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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^2 test. **RESULTS:** 126 patients had clinically suspected myocarditis (≥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±4,6 vs. 63±7,9; p<0,001), longitudinal systolic (S´=6,9±1,3 vs. 9,9±2,1) and diastolic function (E/e´11,9±4,8 vs. 8,7±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]. Endomycardial 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 ± 8.9 years, body mass index BMI 27.8 ± 5.9, dominated by female gender-78 (62%). Mean values of systolic and diastolic pressure on arrival were -127 ± 14/78 ± 11 mmHg. The control group had comparable characteristics: 103 persons with average age 46 ± 12 years, body mass index BMI 29.3 ± 6.4, 53 persons (52%) female. Mean systolic and diastolic pressure on arrival were 136 ± 14/71 ± 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 **TABLE1** [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 ≥1-CRITERIA OF CLINICAL PRESENTATIONS (1-5)	≥ 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 4 .PALPITATIONS AND/OR UNEXPLAINED SYMPTOMS OF ARRHYTHMIA AND/OR PRESINCOPE AND SYNCOPE AND/OR RESUSCITATED PATIENTS	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
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 ≥54mm for women and ≥59mm 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).

$$\mathbf{(\ LVMI\ (g/m^2) = [(TDD + ZZd + IVSd)^3 - TDD^3]\ x\ 1.05\text{-}13.4\ /\ BSA(m^2)}\ [37]$$

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/m² for females and less than 115 g/m² 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 ≥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 ≥0.8 - <1.5, E-DTE deceleration time> 160 ms, mean E/e'≤8);

(b) Grade 1, impaired relaxation (E/A <0.8, DTE> 200 ms, mean E/e'≤8);

(c) Grade 2, Pseudonormalization (E A ≥0.8 and <1.5, DTE 160–200 ms, mean E/e'= 9–12);

(d) Grade 3, Restrictive pattern (E/A ≥1.5, DTE <160 ms, mean E e'≥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 ≥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 Chi² test.

RESULTS

126 patients (GROUP A) had clinically suspected myocarditis (KSVMy with ≥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	%
SYMPTOMS - CLINICAL PRESENTATIONS		
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%
PHYSICAL SIGNS		
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%
ECG CHANGES		
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 cracklesin 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,

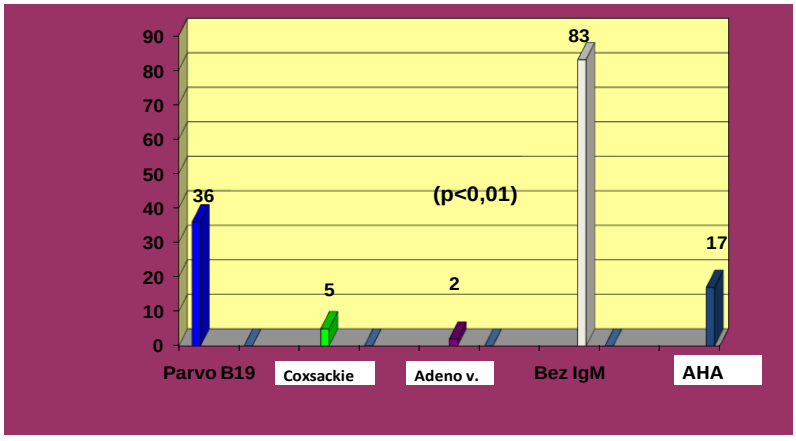
but with clinical and ECG criteria for myocarditis..

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

ECHOCARDIOGRAPHIC PARAMETERS	GROUP A, N=126 patients	PERCENTAGE
Pathological findings on echocardiography	102	81%
Normal echocardiographic findings	24	(19%)
Diastolic dysfunction represented by the ratio -E / e ’≥9	39	(31%)
Severe diastolic dysfunction grade III (E / e´prim ≥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.

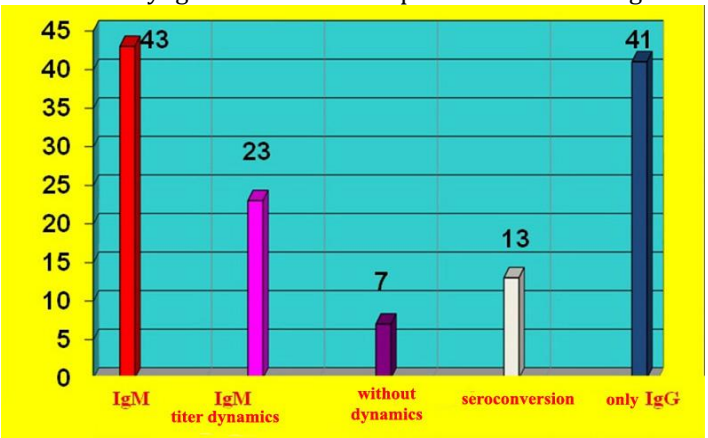
CHART 1. Distribution of IgM serological positivity in 43 (34%) of 126 patients examined for suspected recent virus infection and evidence of autoimmune response via elevated AHA antibodies serum titer



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 IgaM, 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%) POSI-TIVE 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 FUNC-TION (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 MYOCARDI-AL MASS INDEX g/m²	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

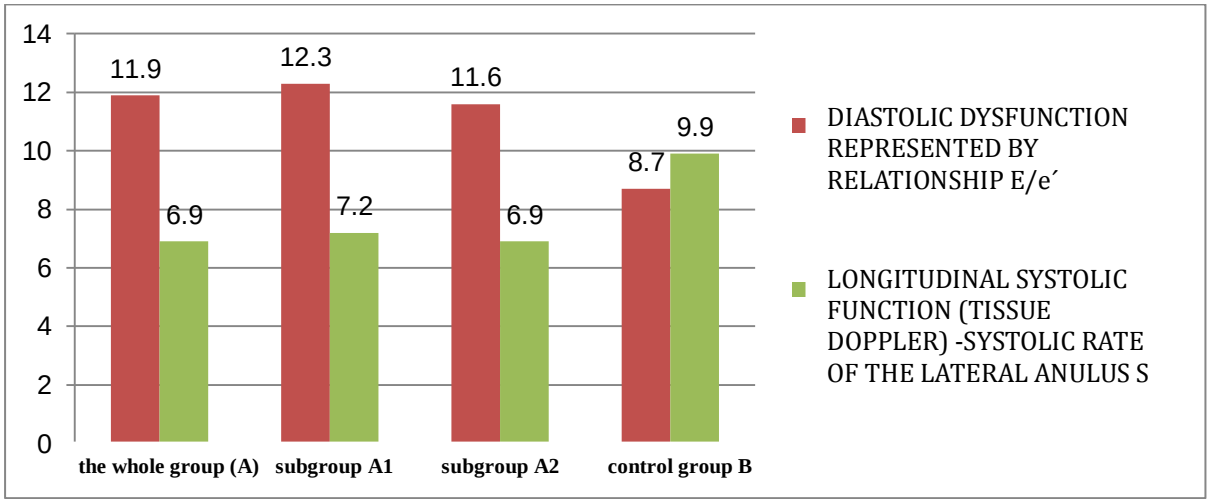
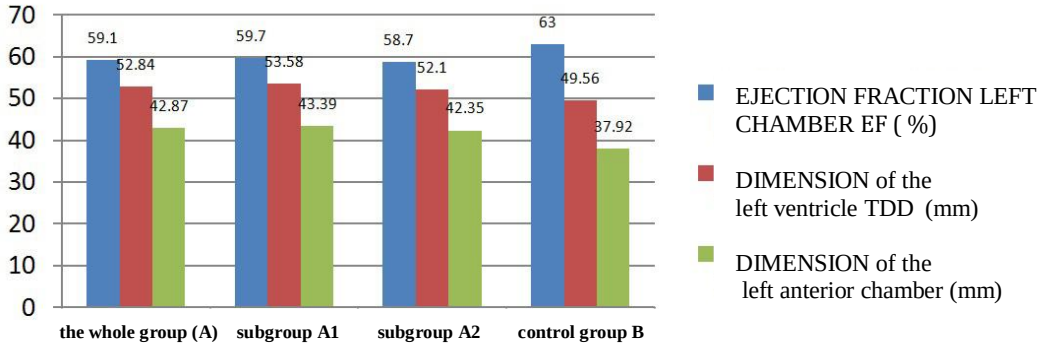


CHART 4. Echocardiographic parameters of left ventricular and atrial systolic function and dimensions in relation to viral serology in clinically suspected myocarditis



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 ± 7.6% vs. 63 ± 7.9%; p <0.001) (Table 4 and Chart 3) including longitudinal systolic function S´ via tissue Doppler 6.9 ± 1.3 cm / s vs. 9.9 ± 2.1; p <0.001 (Table 4 and Chart 4).

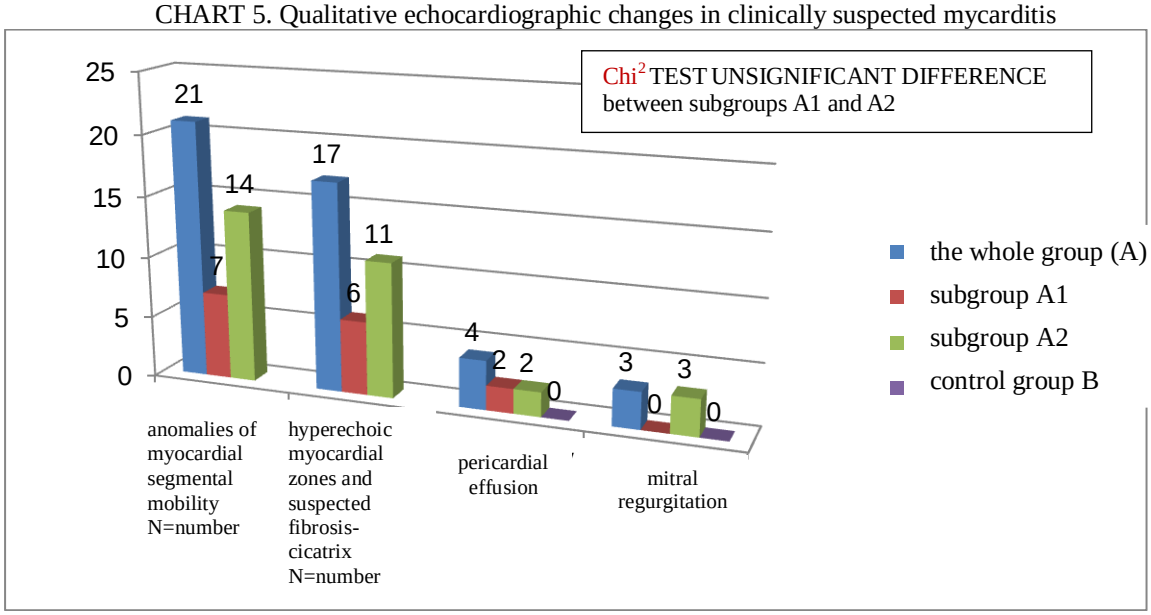
Diastolic dysfunction (E/e´11.9 ± 4.8 vs. 8.7 ± 4.6; p <0.001) shown in Table 4 and Graph 3, was highly significant in the study group vs. control group. The increase in left ventricular telediastolic dimension (TDD, EDD), myocardial mass index (LVMI) and left atrial size (TABLE 4 and CHART 4) was statistically 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 (Xi2 test of insignificant difference).

CHART 5. Qualitative echocardiographic changes in clinically suspected mycarditis



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. Endomycardial 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 palitations 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 electropathological 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´prim≥9 in 39/126 (31%), of which 17 (14%) patients, about half had severe diastolic dysfunction grade III (E / e´prim ≥14). In one series of 147 patients with severely reduced EF (23 ± 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 endomicardial biopsy with histological and immunohistochemical findings in 124 patients aged 40 ± 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 ± 7.6 vs. 63 ± 7.9; p <0.001) and longitudinal systolic function (S´ = 6.9 ± 1.3 vs 9.9 ± 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 ± 4.8 vs. 8.7 ± 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 ± 28.5 g/m^2 vs. 98.1 ± 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 pathognomonicity 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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THE IMPORTANCE OF OPERATIVE HYSTEROSCOPY IN TREATING PATHOLOGIES OF THE UTERINE CAVITY IN INFERTILE PATIENTS

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Abstract: According to the definition of the World Health Organization (WHO), infertility is the inability of a sexually active, non-contracepting couple to achieve pregnancy in one year. One of the causes of sterility is inborn and acquired uterine anomalies. The best visualization of the inside of the uterus is achieved hysterscopically. Hysteroscopy is a minimally invasive surgical procedure and has the greatest significance in the diagnosis and treatment of congenital anomalies of the uterus. It is possible to eliminate and correct most of the congenital anomalies of the uterus, and it also enables the removal of other pathological changes in the cavity of the uterus. The incidence of congenital uterine anomalies in general population is 0.1- 3.5%. Infertile patients have a higher incidence of these anomalies which range from 3-6%, and 5-10% in habitual abortions. The study included 200 infertile patients up to 40 years of age, with performed surgical hysteroscopy due to diagnosed changes in the uterine cavity. The patients were operated at the Department of infertility of the Obstetrics and Gynecology Clinic "Narodni Front" in Belgrade, in 2013. and 2014. The following pathological changes of the uterine cavity, were hysteroscopically removed: submucosal fibroids type 0 and type I. The aim of this paper was to evaluate the success of operative hysteroscopy in the treatment of pathological changes of the uterine cavity in infertile patients, based on the number of relapses in the first six months upon surgery. Relapses occurred in 0.5% of patients during a six-month postoperative course. Complications during hysteroscopic operations were intraoperative and postoperative. There were 1.5% of overall complications in the participants.

Keywords: infertility, hysteroscopy, submucosal fibroids.

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The paper presents the most significant results of the subspecialist paper "The importance of operative hysterscopy in treating pathologies of the uterine cavity in infertile patients", authored by Dr Aleksandar P. Dević under the mentorship of Prof. Mladenko Vasiljević.

INTRODUCTION

Hysteroscopy is a minimally invasive surgical procedure which is of the greatest importance in the diagnosis and treatment of congenital uterine anomalies [1,2]. Hysteroscopic examination is usually performed in the first phase of the menstrual cycle [3]. Hysteroscopy can also be done regardless of the phase of the menstrual cycle if the patient has been previously prepared with oral contraceptives [4]. Hysteroscopy can be diagnostic and operative [5,6]. After hysteroscopic surgeries the fertility rate is significantly improved, as well as the overall percentage of pregnancies and live births, whereas the rate of miscarriages significantly decreases in these patients [7,8]. In our country, the total frequency of infertility is around 15%. The most frequent uterine causes of infertility are congenital anomalies of the uterus and uterine fibroids [9]. The significance of fibroids as the cause of infertility is even greater now due to an increasing number of women who decide to give birth later in life, at the time when uterine fibroids are more frequent [10,11]. The accepted parameters for fibroids being the cause of infertility are the following: subserosal fibroids that are ≥5 cm in diameter, intramural fibroids that are 2-3 cm in diameter and submucosal fibroids that are 1-2 cm in diameter [12]. It has been proven that the

percentage of pregnancies and implantations is significantly lower in patients with intramural and submucosal fibroids even when there is no cavum deformity [13]. The percentage of pregnancies upon myomectomy is up to 60% [14,15].

