause of

DCM in 34% [43]. Accurate prediction of CV risk in the earlier stages of myocarditis is especially important due to the timely identification of high-risk patients [15].

The largest number of published studies rarely involve both initial and follow-up biopsies [44,45,46] and have only outlined the initial finding of EMB at the onset of symptoms. EMB-free series have been diagnosed with chronic myocarditis based on clinical presentation, elevated inflammatory markers, and image characterization in patients with normal coronary angiography [47]. Previous studies have estimated that 30% of DCM develops from myocarditis [45,46,48,49].

Patients with acute myocarditis usually present chest pain, dyspnea, or both, with tachycardia and dysrhythmias. [1,13,50,51,52]. In a recent series of 245 patients with clinically suspected myocarditis, the most common symptoms were fatigue (82%), exercise dyspnea (81%), arrhythmias (55%, supraventricular and ventricular), palpitations (49%), and chest pain at resting (26%) [53]. This is consistent with our results, where arrhythmias and palpitations dominated in 84%, while chest pain was twice as common (66%). Viral prodrome of fever, myalgia and respiratory symptoms occurs in between 20% and 80% of cases, the patient can

easily fail to report prodromes, so one cannot rely on that in the diagnosis.

Of our 126 cases of clinically suspected myocarditis, most had some electrophathological ECG changes-112/126 (89%), and with a normal ECG there were 14/126 (11%) so it cannot be used to rule out myocarditis. However, in these 14 patients there were echocardiographic changes and criteria for clinical presentation. Dysrhythmias have no specificity for myocarditis, while ECG signs of myocardial damage, depression or ST elevation, block of the left branch of the His bundle speak in favour of myocardial lesions, but do not indicate the cause. Estimation of ECG sensitivity for myocarditis is at about 47%, while the specificity is very low [52]. Troponin, for example, has an even lower sensitivity for myocarditis of 34% but a good specificity of over 89% [52].

The analysis of parameters measured by transthoracic echocardiography in the criteria for clinically suspected myocarditis was dominated by left ventricular diastolic dysfunction, represented by the ratio  $E / e'_{\text{prim}} \geq 9$  in 39/126 (31%), of which 17 (14%) patients, about half had severe diastolic dysfunction grade III ( $E / e'_{\text{prim}} \geq 14$ ). In one series of 147 patients with severely reduced EF ( $23 \pm 8\%$ ), 42% had diastolic dysfunction, but these were more severe patients with inflammatory cardiomyopathy. Improvement of diastolic function in 58% of these patients after treatment and follow-up for about 6 months is prognostically important, as is improvement in EF and it carries increasing prognostic value for risk stratification [54]. Global left ventricular systolic dysfunction (EF <50%) was found in only 19/126 (15%) of our patients and all had mild left ventricular dilatation and criteria for inflammatory cardiomyopathy. There was a significantly higher number of patients with systolic dysfunction in the Italian study with biopsy-proven myocarditis in a series of 41 pts [55], where left ventricular systolic dysfunction was present in 69% and regional contractility disorders in 64%, left ventricular hypertrophy due to myocardial edema in 20%, changes in myocardial texture 23%, ventricular thrombus in 15%, and restrictive left ventricular filling pattern in 7%. Most of our patients had a normal ejection fraction of 107 pts or 85%, which is an important prognostic factor in most relevant studies [56,57,58]. In the registry of one German

centre on 210 EMB-proven myocarditis 50% or three times as many than in our results had a reduced ejection fraction, due to the clinical spectrum of severe patients with myocarditis who are sent for EMB. After two years of follow-up and treatment with standard therapy for heart failure, 26% normalized EF and 27% remained with decreased EF [59]. Study by Grün S et al. [56] with a series of 222 consecutive pts with EMB-proven viral myocarditis, gives the mortality rate of 19% with a median of 4.7 years. In general, about 1/4 of patients with EMB-proven viral myocarditis go towards worsening cardiac function and undergo or have a heart transplant or exit. [15]. Outcome predictors vary in various studies with EMB: NYHA class III to IV persistence, left atrial dilatation, and EF improvement within 6 months are independent predictors of long-term outcome [42]. Kinderman I et al. state that high NYHA class, immune signs of inflammation, and lack of beta-blockers in therapy are predictors of poor outcome rather than histological characteristics of the Dallas criteria or the presence of a viral genome [10].

