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Welcome to the Moldovan Medical Journal!

The Moldovan Medical Journal is an international scientific double-blind peer reviewed periodical edition, 6 per year, of the Scientific Medical Association of the Republic of Moldova designed for specialists in the areas of medicine, dentistry, pharmacy, social medicine and public health. From its debut the journal has striven to support the interests of Moldovan medicine concerning the new concepts of its development.

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RESEARCH STUDIES

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The impact of the molecular genetic test on the diagnosis delay and outcome in patients with pulmonary tuberculosis

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Abstract

Background: The aim of the study was the assessment of the impact of the molecular genetic Xpert MTB/Rif assay on the diagnostic delay and the treatment effectiveness in pulmonary tuberculosis, according to the results of the microbiological and molecular-genetic methods, in order to optimize the management of the drug resistant tuberculosis.

Material and methods: A retrospective selective study, which included 226 patients with multidrug-resistant pulmonary tuberculosis (study sample) and 78 patients with drug-susceptible pulmonary tuberculosis (control group), was performed. Patients were divided into 2 subgroups following the results of the molecular-genetic and bacteriological investigations. The study sample was divided in two groups: the 1st group included 85 patients enrolled in the treatment for drug resistant tuberculosis, according to the results of the Xpert MTB test and the 2nd group included 141 patients enrolled in the treatment DOTS plus, according to the results of the conventional cultures.

Results: The impact of the molecular genetic GeneXpert MTB/Rif assay was demonstrated on early diagnosis, adequate treatment according to the drug susceptibility to rifampicin, shorter duration of the treatment, low rate of the side events, optimal treatment compliance and higher treatment effectiveness. The conventional microbiological methods contributed to the late diagnosis of the patients, high rate of the severe and complicated tuberculosis, long duration of the treatment which were reflected on the low treatment effectiveness. However the conventional culture methods allowed the treatment individualization according to the drug susceptibility tests.

Conclusions: Target screening of the population with risks and compulsory investigation by GeneXpert MTB/Rif assay will diminish the rate of severe late detected forms of pulmonary tuberculosis and adapt the treatment according to the drug resistance to rifampicin.

Key words: MDR-TB, Xpert MTB/Rif assay, treatment.

Introduction

Tuberculosis is one of the most important challenges for any health care system [15, 38, 42, 45]. The World Health Organization (WHO) declared tuberculosis a global emergency in 1993. Actually the Republic of Moldova ranks among the 30 countries with the highest burden of multidrug-resistant tuberculosis (MDR-TB), defined as the infection with resistant strains of *Mycobacteria tuberculosis* to at least two most powerful anti-tuberculosis drugs: isoniazid and rifampicin [45]. In 2001 the Republic of Moldova started the national implementation of the DOT (Directly Observed Treatment) strategy in the tuberculosis patient's management recommended by the World Health Organization [10]. Its implementation determined the epidemic extension of primary drug-resistant infection in the population. The rate of the primary MDR-TB, established as an infection with drug resistant strains increased four times in our country during the period 2003-2017 (6.0% – 2003, 9.9% –2004, 13.4% – 2005, 19.4% – 2006, 17.8% – 2007, 24.0% – 2008, 22.2% – 2009, 25.7% – 2010, 26.35% – 2011, 24% – 2012, 25% – 2013, 25% – 2014) and acquired drug-resistance two times (37.5% – 2003, 38% – 2004, 49.6% – 2005,50.8 % –2006, 52.3% – 2007, 59.0% – 2008, 64.85% – 2009, 67.8% – 2010, 63.8% – 2011, 62% – 2012, 62% – 2013, 63%– 2014) [10]. The extension of the drug resistance lowered the treatment effectiveness (2005 – 62%, 2006 – 62%, 2007 – 62%, 2008 – 58%, 2009 – 57%, 2010 – 52%, 2011 – 62%, 2012 – 62%, 2013 – 62%) which was lower in average by 20% compared with targeted 85% recommended by the WHO [4]. The low success rate was directly linked to the high rate of lost to follow-up patients: 2001 – 5,5%, 2002 – 15,6%, 2003 – 10,5%, 2004 – 10,4%, 2005 – 10,9%, 2006 – 11,7%, 2007 – 10,7%, 2008 – 7,4%, 2009 – 6,2%, 2010 – 7,9%, 2011 – 7,7%, 2012 – 5,2% [5]. International researches established high rate of drug resistance among HIV co-infected patients, which lowers treatment outcome and endangers the epidemiological situation [35]. Between 2011 and 2015 the rate of HIV infected among tuberculosis new cases patients increased by 2,6%: 2011 – 5,0%, 2012 – 5,0%, 2013 – 2,2%, 2014 – 5,3%, 2015 – 7,6% in Chisinau and 2011 – 5,1%, 2012 – 5,1%, 2013 – 5,2%, 2014 – 6,5% and 2015 – 7,7% in the Republic of Moldova [5]. The rate of HIV infection in new and relapsed cases increased evidently in Chisinau by 2,3%: 2011 – 4,6%, 2012 – 4,4%, 2013 – 2,9%, 2014 – 5,5% and 2015 – 6,9% and by 2,9% in the Republic of Moldova: 2011 – 5,2%, 2012 – 5,3%, 2013 – 5,9%, 2014 – 7,2% and 2015 – 8,1% [5]. The rate of TB-HIV co-infected cases among died patients showed an increased dynamic in Chisinau by 4,2%: 2011 – 11,9%, 2012 – 13,8%, 2013 – 9,2%,

2014 – 16,0% and 2015 – 16,1% as well as in the entire country by 5,3%: 2011 – 15,4%, 2012 – 12,8%, 2013 – 11,8%, 2014 – 20,8% and 2015 – 20,7% [5].

The diagnosis of MDR-TB, especially in children and HIV co-infected patients represents an important concern in many countries [12, 35, 36, 46]. No much technology is available for an accurate detection of the drug resistant strains of *Mycobacteria tuberculosis* worldwide [29]. So, detected deficiencies represent a major obstacle in the improvement of tuberculosis control and reduction of the global burden [11]. Microscopy alone, although inexpensive, misses one of three patients and detects only those with relatively advanced disease and severe lung destruction [8, 10, 32].

Only 28% of Moldovan patients were reported as smear positive cases in 2016 [5]. So, misdiagnosed cases increase the risk of infection transmission in the healthy population, endangering tuberculosis control at the regional level [11]. In the countries with a high burden of the HIV infection, the sensitivity of the smear microscopy is very low, which contributed to the increasing use of the rapid methods in the diagnosis of tuberculosis [35, 36]. According to the latest researches substantial efforts were made for strengthening the laboratory capacities for the diagnosis of smear-negative cases, TB-HIV co-infected cases and MDR-TB cases [10].

Conventional methods, such as smear microscopy, liquid and solid culture methods require qualified laboratories with important consequences: delayed diagnosis, worsening of the patient's condition, inadequate treatment which can generate additional drug resistance and continuous transmission of infection in the society [33]. In 2010 worldwide were implemented the first – and second – line probe tests which require extensive laboratory infrastructure and should be performed only in the reference facilities [33]. Starting from 2014 a real-time polymerase chain reaction Xpert MTB/RIF test for the *Mycobacterium tuberculosis* gene and the *rPob* gene mutation detection were implemented in the Republic of Moldova [8]. The Xpert MTB/RIF platform was developed by the Cepheid Company (Sunnyvale, USA). More than 50 devices and 12.000 cartridges were distributed in the Moldovan civilian specialized services, 2 in the penitentiary services and 2 in AIDS services. It contributed to an early detection of tuberculosis, precocious treatment, according to the rifampicin sensitivity, improvement of the infection control, increasing the treatment success rate, which are considered the most efficient tools for interrupting the epidemiological chain. [11]. Actual WHO recommendation regarding the Xpert MTB/RIF is to test all adults and children presumed to have pulmonary tuberculosis, with or without HIV co-infection. In addition, WHO established conditional recommendations to test extrarespiratory specimens of the adults and children presumed to have extrapulmonary tuberculosis [46]. The interpretation of the test results must be correlated with laboratory and clinical findings [9]. Published data established a high sensitivity of the Xpert MTB/RIF among culture positive specimens, in an average 97.3% and among smear positive patients – 99.5% [6]. The sensitivity of the Xpert MTB/RIF is slightly decreased in a single sputum sample [7]. Assessing the threshold of the estimated sensitivity it was demonstrated that the biological sample must contain at least 131 colony forming units (CFU) per ml of sample with a confidence interval ranging from 106.2 CFU to 176.4 CFU [6]. The sensitivity depends on the collection procedure, storage, transportation and technical errors. Insufficient volume of the specimen and insufficient concentration of the viable organisms are the most frequent causes of the negative results [9]. The test specificity compared with the non-tuberculosis patients are also high – 97.9% [6]. Despite of the higher sensitivity of the Xpert MTB/RIF test, more than one half of Moldovan patients were diagnosed through the clinical-radiological methods in 2016 [4]. Due to the low cost of the Lowenstein Jenson and BACTEC cultures, conventional methods remain the golden standard for *Mycobacterum tuberculosis* complex detection and drug susceptibility testing on the national standards, instead of highly recommended Xpert MTB/RIF test [10].

The standard treatment for drug-susceptible tuberculosis, according to WHO recommendations in the Republic of Moldova has been used since 2000 as a part of DOT strategy and lasts 6 months in new cases and 8 months in previously treated cases [11]. The treatment of the new drug-susceptible patients consists in a two phase regimen with four first-line anti-tuberculosis drugs: Isoniazid (H), Rifampicin (R), Ethambutol (E) and Pyrazinamide (Z) in the intensive phase and two drugs H and R in the continuation phase [39]. A regimen of eight months consisted of H, R, E, Z and Streptomycin (S) during the intensive phase and H, R and E in the continuation phase is used for the treatment of previously treated cases (relapses, failed and treatment for lost to follow-up cases) [39]. Multidrug-resistant patients are treated using the standard regimen for resistant tuberculosis that consists of second-line anti-tuberculosis drugs during 18-24 months [47]. Second-line anti-tuberculosis drugs used in the treatment of drug resistant tuberculosis are: Fluoroquinolones, Aminoglycosides, Thioamides, Oxazolidinones and new drugs such as Delamanid and Betaquiline. The major contributing factor of the treatment effectiveness represents the right combination of the drugs, according to the *Mycobacteria* susceptibility results [40]. Without an appropriate treatment tuberculosis-related death occurs in average within 2 years [24]. Due to high global epidemiological burden tuberculosis was placed on the 5th place in the top of the causes of death [45].

The major social determinants of tuberculosis and poor outcome are: social and economic inequalities, high levels of internal/external migration, rapid urbanization and population growth in urban areas [22, 25, 27]. Such determinants determine the polarization of the public health interventions, poor housing, low environmental conditions, malnutrition, geographic and cultural barriers in access to the health care [47]. There are several predictive factors for low treatment effectiveness. First of all – the infection with drug resistant and virulent mycobacteria [47]. The risk depends on the spread of the virulent mycobacteria in the community. The second group includes the factors which

increase the disease relapse or recrudescence of the latent tuberculosis infection: phtisiogenic ages (infants less than 5 years, teenagers, elders aged 65 and more), malnutrition, comorbid state and lack of the BCG vaccination [11]. The third group included the determinants of the patient's immune status: HIV infection, immune suppressive drugs (>15mg/day of prednisone for 1 month or more, immune modulators: TNF-α blockers or oral steroids, antineoplastic agents), diabetes, cancer, silicosis, chronic respiratory diseases, gastrointestinal diseases, underweight, phtysiogenic ages, harmful habits (tobacco smoking, alcohol abuse, illicit drug using) [27]. The fourth group includes: the accessibility of the sick people to the tuberculosis screening and health care, treatment compliance and patient's nursing [44]. In this group are also included social related barriers: lack of the social protection and medical insurance, geographic and economic barriers, cultural behaviour or stigma. The social determinants of the poor treatment outcome are strongly correlated with the psychotropic substance abuse: tobacco smoking, alcohol abuse and illicit drug using. Such behaviour habits contribute to multiple treatment interruptions and high rate of death.

Low treatment adherence and high rate of the lost to follow-up patients contributed to the development of a shorter conventional MDR-TB regimen lasting less than 12 months with low costs (<1.000$/patient) [47]. It showed promising results in selected MDR-TB patients and WHO updated its treatment guidelines in 2016 by including the recommendation to use the shorter MDR-TB regimen in patients with non-complicated tuberculosis (excluding extrapulmonary tuberculosis and pregnancy) [47]. Fluoroquinolones and Aminoglycosides are key drugs in the new MDR-TB regimen, however, surveillance of the cross resistance in five high burden countries: Azerbaijan, Bangladesh, Belarus, Pakistan and South Africa established that resistance of Rifampicin is frequently associated with resistance to Pyrazinamide, so the effectiveness of the short regimen might be lower than predicted [45]. Considering the WHO recommendations for aligning the Moldovan procedures for the international quality-assured standards, it was established the importance of the diagnosis algorithm improvement for increasing the treatment effectiveness.

The aim of the study consisted in the comparative assessment of the clinical aspects and the treatment effectiveness in patients with pulmonary tuberculosis diagnosed according to the results of the conventional microbiological methods (solid either liquid media) and molecular-genetic method Xpert MTB/RIF test (susceptible or resistant to Rifampicin forms).

Material and methods

A selective, descriptive and retrospective study was conducted according to a linear model, structured in several stages: purpose, sampling, investigation, data collection and interpretation. A preliminary documented research has been carried out on new cases of pulmonary tuberculosis investigated and treated in the Clinical Hospital of Phtisiopneumology of Chișinău between 2010 and 2014. The investigation methods were used according to the National Clinical Protocol. The patients were investigated by laboratory examinations: general blood and urine analysis, chest X-ray, sputum microscopy for acid fast bacilli, conventional microbiological investigations (smear microscopy, Lowenstein – Jensen culture, BACTEC assay) and innovative platform of the Xpert MTB/RIF test. The statistical processing of the results was done computerized through the Excel program of the Microsoft Office package. For estimating significant differences, the Student test was used (differences were true for $p<0.05$; P – characteristic rate, ES – standard error).

Results and discussion

The study included 304 patients with pulmonary tuberculosis, of which 141 had proved multidrug-resistant tuberculosis (MDR-TB), according to the conventional drug susceptibility tests, 85 had Rifamipicin resistance on the Xpert MTB/RIF test and 78 patients were Rifamipicin susceptible of the Xpert MTB/RIF test. Distributing patients in groups according to the drug susceptibility definition, 226 were diagnosed with MDR-TB and 78 with sensible tuberculosis. The patients were divided into three groups according to the results of the molecular-genetic or bacteriological investigations: in the first group were included 85 patients enrolled in the DOTS plus cohort treated for MDR-TB according to the results of the Xpert MTB test and the second group included 141 patients treated for MDR-TB, according to the results of the drug susceptibility test on conventional microbiological methods (Lowenstein – Jensen culture, BACTEC assay). Both groups should be considered as a study sample constituted from 226 patients with MDR-TB. The third group, defined as the control group, included 78 patients with positive and sensible for Rifampicin Xpert MTB/RIF test and diagnosed with drug susceptible tuberculosis.

Assessing the groups according to the sex distribution was established the predominance of men in all groups. So, in the fist group there were 61 men (71.7%) and 24women (28.3%) cases with a male/female ratio = 2.5/1. In the second group men were more frequently identified than women and their rate was higher compared with the first group: 106 (75.1%) *vs* 35 (24.8%) women with male/female ratio=3/1. In the third (control group) there were 53 men (68%) cases and 25 women (32%) cases with a male/female ratio=2.1/1. The urban residence prevailed in all three groups compared with the rural one. So, in the first group there were 67 urban residents (78.8%), in the second group – 90 (70.2%) and in the third group – 60 (76.9%). Assessing the social vulnerability it was established that poor living conditions prevailed in the MDR-TB sample. In the first group there were 69 (81.2%) socially vulnerable patients, in the second group – 102 (81.2%), and only 28 (35.9%) patients in the third group. The rate of the unemployed patients was high in the first and second groups, 64 (75.3%) and 104 (73.7%) compared with the third group 47 (60.3%) patients. Unqualified workers were in a similar proportion in the first and second groups, 23 (27.1%) and 25 (24.1%) and higher compared with the third group 14 (17.9%) patients. Assessing the level of the school education it was established that completed general

studies presented a similar proportion of patients in the first and second groups 34 (40.1%) and 51 (36.2%) being higher compared with the third group of 14 (17.9%) patients. The professional studies prevailed in the third group – 39 (50.1%) compared with the first and second groups, 24 (28.2%) and 39 (27.7%) patients. Comparing the rate of patients with a low school education, which included graduated primary school, incomplete general school and secondary school it was determined their predomination in the first and second groups compared with the third group. The high level of the school education which included professional and superior studies predominated in the third group compared with the first and second groups. The civil status of the single state person prevailed in the first group 37 (43.5%) compared with the second and third groups, 36 (25.5%) and 26 (33,3%) patients. Married patients predominated in the second group 105 (74.5%) compared with the first and third groups, 48 (56.5%) and 52 (66.7%) patients. Lack of the health insurance predominated in the third group 43 (55.1%) compared with the first and second groups, 43 (50.6%) and 92 (65.2%) patients. Comparing the groups of patients was established a more evident social vulnerability of the MDR-TB patients from the study sample being appreciated through the rate of the unemployed persons, unqualified workers, patients with low level of the school education and lack of the health insurance (tab. 1).