THE AIM

The aim of the paper was to assess the success of operative hysteroscopy in treating pathological changes of the uterine cavity caused by fibroids in infertile patients taking into consideration the number of relapses in the first six months upon surgery and the number of intraoperative and postoperative complications.

THE MATERIAL AND METHOD

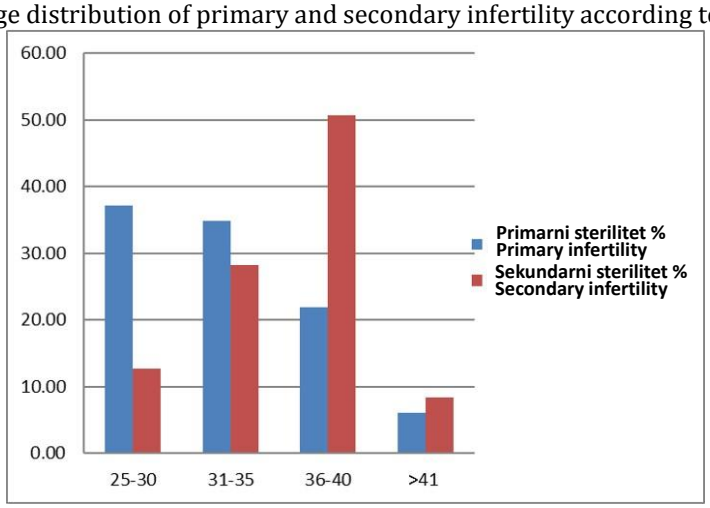
The research included 200 infertile patients of up to 40 years of age who had been previously diagnosed with fibroids in the uterine cavity and thus had an operative hysteroscopy done for removing the fibroids. The patients were randomly selected and they were all operated on during 2013. and 2014. at the Obstetrics and gynecology hospital "Narodni front". A rigid hysteroscope with an outer sheath 9 mm in diameter and a resectoscope containing a bipolar electrode for resecting pathological changes in the uterine cavity was used for performing hysteroscopy [16,17]. Saline solution (0,9% NaCl) was used for the distension of the uterine cavity [18]. The following pathological changes of the uterine cavity were removed: submucosal fibroids type 0 and type I [19]. The success of hysteroscopic surgeries was assessed according to the number of relapses in the first six months upon surgery [20]. In all the patients the following parameters were analyzed: age, occupation, education, the type of infertility, the duration of infertility, the presence of previous miscarriages or labors, ultrasound findings, hysterosalpingography findings, and diagnosed fibroids in the uterine cavity. The decision to perform a hysteroscopic surgery was made according to ultrasound or hysterosalpingography findings [21]. Submucosal fibroids were classified using the European Society for Hysteroscopy's classification as type 0 (pedunculated, i.e. completely located in the uterine cavity), type I (≤50% of the fibroid is located in the myometrium whereas its ≥ 50% is located in the uterine cavity) and type II (≥50% of the fibroid is located in the myometrium and its ≤50% is located in the uterine cavity) [19,22,23]. Hysteroscopic surgeries were performed in the first phase of the menstrual cycle between day 6 and day 12, under general endotracheal anesthesia and after adequate preoperative preparation of the patient [21,16]. The collected data were analyzed using the methods of descriptive statistics (the mean and standard deviation) and analytical statistics (Chi-square test, Mann-Whitney U test and Student's t-test). A database was created on an ASUS X% 1 RL computer using the software package SPSS 10.0 for analyzing the data. The results obtained were presented using figures and tables and they were compared with the results obtained by other authors. According to the collected data certain conclusions were made.

RESULTS

In this part the most significant results are presented through tables and figures. Figure 1 shows the distribution of primary and secondary infertility according to the patient's age.

Figure 1 presents the percentage distribution of primary and secondary infertility according to the patient's age.

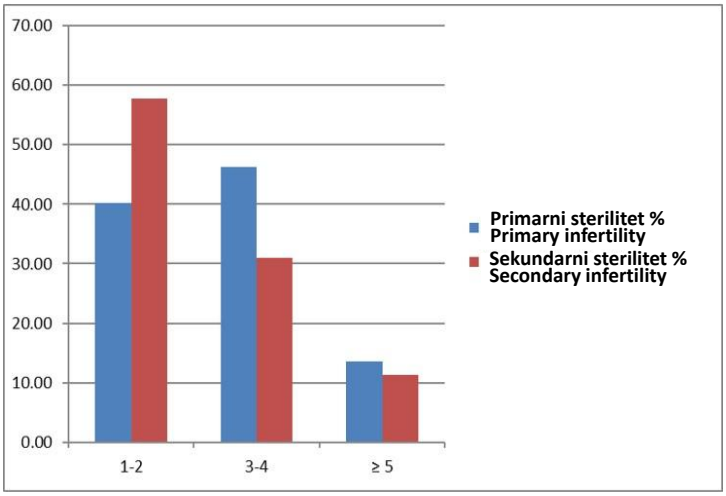
Figure 1. Percentage distribution of primary and secondary infertility according to the patient's age.



There is a large statistically significant difference in the distribution of groups formed according to age between the patients with primary infertility and those with secondary infertility (U=2493.5; p<0.001). Moreover, a statistically highly significant correlation was found between the age groups and the type of infertility (r=0.408; p<0,001), which indicates a significantly more frequent correlation between primary infertility and older patients.

Figure 2 presents the distribution of primary and secondary infertility according to the d uration of infertility in both groups of patients.

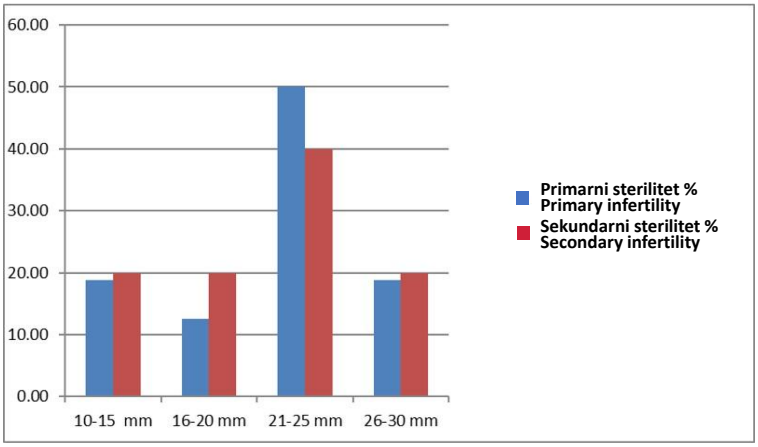
Figure 2. Percentage distribution of primary and secondary infertility according to the duration of infertility in the observed patients.



The duration of infertility in patients with primary infertility is statistically significantly longer when compared to the patients with secondary infertility, (U=3907.5; p<0.05). Besides, a statistically significant correlation was found between the duration of infertility and the type of infertility (r=0.151; p<0,05), which indicates a significantly more frequent correlation between primary infertility and the duration of infertility.

Figure 3 presents the distribution of the size of submucosal fibroids in patients with primary and secondary infertility.

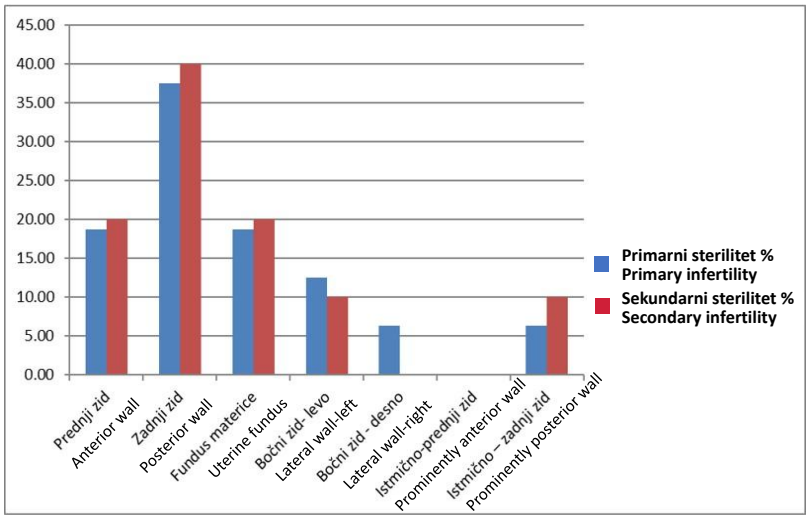
Figure 3. Percentage distribution of the size of submucosal fibroids in patients with primary and secondary infertility.



There is no statistically significant difference in the size of submucosal fibroids between the patients with primary infertility and those with secondary infertility (U=76.000; p>0.05).

Figure 4 presents the distribution of submucosal fibroids according to the location in the uterus in patients with primary and secondary infertility.

Figure 4. Percentage distribution of the location of submucosal fibroids in patients with primary and secondary infertility.



There is no statistically significant difference between the patients with primary infertility and those with secondary infertility concerning certain locations of submucosal fibroids (U=76.500; p>0.05).

Table 1 shows the most frequent complications that occurred during hysteroscopic surgeries.

Table 1. Intraoperative complications of hysteroscopic surgeries

Intraoperative complications	Number and percentage of patients	
	N	%

Bleeding during surgery	1	0.5
Uterine perforation	1	0.5
In total	2	1

Table 2 presents the most frequent postoperative complications of hysteroscopic surgeries.

Table 2. Postoperative complications of hysteroscopic surgeries.

	N	**%**
Creating adhesions after surgery	1	0.5
In total	1	0.5

Table 3 presents the frequncy of relapses in the first six months upon a hysteroscopic surgery of fibroids.

Table 3. The frequency of relapses six months after a hysteroscopic myomectomy

	Number and percentage of patients	
	N	%
Miomektomia	1	0.5

Six months upon a hysteroscopic surgical myomectomy there was a relapse in one patient, p>0.05.

DISCUSSION

Observing the type of infertility in relation with the age of the examined patients, we found that primary infertility was approximately equally represented in patients of 25-30 years of age and those of 31-35 years of age, whereas secondary infertility was most often represented in patients of 36-40 years of age. In most of our patients, primary infertility lasted for 3-4 years, while secondary infertility lasted for 1-2 years. Transvaginal ultrasound is accurate in diagnosing uterine fibroids [23,24]. No abnormalities were found in 10.6% of the patients. There is no statistically significant difference when it comes to individual locations of submucosal fibroids between the patients with primary and secondary infertility and there is no significant difference concerning the size of submucosal fibroids between these two groups of patients [25].

Submucosal fibroids were present in 12.12% of our patients with primary infertility and in 14.08% of the patients with secondary infertility. The size of fibroids was between 21 and 25 mm both in the patients with primary infertility and those with secondary infertility. Other authors also stated that the average size of submucosal fibroids in patients who had undergone hysteroscopic myomectomy was 2.1 cm [25,26]. Most of the authors agree that type 0 and type I submucosal fibroids up to 6 cm in size and type II submucosal fibroids up to 4 cm in size can be removed hysteroscopically [20,25,26]. Trying to answer the question whether the size and location of submucosal fibroids affected the reproductive outcome, women with and without submucosal fibroids were compared and no significant difference was found in the birth rate which was 30.5% in women with fibroids and 33.7% in women without fibroids [15]. The largest meta-analysis to date by Sunkare et al. involved 11 different studies and it found that there was a 21% lower birth rate in the patients with submucosal fibroids with no distortion of the cavity compared to the patients with no fibroids [19,21].

In both groups of the patients submucosal fibroids were most commonly located on the dorsal wall of the uterine corpus, followed by the anterior wall of the uterus and the fundus of the uterus. Fibroids located in the uterine horns are more difficult to reach and remove and are thus connected with a greater risk of complications during surgery [16]. In all our participants resections of submucosal fibroids were performed in a single step. Other authors stated that they had done a complete resection of submucosal fibroids in 92.9% of cases and an incomplete resection in 7.1% of cases [27].

Complications during hysteroscopy can be intraoperative and postoperative. There were complications in three patients. When it comes to intraoperative complications, perforation of the uterus during dilation of the cervix occurred in one patient, and another patient developed

uterine bleeding during fibroid resection. Postoperative complications included adhesion formation after fibroid resection in one patient. In 0.5% of the patients there was a relapse in the first six months upon surgery, a relapse of submucosal fibroid occurred in one patient.

Other authors stated that the percentage of intraoperative complications was around 5.4% and that the risk of uterine perforation was particularly pronounced during resection of type II submucosal fibroids [16]. Uterine rupture during pregnancy and childbirth after a hysteroscopic myomectomy was found in 1% of cases [13]. The pregnancy rate after a hysteroscopic myomectomy was 29.7% [14]. The pregnancy rate was 40% if the fibroid was the only cause of infertility and in 33.3% in it was completely located in the uterine cavity [14].

CONCLUSION

Hysteroscopy is a safe and efficient endoscopic procedure for diagnosis and surgical removal of submucosal fibroids as one of the factors causing pathological conditions of the uterine cavity. Submucosal fibroids which deform the uterine cavity reduce a woman's fertility. Submucosal fibroids type 0 and type I 21-25 mm in size located on the dorsal wall of the uterine corpus were most often resected using the hysteroscopic procedure. The percentage of intraoperative complications was 1%. One patient experienced perforation of the uterus and one patient had uterine bleeding. The percentage of postoperative complications was 0.5%. One patient experienced adhesion formation in the uterus after fibroid resection. The percentage of relapses six months upon surgery was 0.5% as one patient had a relapse of a submucosal fibroid. Through adequate planning and performance it is possible to minimize the risk of complications during hysteroscopic surgeries. Advantages of the hysteroscopic approach include a shorter procedure, a better observation of the cavity, a greater precision, less pain, lower morbidity, the absence of cuts, faster recovery and getting back to work sooner.

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ROLE AND IMPORTANCE OF POSTERIOR MALLEOLUS FIXATION IN TRIMALEOLAR FRACTURES

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ABSTRACT: **Introduction** – ankle fractures are third when it comes to frequency, right after hip and wrist joint fractures. Posterior malleolus fractures are common, comprising 7% to 44% of all ankle fractures, and are very rare on their own; that is a Volkmann triangle fracture. Ankle is a supporting joint in the human body, and fractures generally occur within rotation of the body with different fixed foot positions. The posterior malleolus is a very important structure in distal tibiofibular joint. **Material and methods** - we retrospectively present a group of 21 patients who had a fracture of the ankle and were surgically treated at the Clinic for Orthopedics and Traumatology in Niš during the period from January 2013 to December 2015. The basic criterion for surgical treatment was the size of the fragment, > 25% from tibial joint surface and dislocation >2mm. We systemized all ankle fractures according to the Lauge Hansen system, which is based on the mechanism of injury. We evaluated treatment results according to the Olerud-Molander classification, followed by subjective and objective signs. We systemized posterior malleolus fractures into three types, according to the Haraguchi classification. **Results** - etiological causes of the fractures are: a fall at the same level - sport, skating in 11 (52.3%) patients, a fall from a height in 6 (28.6%) and traffic accident in 4 (21.1%) patients. According to the Lauge Hansen classification, there were 15 (71.4%) patients with a SER-type fracture, 4 (19%) of the PER type, and 2 (9.6%) of the PA type. End result of the treatment was excellent in 13 (61.9%) patients, good in 7 (33.4%) and poor in 1 (4.7%) patient. Post-traumatic arthritis (PTA) was assessed one year after the surgery and level 1 and 2 were present in 12 (57.1%) patients.