Regional systolic dysfunction according to our research was determined in 21/126 (17%) and in these cases cicatrix must be excluded after asymptomatic infarction by stress echocardiographic test by pharmacological or physical load and in inconclusive cases by MSCT or invasive coronary angiography [60].

Echocardiography is an excellent tool for diagnosing and monitoring patients with myocarditis and DCM. Speckle tracking echocardiography (image of myocardial deformity) is of increasing importance in the early stages of myocarditis and detection of progression to cardiomyopathy [50].

The change in the type of myocarditis-causing virus is in line with other studies [7,8,11,12], while one of the few recent studies from Bulgaria finds the serological dominance of Coxsackie virus as a possible cause of myocarditis [61]. Clear dynamics of IgM titer was observed in a small number of patients in 23/126 (18%) persons with Parvo B19 antibody dominance and a decrease in titer with an increase in IgG titer (seroconversion) in 13/126 (10%) patients. Increasing the titer dynamics of circulating antiviral antibodies from acute to subacute and chronic phases may aid Dg viral myocarditis with possible spontaneous recovery [13]. The sensitivity of antiviral antibodies is low

and estimated based on several studies at 25-32% and specificity at 40% [52]. This tells of the active process of infection anywhere in the body and contributes to a possible causal diagnosis only with strong evidence of myocardial involvement through valid ESC criteria for clinically suspected myocarditis. In the most significant study on this topic, Mahfoud F. et al [26] examined the serology of the virus and compared it with PCR findings by endomyocardial biopsy with histological and immunohistochemical findings in 124 patients aged  $40 \pm 15$  years with suspected myocarditis. The viral genome was detected in the myocardium by a polymerase chain reaction. Acute viral infection with cardiotropic viruses was diagnosed by IgM in the initial sample or IgG seroconversion in the next sample. Immunohistochemical signs of inflammation were present in 54 patients. The viral genome was detected in the myocardium of 58 patients (47%). In 20 patients (16%), acute viral infection was diagnosed by serology, which is in line with our result of 18%. But only 5 of 124 patients (4%) had serological evidence of infection with the same virus detected by EMB. The sensitivity of virus serology was only 9% and the specificity was 77%. The lack of correlation between serology and EMB is evidence against the routine use of viral serology in all patients with clinically suspected myocarditis. The sensitivity of viral serology is very low in relation to ECG and echocardiography, and the specificity is moderate, and it should not be used routinely in the evaluation of myocarditis, but in selected cases with ESC criteria where CMR and EMB are not performed. It is known from clinical experience that it is difficult to reassure some patients of not having the "Coxsackie virus in their heart". The mental burden of patients and attachment to "Coxsackie disease", which they are convinced to carry for many years only on the basis of increased serum IgG antiviral antibodies, is counterproductive from the social-medical point of view. Anti-heart antibodies (AHA) do not have an established role, because they occur in other diseases (CAD, genetic CMP) and the sensitivity is similar to viral serology 25-30% and specificity about 40% [52]. However, the pathohistological Dallas criteria itself [52] without immunohistology and PCR have low sensitivity 35 to 50% and good specificity 78 to 89%. Complemented by immunohistochemistry

and PCR identification of the virus genome, the sensitivity is satisfactory 65% to 70% and the specificity 80-100%. Unfortunately even EMB has false negative findings, depending on where the samples were taken and whether technically enough tissue was taken.

A comparison between group A1 and group A2 did not reveal a statistically significant difference in echocardiographic parameters, which means that IgM antibodies and seroconversion do not indicate more severe forms of myocarditis. There have been no studies on this aspect so far.