Table 1

Social and economic state of patients with pulmonary tuberculosis

Economic Social state	1st group	2nd group	3rd group	$p_{1/2}$	$p_{1/3}$	$p_{2/3}$
	n=85(P%)	n=141(P%)	n=78(P%)			
Employed	21 (24,7)	37 (26,3)	31 (39,7)	>0,05	<0,05	<0,05
Unemployed	64 (75,3)	104 (73,7)	47 (60,3)	>0,05	<0,05	<0,05
Low level of education	53 (62,3)	92 (65,5)	23 (29,5)	>0,05	<0,05	<0,05
Optimal level	35 (37,7)	49 (34,5)	55 (70,5)	>0,05	<0,05	<0,05
Single civil state	37 (43,5)	36 (25,5)	26 (33,3)	>0,05	>0,05	>0,05
Married/ in couple	48 (56,5)	105 (74,5)	52 (66,7)	>0,05	>0,05	>0,05

Epidemiological risk factor, established during the household contact with a sick family member was identified in a similar proportion in the second and third groups, 68 (48.2%) and 39 (50.1%) compared with the first group, 25 (29.4%) patients. Migrants were identified in a higher proportion in the third group 12 (15.4%), compared with the first and second groups 10 (11.8%) and 14 (9.9%) patients. Former detainees were more numerous in the second group 28 (19.9%) compared with the first and third groups 13 (15.3%) and 11 (14.1%) patients. Harmful habits were detected in a high proportion in all groups. So, the active smoking prevailed in the second group 117 (83.1%) compared with the first group and third group 63 (74.1%) and 48 (61.5%) patients. Alcohol abusers prevailed in the second group 81 (57.4%) compared with the first group and third groups 38 (44.7%) and 32 (41.1%) patients. A limited number of drug users were diagnosed in all groups: 4 (4.7%) in the first group, 3 (2.4%) in the second group and 1 (1.3%) in the third group. Patients that form risk groups were distributed differently among selected samples. Even statistical difference was not established, contact with a sick family member predominated in the groups diagnosed with drug susceptible tuberculosis through the Xpert MTB/RIF and MDR-TB by conventional methods. No differences were established regarding harmful habits in the studied groups (tab. 2).

Table 2

Distribution of patients in risk groups

Risk groups	1st group	2nd group	3rd group	$p_{1/2}$	$p_{1/3}$	$p_{2/3}$
	n=85 (P%)	n=141 (P%)	n=78 (P%)			
TB contact	25(29,4)	68(48,2)	39(50)	<0,05	<0,05	>0,05
Migrants	10 (11,8)	14 (9,9)	12 (15,4)	>0,05	>0,05	>0,05
Former detainees	13 (15,3)	28 (19,9)	11 (14,1)	>0,05	>0,05	>0,05
Smokers	63 (74,1)	117 (83)	48 (61,5)	>0,05	<0,05	<0,05
Alcohol abuse	38 (44,7)	81 (57,4)	32 (41)	>0,05	>0,05	<0,05
Chronic alchoolism	9 (10,6)	17 (12,1)	0	>0,05	<0,05	<0,05
DIU	4 (4,7±2,3)	3 (2,1±1,2)	1 (1,3±1,3)	>0,05	>0,05	>0,05

Note: DIU – drug injection use;

Assessing the associated diseases, it was established that the comorbid state predominated in the first group 69 (81.1%) compared with the second and third groups, 69 (48.9%) and 58 (74.3%) patients. Distributing patients, according to the nosological groups of the associated diseases was determined the predomination of the gastrointestinal disorders in the first group 36 (42.4%), followed by the second and third groups, 45 (31.9%) and 28 (35.9%) patients. Chronic respiratory diseases were diagnosed in a similar proportion in all groups: 31 (36.5%) in the first group, 39 (27.7%) in the second group and 35 (44.9%) patients in the third group. Diabetes mellitus was diagnosed more frequently in the first group 12 (14.1%) followed by the third and the second groups 9 (11.5%) and 7 (5.1%) patients. Co-infected TB/HIV has been encounted frequently in the first group 4 (4.7%) followed by the third group 2 (2.7%) and 1 (0.7%) patient in the second one.

Detected particularities established the predominance of the passive case-finding in the first and second groups, 68 (80.1%) and 106 (75.2%) patients compared with 38 (49.7%) patients in the third group. Active screening of the risk groups was most frequently used to detect patients in the third group 40 (51.3%) compared with 17 (20.1%) and 35 (24.8%) patients from the first and second groups. Distributing patients according, to the diagnostic delay, it was identified that each fourth patient in the first and second group were diagnosed in more than three months after the disease onset. The early diagnosis till 30 days after the disease onset was established in each fifth patient in the third group 7 (18.4%). Pulmonary infiltrative tuberculosis was

diagnosed in most of the patients in all groups: 79 (93.1%) in the first group, 132 (93.6%) in the second group and 72 (92.3%) patients in the third group. Severe forms in terms of disseminated and fibro-cavitary tuberculosis were diagnosed in a similar proportion in all groups: 6 (7.1%) in the first group, 7 (4.9%) in the second group and 6 (7.7%) in the third group. Complicated tuberculosis was diagnosed in a similar proportion in the first group and third groups, 28 (32.9%) and 24 (30.7%) patients, compared with 24 (17.1%) patients of the second group. Among complications predominated pleurisy in a similar proportion in all groups: 14 (16.5%) in the first group, 21 (14.9%) in the second group and 9 (11.5%) patients in the third group. Hemoptysis predominated in the second group 25 (17.8%), followed by the first and third groups 9 (10.6%) and 9 (11.6%) patients. The most severe complication defined as tuberculosis meningitis was diagnosed in 3 (1.4%) patients of the second group. No such complication was diagnosed in other groups. Lung parenchyma destruction predominated in the second group 137 (97.2%), followed by a similar proportion in the first and third groups, 67 (78.9%) and 58 (74.4%) patients. More than three pulmonary segments were affected by tuberculosis in the majority of patients from all groups: 80 (94.1%) in the first group, 127 (90.1%) in the second group and 48 (61.5%) patients in the third group. Both lungs were involved in two thirds of the first and second groups, 74 (87.1%) and 124 (87.9%) and in each second patient of the third group – 51 (65.4%) cases. So, passive way of finding of symptomatic cases predominated in the MDR-TB sample and active screening of the risk groups in the drug susceptible sample. Even the pulmonary infiltrative tuberculosis was diagnosed in most of the cases, severe forms, involvement of both lungs and complicated tuberculosis were more frequently diagnosed in the sample diagnosed by microbiological conventional methods.

Microscopic smear positive was more frequently distinguished in patients from the second group 96 (68.1%), followed by the first and third groups, 53 (62.3%) and 51 (66.7%) patients. The delay between the diagnosis by Xpert/RIF examination and the beginnig of the treatment was less than 10 days in the first and third groups. In one third of the second group the delay between culture examination and initiation of the treatment, according to the drug susceptibility test constituted in average 2 months, however, in 3 till 5 months the therapy was started in 64 (45.4%) patients and more than 5 months in 46 (32.6%) patients. Each second patient from the study sample with MDR-TB was identified resistant against all first-line anti-TB drugs: Isoniazid, Rifampicin, Ethambutol and Streptomycine: 40 (47.1%) in the first group and 67 (47.5%) patients in the second group. No drug resistant patients were diagnosed by culture methods in the third group, which confirmed the high specificity of the Xpert/RIF test. Sputum conversion under the anti-tuberculosis treatment assessed by microscopic method during the first three months of the treatment for MDR-TB (DOTS-Plus) was distinguished in a higher proportion in the first group compared with the second group: 31 (58.5%) *vs* 21 (21.2%) patients. In most of the cases with drug susceptible tuberculosis included in the third group, 43 (84.2%) patients, the sputum conversion was obtained in the first three months. Sputum conversion during the first three months of the treatment assessed by culture methods was determined in the highest proportion in the third group, 68 (87.2%) patients. In a lower proportion was established the sputum conversion in patients from MDR-TB groups. Comparing the first with the second group, the sputum conversion predominated in the first group 55 (64.8%) vs. 30 (21.3%) patients from the second group. A high proportion of the non-assessed patients, which included died and lost to follow-up patients were established in the second group, due to delayed diagnosis and longer duration of the treatment compared with the first group (tab. 3).

Table 3

Conversion of sputum by culture methods

Sputum conversion	1st group	2nd group	p
	n=85 (P%)	n=141 (P%)	
Till 3 months	55 (64.8)	30 (21.3)	<0,001
3-6 months	39 (45.5)	64 (54.7)	<0,05
6-12 months	2 (2.4)	13 (4.7)	>0,05
More than 12 months	0	1 (0.7)	>0,05
Non-assessed patients	9 (10.6)	45 (31.9)	>0,05

Obtained data confirmed the impact of molecular genetic test for early diagnosis, interruption of the epidemiological chain and the appropriate treatment according to the drug resistance against Rifampicin. The early sputum conversion assessed through the microscope and conventional culture methods had a high relevance in groups diagnosed by the Xpert MTB/RIF assay. It was due to a lower rate of severe forms, lung destruction and an appropriate treatment, according to the drug resistance test to Rifampicin.

The anti-tuberculosis treatment duration in the first group was 24 months in 54 (63.52%) patients and 18 months in 16 (18.8%) patients. A limited number were treated less than 6 months due to a precocious death. So, the Xpert MTB/RIF assay offers the opportunity of an early diagnosis and rapid begining of the appropriate treatment for drug resistant tuberculosis. In the second group the duration of the anti-tuberculosis treatment was long due to a high rate of patients with severe and complicated tuberculosis. So, during 24 months were treated 37 (62.2%) and more than 24 months 76 (53.9%) patients. Treatment in outpatient settings was performed by the every third patient in the first and third groups, 27 (31.8%) and 26 (33.1%) cases. First-line anti-tuberculosis drugs received all patients with drug susceptible tuberculosis from the third group. A few patients from the first and second groups were treated for a limited duration with first-line therapy till obtaining the results of the drug susceptibility test. The standard treatment for MDR-TB was initiated in the first and second groups 83 (97.6%) and 113 (80.1%). However, in every fifth patient of the second group it was individualized according to the drug resistant susceptibility obtained through the conventional culture methods. Data are shown in the table 4.

Table 4

Anti-tuberculosis treatment duration in patients from the study sample

Duration	1st group	2nd group	P
	n= 85 (P%)	n=141 (P%)	
6 months	7 (8,2)	14 (9,9)	>0,05
18 months	16 (18,8)	21 (14,9)	>0,05
24 months	54 (63,5)	37 (26,2)	<0,001
24-30 months	2 (2,3)	74 (52,5)	<0,001
>30 months	0	2 (1,4)	>0,05

Multiple treatment interruptions were established more frequently in the second group 42 (29.9%) followed by the first and third groups 18 (21.2%) and 8 (10.3%) patients. So, it was assessed that long treatment duration diminished the treatment compliance of patients with MDR-TB patients, especially those diagnosed through the conventional microbiological methods. Major adverse drug events were diagnosed in every third patient of the second group 43 (30.5%), followed by the first group and third group 19 (22.4%) and 4 (5.1%) patients. Treatment success more frequently was established in the third group 67 (85.9%), followed by the first group 59 (69.4%) and second group 84 (59.6%). Were lost to follow-up more frequently patients from the second group 21 (14.9%) which was due to multiple treatment interruptions. The rate of patients lost to follow-up in the first and third groups was similar, 9 (10.7%) and 8 (10.3%). Died more frequently in the second group 22 (15.6%), followed by the first group 12 (14.1%) and the third group 1 (1.3%). So, the highest rate of lost to follow-up patients was explained by the long treatment duration and the higher death rates due to severe tuberculosis and high rate of major adverse drug events. Exposed data are presented in the table 5.

Table 5

Anti-tuberculosis treatment outcome in the study sample

Treatment outcomes	1st group	2nd group	p
	n= 85 (P%)	n=141 (P%)	
Treatment success	59 (69,4)	84 (59,6)	>0,05
Including cured	54(63,5)	82(58,1)	>0,05
Including completed the treatment	5(5,9)	2(1,4)	>0,05
Treatment failure	5(5,9)	14(9,9)	>0,05
Lost to follow-up	9(10,6)	21(14,9)	>0,05
Died	12(14,1)	22(15,6)	>0,05

Assessing all related data it was established the predominance of men in all selected groups, which confirmed their social vulnerability. Urban residence identified in two thirds of each group established that the level of the epidemiological control is lower in cities. The social vulnerability was higher in the MDR-TB patients than in drug susceptible cases, demonstrated by the rate of the unemployed persons, with the lack of the social and medical insurance. Epidemiological risk in terms of the tuberculosis contact was more relevant in patients with drug resistant tuberculosis. Such risk conditions as migration, former detention, and harmful habits had no prevalence in drug resistant or susceptible groups. Passive finding of the symptomatic cases predominated in the MDR-TB sample and active screening in the drug susceptible sample. Pulmonary infiltrative tuberculosis was diagnosed in most of the cases, but severe forms, involvement of both lungs and complicated tuberculosis were more frequently identified in the sample diagnosed by microbiological conventional methods. Early diagnosis, infection control and the adequate treatment according to the drug susceptibility to Rifampicin were possible in the groups diagnosed by the Xpert MTB/RIF assay. It permitted the sputum conversion in the first three months in most of the patients. Patients diagnosed through culture methods had more frequently extended radiological processes, both lungs involvement, which contributed to a long duration of treatment, high rate of the adverse drug events and low treatment outcome. The treatment success rate for drug susceptible tuberculosis reached the WHO recommended level 85%. The treatment success level for drug-resistant tuberculosis was low, mostly in the group diagnosed through the conventional microbiological methods. Long duration till the sputum conversion of patients treated for drug resistant tuberculosis demonstrated their epidemiological burden on the healthy population. The low rate of the lost to follow-up patients and died due to the progression of pulmonary tuberculosis in groups diagnosed through the Xpert MTB/RIF assay was due to a shorter period of the anti-tuberculosis treatment.

Conclusions

The molecular genetic Xpert MTB/Rif assay demonstrated an important impact on the diagnosis of pulmonary tuberculosis in terms of earlier diagnosis, adequate treatment according to the drug resistance to Rifampicin, shorter duration of the treatment, low rate of the side events, optimal compliance and higher treatment effectiveness.

The conventional microbiological methods contributed to the late diagnosis of the patients, high rate of the severe and complicated tuberculosis and long duration of the treatment which were reflected on the low treatment effectiveness. However, the conventional culture methods allowed the individualization of the treatment, according to the drug susceptibility tests.

Target screening of the population with risks and compulsory investigation by Xpert MTB/Rif assay will diminish the rate of severe late detected forms of pulmonary tuberculosis and adapt the treatment according to the drug resistance to Rifampicin.

References

1. Barnard M, Warren R, Van Pittius NG. Genotype MTBDRsl line probe assay shortens time to diagnosis of extensively drug-resistant tuberculosis in a high-throughput diagnostic laboratory. Am J Respir Crit Care Med. 2012;186(12):1298-305.
2. Boehme CC, Nicol MP, Nabeta P, et al. Feasibility, diagnostic accuracy, and effectiveness of decentralized use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicenter implementation study. Lancet. 2011;377:1495–505.
3. Boehme CC, Nicol MP, Nabeta P, et al. Feasibility, diagnostic accuracy,