Keywords:– ankle, malleolus posterior, fixation, post-traumatic arthritis.

INTRODUCTION

Ankle fractures are relatively common, with an incidence of 187 fractures in 100,000 (1: 800) people per year [1], i.e. comprising 3.92% of all fractures in the body [2]. Posterior malleolus fractures are very common, comprising between 7% to 44% of all ankle fractures [3,4]; and are very rare on their own, and this is a fracture of the Volkmann triangle [5]. Ankle is a supporting joint in the human body, and fractures generally occur within rotation of the body with the foot fixed in different positions. Destot [6] was the first who described posterior malleolus in 1911, and Henderson [7] was the first who introduced the term trimaleolar fracture in 1932. Trimaleolar ankle fractures have a poorer prognosis than injuries without posterior malleolus fractures, so-called bimalleolar fractures [8,9] - fractured displacement, disorder of articular surfaces congruence, tibiotalar subluxation and instability of the ankle joint occure in this situation.The aim of ankle joint fracture treatment is to reduce and restrain talus in its anatomical position and inserting it into the ankle fork.

Posterior malleolus is a very important structure in the distal tibiofibular joint - providing restriction for distal fibula and stabilizing tibiofibular syndesmosis via posterior inferior tibiofibular ligament (PITFL) and inferior transvers ligament (ITL). Ogilvie-Harris et al. [10] state that PITFL enables 42% of strength and stability of syndesmosis. Integrity of the posterior malleolus and ligamentous adhesions is important for weight transfer, posterior talus stability, and rotational stability. This type of ankle joint fracture is associated with posterior

tuberculum tibia fracture, to which the PITFL is attached.

Indications for internal fixation of posterior malleolus fracture depend on size and degree of fragment dislocation. Lateral radiography is used for fracture diagnostics, although computerized tomography (CT) is increasingly recommended. If the fragment comprises more than 25% of tibial ceiling and there is a dislocation larger than 2mm, then there is instability of ankle joint with associated syndesmosis injury, and persistent posterior talus subluxation – in this case there exists an absolute indication for surgery [11,12]. The images are also used to determine the angle between bimaleolar axis and posterior malleolus fracture line, up to 40°, representing the degree of external tibia rotation [12].

The posterior malleolus needs to be fixed for a number of reasons: it forms a part of articular tibia surface, with a fracture there occurs articular non-congruence of the ankle joint, contact pressure between joint surfaces is disrupted - the larger the fragment is, the larger the pressure [13]. Due to the attachment of PITFL from fibula to posterior malleolus, the non-fixed posterior malleolus eventually leads to secondary fibula dislocation, even though it is fixed, which creates a possibility for postero lateral talus instability. After reduction and posterior malleolus fixation, articular tibia surface is restored, fibula is not shortened, syndesmosis is stable and the patient's rehabilitation is faster [14].

Ankle joint fractures occure when external rotational force, abduction or adduction are applied to the foot that is fixed in supination (in 70% of occurence) or pronation (in 30% of occurence) [15].

The aim of this paper is to present the role and importance of posterios malleolus fixation during surgical treatman of trimaleolar fractures.

MATERIAL AND METHODS

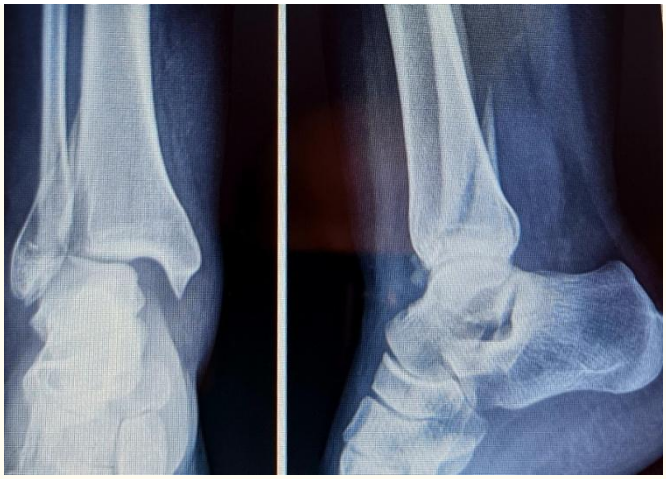
We retrospectively present a group of 21 patients who had ankle joint fracture and were surgically treated at the Clinic for Orthopedics and Traumatology in Niš from January 2013 to December 2015. Fixation of posterior malleolus was also performed, with a minimum follow-up period of 18 months.

Criteria for including patients in this study are: 1) definitive diagnosis of a ankle joint fracture based on clinical and radiological findings, 2) fracture of posterior malleolus, 3) posterior malleolus fragment occupies >25% of tibial joint surface, 4) the fragment is unstable and misplaced > 2mm, 5) reduction and fixation of malleolus have been applied, 6) patients age is from 18 to 70 years, 7) complete clinical monitoring for 3,6,12 and 18 months.

Clinical and radiological examinations are necessary; post-injury and post-surgery CT should be performed, but we did not do it.

Size of the posterior malleolus fragment was determined in LL radiological image. It is obtained by dividing the length of distal articular surface of tibia by the length of the fragment - this is the distance from the fracture line to posterior edge of tibia, expressed in %. Vertical dislocation is measured in LL image, and it is the height of posterior tibia edge step, expressed in mm. We measured the degree of external rotation [12] in the same image (Figure 1).

Figure 1. Antero posterior and lateral radiographic presentation of a luxurious fracture with complete dislocation and trimaleolar fracture



We systematized all ankle fractures according to Lauge Hansen system [16], based on the mechanism of injury. The first word describes foot position, and the other describes movements of talus relative to the extremity. There are five types of ankle joint fractures: supinational external rotational type (SER), supinational adductional (SA), pronational external rotational (PER), pronational abductional (PA) and pronational dorsiflexional (PD) type. The posterior malleolus fractures most commonly occur (>70%) in SER type of fractures in stage IV, and subsequently in PER [17,18].

Haraguchi et al. [19] provided a classification for three types of posterior malleolus fractures.

Type I - posterior outer oblique fracture line, fragment is wedge-shaped and includes posterior outer part of tibial ceiling; appropriate surgical approach is posterolateral;

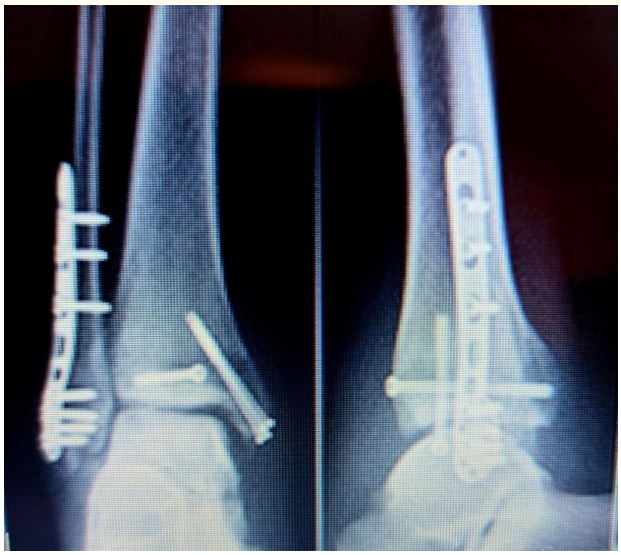
Type II - transversely internal fracture, the fracture line includes tibial noch (fibula dent) down to medial malleolus, and there are usually two fragments; appropriate surgical approach is medial or prolonged medial, with the aim of fixing only the medial fragment which is always larger;

Type III - small flaky fracture, that includes posterior edge of the tibia in the form of flake; this type of fracture does not require surgical intervention.

Having prepared the patient (early surgical intervention should be endeavored), we immediately performed surgery in spinal or I.V. conductive anesthesia with Tourniquet.

For direct reduction and fixation of posterior malleolus posterolateral approach was used and the screw was placed (1 or 2) in postero-anterior (PA) projection; we did not use the plate. Indirect reduction was achieved after osteosynthesis of lateral and medial malleolus with transfixation of syndesmosis; then we made an Rtg image and determined the position of posterior malleolus - if it is good, we place the screw in AP or PA position (Figure 2). In a number of patients, we did not perform fragment fixation due to its size (less than 15% of the tibia joint surface).

Figure 2. Antero posterior and lateral radiographic presentation of combined osteosynthesis of posterior, external and internal maleolus.



After the surgery, we placed a lower leg plaster orthosis for three weeks, and after 6 weeks, we allowed the patients to walk with crutches and lean on the treated leg, with a gradual load increase .

We perform radiological control examination after surgery in order to check the posterior malleolus reduction, and CT scan is also recommended. The reduction can be excellent (articular step is <1mm), when the articular surface is flat,good (when the step is <2mm); and poor reposition (> 2mm), when the surface is uneven [20].

We evaluated treatment results according to the Olerud-Molander classification [21], subjective and objective signs were monitored.

Post-traumatic arthritis (PTA) was determined in Rtg images during the follow-up of patients, and according to the following score: 0 - normal joint, 1 - osteophytes without joint space narrowing, 2 - joint space narrowing with or without osteophytes, 3 - joint space disappearing and deformation (22).

RESULTS

A series of 21 operated patients was presented. There were 12 (57.1%) women and 9 (42.9%) men, 18 to 70 years old (average age 48.8). Etiological causes of fracture are: a fall at the same level - sports, skating 11 (52.3%) patients, a fall from a height 6 (28.6%) and traffic accident 4 patients (21.1%).

The size of posterior malleolus fragment was 27,3% on the average (25% to 34%).

Degree of external rotation, i.e. angle between the two lines was 00 to 400

According to Lauge Hansen classification, there were 15 (71,4%) patients with fracture of type SER, 4 patients (19%) of type PER and 2 patients (9,6%) of type PA.

According to Haraguchi classification of posterior malleolus fracture, there were 12 (57,1%) of patients witi fracture of type I, 6 patients (28,5%) of type II and 3 patients (14,4%) of type III.

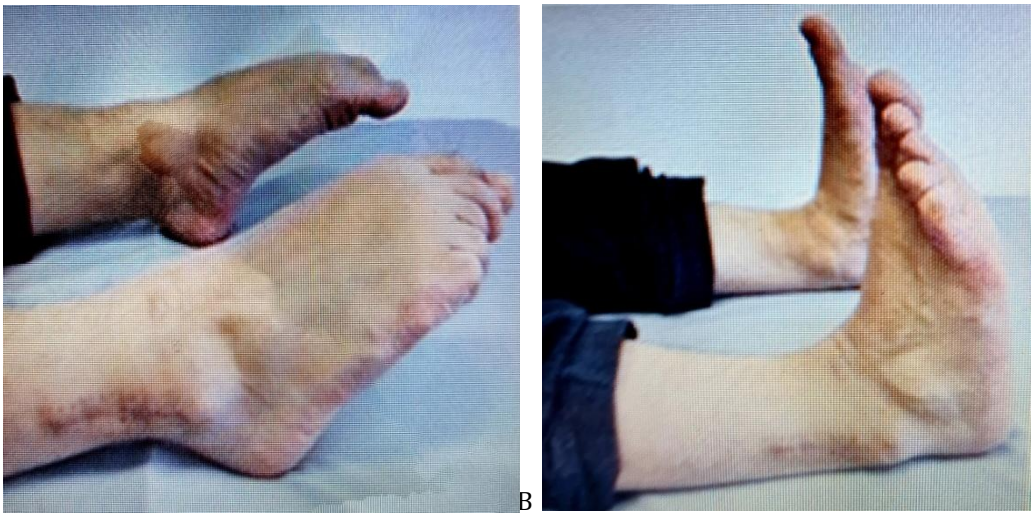
Elapsed time from the moment of injury to surgery was 1,6 days (1-5 days) on the average.

Direct reduction and screw fixation was applied in 6 patients, indirect reduction and fixation in 12, and in 3 patients a fixation of lateral and medial malleolus was applied, and thus a reduction of non-fixed posterior malleolus was achieved.

Postoperative direct reduction was excellent in 5 (83.3%) patients and good in 1 (16.7%). As for indirect fragment reduction, we had excellent results in 6 patients (50%), good in 3, and bad in another 3 (25 %). By comparing these results, it is evident that fracture reduction quality was significantly higher in direct reduction group,

compared to the group where reduction was performed indirectly (p = 0.039).
After follow-up period of 16 months (12 to 18 months) on the average, we evaluated the results according to the Olerud-Molander score. There were excellent results (91% - 100%) in 13 (61.9%) patients, good (61% - 90%)) in 7 (33.4%) patients, and poor (0% - 30%) in 1 (4.7%) patient (Figure 3A,B).

Figure 3. The photograph shows the anterolateral aspect of plantar (A) and dorsal (B) flexion of the foot.



Postoperatively, superficial wound infection occurred in 2 (9.4%) patients – it was treated with antibiotics, parenterally for 4 days, and postoperative thrombophlebitis in 1 (4.7%) patient – it was treated with low molecular Heparin. There were no fractured screws and all fractures healed in up to three months.
Post-traumatic arthritis (PTA) was evaluated one year after surgery and we obtained the following results: grade 0 in 9 (42.9%) patients, grade 1 in 10 (47.7%) patients, and grade 2 in 2 (9.4%)) patients - in total, 12 (57.1%) patients had PTA.

DISCUSSION

Ankle joint fractures are the third in frequency, right after hip and wrist joint fractures. Final functional outcome is better in bimaleolar, compared to trimalleolar fractures. They occur in young people, and with high energy trauma (accident, fall from a height and sport), and in elderly persons low-energy trauma causes fractures due to osteoporosis. In young people there is a risk of developing post-traumatic arthritis cousing changes in the quality of life, due to a reduction of ankle joint function and chronic pain. In elderly patients there is a risk of infection, wound complications and fixation disintegration [11].

Ankle joint fractures are intraarticular, resulting in articular surfaces injury, osteochondral layer disruption, joint surface displacement, and presence of blood and bone content in the joint [23].
From a biomechanical point of view, posterior malleolus plays a significant role in transmission of tibiotalar load. It has a preventive effect on posterior talus displacement, and with fragment size, the risk of posterior talus subluxation increases, especially if the fragment is larger than 25% of tibia joint surface [24,25]. Posterior maleolus is an important structure in distal tibiofibular joint - it provides bone restriction to distal fibula and syndesmosis stability via posterior inferior tibiofibular ligament (PITFL) and inferior transversum ligament (ITL).
Ramsey et al. and Lloyd et al. [26,27] indicate great intraarticular contact pressure in talus displacement as a result of ankle joint injury. Talus displacement by 1mm and external fibula rotation of 3° lead to a 40% decrease of tibiotalar contact, joint incongruence occurs, and decrease of contact surface causes increased pressure per unit of measurement. There is a great deal of stress that damages articular cartilage, which is the main factor in pathogenesis of PTA.

The goal of surgical intervention in posterior malleolus fractures is to achieve articular congruence, to achieve stability and restore ankle joint function. For these reasons, the imperative is to achieve anatomic reduction, and to provide a smooth and flat cartilage surface. Anatomical reduction of articular surfaces is essential factor for good treatment outcome of unstable posterior malleolus fractures [28].