The whole group A of clinically suspected myocarditis in relation to the control group B has statistically highly significantly reduced parameters of global systolic (EF =  $59.1 \pm 7.6$  vs.  $63 \pm 7.9$ ;  $p < 0.001$ ) and longitudinal systolic function ( $S' = 6.9 \pm 1.3$  vs  $9.9 \pm 2.1$ ) which suggests that these subtle changes may lead us to think of myocarditis in everyday clinical practice. In individual distribution, systolic dysfunction is by half less represented than diastolic (15% Vs 31%). Diastolic dysfunction, despite the complexity of the assessment, is even more markedly reduced compared to control group B, when we look at the most representative parameter  $E/e'$  ( $E/e' 11.9 \pm 4.8$  vs.  $8.7 \pm 4.6$ ;  $p < 0.001$ ). Dilatation of the left atrium and left ventricle are highly significantly increased mean values compared to the control group. Myocardial mass and myocardial mass index are possible measures of myocardial edema in myocarditis and are of significantly higher mean values in the examined group vs. control group ( $121.8 \pm 28.5$  g/m<sup>2</sup> vs.  $98.1 \pm 20.2$ ,  $p < 0.001$ ) which is important for making a working diagnosis of clinically suspected myocarditis, monitoring the course of the disease and the effect of treatment. All echocardiographic changes are without pathognomoncity and specificity for myocarditis, but they have good diagnostic sensitivity. The ability of echocardiographic parameters to predict the development of manifest heart failure mortality and adverse CV events in the population of inflammatory cardiomyopathy has been proven in a small number of studies. In patients with clinically suspected myocarditis who have not yet started treatment for heart failure and / or arrhythmias, the association of both ejection fractions and diastolic dysfunction with CV mortality has been confirmed [62,63,64]. Paradoxically in a recent

meta-analysis of Chen WH. and associates the presence of the viral genome does not worsen the long-term prognosis of patients with myocarditis or inflammatory cardiomyopathy. However, virus-positive patients who have not received specific antiviral treatment have a worse prognosis than virus-negative ones. This means that early diagnosis of the presence of a viral myocardial infection improves the patient's prognosis [64].

In this study, we did not have consistent data on the value of the parameters of the cardiac biomarkers Troponin I and T as well as NT-pro BNP, which is an objective shortcoming of this study. Also at that time we did not routinely do a left atrial volume index (LAVI) which is a better indicator of diastolic function than the left atrial size. Echocardiography of myocardial deformation using speckle tracking technology (myocardial strain) will provide a stronger echo tool in the evaluation of clinically suspected myocarditis.

### CONCLUSION

Diagnosis of acute viral myocarditis is not easy to make and is based on the criteria for clinically suspected myocarditis of the European Society of Cardiology (ESC), which include clinical presentations and 4 different diagnostic categories, with a dominant role of ECG and echocardiography in everyday clinical practice with necessary exclusion of other cardiovascular diseases. The whole group of clinically suspected

myocarditis A had highly statistically significantly lower parameters of systolic and diastolic function compared to control group B. Diastolic left ventricular dysfunction dominated in 31% where 17 patients had severe diastolic dysfunction grade III and clinically heart failure with preserved ejection fraction. Regional systolic dysfunction was found in 17% and global left ventricular systolic dysfunction (EF <50%) in 15% with left ventricular dilatation and criteria for inflammatory cardiomyopathy. Changes in myocardial texture - hyperechoic myocardial zone and signs of fibrosis - cicatrix were present in 13% of subjects, and a highly significant increase in left ventricular telediastolic dimension, myocardial mass index and left atrial size. 24 (19%) patients had a normal echocardiographic finding, but with clinical and ECG criteria for myocarditis. However, 81% of patients had some of the echocardiographic pathological changes, which are more specific for diagnosis than ECG changes. A normal ECG and echocardiographic findings cannot be used to rule out a diagnosis of myocarditis. Comparison of subgroups with the presence of antiviral IgM antibody titer dynamics (A1) and without it (A2) did not reveal a statistically significant difference in echocardiographic parameters. The sensitivity of IgM titer to cardiotropic viruses is very low and should not be used in the routine diagnosis of myocarditis.

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