and effectiveness of decentralized use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicenter implementation study. Lancet. 2011;377:1495–505.
4. Centrul National de Management in Sanatate [National Center for Health Management]. Tuberculoza în Republica Moldova: Raport [Tuberculosis in the Republic of Moldova: Report]. Chişinău: The Center; 2017. Romanian.
5. Centrul National de Management in Sanatate [National Center for Health Management]. Indicatori preliminari privind sanatatea populatiei si activitatea institutiilor medico-sanitare pe anii 2016-2017 [Preliminary indicators on population health and healthcare institutions for the 2016-2017 years]. Chisinau: The Center; 2018.
6. Cepheid. Xpert MTB/RIF. Package insert. Cepheid: Sunnyvale, CA; July 2013.
7. Chang K, Lu W, Wang J, Zhang K, Jia S, Li F, et al. Rapid and effective diagnosis of tuberculosis and RIFampicin resistance with Xpert MTB/RIF assay: a meta-analysis. J Infect. 2012;64(6):580–88.
8. Crudu V, Stratan E, Romancenco E, Moraru N, Turcan N, Allerheiligen V, Hillemann A. First evaluation of an improved assay for molecular genetic detection of tuberculosis as well as Rifampin and Isoniazid resistances. J Clin Microbiol. 2012;50(4):1264-9. ISSN: 0095-1137.
9. Crudu V. Principiile de interpretare a rezultatelor testării rezistenţei *M.tuberculosis* prin metode molecular genetice: Xpert®MTB/RIF: Recomandări metodice [Principles for the interpretation of M.tuberculosis resistance test results by molecular genetic methods: Xpert®MTB / RIF: Methodological recommendations]. Chisinau; 2016. Romanian.
10. Crudu V, Romancenco E. Diagnosticul microbiologic al tuberculozei: Ghid [Microbiological diagnosis of tuberculosis: Guide]. Chisinau; 2012. Romanian.
11. Crudu V, Vilc V, Alexandru S, Ignat R. Controlul tuberculozei la nivelul asistenţei medicale primare [Tuberculosis control in primary health care]. Chişinău, 2015. Romanian.
12. Dara M, de Colombani P, et al. Minimum package for cross-border TB control and care in the WHO European region. Eur Respir J. 2012;40(5):1081-90.
13. Faustini A, Hall AJ, Perucci CA. Risk factors for multidrug resistant tuberculosis in Europe: a systematic review. Thorax. 2006 Feb;61(2):158-63.
14. Gupta D, Aggarwal AN, Kumar S, Jindal SK. Smoking increases risk of pulmonary tuberculosis. J Environ Med. 2001;3:5-9.
15. TB CARE I. International standard for tuberculosis care. 3rd ed. Hague: TB CARE I; 2014. 92 p.
16. Jee SH, Golub JE, Jo J, Park IS, Ohrr H, Samet JM. Smoking and risk of tuberculosis incidence, mortality, and recurrence in South Korean men and women. Am J Epidemiol. 2009;170(12):1478-85. doi: 10.1093/aje/kwp308.
17. Jenkins H, Ciobanu A, Plesca V, et al. Risk factors and timing of default from treatment for non-MDR TB in Moldova. Int J Tuberc Lung Dis. 2013;17(3):373-80
18. Kolappan C. Tobacco smoking and pulmonary tuberculosis. Thorax. 2002;57:964-966.
19. Lachmandas E, van den Heuvel M, Damen MS, Cleophas MC, et al. Diabetes mellitus and increased tuberculosis susceptibility: the role of short-chain fatty acids. J Diabetes Res. 2016;2016:15. doi: 10.1155/2016/6014631.6014631
20. Lesnic E, Todorico L. [Risk factors for multidrug-resistant tuberculosis treatment failure]. [Tuberculosis, Lung Diseases, HIV Infection] (Киев). 2017;29(2):18-24. ISSN 2220-5071. Russian.
21. Lesnic E, Todorico L, Niguleanu A, Negru G. The impact of different microbiological methods in the diagnosis of resistant tuberculosis. [Ukrainian Pulmonology Journal]. 2017;(2 Suppl):110-1. ISSN 2220-1026.
22. Lesnic E, Todorico L, Niguleanu A, The socio-economic risk factors of tuberculosis in condition of a high migration in trans-border region. [Bukovinian Medical Herald Journal] (Chernivtsy). 2016;20(4):109-11. ISSN 2220-5012.
23. Lesnic E, Ustian A, Niguleanu A, Malic A, Paladi C. Social features of patients with pulmonary tuebrculosis. [Tuberculosis, Lung Diseases, HIV Infection] (Киев). 2016;25(2):36-40. ISSN 2220-5071.
24. Lesnic E, Rosca V, Niguleanu R, Haidarli I, Popa V, et al. Particularităţile clinico-morfologice ale tuberculozei pulmonare cu evoluţie letală [Clinical morphopathological features of pulmonary tuberculosis with lethal evolution]. Sănătate publică, Economie şi Management în Medicină [Public Health, Economy and Management in Medicine] (Chisinau). 2017:1(71):32-7. Romanian.
25. Lesnic E, Ciobanu S. Determinants of lost to follow-up and tuberculosis patients awareness. The Moldovan Medical Journal. 2017;60(3):23-9.
26. Lesnic E, Niguleanu A, Curocichin Gh. Segregation of tuberculosis patients by social, demografic and economic features on the model of Chisinau city and the role of the community support. Curierul Medical (Chişinău). 2016;59(4):11-7. ISSN 1857–0666.
27. Lesnic E, Niguleanu A, Ciobanu S, Todoriko L. Predictive factors associated with low tuberculosis treatment outcome: a cross sectional study. The Moldovan Medical Journal. 2017;60(2):7-13.
28. Malic A. Molecular–genetic assay results and clinical, radiological features in patients with pulmonary tuberculosis. Curierul Medical (Chişinău). 2016;59(4):2-6. ISSN 1857-0666.
29. Malic A, Ustian A, Lesnic E, Niguleanu A. The predictive factors for positive molecular-genetic assay in patients with pulmonary tuberculosis from chisinau city. Curierul Medical. 2016;59(2):20-7. ISSN 1857-0666.
30. Malic A, Ustian A, Iavorschi, C. Aspectele clinice bolnavilor de tuberculoză pulmonară în dependenţa de rezultatul testului Xpert MTB/RIF [Clinical aspects of patients with pulmonary tuberculosis in dependence of the Xpert MTB/RIF test results]. Revista Sănătate Publică, Economie şi Management în Medicină [Public Health, Economy and Management in Medicine] (Chisinau). 2017;2(72):43-7. ISSN 1729-8687. Romanian.
31. Malic A. Aspectele clinice, microbiologice şi eficacitatea tratamentului a tuberculozei pulmonare resistente = Clinical, microbiological particularities and treatment effectiveness of resistant pulmonary tuberculosis: retrospective, comparative, case series study. Revista de Ştiinţe ale Sănătăţii din Moldova = Moldovan Journal of Health Sciences. 2017;12(2):64-73. ISSN 2345-1467. Romanian, English.
32. Ustian A, Crudu V, Malic A, et al. Aspectele clinico-radiologice, microbiologice şi eficacitatea tratamentului la pacienţi cu tuberculoza pulmonară diagnosticaţi prin Xpert MTB/RIF [Clinical, radiological, microbiological aspects and treatment efficacy in pulmonary TB patients diagnosed by Xpert MTB/Rif]. Buletinul Academiei de Ştiinţe a Moldovei, Ştiinţe medicale. 2016;1(50):73-6. ISSN 1857-0011. Romanian.
33. World Health Orgnization. Policy statement: automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and Rifampicin resistance: Xpert MTB/RIF system. Geneva: WHO; 2011. 36 p.
34. World Health Organization. Brief guide on tuberculosis control for primary health care providers. Geneva: WHO; 2004. 75 p.
35. World Health Organization. TB/HIV Clinical Manual. 2nd ed. Geneva: WHO; 2004. 212 p.
36. World Health Organization. Xpert MTB/RIF increases timely TB detection among people living with HIV and saves lives. Geneva: WHO; 2013 [cited 2017 Oct 16]. Available: http://www.who.int/hiv/topics/tb/note_xpert_mtb_rif/en/
37. World Health Organization. Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and Rifampicin resistance: Xpert MTB/RIF system. Geneva: WHO; 2014.
38. World Health Organization. End TB Strategy. Geneva: WHO; 2014. 20 p.
39. World Health Organization. Treatment of tuberculosis: guidelines. Geneva: WHO; 2014. 420 p
40. World Health Organization. Companion handbook to the WHO guidelines for the programmatic management of drug-resistant tuberculosis. Geneva: WHO; 2014.
41. World Health Organization. Treatment of tuberculosis: guidelines. Geneva: WHO; 2014.
42. World Health Organization. Systematic screening for active tuberculosis. Geneva: WHO; 2013.
43. World Health Organization. Global status report on alcohol and health. Geneva: WHO; 2014. 100 p.
44. World Health Organization. The social determinants of health. Solid facts. Geneva: WHO; 2014.
45. World Health Organization. Global tuberculosis report. Geneva: WHO; 2017.
46. World Health Organization. XpertMTB/RIF assay for the diagnosis of TB, meeting report. Geneva: WHO; 2016.
47. World Health Organization. Treatment guidelines for drug-resistant tuberculosis update. Geneva: WHO; 2016.

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Complex treatment of children with distal malocclusions and osteopathy problems

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Abstract

Background: The aim of the study included interdisciplinary efficiency in treatment children with class II Angle malocclusions in association with osteopathy problems.
Material and methods: there were examined 30 patients of the 6- 12 years old with class II Angle malocclusion in association with osteopathy problems. The patients were divided into two groups, the main group consists of children, who have complex treatment, and in the control group they have just orthodontic treatment.
Results: The results show that patients with complex treatment have potentially positive contributions in anthropometric changes as well as in facial appearance and postural problems. The orthodontic treatment of patients, from the control group, was 1.5 times longer than in the main group. So complex treatment of patients combined with postural problems is much shorter and have a positive influence on musculoskeletal disorders than in the control group.
Conclusions: Efficient complex treatment of children with malocclusion and osteopathic problems influences the duration of orthodontic treatment of class II malocclusion conditioned by distal position of mandible.
Key words: malocclusions, orthodontic treatment, functional therapy, osteopathic treatment.

Introduction

Human body is considered to be a multifunctional and self-regulating apparatus by its nature. "The systems that have the similar direction or having a common origin and location are joined in one word–apparatus. So, the skeletal system and its compounds, organs and muscular system are all can be connected in musculoskeletal system" [1].

All components of stomatognathic system have a morphofunctional relation among them which can be able to change into pathological if one of its elements is disturbed. Among some aspects of stomatognathic system such as mandible position, dentition phase, dental or skeletal occlusion and temporomandibular disorders can be associated with body posture alteration [2,3,4,5]. That's why it is important to have a multidisciplinary approach in treatment of malocclusions in children and it should be taken into consideration by orthodontics in practical as well as in scientific research. "From contemporary point of view, mastication system is a complex interaction and interdependent system of organs participating not just in mastication but in respiration function, in the formation of voice and speech" [6]. Kalvelis D. A. once wrote that "orthodontic is a science which deals with the problems of disorders and regulation of growth and development, as well as alignment the teeth and all stomatognathic system that correlates with changes in shape, involving functional disorders of mastication system and changes in facial appearance of humans" [7]. Orthodontic is a department of dentistry, which studies the diagnosis, prevention and treatment of malalignment of teeth, dental arches and bones [8].

In normal occlusion the centric relation coincides with centric occlusion. In malocclusion this relation is not the same because lower jaw can be situated forward or back (overjet or reverse overjet). It also can be often seen no contact between teeth. We have the concept of normal occlusion which is considered to have maximal contact between teeth and make the mastication system function good [9] (fig. 1).

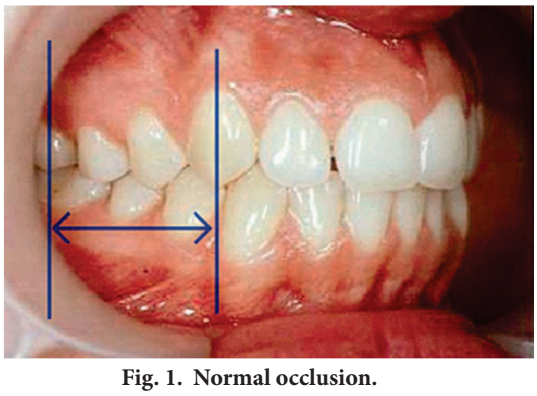

Fig. 1. Normal occlusion.

Law of creativity requires that an idea of an object should consider this object in the same content of signs. That's why orthodontics needs reliable information on the relationships and condition of the musculoskeletal system.

Osteopathy allows determining physically handicapped

disorders and the action of it is to treat this morphofunctional changes.

At the beginning of 20th century the founder of craniosacral osteopathy William Garner Sateland discovered the theory of micro-mobility of cranial bones through the observation and palpation. He revealed the existence of a slow rhythmic impulse inside the skull that is synchronized with a very perceptible sacral movement. Clinical experiments made him confirm that the base physiological complex is the generator of the body's homeostasis considered as a functional unity called by him the primary respiratory mechanism [18].

One of the basic concepts of osteopathic science is the cranio-sacral system. This anatomical and functional system includes brain shells and the place of their attachment, cranial bones and their sutures, spine including the sacrum and coccyx, membranes lining the cerebral canal as well as the impact on these structures [14].

We mostly pay attention to the component of this system called sphenooccipital synchondrosis especially on sphenoid bone which has relation to many cranial bones (fig.2). The forces developed during mastication, swallowing and contact of teeth influence the micro-mobility of sphenoid bone and other cranial bones, but direction of teeth forces influence the movement of each cranial bone and sutures. These processes have an impact on the dura mater which is attached to the bones of the cranium. Long-term action of torque is reflected in the movement of the cerebrospinal fluid along the brain and spinal cord [12,13,17].

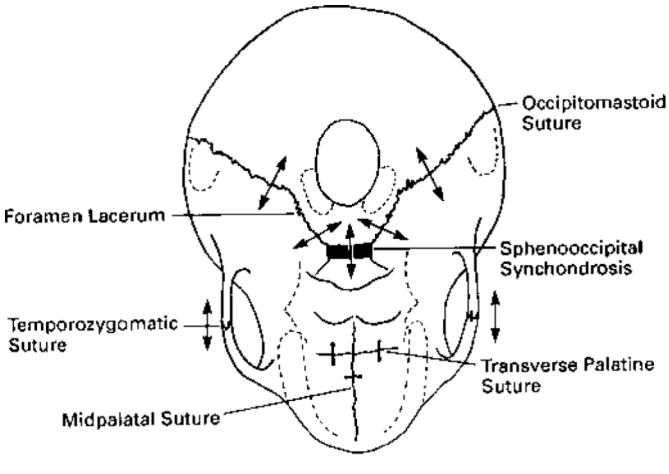

Fig. 2. Sphenooccipital synchondrosis.

Muscles of head and neck functionally integrated and work together with other muscles within the fascial spider web which is also the subject of osteopathy. If changes in the musculoskeletal system occur, there can be pathological tension of the membranes, which makes difficult normal physiological movement [18-20].

Abnormal body posture can make changes in function in all cranial bones up to the compression of the skull and as consequences of the malocclusion. Also we can see reverse action of this system [12].

Occlusion is considered a dynamic interaction of the components of the mastication system, determining the relationship of the teeth. It is a complex action included the teeth, temporomandibular joint (TMJ) and oral-facial muscles. High prevalence of malocclusion among children and adolescents and its effects on body posture, make orthodontic treatment to be indicated in children with osteopathy problems [21, 22].

Every system works relatively to a certain center. We think that the center of occlusion is sphenooccipital synchondrosis, in this way the occlusion is in direct relation to cranial-sacral system.

Anatomic and function aspects of stomatognathic system together with complex neuromuscular relations make an important field of cooperation between orthodontic and orthopedics. So, the interdisciplinary approach is being taken increasingly into account in these special cases [23].

The aim of the study is to evaluate the results of a complex treatment of class II malocclusion in children of 6-12 years old.

Material and methods

This study included 30 patients within the age range of 6-12 years with class II malocclusion, nonskeletal problems. Examining the patients in occlusion can reveal any anteroposterior problems in the buccal and in the anterior relationships (fig. 3). The patients were divided in two groups (study and control group) according to the treatment methods (tab. 1). In the study group which consisted of 12 children, it was made both orthodontic and osteopathy treatment. In the control group that consisted of 18 children it was made just orthodontic treatment (fig. 4). Informed consent was obtained from patients.

Table 1

Distribution of patients within the age range of 6-12 years with class II malocclusion in two groups during examination

Patients with class II malocclusion	Study group	Control group
Complex treatment	20% (6 patients)	30% (9 patients)
Orthodontic treatment	20% (6 patients)	30% (9 patients)

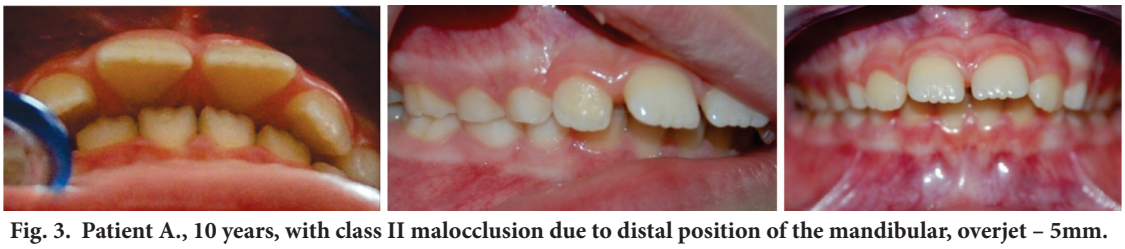

Fig. 3. Patient A., 10 years, with class II malocclusion due to distal position of the mandibular, overjet – 5mm.

Fig. 4. The Frankel II appliance, used in patient with Class II malocclusion in both groups.

Fig. 5. Facial proportion in the frontal and lateral views of the patient A., 10 years old (a) and patient B., 8 years (b) with class II malocclusion due to distal position of the mandible.

Clinical evaluation of the patient was carried out according to the general accepted scheme (interview/questionnaire, facial and dental appearance). A systematic examination of facial appearance was done in the following steps: facial proportions and symmetry in the frontal and lateral views, position of head and body (fig. 5).

It was also made a clinical examination of this patient by osteopathy which assessed interview, posture. The position of the shoulder and pelvic bones was studied (was compared the level of shoulder joints, shoulder blade, crests, presence of lateral rotation of pelvic bones). The deviation of the spinal line from the vertical axis form of back was noted (deformation by kyphosis and lordosis or smoothing of physiological bends) (fig. 6).

For establishing the dominant dysfunction were used active and passive tests by an osteopathic physician. Palpation diagnosis was performed on the skull: sphenooccipital synchondrosis, estimation of primary breathing mechanism, test of suture dysfunction of the bones of the brain and facial skull, test for functional evaluation of temporomandibular joint (TMJ) and muscles which participate in opening and closing of the mandible.

The dental cast analysis evaluates sagittal and transversal dimensions of dental arch, X-rays investigation was made as panoramic radiograph and cephalometric analysis. Cephalometric analysis used Dolphin Imaging program to determine conditionality of malocclusion- insignificant distal position of mandible. Patients who did not have any previous orthodontic treatment were included in research. Exclusion criteria included patients with complicated diseases, cranial-facial trauma, with severe neurological disorders.