Orthopedic reduction and posterior malleolus fixation can be direct and indirect. For direct access and reduction, posterior lateral approach is used, and lateral malleolus can be indirectly reduced [29]. Due to a deep position of posterior malleolus and tendon-neuro-vascular bundle of the area, this approach is very demanding and difficult. Haraguchi et al. [19] recommend this approach for type I posterior malleolus fracture. For type II fracture, a medially extended approach is recommended. Fragment fixation can be direct with screws or a plate [8]. Indirect fixation is performed by placing a screw in AP or PA direction, but only after performing lateral and medial malleolus osteosynthesis, and radiologically checking posterior malleolus position [12]. In posterior malleolus type III fractures, fragment repositioning can be achieved using the principle of ligamentotaxis, because after lateral malleolus repositioning and tibiofibular syndesmosis transfixation, there occurs a spontaneous posterior malleolus reposition – it is pulled to its place by intact PITFL [18,30]. The degree of fixation reduction and stability is the greatest in direct reposition and with the use of an osteosynthesis plate, and lower in indirect reposition and stabilization [31]. Huber et al. [32] report that indirect reduction and stability were achieved in 27% cases, while it was achieved in 83% in direct reduction and stabilization. Our results range within this frame.

Relationship and connection between posterior malleolus and tibiofibular syndesmosis is very important because of PITFL and ITL. These ligaments attach to fibula and posterior malleolus and are very important distal tibiofibular joint structures, as they provide stability to lateral ankle joint side, i.e. lateral part of talus and fibula. If the PITFL is preserved and open reposition and fixation of posterior malleolus is performed, syndesmosis stability will be better than stability in case when transsyndesmal fixation is performed. PITFL complex is the nucleus of tibiofibular syndesmosis stability. The fracture of posterior malleolus alters syndesmosis stability, because of the injury and loss of PITFL function [33]. Rigid fibula fixation and posterior malleolus reduction and fixation can adequately restore ligamentous tension of PITFL, and stabilize syndesmosis without transsyndesmal fixation. Gardner et al [34] found, after performing posterior malleolus repositioning and stabilization on cadavers, that 70% of distal tibiofibular joint stability was achieved, while after transindezmal fixation it was 40%.

Functional outcome of ankle joint fracture associated with posterior malleolus fracture depends on: the size of posterior malleolus fragment, its comminution, the quality of anatomic reposition and fixation stability, and on articular stability [18]. Our clinical results are good and similar to those reported by other authors [14,20,33].

A common late complication in posterior malleolus fractures is post-traumatic arthritis (PTA). The trigger for its formation is the change of articular surfaces, caused by trauma to distal tibia and talus [35]. Risk factors for PTA are: residual articular displacement, joint instability or subluxation caused by an injury, damage on articular surfaces at the moment of injury [36]. Boist and Dust [37] had PTA grades 2 and 3 in 67% of cases in their series, and the results presented in our series of patients are similar to theirs.

CONCLUSION

Posterior malleolus fractures are common in ankle injuries. Posterior malleolus fixation is necessary if the fragment is larger than 25% of tibial joint surface. Direct reduction and fixation should be performed, as the results are better. Fixation restores articular surface, PITFL and syndesmosis stability is achieved. CT helps in classifying the fracture type, and after surgery helps in determining the degree of fragment and joint surface reduction.

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USE OF OFF-LABEL MEDICINES IN PEDIATRIC POPULATION

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Abstract: Modern use of drugs in the treatment of diseases of children and newborns is increasingly based on off-label use of drugs. The lack of adequate formulations for the pediatric population, the lack of appropriate therapeutic parallels for the treatment of children's diseases and the small number of clinical trials involving the pediatric population have contributed to the mass use of these drugs. The use of these drugs implies extrapolation of doses and indications registered for adults to children, although it is known that the pharmacodynamics and pharmacokinetics of children and adults differ significantly. In the past two decades, many legislative and regulatory initiatives have been taken around the world to improve the use of drugs in children. However, children are still prescribed off-label and unlicensed drugs.
The aim of this study was to present a review of the literature in which off-label and unlicensed use in the pediatric population was investigated. Literature was searched through the Google Scholar and Pub Med search engines and using the keywords off label drug, pediatric medicine, use in pediatrics, in the period from May to August 2019. Selected and presented in this article are studies published in the period from 1996 to 2015, which as a subject of research had the use of off-label and unlicensed drugs in the pediatric population. Medicines prescribed for children should be registered for use in the pediatric population and used in accordance with approved indications for children, whenever possible. It is necessary to take measures for more rational use of medicines in pediatrics, which include the collaboration of health workers in order to provide medicines for children that are proven to be effective, high quality and safe to use.

INTRODUCTION

The use of a medicinal product in accordance with the marketing authorization, which defines the formulation, dosage, age, and issued by the relevant regulatory body, is called the use of the medicinal product in accordance with the on-label marketing authorization. The purpose of authorizing a medicinal product is to ensure that the medicinal product is tested for its efficacy, safety and quality. When the drug is prescribed outside the examined indications, the therapy may be less safe, effective and reliable, because it is based exclusively on assumptions and extrapolation. The justification for prescribing these drugs, especially in the pediatric population, due to the large differences between children and adults, even between children of different ages, in terms of pharmacodynamic and pharmacokinetic responses to the drug, is being examined.

Recently, the use of a drug that does not comply with the approved guidelines related to the indication, age, dosage regime or route of administration is becoming more common. Off-label use of drugs includes the use of drugs in higher or lower doses, use for indications not described in the summary of product characteristics, use in children outside the range of years defined by the license, use of alternative routes of administration and use of drugs in indications when contraindicated for a given drug. The use of off-label drugs is mainly related to prevention, diagnosis or therapeutic measures that are in accordance with the relevant legislation, with the primary goal of improving or improving the health condition.

Off-label use of drugs should be distinguished from the use of drugs without a license (off-license). Unlicensed use of drugs is considered to be the use of a drug that is not registered in the Republic of Serbia, but is in other countries, or that is registered, but it should be translated into another formulation or drug that is not registered (eg. for the treatment of rare diseases). Unregistered medicines are medicines that have not been approved by the regulatory body for marketing. Off-label use is considered to be the use of a drug in a way different from the manner described in the marketing authorization: use of the drug for the treatment of an indication not listed in the

summary of product characteristics, use of the drug in the age group outside the permitted range, use of the drug doses of the drug characteristics listed in the summary.

The most common reasons for the use of unregistered drugs are modifications of registered drugs (crushing the tablet to form a suspension), drugs that are registered for use in adults, but the formulation for use in pediatrics requires a special drug permit (adult drug is used in minor doses for children), new drugs that require special permission from the manufacturer (eg. caffeine injection used in case of apnea due to lung immaturity). Use of drugs outside the marketing authorization includes the use of drugs in higher or lower doses, use for indications not described in the summary of product characteristics, use in children outside the age range defined by the license, use of alternative routes of administration and use of drugs in indications when contraindicated allow for a given drug.

Modern use of drugs in the treatment of diseases of children and newborns is increasingly based on the use of off-label drugs due to lack of adequate formulations for the pediatric population, lack of appropriate therapeutic parallels for the treatment of children and almost no clinical trials involving the pediatric population [1-4].

The thalidomide catastrophe (phocomelia in newborns) and the effect of the use of chloramphenicol in children (gray baby syndrome) initiated the process of testing and registration of drugs [5]. The main goal of drug registration is to ensure that the drug is quality, safe and effective. Unfortunately, large number of medicines for children do not have a marketing authorization or marketing authorization [6]. This suggests that for many drugs used in children, evidence derived from pharmacokinetics, adequate dosing, or formulation-related studies is lacking [7,8]. Focusing on other factors influencing the pharmacokinetics and pharmacodynamics of drug dosing has received little attention during drug development in children. As a result, many drugs have been used outside of their licensed recommendations, commonly known as off-label prescribing, which has become an increasingly common prescribing trend in children. Over-the-counter prescribing for children is widespread mainly in systemically administered drugs, but also in locally applied drugs [9].

Several factors leading to off-label prescribing in children have been identified in the past. Subsequently, legislative, regulatory, governmental, and professional initiatives were introduced and implemented globally to obtain better data on the effects of drugs on children and consequently to instruct health professionals to use quality drugs that are effective for children and do not cause harm when used. Initiatives to improve drug use in children were first implemented in the United States from 1994 to early 2000. [10-13]. Almost a decade later, other countries (European Union, Canada, Australia, Japan, China and Korea) as well as international institutions (World Health Organization and the International Council for the Harmonization of Technical Requirements for Pharmaceutical Medicines for Human Use) have joined [14]. Data from the literature show that most initiatives taken in the past have been aimed at encouraging increased research on the use of drugs in children, in order to improve the registration process and enable the safe use of drugs in the pediatric population.

However, despite numerous global initiatives, the number of clinical trials conducted in children is still insufficient, ie. the use of drugs in children is rarely based on evidence from clinical trials [15].

The aim of this study is to provide an overview of the global trend and prevalence of prescribing off-label drugs from 1996 to 2016, and to suggest future directions related to studies related to off-label prescribing in children.

METHODOLOGY

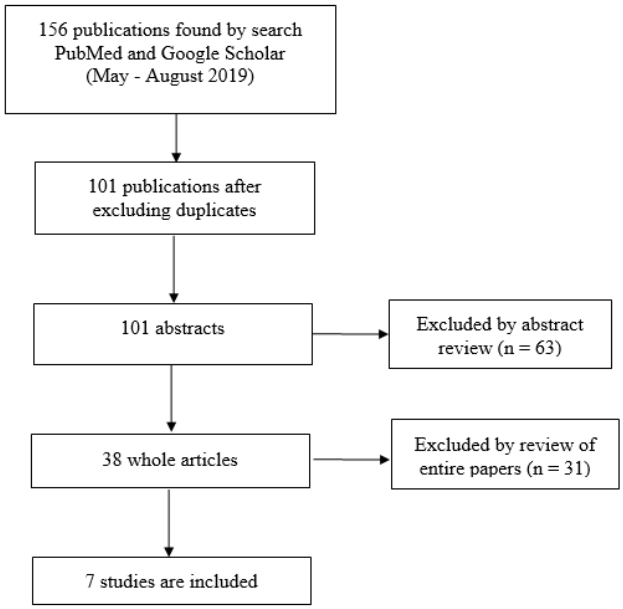
Data collection was performed by electronic search of the PubMed index database and Google Scholar. The literature search and selection protocol has been defined using the PRIZMA method [16]. The corresponding flow diagram is graphically shown in Figure 1. The search was performed in the period from May to August 2019. Selected and presented in the paper are studies published in the period from 1996 to 2015. Searched keywords are: off label drug, pediatric medicine, use in pediatrics. Original research was included which provided data on the extent of use of off-label and unlicensed drugs in the pediatric population as well as one systematic review.

Criteria for inclusion were: 1) published texts in full text in the period from January 1996

to December 2016; 2) articles in Serbian and English; 3) studies showing data on the results of the prevalence of prescribing drugs outside the use permit for children; 4) off label use of cardiac, respiratory, antiallergic, oncological, analgesic drugs and antibiotics

Exclusion criteria were: 1) notes and conferences; 2) off label use of other therapeutic groups of drugs. The title and summary of the articles have been carefully examined to determine the inclusion of the study in this review. The following information was extracted from the eligible studies: 1) study identification; 2) study details (study design, setting, study period, method); 3) defining off-label drug administration; 4) source references; 5) quantification of outcomes; 6) results

Figure 1. PRIZMA diagram



RESULTS AND DISCUSSION

During the research, 101 studies were identified, of which 7 were presented in this article, with the aim of presenting off-label use of drugs in different therapeutic groups: cardiac, respiratory, antiallergic drugs, antibiotics, oncology drugs and analgesics.

In a study conducted at the Department of Pediatric Cardiology, 544 patients participated in the University Children's Hospital in Belgrade and included 2,037 prescriptions, with 102 different drugs, of which 41% were registered drugs, 11% unregistered and 47% prescribed drugs. off -label. Drugs are prescribed off-label: due to age 21% and due to a different dose 26%. The largest number of unregistered and off-label drugs (72%) is prescribed to children aged between 2-11 years. Katopil is the only registered ACE inhibitor for use in the pediatric population and is one of the most prescribed drugs in this study, with one-third of prescriptions being prescribed off-label in relation to the dose of katopil [17].

In a national cohort study conducted in Italy, in the period 2002-2006, medical records of children under 14 years of age were analyzed, and the degree of prescribing drugs belonging to the ATC code R03 - ß mimetics, inhaled glucocorticoids, inhaled anticholinergics, combined formulations, antiallergic drugs, xanthines and leukotriene receptor antagonists. 90% of R03 prescriptions included 11 active substances or combinations. Inhaled glucocorticoids are the most prescribed off-label, with 19% in terms of age and 56% in terms of indications for use. The largest number of off-label drugs was in children younger than 2 years [18].

In the cohort study, conducted in the Netherlands, the largest number of prescribed drugs - off-label and unregistered - was also the largest in the group of children aged 1 month to

2 years. The one-year cumulative risk of off-label and unregistered drugs is 45%, among children with at least one prescription for a respiratory drug [19].

In a prospective study, which lasted from February to March 2000, at the Children's Clinic in Great Britain, in the intensive care and acute care wards, analgesics used in children were classified into those used in accordance with the marketing authorization and those are applied off-label, in accordance with the valid drug registries in the UK. The study included 715 prescriptions, of which 67% were licensed drugs, prescribed in accordance with the summary of product characteristics, and 33% were licensed drugs, but prescribed outside the use permit. Diclofenac, pethidine and morphine are mostly prescribed off-label, while drugs are most often prescribed off-label, in terms of dose. The high percentage of off-label use of this drug, shown in this study, is explained by the fact that diclofenac is not approved for pain therapy in children, but that it has been shown to be effective in adults intra- and postoperatively [20].

The Morais-Almeida M study (2013) showed that the most prescribed off-label drugs were nasal corticosteroids, 76% of the total number of prescription drugs [21], while 22% were off-label antihistamines. In other studies, off-label administration of antihistamines varied between 4.5 -43%. Cetirizine, levocetirizine and loratadine have been most studied in terms of long-term safety when used in the pediatric population. Despite pharmacokinetic studies conducted for next-generation antihistamines, long-term safety studies in children are lacking [22].

A study conducted in three European countries, Italy, Great Britain and Greece, evaluated the off-label use of antibiotics, as the most frequently prescribed drugs for children. The number of prescribed drugs with an unregistered dose was high in all three countries in the neonatology departments, but the number was significantly higher in Italy compared to the United Kingdom. Antibiotics that are most often prescribed outside the recommended dose are aminoglycosides, specifically amikacin and gentamicin. The most common clinical indication for use outside the recommended range is suspected or confirmed diagnosis of sepsis, although significant use of drugs outside the recommended doses in medical prophylaxis was more common in Italy and Greece, compared to the United Kingdom. The most frequently prescribed antibiotics prescribed outside the registered indication are fluoroquinolones in Great Britain and ampicillin and gentamicin in Italy and Greece, while the most common indications were suspected sepsis or diagnosed sepsis.

In the pediatric ward, antibiotics most commonly prescribed outside the registered dose are amoxicillin clavulanate in Italy, cefuroxime in Greece, and gentamicin in the United Kingdom. Doses were higher than recommended in Italy and Greece and lower than recommended in the UK. The most common dosages outside the registered recommendations were indications - sepsis, lower respiratory tract infections and surgical prophylaxis in all three countries, regardless of prevalence. Off-label in terms of dose was most common in the group of children aged 28 days - 23 months [23].

The use of anticancer drugs is precisely described in the drug authorization in terms of the type or subtype of the tumor and the length of treatment. Prescribing anticancer drugs is believed to be often prescribed outside the use permit, while a small number of studies have been conducted, in order to obtain a realistic state. Prospective studies, conducted between 1990 and 2002, indicated a proportion of off-label drug use in children and adults. Most off-label drugs were for palliative care of patients, some were associated with a better clinical effect and in the treatment of specific tumors, they were part of standard therapy [24].

Table 1: Tabelar view of studies presented in this article

Authors/ Article/ Year	Study	Aim of the study	Methods	Results
Bajcetic et al./Eur J Clin Pharmacol/ 2005	Off-label and unregistered drugs in pediatric cardiology	Scope and nature of prescribing off-label drugs in pediatric cardiology, in hospitalized patients	Prospective study; patient records	The problem of off-label and unregistered drugs is in line with the lack of adequate formulations globally
Jong, Eland et al./ Eur Respir J/2004	Unregistered and off-label resorption drugs prescribed to the pediatric population	Unregistered and off-label respiratory drugs prescribed to children, the Netherlands	Cohort, national study; data were collected from a computerized database of children's medical records	A large percentage of respiratory drugs prescribed to children are unregistered or registered but prescribed off label
Baiardi et al./Acta Pediatrica/ 2009	Use of the drug in accordance with the marketing authorization and off-label use of respiratory drugs in the pediatric population in Italy	To determine the degree of prescription of respiratory drugs (ATC code: R03) in Italy and to assess the extent of use of off-label drugs, in relation to the dose or indication	Cohort study	There is a need to conduct quantitative studies, with the aim of increasing current knowledge about registered medicines and to review the registration process and regulatory procedures in order to reduce off-label use of medicines
Conroy et al /Peadiatric Anaesthesia/ 2001	Use of off label and unregistered analgesics in the management of pain therapy in pediatrics	Document the incidence and nature of the use of unregistered and off-label analgesics in children	Prospective study; a questionnaire was used as a tool	67% of drugs were registered; 33% is registered, but the application is off-label; the study did not identify the use of unregistered drugs
Silva et al./ WAO Journal/ 2014	Prescribing off-label drugs in the treatment of allergic diseases in children	A review of the literature aimed at describing and discussing the off-label use of drugs in the therapy and control of allergic drugs in children	Review article	There is a need for a new proposal to highlight the priority for pediatric clinical research, which could meet all the needs of the pediatric population, especially in the field of allergies and respiratory diseases.
Porto et al./Eur J Clin Pharmacol/ 2010	Use of antibiotics off-label in three European countries in children	The aim was to evaluate off-label antibiotic use in three European countries - the UK, Italy and Greece	Antibiotic prescriptions were evaluated for all hospitalized patients in the neonatal intensive care unit: 2 hospitals in the UK, one hospital in Italy and one hospital in Greece	Off-label drug use is usually dose- or indication-related, rarely for years. the only antibiotics identified that have been used off the label, and related to age are: meropenem for neonatal and quinolones and linezolid for older children, which is a priority for future studies
Leveque /Lancet Oncol/ 2008	**Off-label use of anticancer drugs**	**The scope of off-label prescribing of oncology drugs**	**A review of prospective studies in the period 1990-2002**	**Percentage of off-label drug use in children and adults 6-33.2%**

CONCLUSION

According to the analysis of the literature, the prevalence of prescribing off-label and unregistered drugs in the pediatric population is evident and very widespread in the past in intensive care units.

Medicines prescribed for children should be registered for use in the pediatric population and used in accordance with approved indications for children, whenever possible. Although there are indications that the use of off-label and unregistered drugs has more benefits than the risk that the use of that drug poses, this leads to an increasing use of these drugs even when such use is not justified, ie. it may be less effective or harmful.

The lack of indications for use in children, in relation to the dose or inadequate formulation for the pediatric population may prevent children from receiving effective therapy or may lead to errors in the routes of administration of the drug.

The increase in the prevalence of off-label drug use suggests that legislative, regulatory initiatives are not sufficient to improve drug use in children. Aspects of behavior and knowledge related to off-label prescribing as well as efforts to integrate evidence into practice must also be assessed and consolidated as part of a joint effort to reduce prescribing gaps for children.

It is necessary to take measures for a more rational use of medicines in pediatrics, which include the collaboration of health workers in order to provide medicines for children that are proven to be effective, high quality and safe to use.

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Vol. 46 (2021) No. 2 TIMOK MEDICAL GAZETTE Review article

ULTRASOUND CHARACTERISTICS OF NODULES IN THE THYROID GLAND

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Summary: Nodules in the thyroid gland are very common and can be found in 50-68% of adults in the general population. Only about 5% of these nodules are malignant and require treatment. They usually do not give any discomfort. When they are discovered, they should be assessed on the basis of clinical, echosonographic and cytological findings, and if necessary, using additional diagnostic methods, and make a decision on the need for treatment. Based on the ultrasound characteristics of the nodule, it is decided whether further diagnosis is needed, in terms of aspiration puncture with a thin needle (FNA) and cytological examination, after which a decision is made on further procedure. Ultrasound is the initial diagnostic method for the detection of thyroid nodules. In addition to the presence of nodules, it accurately determines the size, location and number of nodules in the thyroid gland (thyroid). This non-invasive screening method is safe, harmless and can be repeated. FNA is a very important diagnostic method, but its performance must be selective, since systematic puncture of all nodes, regardless of size or appearance, is not recommended. It is important that the indications for FNA be based on clinical characteristics, as well as on echosonographic stratification of the risk of malignancy.

Key words: thyroid nodules, ultrasound examination, thin needle puncture.

INTRODUCTION

Nodules in the thyroid gland are very common and can be found in 50-68% of adults in the general population. Only about 5% of these nodules are malignant and require treatment. They usually do not give any discomfort. When they are discovered, they should be assessed on the basis of clinical, echosonographic and cytological findings, and, if necessary, using additional diagnostic methods, and make a decision on the need for treatment. Based on the ultrasound characteristics of the nodule, it is decided whether further diagnosis is needed, in terms of thin needle aspiration puncture (FNA) and cytological examination, after which a decision is made on further procedure [1-5].

Currently, FNA is the most effective method for determining the nature of the node. However, many nodules are benign, and even malignant nodules, especially those smaller than 1 cm, often show indolent and non-aggressive behavior. Therefore, not all detected nodes require FNA. A reliable non-invasive method to detect nodes indicated for FNA would be highly desirable [6]. Ultrasound is the initial diagnostic method for the detection of thyroid nodules. In addition to the presence of nodules, it accurately determines the size, location and number of nodules in the thyroid gland (thyroid).

This non-invasive screening method is safe, harmless and can be repeated [7]. Assessing the risk of malignancy is very important in patients with glandular nodules in order to identify those nodules that need to be punctured with a thin needle. The main disadvantage of this examination is that it largely depends on the doctor performing the examination [8]. Therefore, an attempt was made to find a formula for risk assessment in relation to ultrasound characteristics and standardization of ultrasound description, in order to reduce the subjectivity of the examiner. Koike E. et al. from the Noguchi Thyroid Clinic and Hospital Foundation from Japan in 2001. set the formula for the prediction of thyroid nodule malignancy based on 5 ultrasound characteristics of the nodule: margins, shape, echogenicity, echostructure and calcification [9,10]. As no characteristic can reliably predict malignancy, the use and combination of several traits or characteristics is advised. One such

system, that is, a way of combining and scoring several properties of a node in the thyroid gland, was published in 2009 by Horvath et al. as the Thyroid Imaging Reporting and Data System (TIRADS). It consists of a scale of 6 characteristics for stratification of malignancy risk. Subsequently, similar recommendations were issued by the Korean Thyroid Radiology Society, the American Thyroid Association, the American Association of Clinical Endocrinologists, the American College of Endocrinology, and the Italian Association of Clinical Endocrinologists [8].

In 2015, the American College of Radiology (ACR) issued instructions for access to the most common thyroid nodules and gave instructions for standardizing the ultrasound examination of the thyroid gland. Thyroid Imaging Reporting and Data System (ACR TI-RADS) [6].

Based on a review of the literature, the American Association of Clinical Endocrinologists, the American Thyroid Association and Korean guides, in 2017 a new EU-TI RADS (European Thyroid Imaging Reporting and Data System) classification was formed to assess thyroid nodules and decide on a possible FNA nodule [7]. .

In the following, the European Thyroid Imaging Reporting and Data System (EU-TI RADS) and the American College of Radiology (ACR), Thyroid Imaging Reporting and Data System (TI-RADS), ACR TI-RADS will be described.

GUIDELINES FOR STANDARDIZATION OF ULTRASOUND EXAMINATION OF THE THYROID GLAND EU-TI RADS

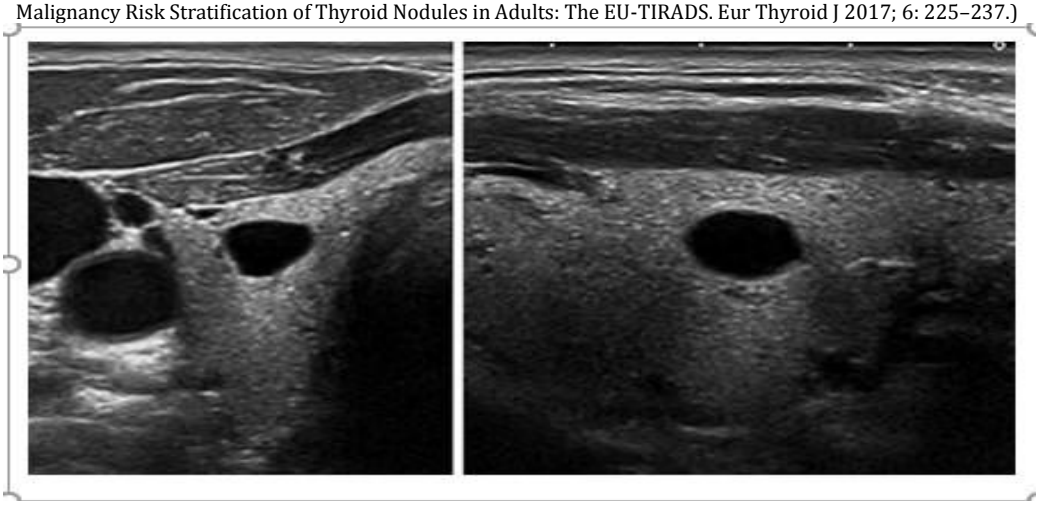
Category EU-TIRADS 1, is a category, ie thyroid gland (thyroid) that does not contain nodules.

Benign category (EU-TIRADS 2), risk of malignancy close to 0%.

This category includes completely anechoic nodules (cysts) and completely spongiform nodules.

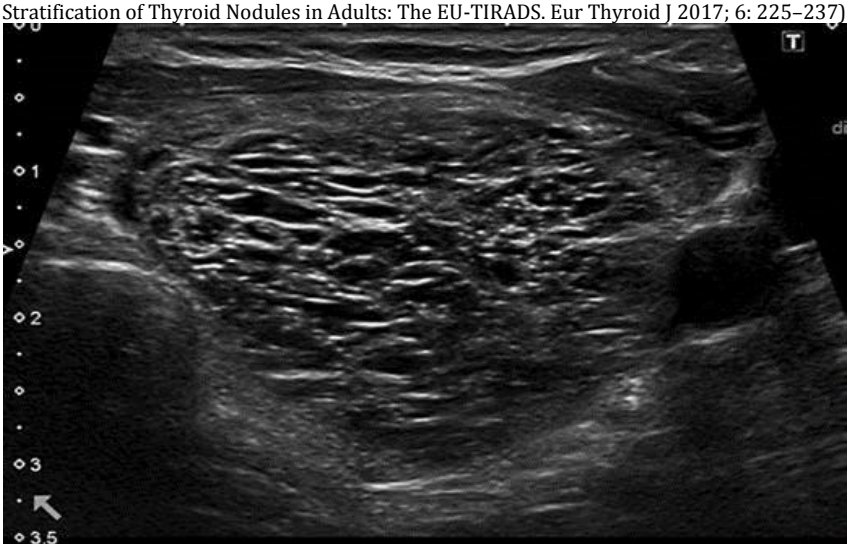
Pure cystic changes, cysts, are characterized by the absence of wall thickening, posterior signal amplification as well as the absence of a solid component, regardless of their size. Figure 1.

Figure 1. Completely cystic nodule (From: Gilles R. et all. European Thyroid Association Guidelines for Ultrasound Malignancy Risk Stratification of Thyroid Nodules in Adults: The EU-TIRADS. Eur Thyroid J 2017; 6: 225–237.)



This category also includes cysts that are divided into separate sections by fibrous septa. The presence of echogenic material within the cyst is often encountered and may correspond to either a clot of fibrin, a colloid, or a true solid component, which may be distinguished by the use of Doppler. If there is a suspicion regarding the existence of a solid component inside the cyst, such a node should be classified as low risk. Spongiform nodules are composed of tiny cystic spaces that cover the entire nodule, with the size of the nodule not playing a role in assessing the risk of malignancy. Small cystic spaces are separated by numerous isoechoic septa. Figure 2. If cystic spaces do not fill the entire node, the node should be classified as a low-risk node. Pure cystic changes and completely spongy nodules should be considered benign. FNA is not recommended for these changes, regardless of their size, and even for such benign cystic nodules, ablation with ethanol is recommended as the therapy of first choice [8,11].

Figure 2. Spongiform node. (From: Gilles R. et all. European Thyroid Association Guidelines for Ultrasound Malignancy Risk Stratification of Thyroid Nodules in Adults: The EU-TIRADS. Eur Thyroid J 2017; 6: 225–237)



Low risk category (EU-TIRADS 3), where the risk of malignancy is 2-4%.

These nodes are characterized by an oval shape, smooth edges (margins), as far as echogenicity is concerned, these nodes are isoechoic or hyperechoic, without any high-risk characteristics. Figure 3, isoechoic nodule, Figure 4 hyperechoic nodule. Nodes with these characteristics have a low risk of malignancy and FNA for nodes> 20mm should be considered. The 20 mm threshold was chosen based on the argument that distant metastases are rarely found in follicular carcinomas <2 cm [12]. Grouped and associated nodes (polynodose goiters) of these characteristics should be included in this category, and FNA should be considered if one or more nodes are> 20 mm. It should be noted that a completely homogeneous isoechoic nodule may correspond in less than 4% of cases to follicular carcinoma or follicular variant PTC [13,14]. However, even minimal cystic changes in the nodule favor benignity [15]. So oval-shaped nodules, which are isoechoic or hyperechogenic with smooth margins and without high-risk characteristics, should be classified as low-risk. FNA is usually only recommended for nodes> 20 mm [8].