Patients from both groups were treated with functional appliance Frankel II and the time of wearing this appliance lasted 8 months in the study group, 12 months in the control group. Also the patients performed osteopathic correction once in two weeks for two months, then once in three-four weeks using the main technique: technique of compression of the fourth ventricle CV-4; technique of venous sinus

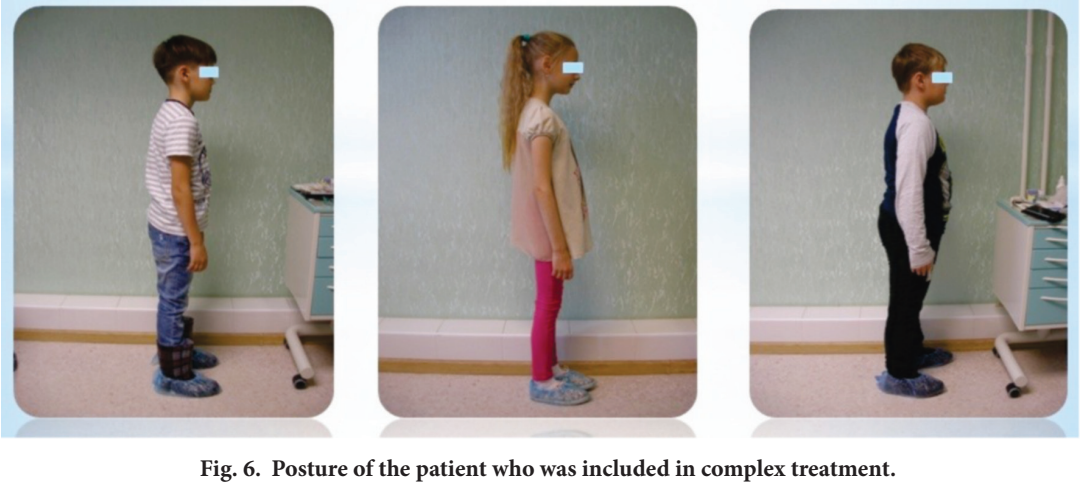

Fig. 6. Posture of the patient who was included in complex treatment. (Both orthodontic and osteopathy treatment).

drainage; decompression technique of sphenooccipital synchondrosis; elimination of suture dysfunction; technique of correction of muscles of cervical part; myofascial relaxation of mastication muscles, technique of TMJ on upper and lower jaw. After each procedure the system of the body was balanced along the craniosacral axis. The protocol of this study was approved by the Human Research Ethics Committee in State University from Samara. The parents were informed about the procedures of the study and provided their written informed consent prior to child's participation in the research.

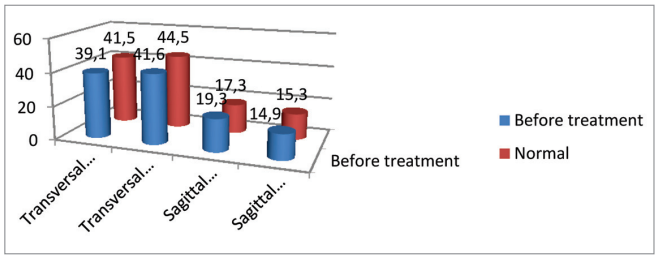

Fig. 7. Changes in dimensions of dental arches before complex treatment of class II malocclusion in patients of 6-12 years compared to normal.

Results and discussion

The testing osteopathy revealed the following dysfunctions:

- Sphenooccipital synchondrosis, in 100% in study and control groups
- Primary breathing mechanism in 100% in study and control groups
- Deregulation of 4th horizontal lines in 100% in study and control groups
- Hyoid bone in 85% of patients from study and control groups

So, after the osteopathy testing was made, the same initial level of certain dysfunction was identified in study and control groups. Complex treatment was conducted in the study group while the control group underwent just orthodontic treatment.

Changes in dimensions of dental arches that were seen before complex treatment of class II malocclusion in patients of 6-12 years old in both groups are identical to normal and are shown in figure 7.

After 8±0.3 months of complex treatment there was a positive evolution in changes of the anthropometric indicators (fig. 8) as well as in facial appearance and postural problems (fig. 9).

Patients from study groups were treated by correction of the posture under the scheme that was described above, once in two weeks during two months, continued once in a week. In 50% of patients from the study group were noted reduced cases of sphenooccipital synchondrosis disorders (p=2.1; p<0.05) compared to the control group (p=3.1; p<0.001). Also there were changes in primary breathing mechanism in 45% of patients from the study group (p=1,97; p<0.05), but in the control group – in 85% of cases (p=2,58; p< 0,01), it was also reduced dysfunction of hyoid bone in 35% of cases (p=1,0; p>0,05), but in the control group in 75% of cases (p=2,58; p<0,01).

Consequently, making the diagnosis correctly, determining the conditionality of malocclusion, making up a complex treatment plan and choosing orthodontic device in both study and control groups lead to decrease of the function disorders in human body.

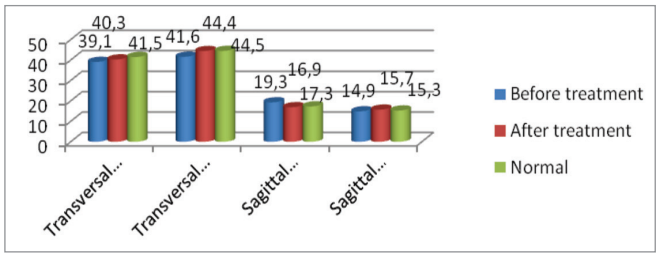

Fig. 8. Changes in anthropometric measurements on models before and after complex treatment of distal occlusion in patients of 6-12 years old compared to normal.

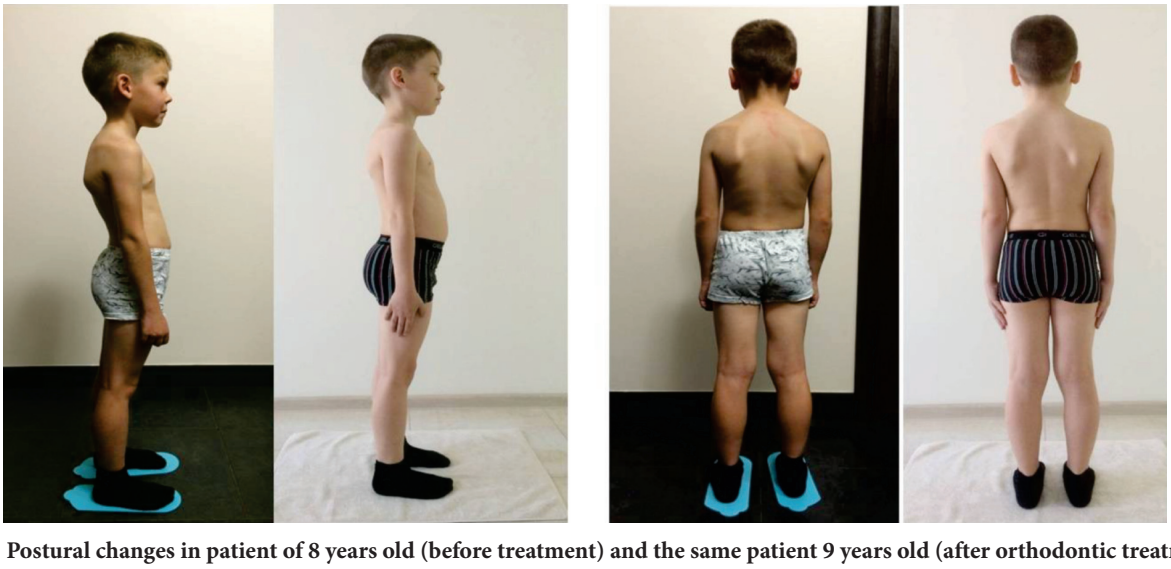

Fig. 9. Postural changes in patient of 8 years old (before treatment) and the same patient 9 years old (after orthodontic treatment).

The results showed that after 12,0±0,5 months was observed positive evolution in changes of anthropometric measurements as well as in osteopathic disorders. Control group established the same changes in anthropometric measurements because the initial data were identical.

The timing of treatment was different in both groups: the control group didn't have any help from osteopathic doctor and as a result the time of orthodontic treatment increased (p=2,61; p<0,01).

The statements of scientific research confirmed all the processes of the relations and dependencies in the body. The problems of malocclusions are eliminated faster, in less time with positive solution of osteopathic problems. At the same time, just orthodontic treatment of distal malocclusion in children 6-12 years old also received positive solution in dysfunctional osteopathic disorders, but it was more time consuming.

Conclusions

1. Osteopathic treatment really influences in reducing the time of distal malocclusion treatment, dental-alveolar form due to insignificant distal position of the mandible.

2. Making correctly diagnosis, determining the conditionality of malocclusion, planning a complex treatment, choosing orthodontic appliances in both groups lead to reducing the cases of dysfunctions in body.

References

1. Mikhailov SS. Anatomiia cheloveka [Human's anatomy]. Moscow: Meditsina; 1984. 704 p. Russian.
2. Sakaguchi K, Mehta NR, Abdallah EF, Forgione AG, Hirayama H, Kawasaki T, et al. Examination of the relationship between mandibular position and body posture. Cranio. 2007;25(4):237-49.
3. Ishizawa T, Xu H, Onodera K, Ooya K. Weight distributions on soles of feet in the primary and early permanent dentition with normal occlusion. J Clin Pediatr Dent. 2005;30(2):165-8.
4. Nobili A, Adversi R. Relationship between posture and occlusion: a clinic and experimental investigation. Cranio. 1996;14(4):274-85.
5. Perinetti G. Temporomandibular disorders do not correlate with detectable alterations in body posture. J Contemp Dent Pract. 2007;8(5):60-7.
6. Kurliandskii VIu. Zubocheliustnye anomalii u detei i metody lecheniia: Ortodontiia. [Dentofacial anomalies in children and treatment methods: Orthodontics]. Moscow: Medgiz; 1957. 222 p. Russian.
7. Kalvelis DA. Ortodontiia. Zubo-cheliustnye anomalii v klinike i eksperimente. [Orthodontics. Tooth- maxillary anomalies in the clinic and experiment]. Leningrad: Meditsina; 1964. 238 p. Russian.
8. Petrovskii BV, editor. Entsiklopedicheskii slovar' meditsinskikh terminov. T.2. [Encyclopaedic dictionary of medical terms]. Moscow: Sovetskaia entsiklopediia; 1983. 500 p. Russian.
9. Persin LS. Vidy zubocheliustnykh anomalii i ikh klassifitsirovanie [Types of dentoalveolar anomalies and their classification]. Moscow: MGMSU; 2006. 23 p. Russian.
10. Baroeva IV, Kolesov MA, Persin LS, Slabkovskaia AB. [Modification of the Persin appliance for treatment of patients with distal occlusion]. Ortodontiia. 2014;4(68):44-50. Russian.
11. Postnikov MA. Ortodonticheskoe lechenie patsientov s anomaliiami okkliuzii v sagittal'nom napravlenii i chastichnoi adentiei. [Orthodontic treatment of patients with anomalies of occlusion in the sagittal direction and partial adentia]. Samara: Ofort; 2015. 120 p. Russian.
12. Amigues J-P. La pratique ostéopathique: L'articulation temporomandibulaire [Osteopathic practice: The temporomandibular joint]. Aix en Provence: Verlaque; 1990. 240 p. French.
13. Magoun HI. Osteopathy in the cranial field. Kirksville: Journal Printing Co; 1976. 380 p.
14. Carlson J. Fiziologicheskaia okkliuziia [Physiologic occlusion]. Moscow: Midwest Press; 2009. 218 p. Russian.
15. Klozad MA, Daraian B. [Osteopathic concept of occlusion]. 2007. 25 p. Russian.
16. Retzlaff EW, Michael DK, Roppel RM. Cranial bone mobility. J Am Osteopath Assoc. 1975;74(9):869-73.
17. Upledger JE, Vredevoogd JD. Kraniosakral'naia terapiia. Ch.1. [Craniosacral Therapy. Part 1]. St. Petersburg: Severnaia Zvezda; 2010. 390 p. Russian.
18. Postnikov MA. [Optimization of orthodontic treatment of children with mesial occlusion during the shift and after the change of teeth] [dissertation]. Samara (Russia): Samara State Medical University; 2016. 316 p. Russian.
19. Postnikov MA, Stepanov GV, Pankratova NV, Kirilin MM, Malkina VD. [The use of cephalometric analysis in the computer program "Dolphin Imaging" in planning the treatment of patients with maxillofacial anomalies]. Ortodontiia 2017;1(77):16-27. Russian.
20. Bredikhin AV, Bredikhin KA, Chekha OA. [Craniosacral system, its components and signs of dysfunction]. Meditsinskie novosti. 2014;(11):43-49. Russian.
21. Brunelle JA, Bhat M, Lipton JA. Prevalence and distribution of selected occlusion characteristics in the US population, 1988-1991. J Dent Res. 1996;75 Spec No:706-13.
22. Perinetti G, Cordella C, Pellegrini F, Esposito P. The prevalence of malocclusion traits and their correlations in mixed dentition children: results from the Italian OHSAR Survey. Oral Health Prev Dent. 2008;6(2):119-29.
23. Korbmacher H, Eggers-Stroeder G, Koch L, Kahl-Nieke B. Correlations between anomalies of the dentition and pathologies of the locomotor system – a literature review. J Orofac Orthop. 2004;65(3):190-203. doi:10.1007/s00056-004-0305-3.

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Tuberculosis evolution and treatment outcome in drug addicted patients

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Abstract

Background: Drug injection is considered an important issue for the public health of the Republic of Moldova. In the RM the intravenous drug users (IVDU) are the key population for HIV infection, B and C hepatitis, TB and sexual transmitted diseases. The aim of the study was to assess the tuberculosis evolution and treatment outcome in intravenous drug addicted patients.

Material and methods: A retrospective selective, descriptive study targeting socioeconomic, demographic, economic and epidemiological peculiarities, case-management, diagnosis of radiological aspects and microbiological characteristics of 233 patients with pulmonary tuberculosis registered in Chisinau city from 2012 to 2016 among them 48 IVDU was performed. The results were compared with a sample of 34 IVDU.

Results: Men were predisposed for drug addiction and the age is younger in addicts with tuberculosis. Socioeconomic vulnerability is extended in the selected groups; however, the poverty deepness was more important in addicted patients with tuberculosis. Close contact with a sick patient predominated in addicts with tuberculosis, but nobody assessed the contact in addicts without tuberculosis. Associated diseases predominated in addicts with or without tuberculosis, more frequently were: HIV infected individuals, viral hepatitis and neurological disorders.

Conclusions: Risk factors for tuberculosis in drug addicts were: unemployment and associated lack of health insurance, patient's homeless state, middle adulthood and the immune suppressive condition – HIV infection. Major disease-related characteristic in IVDU patients was the presence of severe destructive forms of tuberculosis and "loss to follow-up" as outcome.

Key words: tuberculosis, drug use, addiction, risk factors.

Introduction

Drug use has important criminal and social implication in the Republic of Moldova (RM) [14, 17, 21, 23, 30, 31]. According to the national epidemiological data in 2009 on the left side of the territory of the RM were diagnosed 1333 new cases of drug users, among them 6.5% were women. The RM is an agrarian country and the production of the vegetable drugs from poppy and hemp raw material is affordable [31]. Despite this, the prices for opium, marijuana, heroin, cocaine and other drug extracts is continuously increasing. The total number of drug-related crimes during the period 2008-2010 decreased (2008-2103 crimes, 2009-1865 crimes, 2015-745 crimes), but the number of crimes related to the narcotics trade constituted only 15% [12]. So, it can be deducted that most of the trials are related against drug users and not against the drug trafficking. [31] The development of new narcotic and new generation psychotropic substances has serious health consequences. The prevalence of HIV infection among Moldovan intravenous drug users (IVDU) reached 21.0% in 2008 [3]. The globalization of the illicit drug use determined the development and improvement of the legislative basis for preventing consumption, combating illicit drug trafficking, also counseling and treatment of the drug addiction [27, 28, 35]. An important step in the prevention of the consumption and combating drug trafficking was the approval of the National Anti-drug Strategy for 2011-2018 [12]. The strategy was developed according to the European Anti-drug Strategy, Convention on Narcotic Drugs, Convention against Illicit Traffic of Narcotic Drugs and Psychotropic Substances, Declaration about the Principles on the Drug Demand Reduction [27, 28]. The implementation of the Strategy among young IVDU diminished the rate of died people due to the overdoses from 131 in 2002, to 8 in 2008 and 12 in 2009. However, the rate of IVDU with HIV infection reached 8.5%, with viral hepatitis – 9.1%, with TB 1.24% and sexual transmitted infection 12.7% in 2013 [17]. The IVDU have various barriers in the accessing health care and getting an effective treatment for their addictive conditions and associated diseases [9]. High degree of stigma and social marginalization contribute to the lack of the short-term residential treatment, therapeutic communities and rehabilitation services [34]. Due to highly repressive policies the IVDU are frequently incarcerated [31]. Prisons represent an extensive infectious reservoir [8, 29]. Specific needs of the key population IVDU are underestimated contributing to gaps in management of the diagnosis and treatment of tuberculosis [5, 35]. In several high tuberculosis countries the systematic screening of drug users through the chest X-ray and interferon-gamma assay is an important action in the control of the disease at the national level [7, 10, 13, 18].