Figure 3. Isoechogenic nodule. (From: Gilles R. et all. European Thyroid Association Guidelines for Ultrasound Malignancy Risk Stratification of Thyroid Nodules in Adults: The EU-TIRADS. Eur Thyroid J 2017; 6: 225–237)

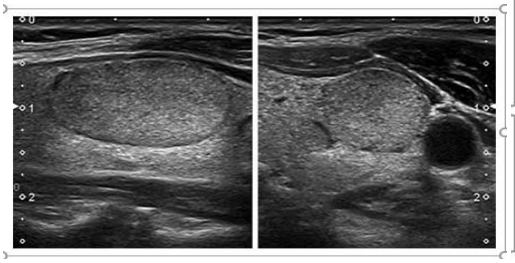
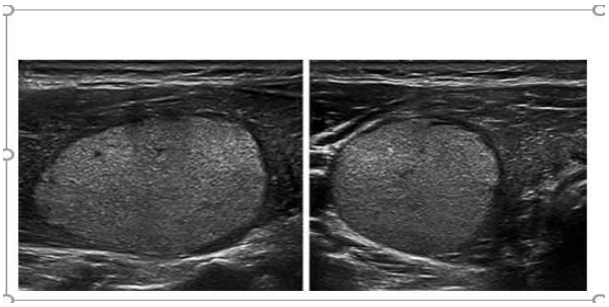


Figure 4. Hyperechogenic nodule. (From: Gilles R. et all. European Thyroid Association Guidelines for Ultrasound Malignancy Risk Stratification of Thyroid Nodules in Adults: The EU-TIRADS. Eur Thyroid J 2017; 6: 225–237)

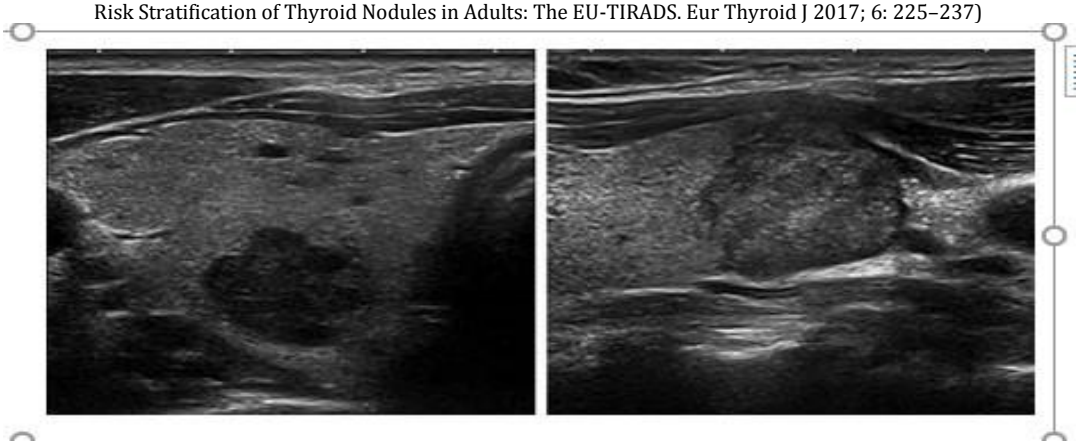


Medium risk category (EU-TIRADS 4) where the risk of malignancy is 6–17%.

These nodules are characterized by an oval shape, smooth edges, mild to moderate hypoechoicity, without other high-risk features. Figure 5. The difference between the low and medium risk category lies in the echogenicity of the solid component of the node. In the case of heterogeneous echogenicity of a solid component, the presence of any hypoechoic change classifies the node into a medium-risk category. The presence of a thin halo, partially cystic changes, comet-type artifact, peripheral vascularity, reduce the risk of malignancy.

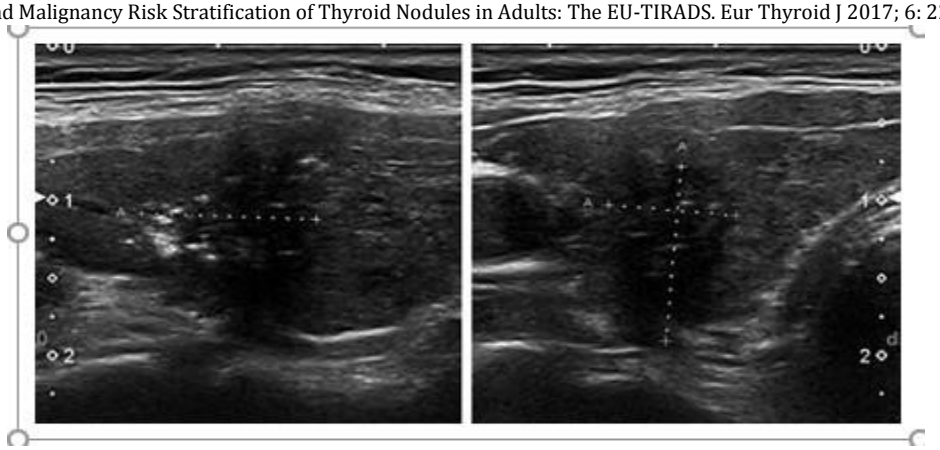
Hypoechoic nodes should be classified as moderate risk, including those with cystic areas, bearing in mind that the risk is lower in partially cystic nodes than in completely compact nodes. Characteristics such as discontinuous peripheral margins, peripheral macrocalcifications, dense halo, predominantly central vascularization may increase the risk of malignancy. In this group, the threshold for FNA is recommended for nodes larger than 15mm [8].

Figure 5. Hypoechogenic nodule. (From: Gilles R. et all. European Thyroid Association Guidelines for Ultrasound Malignancy Risk Stratification of Thyroid Nodules in Adults: The EU-TIRADS. Eur Thyroid J 2017; 6: 225–237)



High risk category (EU-TIRADS 5), where the risk of malignancy is 26–87% The characteristic of these nodes is the presence of at least one of the following characteristics, which belong to the characteristics (features) of high risk: not oval shape (higher than wider), irregular edges, microcalcifications and marked hypoechoicity. Figure 6.

Figure 6. Nodus from the high risk category. (From: Gilles R. et all. European Thyroid Association Guidelines for Ultrasound Malignancy Risk Stratification of Thyroid Nodules in Adults: The EU-TIRADS. Eur Thyroid J 2017; 6: 225–237)



All these characteristics show high rates of specificity (83–84%), but also low rates of sensitivity (26–59%). Pronounced hypoechoicity has the lowest sensitivity of the four described characteristics and is specific only if the node is compact, because a highly hypoechoic node may be the remnant of a previous cyst. In a partially cystic node, microcalcifications are the best predictor of malignancy, while other features appear less significant. At the same time, the presence of several anomalies, jagged intermittent, edges with spicules, lobulations, dotted echogenic foci, non-oval shape, increase the risk of malignancy. Nodes with such properties that are larger than 10 mm should undergo FNA, except in inoperable patients for any reason or a short lifespan is expected, due to the existence of other comorbidities [8]. In case of a benign cytological result, FNA of such a node, the puncture should be repeated within 3 months in order to reduce the number of false negative findings. In the case of nodules smaller than one centimeter with high-risk ultrasound characteristics, it is recommended to actively monitor the nodules as well as to treat pathological lymph nodes in the neck and the symptoms and signs that the patient himself reports. It is known that few or none of these patients will develop distant metastases, ie that mortality is negligible even if the nodule corresponds to cancer, in the case of subsantimetric dimensions of the nodules [8]. Patients with subcentimeter nodules and very suspicious ultrasound characteristics but without abnormal lymph nodes in the neck should be presented with the possibility of active surveillance as one option or FNA under ultrasound control.

EU-TIRADS scoring is also useful in multinodular thyroid gland for selecting nodes that are candidates for FNA. During the echosonographic examination, ultrasound high-risk nodes should be identified, observed, described, and FNA suggested if the node is larger than 10 mm.

Identify medium risk nodes; describe only those nodes larger than 5 mm and for FNA suggest those larger than 15 mm. Identify low-risk nodes; describe only those larger than 10 mm and suggest for FNA for those larger than 20 mm. If there are more nodes, more than three, describe in detail only those that are suspicious (according to the previous risk and size criteria), record the others [8].

SIGNIFICANCE OF OTHER ULTRASOUND CHARACTERISTICS

Shape, margins, echogenicity, composition and microcalcifications are the basic characteristics that enable EU TIRADS classification. However, some of the ultrasound features can be used to further assess and classify risks and modulate indications for FNA.

Suspicious lymphadenopathy

Ultrasound assessment of cervical lymph nodes should be performed in all patients with thyroid nodules, especially in those with medium and high risk.

In suspected lymph nodes, lymph node FNA should be performed for cytological analysis as well as for determination of thyroglobulin and calcitonin [8].

Extrathyroid propagation, proliferation and invasion of surrounding tissue

Propagation into adjacent structures and disruption of thyroid capsule continuity may be considered a specific feature for invasive

malignancy. Adherence to the capsule, ie close contact with the capsule, has less specificity for macroscopic extrathyroid invasion and spread, through the capsule. The presence of an unaltered thyroid parenchyma, 2 mm between the nodule and the continuous, compact thyroid capsule, indicates that there is almost no macroscopic extrathyroid expansion and invasion while reducing the risk of microscopic capsule invasion and extrathyroid expansion. The discontinuity of the capsule, the adhesion of the capsule and the bulge of the capsule must be emphasized in the report, ie the ultrasound description, due to the possible invasion of the capsule and extrathyroid expansion [8].

Macrocalcifications and hyperechoic points (foci)

Macrocalcificationscan be defined as echogenic foci (points) larger than 1 mm with the existence of posterior shading (acoustic window).

1. Isolated central intranodularmacrocalcifications are not necessarily associated with malignancy, ie they do not inevitably indicate malignancy.
2. Isolated macrocalcification, which almost completely fills the calcified node, has a low risk of malignancy.
3. Calcifications on the periphery, peripheral calcifications (peripheral or curvilinear) or (picture of broken egg shell) along the periphery of the node, increase the risk of malignancy if their continuity is interrupted [8].

Hyperechoic points (foci, spots)

These changes correspond to perimilimeter hyperechoic changes and can be caused by:

1. Colloidal crystals or remnants of fibrin that create artifacts (reverberations), comet tails and are almost always a sign of benign change.
2. Posterior acoustic amplification (posterior, posterior wall of the cyst, iemicrocystic area) is mainly seen in high-frequency probes and is a feature that indicates benignity.
3. True microcalcifications correspond to psammomic bodies around which there are multiple round echogenic foci up to 1 mm in size without the existence of posterior shading (acoustic headlight) and they are always placed in a solid, homogeneous component of the node. Microcalcifications largely suggest malignancy.
4. Hyperechogenic spots of indeterminate significance that cannot be classified with certainty in the previous three categories. Rather linear than round and without microcystic cavities and comet tail artefacts.

Isolated macrocalcifications are not specific for malignancy. Their presence should be correlated with other ultrasound characteristics. Echogenic spots of comet tail appearance suggest benignity. True microcalcificationsshould be distinguished from other echogenic spots and such nodules should be subjected to FNA [8].

Halo

The halo is thought to correspond to the nodule capsule or surrounding blood vessels, or to sometimes correspond to the surrounding compressed parenchyma. A thin halo reduces the risk of malignancy (0.3mm), while a thick halo or absence of halo increases the risk of malignancy. However, a clear definition of thin and thick halo cannot be given [8].

Vascularization

As for vascularity, the description of vascularity with the help of color Doppler is often used in clinical practice. Malignant nodules are more likely to have type III vascularity, while benign nodules show type I and II vascularity. Type I vascularity, denotes absent or scarce vascularity. Type II, denotes present perinodal and scarce intranodal vascularization and type III denotes scarce perinodal and pronounced intranodal vascularization.

However, it is very important that the intranodular signal increases with the size of the benign nodule. Vascularity as a criterion remains for the assessment of nodules remains controversial, mainly because the assessment of vascularity largely depends on the equipment and settings of the ultrasound apparatus and because it largely depends on the subjective assessment of the examiner. Therefore, the ETA working group does not recommend the inclusion of vascularity in the assessment in the TIRADS score [8].

Node growth

Regarding the growth of thyroid nodules, the published results suggest that the growth of nodules cannot accurately distinguish between benign and malignant lesions. Thus, determining the growth of nodules is not recommended as a criterion for distinguishing malignant and benign nodules [8].

The EU-TIRADS scoring system is based on the presence of ultrasound characteristics that are highly suspected of malignancy. This system includes five categories, ultrasound findings. The first category involves the absence

of thyroid nodules, the other four include benign, low-suspicion, moderate-suspicion, and high-suspicion categories. Compared to other risk scoring systems, the main advantage of EU-TIRADS is the facilitation of scoring in the use of specific ultrasound features to detect high-sensitivity thyroid cancer which should allow for the reduction of unnecessary FNA procedures [8].

Very few nodules will require invasive processing that includes cytology and molecular testing (FNA). An ultrasound examination with an assessment of clinical risk factors will be sufficient for an initial monitoring and diagnostic strategy. This is especially important for weak, elderly people, with comorbidities, because they are unlikely to be endangered by the thyroid tumor itself, and excessive diagnosis and interventions can do more harm than good. The goal is to identify the best strategy for the individual in terms of disease outcome and quality of life, avoiding the pitfalls of over-diagnosis and over-treatment [16].

ACR TI-RADS
THYROID IMAGING REPORTING AND DATA SYSTEM ACR TI-RADS

In this system, when evaluating the node, it is necessary to determine (score) each of the characteristics or ultrasonic properties of the node, which will be listed later, after which points are added. The total number of points determines the level of ACR TI-RADS score, which ranges from TR1 which is benign to TR5 which is a highly suspicious finding for malignancy. Recommendations for FNA and ultrasound monitoring of the node are based on the level of the number of points and its maximum diameter. Ultrasound, characteristics or properties to be scored are the composition of the node (composition), the echogenicity of the node, the shape of the node, the margins or edges of the node and the echogenic points or foci [6].

Composition

Nodes that are cystic or almost completely cystic do not bring any points, because they are almost always benign. Similarly, spongy material is highly associated with benign characteristics, regardless of other characteristics. However, the spongy node must be composed of at least 50% of small cystic spaces. Nodes should not be characterized as spongy only on the basis of the presence of several scattered cystic elements in a solid node. Mixed cystic solid nodules are categorized as predominantly solid and predominantly cystic. A solid component that is eccentrically placed and has a sharp angle in relation to the wall of the node is suspicious as well as a solid component that is hypoechoic, with lobulations and point echogenic foci. Completely cystic, predominantly cystic and spongy nodes are scored from zero points. Mixed, cystically solid nodes are scored with one point, and predominantly, ie mostly solid with two points [6].

Echogenicity

This feature refers to the reflectivity of the nodule in relation to the surrounding thyroid tissue, except for very hypoechoic nodules where muscles attached to the hyoid bone are used as a basis for comparing echogenicity. This category also includes anechoic changes with zero points, which refers to cystic or almost cystic nodes, and extremely hypoechoic nodes to which three points would be awarded due to their very hypoechoic picture. Anechoic nodes get zero points, isoechoic and hyperechoic one point, and hypoechoic two points, while highly hypoechoic nodes get three points [6].