Tuberculosis represents one of the 10 leading causes of death globally, according to the WHO Global Tuberculosis Report [40]. After the Millennium Summit, that was held in 2000, the 191 member states of the United Nations Organization adopted the 8 Millennium Development Goals (MDGs), which targeted the main directions of global efforts [32]. A major influence obtained the activities for

reduction of extreme poverty (Objective 1), fighting HIV/AIDS, malaria and tuberculosis (objective 6). Despite of important progress in obtaining the results, on 15 September 2015 there were adopted new 16 objectives: Sustainable Development Goals (SDGs), oriented to the poverty alleviation, reducing inequalities, good health, well-being, and other objectives to be realized in the next 15 years [33]. The comprehensive approach to tuberculosis was identified in several objectives: to put an end to poverty and hunger, fight HIV/SIDA, malaria and tuberculosis, to provide sustainable development of communities (target – treatment support of patients with HIV/AIDS, tuberculosis, etc.). It demonstrated the importance of activities implemented to combat the poverty-related diseases and contributing conditions, one of them – the drug use [38]. As an immediate feedback to SDGs World Health Organization adopted the End TB Strategy in 2014 that targets the decrease with 90% of death cases due to tuberculosis and decrease with 80% incidence till 2030, comparing with 2015 [37]. The last WHO report estimated the RM remains one of the highest burdened countries by drug resistant tuberculosis with an estimated rate of 26% among new cases and 56% among previously treated cases [43]. The social determinants of tuberculosis were well recognized [39, 40, 41]. It was identified a strong relationship between social vulnerability and psychotropic substance abuse: tobacco smoking, alcohol abuse and drug use. [39] Addiction is a compulsive need for use of a habit-forming substance (nicotine, alcohol, or heroine) characterized by tolerance and well-defined symptoms upon withdrawal [9, 21, 39]. From the physiopathology point of view, addiction affects neurotransmission and interactions within reward structures of the brain, including the *nucleus accumbens, anterior cingulate cortex*, basal forebrain and *amygdale*. It affects neurotransmission and interactions between cortical and *hippocampus* circuits and brain reward structures, such as the memory of previous exposures to rewards (ex. alcohol, drugs, nicotine, food, sex). It leads to a biological and behavioral response to external triggers which engage the person in addictive behaviors. The frontal cortex of the brain and underlying white matter connections are fundamental in the manifestations of altered impulse control, judgment, and the dysfunctional pursuit of rewards. The frontal lobes are important in inhibiting impulsivity and in assisting individuals to appropriately delay gratification. Frontal lobe morphology, connectivity and functioning are still in the process of maturation during adolescence and young adulthood. The early exposure to substance use of a young person is a significant factor in the development of addiction. Addiction is characterized by: 1. inability to consistently abstain; 2. impairment in behavioral control; 3. craving or increased "hunger" for nicotine, alcohol, drugs or rewarding experiences; 4. diminished recognition of significant problems and interpersonal relationships; 5. dysfunctional emotional response. During addiction there is a significant impairment in executive functioning [35]. It manifests in problems with perception, learning, impulse control, compulsivity, and judgment. People with addiction often manifest a lower readiness to change their behaviors. The developing of frontal lobes of adolescents and young adults may contribute to disturbance of executive functioning and predispose young people to engage in "high risk" behaviors, including engaging in smoking, alcohol or other drug use. Addiction is more than a behavioral disorder. Features of addiction include aspects of a person's cognitions, emotions, and interactions with others. It consists in period of relapse and remission. Clinical interventions are difficult to perform and are quite ineffective. Close monitoring of the behaviors of the individual and contingency management can contribute to positive clinical outcomes. The engagement in health promotion activities which underline personal responsibility and accountability, connection with others, and personal growth also contribute to the patient's recovery. It is important to note that addiction can cause disability or premature death, especially when is left untreated or is treated inadequately. Self-management with mutual support and professional care provided by trained staff is very important in the person's recovery from addiction [19, 21, 35]. Drug addiction is a chronic brain disease that causes the compulsive behavior to seek and use the substances. The addiction changes the decision-making capacity of the person and it should be treated like any other illness. Withdrawal syndrome is a pathological condition directly related to addiction, which occurs when the addicted consumer drops the doses. If dependence is strong, the withdrawal can manifest violent: hypertension, palpitations, deep sweating, muscle pain, joint pain, headache, vertigo, vomiting, psychomotor excitation, convulsions, anxiety, insomnia, psychological and physical discomfort. Methadone is a synthetic opioid with pharmacological effects similar to morphine products. Methadone is bioavailable consumed by oral or injected way. The main effect of methadone is expressed by receptors and is similar to that produced by endogenous opiates such as encephaline and endorphin. Methadone increases the elimination of neuromediators: acetylcholine, noradrenaline and dopamine. Methadone decreases the desire to use heroin and other opiates, eliminates the symptoms of the withdrawal and blocks the euphoric effects of other drugs or substances [21].

Genetic factors account one half of the likelihood that an individual will develop drug addiction. However the interaction of the environmental factors increases the predisposition for addiction. Culture also plays an important role for increasing the possibility that the persons with biological vulnerabilities develop addictions. Other factors that contribute to the appearance of addiction, leading to its characteristic bio-psycho-socio-spiritual manifestations are: 1. Biological failure in the function of reward circuits; 2. Repeated engagement in drug use or other addictive behaviors (alcohol, tobacco smoking), causing neuro-adaptation and engagement in addictive behaviors; 3 Cognitive and affective distortions, which impair perceptions and fail the ability to deal with feelings, resulting in significant self-deception; 4. Disruption from the healthy social supports; 5. Appearance of the problems in interpersonal relationships

which impact the development of addictive behavior; 6. Exposure to trauma or stress that determines an individual's coping abilities; 6. Distortion in meaning, purpose and values that establish attitudes, thinking and behavior; 7. distortions in the connection with person's self and community; 8. Co-morbid psychiatric disorders in persons who engage in substance use or other addictive behaviors [5, 36].

The socioeconomic and political crisis through which passes the RM determines the increasing of the social vulnerable population, among which the addictive habits are very frequent [3, 23, 30, 31]. The health-related risks in addicts are highly expressed. It was established a 55 times higher risk to develop tuberculosis among persons with alcohol use disorders, 10 times higher risk to develop tuberculosis among tobacco smokers and non-estimated high risk in IVDU population. The susceptibility for tuberculosis is determined by: 1. The social mixing patterns of addicts; 2. Weak immune system due to associated conditions (malnutrition, HIV-infection, gastro-intestinal disorders, etc.) leading to reactivation of the latent TB infection; 3. Toxic effects of drugs on different organs; 4. Micro- and macronutrient deficiency.

So, the aim of the study was to assess the tuberculosis features and treatment outcome in the intravenous drug addicted patients.

Objectives were: 1. Assessment of the tuberculosis extension among the population of the IVDU from the Republic of Moldova and its epidemiological evolution; 2. Assessment of general, socioeconomic and epidemiological risk factors of IVDU with tuberculosis and comparing with two control samples of IVDU and pulmonary tuberculosis; 3. Evaluation of the case-management, diagnosis, radiological aspects and microbiological characteristics of IVDU with tuberculosis comparing with a control sample of pulmonary tuberculosis; 4. Assessment of the specialized health care accessibility of the IVDU with tuberculosis comparing with the control sample of pulmonary tuberculosis.

Material and methods

It was performed a retrospective selective, descriptive study targeting social, demographic, economic and epidemiological peculiarities, case-management, diagnosis, radiological aspects and microbiological characteristics of 233 patients with pulmonary tuberculosis registered in Chisinau during the period 2012 to 2016. Among them, 48 were established being IVDU and 185 patients had no drug addiction. The results were compared with a sample of 34 IVDU without tuberculosis or a history of a previous treatment for tuberculosis. The electronic system for monitoring and follow-up of tuberculosis cases (SIME TB) was used for the selection. Data were extracted from the statistic templates F089/1-e "Declaration about patient's established diagnosis of new case/relapse of active tuberculosis and restart of the treatment and its outcomes" and F090/e "Declaration and follow up of multidrug-resistant tuberculosis". Inclusion criteria in the study group (SG): age more than 18 years old, tuberculosis and associated intravenous drug addiction, signed informed consent allowed the selection of 48 patients. In the 1st control group (1st CG) were selected 185 new diagnosed cases with pulmonary tuberculosis without associated drug addiction, also in the past. Assessing the type of the drug addiction it was established that 45 (93.7%) of the IVDU from the SG had mental illness and behavior disorders due to the opioid use and 3 (6.3%) had mental illness and behavior disorders due to multiple drug use of psychoactive substances. In the 2nd CG 14 (41.2%) patients had mental illness and behavior disorders due to the opioid drug use, 16 (47.1%) had mental illness and behavior disorders due to multiple drug use of psychoactive substances and 4 (11.7%) patients had mental illness and behavior disorders due to the non-opioid substances use. Most of patients, 21 (61.7%) cases, were previously treated. Each second patient had a relapse – 10 (47.7%), 3 (14.3%) were after loss to follow-up, 7 (33.3%) had methadone addiction and 1 (4.7%) required the preventive treatment. Each third patient from the 2nd CG, 12 (35.3%), was hospitalized for the first time for the treatment of the withdrawal syndrome. Each second patient of the 2nd SG (21 (61.7%) had more than 10 years of drug use and the rest of the group had a shorter life history of addiction. Most of them, 26 (76.5%) cases, directly addressed to the narcological specialized institution and 8 (23.5%) patients by the third parts (relatives, police). Assessing the drug types, most of them – 14 (41.2%) used heroin, 10 (29.4%) a combination of drug with heroin and 10 (29.4%) other types of drugs.

All patients with tuberculosis were investigated and treated according to the National Clinical Protocol 123 "Tuberculosis in Adults" [22]. The "new case" was considered the patient never treated for TB or has taken anti-TB drugs less than one month. Previously treated were considered patients with relapse, treatment failure and loss to follow-up. For assessing the socioeconomic barriers and health care accessibility of the IVDU the 2nd control group (2nd CG) was selected and was composed of 34 patients. The investigational schedule included demographic, social and epidemiological data: sex (male/female ratio), age (distribution in age groups), demographic characteristics (urban/rural residence), educational level, socio-economic status (employed, unemployed, retired, disabled, student), health insurance status (lack of insurance), migration and detention history, presence of high risk (close contact with an infectious source, comorbidities, health care seeking behavior, way of the patient's detection). Enrolled patients from the SG were selected in the period 01.01.2012-31.12.2016. Patients from the 1st CG were enrolled during the period 01.01.2015-31.12.2015. For patients from the 2nd CG the period of selection – during 01.01.2017-31.05.2018. The samples of patients with tuberculosis were managed in the frame of the clinical services of the Municipal Clinical Hospital of Pneumophtisiology of Chisinau and the samples of IVDU were managed in the Republican Narcological Dispensary of the Republic of Moldova. Statistic analysis was carried out using the quantitative and qualitative research

methods [24, 26]. Statistical survey was performed using Microsoft Excel XP soft.

Results

According to the data obtained from the monitoring and follow-up of cases system during the period 2011-2018 it was established an important fluctuation of registered tuberculosis among Moldovan drug users. In 2011 were registered 56 cases, 2012 – 48 cases, 2013 – 71 cases, 2014 – 70 cases, 2015 – 43 cases, 2016 – 47 cases and in 2017 – 55 cases [2]. Distributing selected patients, according to the sex it was established the statistical predominance of men in all three groups, with the highest rate in the 2nd CG. So, men were 33 (97.1%) in the 2nd CG, 42 (87.5%) in the SG and 138 (74.6%) in the 1st CG. The male/female ratio was 7/1 in the SG, 2.9/1 in the 2nd CG and 33/1 in the 1st CG. Men predominated statistically in the 2nd CG compared with the SG and the 1st CG. Repartition of the patients into age groups, according to the WHO recommendation identified that the largest subgroup in the SG was 35-44 years, followed by the 25-34 years in the SG. In the 1st CG predominated the 35-44 years group, followed in the same proportion of those with 45-54 years and 25-34 years old. In the 2nd CG the majority of patients were between 25-34 years old and every third patient was between 35-44 years old. Comparing the groups was identified statistical predominance of the young group of 25-34 years old in the 2nd CG compared with the SG and the 1st CG, as well in the SG compared with the 1st CG. The middle adulthood group of 35-44 years old predominated in the SG compared with the 1st CG and the 2nd CG. Patients aged between 45-55 years old predominated in the 1st CG compared with the 2nd CG and the SG. Obtained data demonstrated that the age for acquiring tuberculosis is younger in addicted patients than in those without drug addiction. The patients with tuberculosis had urban residence in two thirds of the groups (SG and 1st CG). The majority of the 2nd CG had urban residence. The statistical predominance of the urban residence was identified in the 2nd CG compared with the SG and the 1st CG. Homeless patients were in a similar proportion in both groups with tuberculosis (SG and the 1st CG) which statistically predominated compared with the 2nd CG (tab. 1). So, distributing patients according to the biological characteristics it was argued that young IVDU men, from urban areas had high probability to get tuberculosis.

Distributing patients according to the economic status, it was established that employed persons, who are contributing to the health budget by paying taxes, health insurance policy and social taxes predominated in the 2nd CG compared with the study group. As to health insurance and social security more frequently had them patients from the same group. Unemployed patients statistically predominated in the SG

Table 1

Distribution of patients by sex, age and demographic data

Indices	Sex Age Residence	Study group	1st control group	2nd control group
		N=48 (P%)	N=185 (P%)	N=34 (P %)
Sex	Men	42 (87.5) *	138 (74.59) ●●●	33 (97.1)
	Women	6 (12.5) *	47 (25.41)●●●	1 (2.9)
Age groups	18-24 years	0***	24 (12.97)	3 (8.8)
	25-34 years	20 (41.7) ** #	36 (19.46) ●●●	20 (58.8)
	35-44 years	27 (56.2)*** #	52 (28.11)	10 (29.4)
	45-55 years	1 (2.1) **	42 (22.73) ●●●	1 (2.9)
	>55 years	0***	31 (16.4) ●●●	0
Residence	Urban	35 (72.9) #	139 (75.13) ●●●	31 (91.2)
	Rural	5 (10.2) **	46 (24.86)●●●	3 (8.8)
	Homeless	8 (16.7) #	29 (15.68) ●●●	0

Note: Applied statistical test: paired simple T-test, P – probability.

Statistically significant differences between: study group IVDU-TB compared to the 1st control group TB: * – p<0.05;** – p<0.01; *** – p<0.001; study group IVDU-TB compared to the 2nd control group TB: # p<0.05; ## – p<0.01; ### – p<0.001; the 1st control group TB compared to the 2nd control group: ● – p<0.05; ●● – p<0.01; ●●● – p<0.001;

Table 2

Distribution according to the socioeconomic data

Indices	Economic state	Study group	1st control group	2nd control group
		N=48 (P%)	N=185 (P%)	N=34 (P%)
Economicaly stable	Employed	2 (4.7) ##	25 (13.51) ●●	10 (29.4)
	Insured	8 (16.7) ***###	46 (24.8)	12 (35.3)
Economicaly vulnerable	Disease disabled	3 (6.3)	14 (7.57)	3 (11)
	Retired	0	15 (8.11)	1 (2.9)
	Students	0	7 (3.78)	0
	Unemployed	43 (89.6) ***###	124 (67.03)	20 (58.8)
	Lack of health insurance	40 (83.3) *** ###	139 (75.13*)	22 (64.7)

Note: Applied statistical test: paired simple T-test, P – probability;

Statistically significant differences between: study group IVDU-TB compared to the 1st control group TB: * – p<0.05;** – p<0.01; *** – p<0.001; study group IVDU-TB compared to the 2nd control group TB: # p<0.05; ## – p<0.01; ### – p<0.001; the 1st control group TB compared to the 2nd control group: ● – p<0.05; ●● – p<0.01; ●●● – p<0.001;

compared with the 1st CG and the 2nd CG. The same rate was established regarding the patients who lacked the health and social insurance. Despite the predominance of the disabled patients, retired and students in the 1st SG compared with other groups, the statistical significance was not achieved. So, the socioeconomic vulnerability was identified in all selected patients, but its deepness was more evident in the IVDU patients with tuberculosis (tab. 2).

Assessing the educational level, it was established that most of the patients from the SG and the 1st CG graduated upper secondary level of the education. The rate of patients with upper secondary level statistically predominated in the SG and the 1st CG compared with the 2nd CG. Primary and low secondary education statically predominated in the 2nd CG compared with the SG and 1st CG. Secondary technical vocational education predominated in the 1st CG compared with the SG and the 2nd CG, but the statistical difference was not achieved. Bachelor studies predominated in the 2nd CG but the statistical difference was not achieved either. Exposed data are revealed in the table 3.

Hierarchy of risk groups, according to the widest rate of patients identified that the major social characteristics of patients from all three groups were the vulnerable economic state and living in poor conditions. Extreme poverty, expressed by homelessness was identified only in the study group and the 1st CG. History of migration during the last year statistically predominated in the 1st CG compared with the SG and the 2nd CG. History of imprisonment statistically predominated in the SG compared with the 1st CG. Close infectious contact, established as being a member of a family TB clusters statistically predominated in the SG compared with the 1st CG. There were no available data for the 2nd CG.

The review of comorbidities in 3 substances users established a high prevalence of HIV infection, hepatitis C and sexual transmitted diseases in most of the researches [15, 16, 29].

Table 3

Distribution according to the last graduate level

Education	Study group	1st control group	2nd control group
	N=48 (P%)	N=185 (P%)	N=34 (P %)
Primary &low secondary education	10 (20.8) ###	46 (24.8)●●●	22 (64.7)
Upper secondary education	27 (56.2) ###	91 (49.2)●●●	1 (2.9)
Secondary technical vocational education	7 (14.6)	36 (19.5)	3 (8.8)
Bachelor studies	4 (8.3)	12 (6.5)	4 (11.7)

Note: Applied statistical test: paired simple T-test, P – probability;

Statistically significant differences between: study group IVDU-TB compared to the 1st control group TB: * – p<0.05;** – p<0.01; *** – p<0.001; study group IVDU-TB compared to the 2nd control group TB: # p<0.05; ## – p<0.01; ### – p<0.001; the 1st control group TB compared to the 2nd control group: ● – p<0.05; ●● – p<0.01; ●●● – p<0.001;

Table 4

Distribution according to the risk groups

Category	Risks	Study group	1st control group	2nd control group
		N=48 (P%)	N=185 (P%)	N=34 (P %)
Socioeconomic	Poverty	29 (60.4)	106 (57.29) ●●●	14 (41.2)
	Homelessness	8 (16.7) ###	29 (15.68) ●●●	0
	Migration	2 (4.2)	24 (12.97) ●●●	0
	History of detention	16 (33.3) ***	2 (1.1)	7 (20.6)
Biological	Close contact	15 (31.2) ***	15 (8.11)	NA
	Associated diseases	48 (100) ***	50 (27.03) ●●●	31 (91.2)
	HIV-infection	21 (43.7) ***###	11 (5.94)	1 (2.9)
	Chronic alcoholism	3 (6.2) ***	13 (7.03) ●●●	12 (35.2)
	CRD	8 (16.7)	15 (8.2)	7 (20.6)
	GID	10 (20.8) ###	4 (2.1) ●●●	28 (82.3)
	Viral hepatitis	2 (4.6) #	2 (1.1) ●●●	8 (23.5)
	Mental disorders (excluding IVDU)	1 (2.1) ###	4 (2.16)	31 (91.2)
	Neoplasm	1 (2.1)	1 (0.5)	0
	Renal diseases			4 (11.7)
	Others	2 (4.6)		

Note: Applied statistical test: paired simple T-test, P – probability; NA-non available, CRD-chronic respiratory diseases, GID-gastrointestinal diseases.