Shape

Higher than wider (ovoid) is a non-sensitive but highly specific indicator of malignancy. This property is estimated in the axial plane by comparing the height and width of the node measured horizontally and vertically in the transverse section. A higher than broader configuration is usually obvious and rarely requires formal measurements. This shape got three points, the oval shape zero points [6].

Edges

Smooth and clear edges of the node reduce the risk of malignancy, the edges (margins of the node) with such characteristics get zero points. For nodes where we cannot estimate the edges, we classify them in the category of nodes with a poorly defined edge of the node, and that category gets zero points. A lobed or irregular margin refers to a serrated or needle-like edge, with or without protrusions in the surrounding parenchyma, and this characteristic of the node is scored with two points. Propagation beyond the thyroid gland is classified as extensive or minimal and is scored with three points. Extensive extrathyroid spread, which is characterized by invasion of the surrounding soft tissue or vascular structures, is a highly reliable sign of malignancy and is one of

the unfavorable prognostic signs. Minimal invasion may be echosonographically suspicious if we have little thyroid parenchyma between the nodule and the thyroid capsule or there is swelling (bulge) of the contours and loss of echogenicity of the thyroid border [6].

Echogenic foci

The comet's tail artifact is an echogenic focus with V-shaped echoes whose depth is greater than 1mm. They are found in cystic components and are characteristic of benignity, so that for this characteristic the node received zero points. Macrocalcifications are rough echogenic foci accompanied by acoustic shadows. For their existence, one point was awarded. Peripheral calcifications located along the entire margin or along one part of the margin receive two points. Some authors have drawn attention to intermittent peripheral calcifications with bulging soft tissue, as suspected malignancies. For nodes with calcifications that cause a strong acoustic shadow that prevents or limits the assessment of internal characteristics, especially echogenicity and composition, it is best to assume that the node is solid and assign 2 points for composition and one point for echogenicity [6].

Point (punctiform) echogenic foci are smaller than macrocalcifications and they are without acoustic shadow. For their existence, the node received three points. In solid constituents of thyroid nodules, they may correspond to psammomatous bodies (calcifications) that are associated with papillary carcinomas, and are therefore considered highly suspicious, especially in combination with other suspicious properties. This category includes echogenic foci that are associated with small comet tail artifacts in solid node components, as opposed to the large comet tail artifacts listed earlier. Significantly, small echogenic foci can be seen in spongy nodules, where they probably represent the posterior walls of small cysts. They are not suspicious in this case and should not be given any points [6].

Additional benign phenomena

Several ultrasound findings have been described as characteristic of benign changes with a high degree of reliability. These findings include the existence of uniform hyperechogenicity (white knight), as well as the variegated appearance of hyperechogenic areas, divided by hypoechoic bands resembling giraffe skin, both present in Hashimoto's thyroiditis.

Node size as an indication for FNA

In a 2005 publication, Machens et al. [17] reported that the cumulative risk for distant metastases for papillary and follicular thyroid cancers increased significantly for nodules larger than 2 cm. So he suggested a biopsy of nodules larger than 2 cm. Machens et al. Based their analysis on tumor size in resected samples rather than ultrasound. Subsequent studies have shown a significant lack of concordance between sonographic and pathohistological sizing, with the tendency of ultrasound toresult in larger measurements [18].

ACR TI-RADS is in accordance with most other guidelines in the recommended FNA for highly suspicious nodes of 1 cm or larger. That is, for slightly suspicious and moderately suspicious nodules larger than 2.5 and 1.5 cm. Biopsy is usually not indicated in a gland that is interspersed with multiple confluent nodules of similar characteristics [6].

Ultrasound report

For the ultrasound report, the exact dimension of the thyroid nodules is very important, since the maximum dimension of the nodule determines whether a given node should be biopsied or monitored.

Nodes should be measured in three planes. Maximum dimension in axial projection, maximum dimension in perpendicular projection in relation to the previous measurement, maximum longitudinal dimension in sagittal plane. The measurement should include a halo node if present. A calculation can also be used, which determines the volume. In addition to the dimensions, it is necessary to describe the ultrasonic characteristics, previously listed, on the basis of which the scoring is performed. It should be described whether the node touches the trachea or whether it is close to the tracheoesophageal groove (the place of the recurrent laryngeal nerve). An accurate description of the location of the nodes on the sonograms is equally important, especially when the gland is heteroechoic or multiple nodes are present. In the polynodose gland, describe accurately and in detail only the nodes that meet the criteria for FNA, only the others. As far as FNA is concerned, a biopsy of more than two nodes is not recommended, puncturing the most successful nodes. The decision to repeat a biopsy is usually made by physicians who monitor the patient

based on previous FNA results from the Bethesda system for thyroid cytopathology [18].

Definition of growth

Criteria for significant growth depend on the size of the node, which must also take into account the variability of measurements.
Significant magnification is defined as a 20% increase in at least two node dimensions and a minimum increase of 2mm, or a 50% or greater volume increase [6].

Tracking time

There is little agreement in the literature about the optimal time to monitor nodules, since the degree of growth does not reliably distinguish benign from malignant nodules. Examination intervals shorter than one year are not recommended, except for proven malignancies under active supervision, which may require more frequent monitoring. It is advisable to determine the monitoring intervals in relation to the number of points assigned to the node. For a TR5 lesion, we recommend monitoring once a year for 5 years. The first, second, third and fifth years should be done for TR4 controls. For TR3 controls can be performed in the first, third and fifth years.
Monitoring can be stopped after five years, if there are no changes in size, because stability in this time interval reliably indicates that the node behaves benignly, which is valid for all categories of nodes [6].

There are no published data for the treatment of nodules that increase significantly, if their size is still below the threshold for FNA and remain in the same number of ACR TI-RADS points for almost five years, but their monitoring is still necessary. If the ACR of the TI-RADS node increases during monitoring, the next control should be done in one year, regardless of its initial level [6].

Assessment of cervical lymph nodes

The suspicious finding is suggestive in spherical lymph glands, hyperechoic glands, loss of normal echogenic hilus, presence of more pronounced peripheral flow or vascularization than hilus. Heteroechogenicity with cystic components and point echogenic foci that may represent microcalcificationsis also a suspicious finding [6].

Categorization (scoring) of nodes after scoring

After scoring the ultrasonic properties of the nodes, the nodes are categorized as, TR1-TR5. TR1, benign nodules with 0 points TR2, non-suspicious nodes with 2 points, where FNA is not recommended for these nodes, TR3 minimally suspicious nodes with 3 points, where FNA is advised for nodes larger than 2.5 cm and for nodes larger than 1.5cm tracking, TR4 moderately suspicious nodes with 4-6 points, with FNA recommended for nodes larger than 1.5cm and for nodes larger than 1cm tracking and TR5 highly suspicious nodes having more than 7 points, with FNA is advised for nodes larger than 1 cm and monitoring for larger than 0.5 cm [6].

In addition to the ultrasound appearance of the nodule, other factors must be taken into account when deciding on FNA. TSH should be measured in all patients to rule out the possibility of a hyperfunctional node. Such lesions do not require a biopsy, because they are practically always benign. Risk factors for malignancy are exposure to ionizing radiation during childhood accidentally or for medical reasons, positive family history of thyroid malignancy, occurrence of nodules in children and the elderly, clinical features, nodules that are firm, hard, fixed to the substrate and the environment, grow rapidly. It has recently been confirmed that node location is also an independent risk factor for malignancy. Nodes located in the isthmus carry a higher risk of malignancy, while those located in the lower third of the lobe carry the lowest risk compared to those from the middle or upper lobes. These factors are not usually classified as a stratification algorithm, but may influence a definitive attitude in joint decision-making with patients about further diagnostic and therapeutic procedures [19].

Conclusion

Certain ultrasound properties, the characteristics of nodules in the thyroid gland, can significantly indicate malignancy and are used as criteria for FNA. The features with the greatest diagnostic significance for predicting malignancy are the shape of the nodule, higher than wider in the transverse section, ie ovoid appearance, the presence of small calcifications in the nodule, irregular margins, while the spongy and cystic appearance of the nodule and the presence of halo around the nodule significantly indicate benignity. Node size is an unreliable parameter for estimating nodes. These ultrasound properties have different sensitivity and specificity, but unfortunately

none of them is enough for certain rejection or confirmation of malignancy. FNA is a very important diagnostic method, but its performance must be selective since systematic puncture of all nodes, regardless of size or appearance, is not recommended. It is important that the indications for FNA be based on clinical characteristics, as well as on echosonographic stratification of the risk of malignancy.

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Vol. 46 (2021) No. 2 TIMOK MEDICAL GAZETTE Short review article

THE MOLECULAR MECHANISM BY WHICH VITAMIN D PROTECTS AGAINST COVID-19

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Abstrakt: The SARS-CoV2 virus, which causes COVID-19, exerts its pathophysiological effect by intensively binding to the angiotensin-converting enzyme 2 receptor (ACE2) on the host cells. By blocking the ACE2 receptor, the physiological functions of the cell are inhibited, which are important for the normal function of various organs, and especially for the protection of the lungs. Therefore, the number of functionally active ACE2 receptors is extremely important for the body's resistance to COVID19. More receptors equal greater resistance of the host. An increased number of ACE2 receptors gives the body more time to mobilize an adequate immune response. Experience to date from the immediate fight against COVID19 has confirmed this rule: (A) women are generally more resistant (the ACE2 receptor gene is on the X chromosome, and women have two X chromosomes), (B) younger people are more resistant to the virus (ACE2 expression decreases with age), (C) patients with chronic diseases are more sensitive (have a reduced number of ACE2). Therefore, an increase in the number of ACE2 receptors is extremely important for the body's protective power in the fight against the SARS-CoV2 virus. Vitamin D increases the expression of the ACE2 gene, which increases the number of ACE2 receptors, which can be of significant aid in the fight against COVID-19.

Keywords: COVID-19, ACE2, Vitamin D

Molecular mechanism of the pathophysiological action of the SARS-CoV2 virus

Man has long lived alongside corona viruses and there has never been a pandemic of this magnitude. However, the new corona called SARS-CoV2 which first appeared in Wuhan (China), rapidly spread across the planet and evolved into a serious pandemic.

The question is why did SARS-CoV2, unlike other previous coronaviruses, become so pathogenic? What caused this virus to become so deadly and infectious?

There is already an answer to this question. The key point is that the new corona virus SARS-CoV2 has acquired a new mutation in its S protein that allows it to bind almost 1000 times more intensively to the ACE2 receptor [1]. The new mutation is in a part of the S protein called the furin cleavage site, which results in easier opening of the S protein and more intense binding to the ACE2 receptor, which is the gateway for the virus to infect cells.

So, the pathological effect of SARS-CoV2 virus is caused by the intensive binding to the ACE2 receptor, also by inactivation of the ACE2 receptor. That is, by knocking the ACE2 receptor out of function2. This coincides perfectly with patients who have ACE2 receptor dysfunction. Consequences of ACE2 receptor dysfunction are: Severe Acute Respiratory Syndrome, Heart Disease, Hypertension, Essential, Kidney Disease, Myocardial Infarction, Intracranial Aneurysm, Malaria, Vascular Disease, and Cardiovascular System Disease… [2]

The number of ACE2 receptors is crucial when it comes to the host's response to the SARS-CoV2 virus

The body's ability to fight the virus increases with an increase in the number of functioning ACE2 receptors. Taking this into consideration, we can conclude that people who have fewer ACE2 receptors are more likely to develop serious symptoms. The current

experience from the fight with COVID19 only confirms this rule.

(A) Women are more resilient due to the fact that they have more ACE2 receptors [3]. The gene that codes the ACE2 receptors is located on the X chromosome. Some experts will argue that one X chromosome is inactivated in women (Barr body). However, 15-25% of the genes located on the inactivated X chromosome escape the process of inactivation and remain active [4], also during embryonal development both X chromosomes are active.

(B) Children and the young are usually asymptomatic or have mild symptoms, while a more serious cases of COVID19 are usually present in the elderly.

This is due to the fact that the production of ACE2 receptors drops with age.

Scientists who have analyzed the ACE2 receptor in 30 different tissues (over 1000 patients) have concluded that the expression of the ACE2 receptor is significantly reduced in those over the age of 60 [3]. This is in line with the results from the field, where younger patients usually have mild symptoms, while on the other hand the elderly have far worse symptoms and more often than not succumb to the disease.

(C) People with chronic illnesses, especially type 2 diabetes are far more sensitive to the SARS-CoV2 virus and have worse symptoms.

The same group of scientists that have found a decrease in the number of ACE2 receptors with age have also found that patient with type 2 diabetes have a significant reduction in the number of ACE2 receptors [3]. Examples from the field show that patients with type 2 diabetes often develop worse symptoms.

How Vitamin D works as a preventative and protective measure?

Vitamin D works as a preventative measure in the fight against COVID 19 by increasing the expression of the ACE2 receptor. Vitamin D is a liposoluble vitamin (with a steroid structure) that has to bind to its VDR (Vitamin D Receptor) receptor within the cell nucleus in order for it to have an effect. The VDR belongs to a superfamily of ligand-inducible transcription factors. Vitamin D regulates the expression of a large number of genes including the gene that codes the ACE 2 receptor. Vitamin D increases the expression of the ACE2 gene [5,6]. Due to this increased expression the amount of ACE2 receptors also increases.

Conclusion:

From the information mentioned above we come to the conclusion that Vitamin D has a protective effect in the case of infection with the novel coronavirus. This protective effect is especially apparent in the elderly and in people with chronic disease.

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BOOK REVIEW: Healing war wounds of the extremities

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At the beginning of 2018, full professor dr. Zoran Golubović, an orthopedic surgeon from the Medical Faculty of the University of Niš, published the monograph "Treatment of war wounds of the extremities". This monograph is a specific publication in the field of war injuries to the extremities. In it, the basic principles of the occurrence and consequent surgical treatment of the mentioned war injuries are presented in a professional and clear way.

The monograph is dedicated to fighters, wounded, doctors, medical technicians as well as other medical and non-medical staff who participated in the treatment and care of the wounded.

The monograph has a general and a special part.

The mechanisms of injuries and the basic principles of treatment of injuries inflicted by high and low initial velocity projectiles are presented in general. The chapter is illustrated with photographs and X-rays taken after the wound and during treatment.

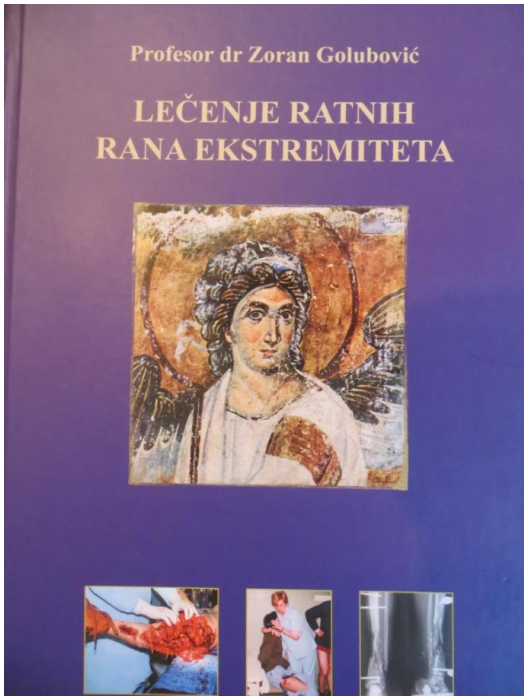
The special part shows gunshot wounds to the feet, lower legs, thighs, pelvis, spine, hands, forearms, upper arms, collarbones and extremity joints. Along with each of the mentioned regions, individual cases and ways of their treatment are described. In that way, more than 80 wounded were shown with characteristic injuries of the mentioned regions. As a special chapter, war polytrauma, blast and crash injuries, as well as complications such as infections, gas gangrene, non-healing of gunshot fractures and others are treated.

The monograph has 467 pages and 1066 pictures, tables and graphs.

Along with the used literature, the names of health workers who participated in the treatment of the wounded are also listed.

The monograph is intended for medical students, general practitioners, specialists in surgery, orthopedics, vascular, plastic and reconstructive surgery, and other health professionals. Its goal is to provide basic and most important information from war surgery, diagnostics and treatment of war injuries of the extremities and to present the acquired experiences of importance for war and peacetime traumatology.

Prof. Dr. Dragan Nikolic, orthopedist.



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Timok medical GAZZETTE publishes previously unpublished scientific and professional papers bilingually, in Serbian and English language from all fields of medicine and related branches. Original papers, patient case reports, review articles, medical and health history articles, book and journal reviews, editorial letters and other medical information are received for publication. The authors propose a category of their work and the Editorial Board reserves the right to change the category with the consent of the author.

Manuscripts should be prepared in accordance with the Vancouver Recommendations: UNIFORM REQUIREMENTS FOR MANUSCRIPTS SUBMITTED TO BIOMEDICAL JOURNALS, recommended by ICMJE (International Committee of Medical Journal Editors - Ann Intern Med. 1997; 126: 36-47), or in accordance with the Serbian languageversion JEDNOBRAZNI ZAHTEVI ZA RUKOPISE KOJI SE PODNOSE BIOMEDICINSKIM ČASOPISIMA, Serbian Archives of Medicine, 2002; 130 (7–8): 293. The digital version is freely available on the ICMJE website, www.icmje.org, as well as at www. tmg.org.rs/saradn.htm

When writing atext in English, one should adhere to the American English language standard and use short and clear sentences. Manuscripts received by the editorial staff are not expected to contain results already published by authors in another journal or similar publication. The original manuscript must be accompanied by the certificate of authorship (you can download the form at: www.tmg.org.rs), scanned signatures of all authors of the article.

The editorial board sends all the papers for peer review - usually two reviewers. Proceedings in supplements are not peer reviewed.

In works where the described patient may be identified, the utmost care should be taken to avoid any details that can identify him/her or obtain written consent for publication from the patient himself or his immediate family. When consent exists, it should be stated in the article.

If the paper receives positive anonymous reviews (2 reviewers) it will be accepted for publication. After receiving a positive review, in order for the paper to be published in electronic version on the website www.tmg.org.rs and printed, it is necessary to pay a fee for the cost of editing the article, proofreading and printing costs for the Timok medical journal **only for the first author**, which amounts to four thousand dinars (4000 RSD) paid to the current account.

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TECHNICAL REQUIREMENTS

The manuscripts are to be submitted exclusively in electronic form, bilingually (starting with volume 45), in Serbian (preferably Cyrillic) and in English. Papers submitted only in Serbian or English only will not be considered. Send the manuscripts in electronic form to: tmglasnik@gmail.com

The electronic format of the manuscript should be in Microsoft Office Word (with a .doc or .docx extension) and should include a final version of the manuscript. All text, references, tables and titles of tables and images and legends of images should be in one document. It is best to form the filename by the first author's last name, one keyword and type of work (for example: paunkovic_tiroidea_originalni.doc).

Use the Times New Roman font, 12p size. Write the paragraph so that only the left alignment is straight. Do not divide words into syllables at the end of the line. Insert only one blank space after the punctuation mark. Allow the titles and subheadings to be aligned with the left edge. Use bold, italic, sub, and superscript and underlined letters only where necessary. **Tables, images and charts should be inserted in the text where they should appear in the paper**. Acceptable formats for tables, charts, illustrations, and photos are doc, xls, jpeg, gif, and npg.

TYPES AND SCOPE OF MANUSCRIPTS

The title of all types of articles is followed by Summary (up to 300 words) and keywords (3 to 8).

The Original Paper (work) is a systematically published research of a problem according to scientific criteria and a clear aim of the research. **The integral parts of the paper are: a) introduction**- (the aim of the paper as the last paragraph of the introduction); **b) material and methods**; **c) results; d) discussion; e) conclusion; f) literature**. The length of the text is limited to 3500 words, with a maximum of 5 tables, charts, or pictures (up to 12 pages of text).

A Review Article covers a systematically addressed specific medical problem, in which the author made some contribution, visible on the basis of self-citations. **Integral parts of the paper are: a) introduction**- (the aim of the review paper as the last paragraph of the introduction); **b) the text of the review of literatureonthe problem , with subtitles**; **c) conclusion; d) literature**. The review article is usually commissioned by the Editorial Board, but non-commissioned manuscripts are also considered. Contact the Editorial Board before writing a review article. Text length can be up to 5000 words (18 pages).

A Case Report (patient presentation**)** sheds light on individual cases ofmedical practice. It usually describes one to three patients, or one family. The integral parts of the paper are: **a) introduction**- (the aim of the paper as the last paragraph of the introduction); **b) presentation of the patient; c) discussion and d) conclusion**. Unlike the original research, omit the section on methodology and results. The text is limited to 2500 words, max 4 tables, or 4 pictures and up to 25 references (up to 6 pages of text in total). Patient names, initials, or medical history numbers should not be used, especially in the illustrations. Casereportsmust not have more than 5 authors

Articles in the history of medicine and health culture shed light on certain aspects of medical practice in the past. Text length can be up to 2500 words (6 pages). These and the articles statedbelow do not have a prescribed structure, such as original papers, case reports, and review articles. Short contributions from the field of medical practice (diagnostics, therapy, remarks, suggestions and opinions on methodological problems, etc.) are published, too, as well as presentations from various medical meetings, symposia and congresses in the country and abroad, book reviews and articles from foreign journals up to 1000 words, 1-2 tables or images, up to 5 references (up to 3 pages of text). Editorial letters have up to 400 words, or 250 words if they contain comments on published articles. By order of the editorial board, or in agreement with the editorial board, works of didactic character are published.

If the work is part of a master's thesis, or a doctoral dissertation, or is done in the framework of a scientific project, this should be **clearlyindicated in the note after the abstract and before the text**. Also, if the work has been previously announced at a professional meeting, state the official name of the meeting, the venue and time of the event, whether the work has been published and how it has been published (eg the same or a different title or abstract).

ETHICAL CONSENT. Manuscripts on human research should include a statement in the form of a written consent of the persons interviewed in accordance with the WMADeclaration of Helsinki and the approval of the responsible ethics committee that the research can be carried out and is in accordance with legal standards. Experimental research on human material and animal testing should include a statement from the ethics committee of the institution and be in accordance with legal standards. Information on this must be provided in the section

AUTHORSHIP. All persons listed as authors of the work should qualify for authorship. Each author should have participated sufficiently in the work on the manuscript to be able to take responsibility for the entire text and the results presented in the work. Authorship is based solely on: making a significant contribution to the concept of the work, obtaining results or analyzing and interpreting the results; the planning of the manuscript or its critical revision of considerable intellectual importance; the final refinement of the print version of the manuscript. Authors should attach a description of the contributions individually for each co-author within the Submission Letter form. Financing, collecting data or generally overseeing a research team cannot by itself justify authorship. All other contributors who are not the authors of the manuscript should be listed onthe

acknowledgementpage, with a description of their contribution to the work, with written consent, of course.

STATEMENT OF CONFLICT OF INTEREST. The manuscript is accompanied by a signed statement in the form of a Submission Letter stating the authors of each possible conflict of interest or lack thereof. For more information on the different types of conflicts of interest, visit the World Association of Medical Editors' Association (WAME; http://www.wame.org), entitled "Conflict of Interest Statement Policy".At the end of the paper, below the Remarks section, in a separate section Conflict of Interest,each possible conflict of interest or its absence should be declaredfor each author individually (full name of the author or initials) For example Zoran Petrovic: Krka (lecturer) Ljiljana Aleksic: none. Mila Bastac: Pfizer, Sanofi, Bristol-Meyers Squibb (lecturer, honorary consultant, researcher on ascientific project).

PLAGIARISM. As of January 1st, 2019, all manuscripts are subjected to plagiarism / autoplagiarism through the SCIndeks Assistant-Cross Check (iThenticate). Papers containing plagiarism or self-plagiarism will be rejected and the authors sanctioned.

ABBREVIATIONS. Use only when necessary, for very long names of chemical compounds, that is, abbreviations that are already recognizable (standard abbreviations, such as DNA, AIDS, HIV, ATP). For each abbreviation, the full term should be stated when first quoted, unless it is a standard unit of measure. Do not use abbreviations in the title. Avoid using abbreviations in the abstract, but if necessary, explain each abbreviation when first referenced in the text.

ACKNOWLEDGEMENTS. List all contributors who contributed to the creation of the work but did not meet the criteria for authorship, such as those providing technical assistance, writing assistance, or managing a department that provides general support. Financial and material assistance, in the form of sponsorships, scholarships, gifts, equipment, medicines and more, should also be listed

MANUSCRIPT PREPARATION

The text of the paper contains first and foremost the title of the paper, in the following lines: full names of the authors and all co-authors; the name, place and address of the institutions from which the author and co-authors come (in parentheses, associate the names of the authors); possibleacknowledgementfor help with elaborationofthe paper;

It is obligatory to submit:

-proposal of the manuscript category (original work, review article, case report, etc.);

-**first and last name, year of birth of the author and all co-authors**;

-**full address, telephone and fax numbers, as well as the author's e-mail for correspondence**.

The following is a SUMMARY (Abstract), up to 300 words is best. A summary cannot have footnotes, tables, images, or references. A summary of **the original papers** should include: Introduction (state the objective in the last sentence), **Material and methods, Results and Conclusions**. Write each of the segments listed at the beginning of the sentence in bold. Provide the most important results (numerical values) of the statistical analysis and the levelofsignificance. The conclusion must not be general, but must be directly linked to the results of the work. **For case reports, the summary** should have the following parts: **Introduction** (state the objective in the last sentence), **Case report, Conclusion**. For other types of papers the summary has no specific structure.

The summary must not contain any claims that are not contained in the text of the article. It must be written in such a way that even an educated nonexpert can understand the content of the article. After the summary, write 3 to 8 keywords. The words in the title should not be repeated and the keywords should be relevant or descriptive and in accordance with MESH rules (available at https://www.nlm.nih.gov/mesh).

The next part of all the papers is an **INTRODUCTION** (with a subtitle of the same name), which must be brief, with a brief overview of the literature on the problem in question, and with a clear statement of **the purpose of the article** in a separate paragraph at the end of the introduction.

MATERIALS AND METHODS (with the same subtitle) must contain sufficient information to enable other researchers to repeat similar research without further information. Patient names and medical history numbers should not be used nor other details to help identify patients. The names of the apparatuses, software and statistical methods used must be indicated.

Show the **results** (with the subtitle of the same name in BOLD) clearly and concisely. You should not display the same data both in tables and charts.

DISCUSSION (with the subtitle of the same name) should discuss the interpretation of the results, their meaning in comparison with other, similar research and in accordance with the hypotheses of the research. The results already written should not be repeated.

CONCLUSION (with the subtitle of the same name) should be given in a separate chapter.

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REFERENCES

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Journal articles

Standard journal article:

Gao SR, McGarry M, Ferrier TL, Pallante B, Gasparrini B, Fletcher JR, et al. Effect of cell confluence on production of cloned mice using an inbred embryonic stem cell line. Biol Reprod. 2003; 68 (2): 595-603.

Organization as author:

WHO collaborative study team on the role of breastfeeding on the prevention of infant mortality. Effect of breastfeeding on infant and child mortality due to infectious diseases in less developed countries: a pooled analysis. Lancet. 2000; 355: 451–5.

No authors listed:
Coffe drinking and cancer of the pancreas [editorial]. BMJ. 1981; 283 628.

A volume with a supplement:
Magni F, Rossoni G, Berti F. BN-52021 protects guinea pig heart anaphylaxis. Pharmacol Res Commun. 1988; 20 Suppl 5: 75–8.

Books and other monographs

The author is a person (s):
Carlson BM. Human embryology and developmental biology. 3rd ed. St. Louis: Mosby; 2004.

Editor (s) as authors:
Brown AM, Stubbs DW, editors. Medical physiology. New York: Wiley; 1983.

Chapter in a book:
Blaxter PS, Farnsworth TP. Social health and class inequalities. In: Carter C, Peel JR, editors. Equalities and inequalities in health. 2nd ed. London: Academic Press; 1976. p. 165–78.

Meeting announcements: Harris AH, editor. Economics and Health: 1997: Proceedings of the 19th Australian Conference of Health Economists; 1997 Sep 13-14; Sydney, Australia. Kensington, N.S.W.: School of Health Services Management, University of New South Wales; 1998.

Conference Articles:
Anderson JC. Current status of chorion villus biopsy. In: Tudenhope D, Chenoweth J, editors. Proceedings of the 4th Congress of the Australian Perinatal Society; 1986: Brisbane, Queensland: Australian Perinatal Society; 1987. p. 190-6.

Dissertation:
Cairns RB. Infrared spectroscopy studies of solid oxygen. Dissertation. Berkley, California: University of California, 1965.

Electronic material

Article in aninternetmagazine:
Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs. 2002; 102 (6). Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htm

Article published electronically before the printed version:
Yu WM, Hawley TS, Hawley RG, Qu CK. Immortalization of yolk sac-derived precursor cells. Blood. 2002-Nov-15; 100 (10): 3828-31. Epub 2002 Jul 5.

CD-ROM:
Anderson SC, Poulsen KB. Anderson's Electronic Atlas of Hematology [CD-ROM]. Philadelphia: Lippincott Williams & Wilkins; 2002.

Online monograph:
Foley KM, Gelband H, editors. Improving palliative care for cancer [monograph on the Internet]. Washington: National Academy Press; 2001 [cited 2002 Jul 9]. Available from: http://www.nap.edu/books/0309074029/html/.

Website:
Cancer-Pain.org [homepage on the Internet]. New York: Association of Cancer Online Resources, Inc.; c2000-01 [updated 2002 May 16; cited 2002 Jul 9]. Available from: http://www.cancer-pain.org/.

Part of awebsite:
American Medical Association [homepage on the Internet]. Chicago: The Association; c1995-2002 [updated 2001 Aug 23; cited 2002 Aug 12]. AMA Office of Group Practice Liaison; [about 2screens]. Available from: http: //www.ama-assn.org/ama/pub/category/1736.html

NOTE. A paper that does not meet the requirements of this guide cannot be referred for review and will be returned to the authors for completion and correction. Adhering to the preparation instructions will significantly shorten the time of the entire process until the paper is published, which will positively affect the quality of the articles and the regularity of the publication of the journal.

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