Statistically significant differences between: study group IVDU-TB compared to the 1st control group TB: * – p<0.05; ** – p<0.01; *** – p<0.001; study group IVDU-TB compared to the 2nd control group TB: # p<0.05; ## – p<0.01; ### – p<0.001; the 1st control group TB compared to the 2nd control group: ● – p<0.05; ●● – p<0.01; ●●● – p<0.001;

Comorbid patients statistically predominated in the SG and the 2rd group compared with the 1st CG. Among associated diseases, HIV infection was established in every second patient of the SG, which statistically predominated compared with the 1st CG and the 2nd CG. Chronic alcoholism statistically predominated in the 2nd CG compared with the SG and the 1st CG. Chronic respiratory diseases predominated in the SG and the 2nd CG. Gastro-intestinal disorders predominated in the 2nd CG compared with SG and the 2nd CG. Viral hepatitis predominated in the 2nd CG compared with SG and the 1st CG. Mental disorders predominated in the 2nd CG compared with the SG and the 1st CG. In this subgroup were not included illnesses due to the drug addiction. No other statistical differences were established among groups regarding the associated diseases. Data were revealed in the table 4.

Studying case-management it was identified that general medical staff in terms of the general practitioners, was involved in the detection of one half of the 1st CG and one third of the SG. The rate of high risk groups investigated through active screening was low in both groups, but the value was lower in the SG. Despite that drug users are a high risk group target by the screening procedures their proportion detected by active screening was lower compared with the general population. Detected by the specialists as symptomatic cases were in a higher proportion in the SG compared with the control group, but the statistical difference was not achieved. Direct addressing to the specialized services for diagnosis and treatment of tuberculosis was higher in the SG and demonstrated multiple barriers for accessing the healthcare at the primary level. Other ways of detection predominated in the control group and were used for diagnosis of patients hospitalized in somatic clinical hospitals. Obtained data established a lower accessibility of the health care services by the IVDU, due to their social vulnerability. The accessing of the tuberculosis specialized institutions was obtained by independent addressing avoiding the primary health care staff in a large proportion of cases. Information is exposed in the table 5.

Table 5

Case-management characteristics of tuberculosis patients

Health level	Detection ways	Study group	1st control group
		N=48 (P%)	N=185 (P%)
PHC	Detected by GPs-symptomatic	16 (33.3) ***	103 (55.67)
	Detected by GPs-screening of HRG	5 (10.4)	28 (15.13)
Ambulatory specialized level	Detected by SP-symptomatics	13 (27.1)	34 (18.38)
	Detected by SP-screening of HRG	4 (8.3)	7 (3.78)
Hospital level	Direct addressing	10 (20.8) **	10 (5.41)
Others	Other ways	0	3 (1.62)

Note: Applied statistical test: paired simple T-test, P – probability; GP-general practitioner, SP-specialist, HRG-high risk group. Statistically significant differences between: study group IVDU-TB compared to the 1st control group TB: * – p<0.05;** – p<0.01; *** – p<0.001.

Distributing patients according to the registered case type was identified that every second patient was a "new case" – 23 (47.9%) and every fourth was "relapse" – 12 (25%). In a similar rate were patients included in the treatment after a previous loss to follow-up and after a treatment failure – 6 (12.5%) patients for each category type. Diagnosed and transferred from abroad was 1 (2.1%) patient. Following the diagnosis only 46 (95.8%) patients started the treatment. One patient died and diagnosis was established after his death and one patient was transferred for treatment abroad (2.1% for each category type). The standard treatment for new drug-susceptible tuberculosis in the RM is used since 2000 and lasts 6 months, according to the WHO recommendation [22, 42]. It consists in a two phases regimen with four first-line drugs: isoniazid (H), rifampicin (R), ethambutol (E) and pyrazinamide (Z) in the intensive phase and two first-line drugs: isoniazid and rifampicin in the continuation phase. For previously treated cases was used a two phase regimen which lasts 8 months: 3 months with H, R, E, Z and streptomycin and 5 months with H, R and E. Patients with rifampicin-resistant or MDR-TB were treated with second-line drugs for 18 months or more divided in two phases The regimen composition during the intensive phase lasts 6 months and included kanamycin (Km) or capreomycin (Cm), levofloxacin (Lfx), para-amino salicylic acid (PAS), ethionamide (Eto), cycloserine (Cs) and pyrazinamide (Z) and for continuation phases during 12-18 months of Lfx, PAS, Etho, Cs and Z.

Identifying the clinical radiological forms of pulmonary tuberculosis it was established that infiltrative opacities were identified in a similar proportion in both groups. Destructive forms of pulmonary tuberculosis were identified in a similar proportion in both groups, however the severest destructive form defined fibro-cavernous tuberculosis was diagnosed only in the study group. Appreciating clinical radiological forms it was established that the majority of cases had pulmonary infiltrative tuberculosis, however statistical predominance was identified in the control group. Other radiological forms: disseminated tuberculosis and fibro-cavernous tuberculosis prevailed in the study group, but the statistical difference was obtained only for the fibro-cavernous tuberculosis. Distributing patients according to the number of the affected lungs, it was established that both lungs were involved in the majority of both groups, but the predominantly in the study group. No differences were established regarding the rate of patients with parenchimal destruction in one or both lungs. Extensive forms, affecting 3 and more segments were diagnosed in a similar proportion in both groups (tab. 6).

When assessing the laboratory features of the enrolled pulmonary tuberculosis cases, it was identified that one half of patients were microscopic positive for acid-fast-bacilli in both groups. A similar proportion was identified to have

Table 6

Clinical forms and radiological features of tuberculosis patients

Index	Radiological features	Study group	1st control group
		N=48 (P%)	N=185 (P%)
Clinical forms of pulmonary TB	PIT	30 (62.5) ***	170 (91.89)
	PDT	7 (14.6)	15 (8.11)
	FCVT	7 (14.6) ***	0
Extrapulmonary TB	Bone and joints TB	3 (6.25)	NA
	Pleurisy	1 (2.1)	NA
Localization	One lung	9 (18.7) ***	80 (43.24)
	Both lungs	39 (81.2) ***	105 (56.75)
Extensiveness	Limited	20 (41.7)	79 (42.7)
	Extensive	28 (58.3)	106 (57.3)
Radiological features	Infiltration	20 (41.7)	79 (42.71)
	1 lung destruction	18 (37.5)	80 (43.24)
	2 lungs destruction	10 (20.8)	26 (14.05)

Note: Applied statistical test: paired simple T-test, P – probability; PIT-pulmonary infiltrative tuberculosis, PDT-pulmonary disseminated tuberculosis, FCVT-fibro-cavernous tuberculosis. NA-non-available; Statistically significant differences between: study group IVDU-TB compared to the 1st control group TB: * – p<0.05;** – p<0.01; *** – p<0.001.

Table 7

Microbiological features of tuberculosis patients

Microbiological characteristics N=48 (P%)		Study group	1st control group
		N=185 (P%)	
Mo	Microscopic positive	20 (41.7)	101 (54.5)
	Culture positive	24 (50)	99 (53.51)
	GeneXpert performed	25 (52.1)	185 (100)
	GeneXpert MTB/Rif positive	13 (27.1)	185 (100)
	GeneXpert MTB/Rif positive susceptible	4 (8.3)	147 (79.46)
	GeneXpert MTB/Rif positive rezistent	9 (18.7)	38 (20.54)
M0, M2/3	Poliresistance by DST	1 (2.1)	14 (7.56)
	MDR-TB by DST	8 (16.7)	36 (19.46)
	Susceptible by DST	40 (83.3)	135 (72.97)
Other forms	XDR-TB	1 (2.1)	0

Note: N/A- non available; DST-Drug susceptibility testing, M0 beginning of the treatment, M2/3 the end of the intensive phase of the treatment; MDR-multidrug resistant tuberculosis, XDR-TB extensively drug-resistant tuberculosis; Statistical assessment was not performed due to including criteria in the control group.

positive bacteriological results (culture on solid Lowenstein-Jensen or liquid MGIT BACTEC). The molecular genetic assay was performed in one half of the study group and in the entire control group. The differences between groups were determined by the period of the selection. The molecular genetic test was implemented in 2014 in the RM. The sensibility to the rifampicin through GeneXpert MTB/Rif assay was established positive in two thirds of the control group. Drug sensitivity testing identified mono- and poliresistance in both groups in a similar proportion. In a similar proportion were identified patients resistant to rifampicin through GeneXpert MTB/Rif assay or conventional drug sensitivity test. The drug resistance was established in a similar proportion using the drug sensitivity testing and GeneXpert MTB/Rif assay in both groups. Every fifth patient from both groups had MDR-TB. Established data were exposed in the table 7.

All selected patients were treated according to the National Clinical Protocol "Tuberculosis in adults". The standard treatment for drug susceptible tuberculosis was used for treatment of a similar proportion of both groups: 35 (72.9%) and 149 (80.54%) patients respectively. Every fifth patient from the study group was treated as previously treated cases – 9 (18.7%) patients. Even the rate of MDR-TB was in average similar in both groups, only a minor proportion of patients from the SG started the treatment for drug resistant tuberculosis – 4 (8.33%) in the SG compared with 36 (19.46%) in the CG. It is important to emphasize that the standard treatment for MDR-TB could be started only if the therapeutic compliance of the patient is established and the clinical tolerance is acceptable. Assessing treatment outcome, it was established a high statistical difference in the treatment success, which predominated in the control group 158 (85.4%) compared with 21 (43.7%) in the study group. The rate of patients lost to follow-up statistically predominated in the study group 10 (20.8%) compared with 2 (1.08%) in the control group. The rate of died patients predominated in the study group 8 (16.7%) compared with 11 (5.95%) in the control group, but the statistical difference was not achieve (tab. 8).

Table 8

Treatment outcome of tuberculosis patients

Index	Radiological features	Study group	1st control group
		N=48 (P%)	N=185 (P%)
Available final outcomes	Treatment success	21 (43.7) ***	158 (85.4)
	Treatment failure	1 (2.1)	2 (1.08)
	Loss to follow-up	10 (20.8) ***	2 (1.08)
	Death	8 (16.7)	11 (5.95)
Non available final outcomes	Still continuing	8 (16.7)	12 (6.48)

Note: Applied statistical test: paired simple T-test, P – probability.

Statistically significant differences between: study group IVDU-TB compared to the 1st control group TB: * – p<0.05;** – p<0.01; *** – p<0.001.

All the patients of the 2nd CG were managed and treated with the standard treatment of the drug addiction according to the National Clinical Protocol [21]. Most of them – 26 (76.4%) were hospitalised and treated being supported by the national insurance company and 8 (23.5%) – supported by private costs. The methadone was used to treat the

Table 9

Risk factors for tuberculosis among IVDU patients

Factors		Statistical indices			
		RR	OR	Pearson's	AR(%)
Age	35-44 years	1,56 (1,08-2,26)	3,08 (1,21-7,84)	**0,01**	**48**
Social economical features	Unemployment	2,59 (1,2-5,6)	6,02 (1,91-19,07)	**0,0011**	**34**
	Lack of insurance	0,0015	3,22 (1,15-9,02)	**0,001**	**54**
	Homelessness	1,6 (1,18-2,21)	5,67 (0,74-43,3)	**0,05**	**100**
	HIV infection	2,1 (1,58-2,84)	27,10 (3,24-203,31)	**4,0E-5**	**93**

Note: RR-relative risk, OR-odds ratio; AR-attributable risk.

Table 10

Risk factors for death due to the tuberculosis progression

Factors		Statistical indices			
		RR	OR	Pearson's	AR(%)
Radiological characteristics	Destructive forms of TB	1,57 (1,13-2,02)	5,63 (1,6-48,12)	**0,05**	**100**
	Both lungs involvement	2,67 (1,36-5,26)	3,32(1,51-7,21)	**0,001**	**30**
Treatment outcome	Loss to follow-up	4,84 (3,3-7,2)	24,08 (5,01-114,3)	**0**	**95**

Note: RR-relative risk, OR-odds ratio, AR-attributable risk, N/A-non available.

withdrawal syndrome 8 (23.5%) patients. Each third patient was treated with the standard regimen associated with other drugs due to disease complications. So, in 9 (26.5%) were associated neuroleptic drugs, in 8 (23.5%) antidepressant drugs and 7 (20.6%) antibiotics. Antibiotics were used for prevention of nosocomial infections due to subclavian venous catheter placement. The average duration of the treatment during the hospitalisation was 21 days. Most of the IVDU 31 (91.2%) patients were therapeutically compliant and 3 (8.8%) were lost to follow-up. The withdrawal syndrome was successfully treated in 31 (91.2%) patients. No deaths were registered. The working capacity and performances were restored in 29 (85.3%) cases and diminished in 5 (14.7%) patients. The drug use consequences were identified in 28 (82.3%) patients. It is important to emphasize that in one patient could be several consequences and sequellae. So, the diminished mnestic capacities were identified in 28 (82.3%), lack of peripheral venous access – in 20 (8.4%) and postinjectional signs – in 18 (52.9%) cases.

An important research outcome represents the relative risk (RR), odds ratio (OR) and the attributable risk (AR) indices for identifying the priority interventions in the frame of high risk groups [24,16]. In the table 8 were represented only risk factors and features which predominated and exposed statistical difference between the study group and the 2nd CG. It was established that major risk factors for tuberculosis in drug addicted were: unemployment and associated lack of health insurance, patient's homeless state and the immune suppressive condition – HIV infection and middle risk factors was the middle adulthood. Attributable risk established the hierarchy of risks: homelessness, HIV infection, lack of health insurance, middle adulthood and unemployment (tab. 9).

The next table reflects the assessment of the disease-related features which statistically predominated in the group of IVDU with tuberculosis compared with patients with tuberculosis without drug addiction. It was established that major disease-related characteristic in IVDU patients is the severe destructive forms of tuberculosis. The major treatment outcome was the "loss to follow-up". Both lung involvements were assessed as a disease-related characteristic with middle degree impact (tab. 10).

Conclusions

Men were predisposed for drug addiction; however, women frequently developed tuberculosis.

The age for acquiring tuberculosis is younger in addicted patients than in those without drug addiction.

Socioeconomic vulnerability is extended in the groups of patients with tuberculosis, with or without addiction, however the poverty deepness was more important in addicted patients.

Low level of education predominated in drug addicts.

Close contact with a sick patient predominated in addicted patients with tuberculosis, but nobody assessed the infectious contact in addicted patients without tuberculosis.

Associated diseases predominated in addicts with or without tuberculosis, more expressed were: HIV infected individuals, viral hepatitis and neurological disorders.

Risk factors for tuberculosis in drug addicted were: unemployment and associated lack of health insurance, patient's homeless state, middle adulthood and the immune suppressive condition – HIV infection.

Major disease-related characteristic in IVDU patients was the presence of severe destructive forms of tuberculosis and "loss to follow-up" as a final outcome.

References

1. Altice FL, Azbel L, Stone J. The perfect storm: incarceration and the high risk environment perpetuating transmission of HIV, hepatitis C virus, and tuberculosis in Eastern Europe and Central Asia. Lancet. 2016 Sep 17;388(10050):1228-48.
2. Centrul Național de Management în Sănătate [National Centre for Health Management]. Indicatori preliminari privind sănătatea populației și activitatea instituțiilor medico-sanitare pe anii 2014-2015 (format prescurtat) [Preliminary indicators on population health and on medical and healthcare institutions activity 2014-2015]. Chisinau: The Centre; 2016. 216 p. Romanian.
3. Scutelniciuc O, Cantarji V, Plesca V, Cotelnic-Harea T, Cojocaru O, Costin T. Cunoștințele, atitudinile și practicile populației generale (15-64 ani) cu referire la HIV/SIDA în R.Moldova [HIV/AIDS related knowledge, attitudes and practices of general population (15-64 years old) of the Republic of Moldova]. Chisinau; 2010. 57 p. Romanian.
4. De Vries G, Van Herst RA. From contact investigation to tuberculosis screening of drug addicts and homeless persons in Rotterdam. Eur J Public Health. 2006;16(2):133-6.
5. Deiss R, Rodwell T, Garfein R. Tuberculosis and illicit drug use: review and update. Clin Infect Dis. 2009;48(1):72-82. doi: 10.1086/594126.
6. Friedland G. Infectious disease comorbidities adversely affecting substance users with HIV: Hepatitis C and tuberculosis. J Acquir Immune Defic Syndr. 2010;55 Suppl 1:S37-42.
7. Grimes CZ, Hwang LY, Williams ML. Tuberculosis infection in drug users: interferon-gamma release performance. Int J Tuberc Lung Dis. 2007;11(11):1183-9.
8. Getahun H, Gunneberg C, Sculler D, et al. Tuberculosis and HIV in people who inject drugs: evidence for action for tuberculosis, HIV, prison and harm reduction services. Curr Opin HIV AIDS. 2012;7(4):345-53.
9. Getahun H, Baddeley A, Raviglione M. Managing tuberculosis in people who use and inject illicit drugs. Bull World Health Organ. 2013;91(2): 154-6.
10. Goetsch U, Bellinger OK, Buettel KL. Tuberculosis among drug users and homeless persons: impact of voluntary X-ray investigation on active case finding. Infection. 2012;40(4):389-95.
11. Hibell B, Guttormsson U, Ahlström S, et al. The 2011 ESPAD Report: Substance use among students in 36 European countries. Stockholm: ESPAD; 2012. 390 p.
12. Guvernul Republicii Moldova [Government of the Republic of Moldova]. Hotărârea nr. 1208 din 27.12.2010, cu privire la aprobarea Strategiei naționale antidrog pe anii 2011-2018 [Decision no. 1208 of 2010 December 27, on the approval of the National Drug Strategy for 2011-2018]. Monitorul Oficial al Republicii Moldova. 2011 Jan 7;(1-4):art. no. 1. Romanian.
13. Hwang LY, Grimes CZ, Beasley RP. Latent tuberculosis infections in hard-to-reach drug using population-detection, prevention and control. Tuberculosis (Edinb). 2009;89 Suppl 1:S41-5.
14. [National Centre for Health Management of the Republic of Moldova]. Studiu de evaluare a cunoștințelor și practicilor populației Republicii Moldova privind consumul de alcool, droguri și tutun (KAP) [Study of knowledge, attitude and practice of general population of the Republic of Moldova related to alcohol, drugs and tobacco consumption]. Chisinau: The Centre; 2012. 80 p. Romanian.
15. Kresina TF, Khalsa J, Cesari H, et al. Hepatitis C virus infection and substance abuse: medical management and developing models of integrated care – an introduction. Clin Infect Dis. 2005 Apr 15;40 Suppl 5:S259-62.
16. Larney S, Peacock A, Leung J, et al. Global, regional and country level coverage of interventions to prevent and manage HIV and hepatitits C among people who inject drugs: a systematic review. Lancet Glob Health. 2017;5(12):e1208-e1220.
17. [National Centre for Health Management of the Republic of Moldova, National Drug Observatory]. Consumul și tarficul ilicit de droguri în Republica Moldova: raport anual statistic [Consumption and illicit drug use in the Republic of Moldova: statistical annual report]. Chișinău: The Centre; 2014. 79 p. Romanian.
18. Paquette K, Cheng MP, Kadatz MJ, et al. Chest radiography for active tuebrculosis case finding in the homeless : a systematic review and meta-analysis. Int J Tuberc Lung Dis. 2014;18(10):1231-6
19. Prelipceanu D, Cicu G. Ghid clinic de tratament substitutiv al dependenței de opiacee [Guidelines for opioid substitution treatment of opioid addiction]. Bucuresti; 2010. 106 p. Romanian.
20. Guvernul Republicii Moldova [Government of the Republic of Moldova]. Hotărârea nr. 1171 din 21.12.2010, privind aprobarea Programului național de control al tuberculozei pentru anii 2011-2015 [Decision no. 1171 of 2010 December 21, on the approval of the National Tuberculosis Control Program for 2011-2015]. Monitorul Oficial al Republicii Moldova. 2010 Dec 31;(259-263):art. no. 1316. Romanian.
21. Oprea M, Vasiliev T, Marandici A, et al.; [Ministry of Health of the Republic of Moldova]. Tratamentul farmacologic cu metadonă al dependenței de opiacee: Protocol clinic național [Pharmacological treatment with methadone of opioid addiction: National clinical protocol]. Chisinau: The Ministry; 2015. 78 p. (PCN-225). Romanian.
22. Domente L, Alexandru S, Iavorschi C, et al.; [Ministry of Health of the Republic of Moldova]. Tuberculoza la adult: Protocol clinic național [Tuberculosis in adults : National clinical protocol]. Chisinau: The Ministry; 2015, 140 p. (PCN-123). Romanian.
23. Scutelniciuc O, Bivol S, Slobozian V, et al. Studiul comportamental și de seroprevalență în R. Moldova : raport 2007 = Behavioural and seroprevalence study in Moldova : report 2007. Chisinau ; 2010. 214 p. Romanian, English.
24. Spinei L, Lozan O, Badan V. Biostatistica [Biostatistics]. Chișinău; 2009. 186 p. Romanian.
25. Stone K. Reviewing harm reduction for people who inject drugs in Asia: the necessity for growth. Harm Reduct J. 2015 Oct 16;12:32.
26. Tintiuc D, editor. Biostatistica și metodologia cercetării științifice [Biostatistics and scientific research methodology]. Chisinau: Medicina; 2011. 344 p. Romanian.
27. United Nations. Convention on Psychotropic Substances of 1971 signed in Vienna, Austria.
28. United Nations. Single Convention on Narcotic Drugs, 1961.
29. Vagenas P, Axbel L, Polonsky M, et al. A review of medical and substances use co-morbidities in Central Asian prisons : implications for HIV prevention and treatment. Drug Alcohol Depend. 2013;132, Suppl 1:S25-31. doi: 10.1016/j.drugalcdep.2013.07.010
30. Vacarciuc O, Costin T.; [National Centre for Health Management of the Republic of Moldova, National Drug Observatory]. Rezultatele studiului național privind consumul de alcool, droguri și tutun în rândul elevilor din Republica Moldova (ESPAD 2011) [Results of the national study on alcohol, drug and tobacco consumption among students in the Republic of Moldova]. Chisinau: The Centre; 2011. 17 p. Romanian.
31. Vasiliev T, Stoev I. Narcomania: situația medico-socială și judiciară în Republica Moldova [Drug addiction: the medical-social and judicial situation in the Republic of Moldova]. Chișinău: Pontos; 2001. 400 p. Romanian.
32. United Nations Statistics Division. Millennium Development Goal Indicators Database [Internet]. New York: United Nations; ©2006. [cited 2018 May 20]. Available from: https://millenniumindicators.un.org/unsd/mi/mi_goals.asp
33. United Nations Statistics Division. Sustainable development goal indicators database. [Internet]. New York: United Nations; ©2018. [cited 2018 May 20]. Available from: https://unstats.un.org/sdgs/indicators/database/
34. Van Hest NA, De Vries G, Smit F, at al. Estimating the coverage of a targeted mobile tuberculosis screening programmes among illicit drug users and homeless persons with truncated models. Epidemiol Infect. 2008;136(5):628-35.
35. World Health Organization. Substitution maintenance therapy in the management of opioid dependence and HIV/AIDS prevention. Geneva: WHO; 2004. 33 p.
36. Dye C, Lönnroth K, Jaramillo E, Williams BG, Raviglione M. Trends in tuberculosis incidence and their determinants in 134 countries. Bull World Health Organ. 2009 Sep;87(9):683-91.
37. World Health Organization. The global plan to stop TB 2011-2015: transforming the fight towards elimination of tuberculosis. Geneva: WHO; 2010. 96 p.
38. World Health Organization. Global tuberculosis control 2011. Epidemiology, strategy, finances. Geneva: WHO; 2011. 258 p.
39. World Health Organization. Commission on Social Determinants of Health. Action on the social determinants of health. Geneva: WHO; 2005. 50 p.
40. Blas, E, Sivasankara Kurup, A; World Health Organization. Equity, social determinants and public health programmes. Geneva: WHO; 2010. p. 219-241.
41. World Health Organization. Global tuberculosis report 2015. Geneva: WHO; 2015. 192 p.
42. World Health Organization. Treatment of tuberculosis: guidelines. Geneva: WHO; 2014. 420 p.
43. World Health Organization. Global tuberculosis report 2017. Geneva: WHO; 2017. 249 p.

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Obliteration and reconstruction of the mastoid cavity with auto osteomatrix forte autograft

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Abstract

Background: Over the past decades, there have been a multitude of reports on the effectiveness of various techniques for the reconstruction and obliteration of the mastoid cavity.

Material and methods: A comparative study on the meatotympanoplasty effectiveness using the auto osteomatrix forte autograft associated with temporal fascia and mastoid obliteration with pediculated musculoperiosteal flap in 58 patients after total tympanomastoidectomy and 59 patients after revision surgery of the evidation cavity. The patient batches were similar according to age, gender and living environment.

Results: 12 months postoperatively, in all patients of both study groups was determined: significant decrease of complaints, ameliorated anatomical outcomes (small, clean and dry evidation cavity), eradication of the evidation cavity pathology, improvement of the auditory functional results (statistically significant increase in the perception of voice on whispering and speech, airway and bone conduction), improvement of the vestibular function and life quality.

Conclusions: Meatotympanoplasty with the use of the auto osteomatrix forte autograft when associated with the temporal fascia and the mastoid dislocation with pediculated musculoperiosteal flap is a feasible and safe technique for pathology eradication involving the evidation cavity. The use of the autologous bone for reconstruction is effective for the stability of the external auditory canal and for the tympanic membrane. It preserves and / or restores hearing, and has a lot of advantages: is immediately available in primary and revision surgeries, is economical, does not cause inflammation processes in the body, contributes to the growth of the new bone, reduces the risk of infection, resorption and subsequent degradation and improves life quality.

Key words: mastoidectomy, reconstruction, obliteration, auto osteomatrix forte autograft.

Introduction

The objectives of mastoidectomy in chronic suppurative otitis media with cholesteatoma are: eradication of the disease, obtaining dry ears, prevention of recurrent pathology and the possibility to restore the affected sonic transmission mechanism. Canal wall down mastoidectomy (CWD) is one of the commonly used surgical techniques for the effective eradication of the middle ear disease. This approach is aggressive with the removal of affected anatomical structures. Thus, it leads to an open cavity formation, in this way changing middle ear anatomy and physiology. Although most patients experience postoperative minor short-term problems, there are a considerable number of patients with chronic disorders associated with the evidation cavity persistence [1].

Otorrhea and recurrent infections are the commonest causes of frequent complaints of patients with mastoid cavities. Other frequent complaints include water intolerance with the development of infections, frequent need for otomicroscopic treatment and removal of pathological secretions, dizziness caused by exposure to water or air, barometric induced dizziness, and hearing loss with the inability of wearing the traditional hearing aids [1].

Reconstruction techniques of the posterior wall of the external auditory canal (EAC) and the methods of mastoid cavity obliteration appeared as an effort to avoid the disadvantages of CWD mastoidectomy techniques. Reconstruction procedures of the posterior wall of the external auditory canal (EAC), mastoid cavity obliteration and ossiculoplasty may be performed primarily during the same procedure along with CWD mastoidectomy or as a secondary procedure for revision of the mastoid cavity with chronic otorrhea and hypoacusis [1].

So far, the otosurgeons do not have a unanimous opinion on the optimal material used to restore ear structures after the aggressive surgery. Both organic and inorganic materials currently used in reconstruction surgery, along with indisputable advantages, present serious disadvantages. More and more specialists tend to use materials obtained from bone and autologous cartilage tissue which possess osteoinductive properties [1].

Over the last few decades, there have been a large number of reports on the effectiveness of a variety of mastoid cavity techniques. The commonest applied and popular techniques are local flaps (muscle, periost or fascia), free autologous grafts (bone, cartilage, fat, fascia) or alloplastic grafts (hydroxyapatite, silicon, synthetic bones) [1].

The study aim is to evaluate the efficacy of meatotympanoplasty with the use of the auto osteomatrix forte graft (AOMF) associated with temporal fascia and mastoid obliteration with pediculated musculoperiosteal flap in patients after total tympanomastoid dissection (ETMT) in comparison to patients after revision surgery of the evidation cavity.

Material and methods

To determine the effectiveness of the use of autogenous bone grafts with AOMF osteoinductive properties in reconstruction surgery after ETMT the prospective cohort study was conducted which included 2 batches of patients:

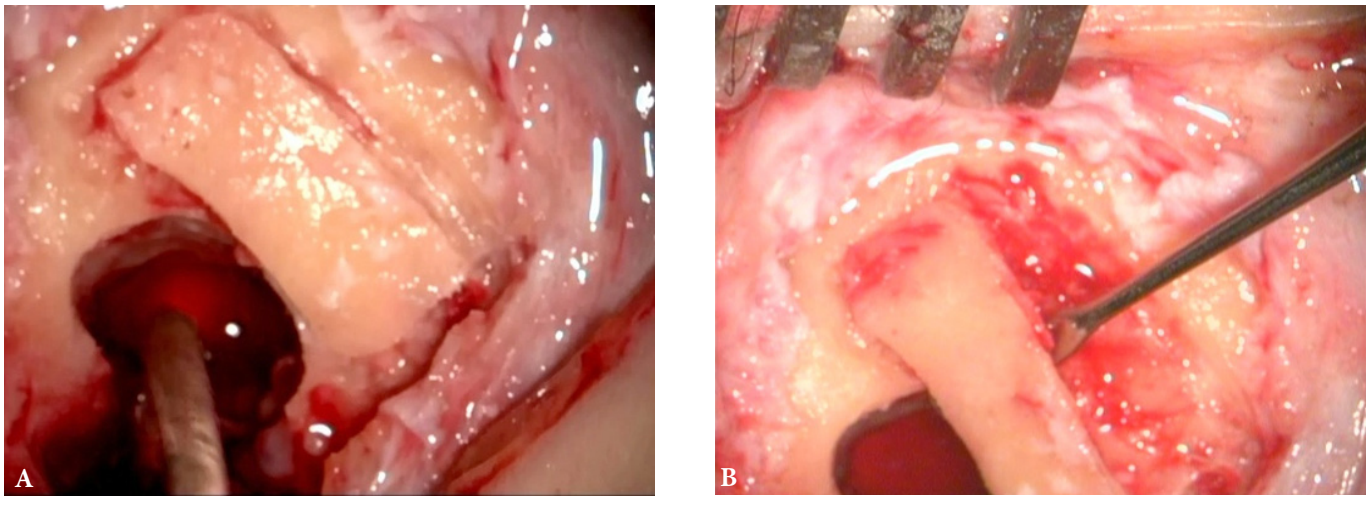

Fig. 1. A, B – sampling of the fragment from the cortical layer of mastoid apophysis.

- Group of patients with ETMT and reconstruction by means of the elaborated method included 58 patients: 28 (48.3%) women and 30 (51.7%) men with mean age of 41.83 ± 1.2 years from 24 up to 60 years old) – the baseline lot (LB);
- Group of patients with revision surgery of the evidation cavity and reconstruction by means of the elaborated method constituted 59 patients: 30 (50.8%) women and 29 (49.2%) men with mean age of 40.44 ± 1.3 years (from 18 up to 61 years old) – control group (LM).

From the study were excluded: patients with malignant ear cancer, patients with congenital disorders, patients that were not fully investigated preoperatively or postoperatively according to the study protocol, patients that did not attend the medical check-ups on the schedule, patients that refused to participate in the study or abandoned it.

All patients were treated at the Department of Otorhinolaryngology of the Republican Clinical Hospital in Chisinau, during 2005-2014.

We applied meatotympanoplasty with the use of auto osteomatrix forte graft associated with temporal fascia and mastoid obstruction with pediculated musculoperiosteal flap. The first variant is used in patients that were not previously operated by OMCS. Firstly the mastoid plane is opened within the limits of the Chipault triangle. The rectangular bone fragment with the dimensions of 1.5x2.5 cm and the thickness of 1 cm (fig. 1 A, B) is cut from the cortical layer. After that typical ETMT is performed (fig. 2). When EAC postero-superior wall is removed, 2 fine crests are cut into their upper and lower sides. EAC plastic surgery of the skin is not performed. The postoperative cavity is treated with Mirocel or with mesh gauzes with antibacterial ointment. The plaque remains opened during 3-5 days (time required to prepare the OMF autograft from the bone fragment of the cortical layer, extemporary prepared). Daily, on the postoperative cavity are performed measures for rehabilitation and removal of pathological secretions with microscopic revision. For this are applied dressings, swabbing with Mirocel, or treatment with mesh gauzes with antibacterial ointment. The reconstruction stage is performed on the 4th up to 6th day (depending on the preparation of the OMF autograft). The prepared autografts have sufficient elasticity and strength to provide the shape and position required to model the EAC posterior wall. In addition, it offers the possibility of bonding the fascicular graft to reconstruct the eardrum. On the margins of OMF grafts, longitudinal incisions of 1.5-2 mm are made. After attaching the fascicular graft, the EAC postero-superior wall is reconstructed. The bonding of OMF grafts is achieved by using the upper and lower ridges prepared during the rehabilitation stage (fig. 3). In the mastoid cavity is introduced a 5 mm diameter policlorvinil perforated drainage tube and the mastoid cavity is obliterated by placing the pediculated musculoperiosteal flap taken from the retroauricular area (fig. 4). EAC is swabbed with Mirocel or treated with mesh gauzes with antibacterial ointment. Plaque is sutured layer by layer.

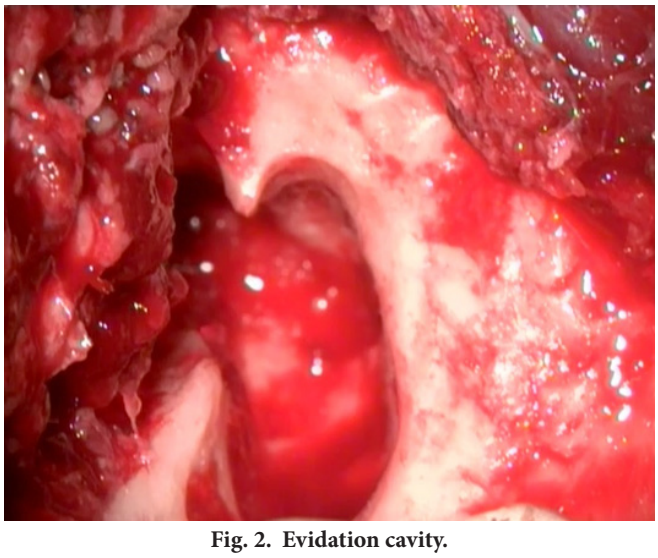

Fig. 2. Evidation cavity.
Mastoid cavity after radical mastoidectomy.

The second variant is used in patients with ETMT antecedents and with MUO. Until the revision of the evidation

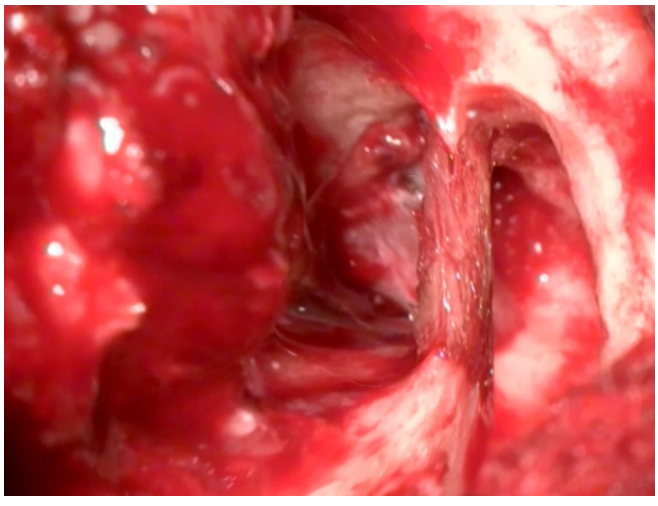

Fig. 3. Reconstruction of the postero-superior wall of the external auditory canal with the osteomatrix forte graft.

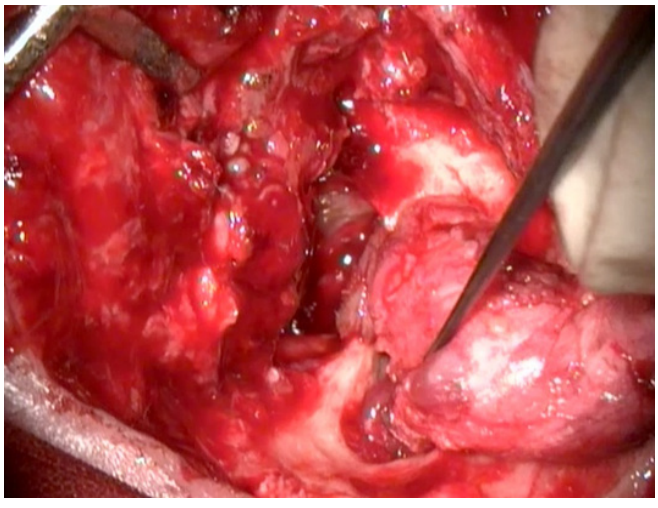

Fig. 4. Mastoid obliteration with pediculated musculoperiosteal flap.

cavity, a bone fragment from the cortical layer is cut to prepare the OMF autograft. After revision, the mastoid cavity is swabbed with Mirocel or treated with mesh gauzes with antibacterial ointment. The plaque remains opened during 3-5 days (the time required to prepare the OMF autograft). Daily, on the postoperative cavity are performed procedures for rehabilitation and removal of pathological secretions with microscopic revision. For this are applied dressings, swabbing with Mirocel, or treatment with mesh gauzes with antibacterial ointment. The reconstruction stage is performed on 4th up to 6th day (depending on the preparation of the OMF autograft). The bonding of the OMF graft and mastoid obliteration is similar to the first variant. Plaque is sutured layer by layer. EAC is treated swabbed with Mirocel or mesh gauzes with antibacterial ointment.

To achieve the study objectives, patients underwent a complex clinical and paraclinical examination program according to pre-established research designs (at the onset of the study and in dynamics at 1, 6, and 12 months postoperatively). In the study were applied the following methods of investigation: clinical, functional, instrumental, imagistic, and microbiologic.

Patient examination protocol included: (1) history of the disease taking into consideration the duration and severity of the symptoms (otorrhea, hearing loss, tinnitus, dizziness, otalgia, cephalalgia) and previous surgical procedures, (2) otomicroscopic and /or otoendoscopic examination, (3) complete audiological evaluation (audiometry, instrumental audiometry with diapason, Liminal pure-tone audiometry, acoustic impedancemetry) (4), vestibular function test, (5) temporal bone imaging examination by high resolution computerized tomography, life quality assessment by applying the Chronic Ear Survey.

Primary data processing was performed with the help of "Statistical Package for the Social Sciences" program, version 20.0 for Windows (SPSS, Inc., Chicago, IL, 2011) using descriptive and inferential statistics. The X^2 method was used with Yates correlation or Fisher's exact method to compare discrete variables, unifactorial variance analysis with the application of post-hoc analysis test, the difference between the mean values in the study batches, correlation analysis to determine the relationship between variables, power and direction.

Results

Comparative examination of patients from LB and LM groups did not reveal significant statistic differences according to: age (40.44±1,3 years and 41.83±1,2 years, respectively; p>0.05), gender (50.8% and 48.3% women, p>0,05; 49.2% and 51.7% men, p>0.05), living environment (52.5% and 56.9% rural area, p>0.05; 47.5% and 43.1% urban area, p>0.05), rate of unengaged people(8.5% and 13.8%, respectively; p>0.05), smoking (37.3% and 46.6%, respectively; p>0.05) and alcohol consumption (28.8% and 39.7%, respectively; p>0.05).

During the preoperative examination, patients from LB group frequently complained of headaches (94.8% and 78.0%, p <0.05), otalgia (94.8% and 78.0%, p <0.05) and tinnitus, 1% and 90.8%, p <0.01). Patients' general state was similar in both study groups. General state of moderate severity constituted 71.2% in LM patients and 81.0% in LB patients (p> 0.05).

Hearing test revealed reduced and similar incidence of EAC narrowing in patients from both study groups: 8.5% of LM patients and 6.9% of LB patients (p> 0.05). Although pathological eliminations were identified in all patients in both groups, in LB patients were revealed frequent statistically significant pathological eliminations with fetid odor (49.2% and 100.0%, p <0.001), and in LM – odorless pathological eliminations without fetid odor (50.8% and 0%, p <0.001).

During the objective exam, the postero-superior wall was absent statistically significantly more frequently in LM patients (100.0% and 0%, p <0.001) was normal (0% and 89.7%, p <0.001) and protruded (0% 10.3%, p <0.05) more frequently in LB. the evidation cavity was recorded only in LM patients. The tympanic membrane (TM) was presented

with perforation and localization in par sensing and pars flaccida in all patients from both study groups.

Granulations were present in all patients from both study groups. Polyps (22.0% and 43.1%, p <0.05) and cholesteatoma (39.0% and 100.0%, p <0.001) presented statistically significant incidence in LB patients.

Hearing test revealed: mean value of voice perception on whistling (1.95 ± 0.08 m and 1.18 ± 0.04 m, p <0.001), mean voice perception during speech (4.24 ± 0 , 09 m and 3.37 ± 0.07 m, p <0.001), an average decrease of air conduction duration (49.41 ± 1.1% and 34.14 ± 0.8%, p <0.001) and an average decrease in bone conduction (84.41 ± 1.0% and 60.69 ± 0.6%, p <0.001) significantly higher in LM.

Thus, during the preoperative examination, patients from LB group presented more statistically significant incidence of otorrhea with fetid odor, polyps and cholesteatoma. Patients from LM group presented pathological elimination without fetid odor, and statistically significantly higher audiometry tone values.

One month postoperatively, the majority of complaints in patients from both study groups were significantly reduced. Although, all patients complained of hearing loss in both study groups, patients from LB group complained of more pronounced statistically significant hearing loss (86.4% and 98.3%, p <0.05), and patients from LM group complained more frequently of moderate hearing loss (13 , 6% and 1.7%, p <0.05). The general condition was similar in patients from both study groups.

Hearing test revealed statistically significantly higher incidence of moderate otorrhea (22.0% and 54.4%, p <0.001) and mild otorrhea (67.8% and 40.4%, p <0.01) was more rare in LB patients. The objective examination revealed normal postero-superior wall incidence. Perforated and absent integral MT was similar. Cholesteatoma, granulations and adhesions were not found in patients from both study groups. The evidation cavity was closed without pathologies in all patients from both study groups.

Hearing test revealed: mean value of voice perception on whistling (1.96 ± 0,09 m and 2.88 ± 0,05 m, p <0.001), average voice perception during speech (4.34 ± 0,1 m and 4.98 ± 0.02 m, p <0.001), mean loss of air conduction duration (44.58 ± 1.7% and 54.83 ± 0.7%, p <0.001) and mean loss of osseous conduction duration (77.29 ± 1.5% and 88.97 ± 1.5%, p <0.001) presented higher statistically significant values in LB patients.

Thus, 1 month postoperatively, patients from LM group complained more frequently of moderate hearing loss and patients in LB group – of pronounced hearing loss. The objective examination revealed more frequent moderate forms of otorrhea, and otorrhea in insignificant amount was statistically significantly less rare in LB patients. Improvement of hearing function presented higher statistically significant values in patients from LB group.

Six months postoperatively, the complaints frequency continued to decline. The complaints frequency and patients' general condition were similar in both study groups. The general condition of the majority of patients was satisfactory.

The hearing test did not reveal statistically significant differences in the incidence and characteristics of pathological eliminations. The evidation cavity was closed without pathologies in all patients from both study groups.

Hearing test revealed: average voice perception on whispering (2.42 ± 0,1 m and 3.45 ± 0.09 m, p <0.001), average voice perception during speech (4.60 ± 0,1 m and 5.95 ± 0.04 m, p <0.001), mean value of air conduction loss (58.47 ± 0.6% and 65.69 ± 1.5%, p <0.001) and the mean value of osseous conduction loss (87.29 ± 0.9% and 92.76 ± 0.3%, p <0.001) were statistically significantly higher in patients from LB group.

Thus, 6 months postoperatively, the complaints frequency continued to reduce, and their incidence was the same in both study groups. The objective examination did not reveal statistically significant differences in the frequency and characteristics of pathological eliminations, postero-superior wall, MT, and pathology of the cavity was not found in patients in both study groups. The hearing test showed statistically significantly higher improvement in LB patients.

Although 12 months postoperatively, hypoacusis was reported in all patients from both subgroups of study, insignificant hearing loss was more common in patients from LB group (11.9% and 41.4%, p <0.001), acute hypoacusis (22.0% and 0%, p <0.001) – was statistically significantly more frequent in LM patients. The general condition was satisfactory in the majority of patients from both study groups.

The hearing test did not reveal a narrowing of the EAC and did not reveal statistically significant differences in the incidence and characteristics of the otorrhea and the MT state. The condition of the cavity was shown to be closed and without pathological changes in all patients from both study groups.

The hearing test revealed the average voice perception on whispering (3.32 ± 0.1 m and 3.74 ± 0.09 m, p <0.01) and the mean value of air conduction loss (62.71 ± 1.7% and 67.24 ± 1.2%, p <0.05) were statistically significantly higher in LB patients.

Thus, 12 months postoperatively, hearing loss was present in all patients in both subgroups of study. Insignificant hearing loss was more common in LB patients, and significant hearing loss was statistically significantly more frequent in LM patients. The mean value of voice perception on whispering and the mean value of air conduction loss were statistically significantly higher in LB group.

Discussion

The management of open mastoid cavity has two options: conservative and revision surgery with correction (surgery for elimination of mastoidectomy adverse effects) [4].

The procedure for the revision surgery of the evidation cavity includes the complete elimination of the disease and methods for the prevention of recurrent diseases. It also includes the reconstruction of EAC posterior wall

with meatoplasty, mastoid obliteration procedures, reconstruction of the tympanic membrane (tympanoplasty), reconstruction of the osicular chain (osiculoplasty), and meatoplasty [3].

Mercke suggested an innovative procedure in which the EAC posterior wall is temporarily removed, in order to avoid the disadvantages and combine certain features of CWD mastoidectomy. This procedure facilitated the complete removal of the impairment. Subsequently, it is followed by the reconstruction using autologous grafts (bone, cartilage) or alloplastic grafts (HA cement, ionomeric glass cement, titanium plates). This technique, called the canal wall reconstruction, with a single step for the removal of cholesteatoma and reconstruction of the EAC wall, had long acceptable results [5, 6].

Many techniques and materials for grafting (autologous, allogeneic, heterologous or synthetic biocompatible) have been used in the reconstruction of the posterior wall of EAC and mastoid obliteration, but none of these proved to be ideal for obliteration. Patient's tissues are considered to be selective materials in reconstruction [2, 7, 8, 9].

In our study, we compared the efficacy of meatotympanoplasty with the use of AOMF autograft associated with temporal fascia and mastoid dislocation with pediculated musculoperiosteal flap in 58 patients after ETMT and in 59 patients after revision surgery of the evidation cavity. The patient batches were similar depending on age, gender, living environment, rate of unemployed people, smoking and alcohol consumption.

Overall, the dynamic evaluation found more significant improvement in LB patients in comparison to LM patients. These results coincide with the specialized literature data. Kronenberg and co-authors reported the results of a retrospective case study of 49 consecutive patients (31 children and 18 adults) with the reconstruction of the EAC posterior wall with autologous bone paste and obliteration of the mastoid cavity with tympanomeatal flap. After an average 28 months follow-up, 30 primary operated patients achieved impermeable ears in 85.7% of cases, dry ears in 90% of cases, intact MT in 93.3% of cases, and recurrent cholesteatoma in 10.0% of cases, compared to 64.7%, 73.7%, 73.7% and 15.8%, respectively, in 19 patients with secondary intervention for the revision of the cavity [11].

A significant complaints reduction (headache, otalgia, nausea, dizziness, and general weakness) was determined in patients from both study groups, 1 month postoperatively. Although all patients had hearing loss in both groups, there was statistically significant increase of mild and moderate hypoacusis and statistically significant reduction of acute hypoacusis. However, the modification rate was higher in patients from LB group. Tinnitus and otorrhea were significantly statistically reduced after 6 months postoperatively. Although at the objective structured primary examination the general condition was more severe, the improvement rate was higher in LB patients.

The hearing test revealed, in patients from both study groups, similar incidence of: narrowed EAC correction, otorrhea reduction, disappearance of adhesions, granulations and cholesteatoma, 1 month postoperatively. The exanimation of the evidation cavity found, 1 month postoperatively, a closed cavity in all patients from both study groups. 1 month postoperatively almost all pathologies of evidation cavity were eradicated, in all patients from both study groups. Exception presented desquamation of the epidermis and mucositis that significantly statistically decreased 1 month and 6 months postoperatively and completely disappeared after 12 months postoperatively in patients from LM group.

Patients from both study groups showed a statistically significant increase of voice perception on whistling and speech and duration of airway and bone conductions. But the growth rate was also higher in LB group.

In both study groups the tuning fork samples revealed mixed hypoacusis in all patients. Vestibular apparatus examination revealed a statistically significant improvement. Subjective examination using the Likert - CES scale found a statistically significant life quality improvement. Statistically significantly increased the subscale score of activity limitation / restriction, symptom subscale score, medical resource utilization subscale score, and total score. However, the rate of life quality improvement was higher in LM patients.

Few authors studied the benefits of mastoid obliteration with the reconstruction of the external auditory bone canal, in particular the improvement of life quality after this procedure. A recent observational retrospective study was conducted to determine whether the obliteration of the mastoid cavity with autologous cranial bone graft after CWD mastoidectomy improves the life quality. Primary obliteration was performed in 46 patients and secondary obliteration in 12 patients. The Glasgow Benefit Inventory questionnaire was used to estimate the life quality. The overall score and subscale scores improved significantly. These results were similar to our study data. The authors concluded that the mastoid cavity obliteration with the reconstruction of the EAC posterior wall with an autologous cranial graft provides a significant benefit to life quality for both patients after primary and secondary interventions [14].

Thus, meatotympanoplasty with the use of auto osteomatrix forte autograft associated with temporal fascia and pediculated musculoperiosteal flap is a feasible and safe technique for eradicating the pathology of the middle ear and mastoid cavity. Our preliminary results indicate good overall results for the stability of the external auditory canal and MT, the preservation and / or improvement of hearing and cholesteatoma.

Similar results have been reported in many publications in the specialized literature. Mokbel and Khafagy presented a prospective case study of 100 adult patients (55 males and 45 females) aged between 17-55 years with chronic unilateral otitis media with or without cholesteatoma, without mastoidectomy antecedents, with 12 months minimum follow-up period. The authors carried out CWD mastoidectomy, extensive meatoplasty, anterior and inferi-

or canalplasty, tympanoplasty, obliteration with bone paste covered by musculoperisteal flap based on three pedicles (anterior, lower and upper). The flap and the exposed bone were covered with temporal fascia and skin grafts to accelerate epithelialization and to obtain a dry cavity. The reconstruction of the osseous chain was not performed. Twelve months postoperatively, 78% of patients had a completely dry cavity, 16% had intermittent otorrhea and 6%- persistent otorrhea [10].

Mobashir and co-authors reported the results of the study performed on 31 patients (24 men and 7 women) with mean age 26.7 years (15 to 36 years old) with primary acquired cholesteatoma. The postoperative follow-up constituted 15.3 months. The surgical technique included complete cortical mastoidectomy, extended posterior tympanometry, removal of the posterior bone wall of the EAC, dissection and eradication of cholesteatoma, reposition of the EAC wall segment in the anatomical area, osiculoplasty (in 26 patients), reconstruction of the atical lateral wall with autologous bone paste. Postoperatively, all patients presented a completely healed outer ear canal of almost normal size, shape and contour. The posterior wall was stable and the bone paste turned into a consistent newbone with the texture and integrity of a normal bone canal. None of the patients showed residual perforation of MT, otorrhee, myringitis or prosthesis extrusion [6].

Ghiasi, in a prospective longitudinal study with 28 months average follow-up, evaluated 56 ears of 48 patients with chronic cholecystectomy. After CWD mastoidectomy, mixed obliteration of mastoid cavity with autologous bone paste was performed. The bone graft was covered with a posterior section of the temporal fascia and Pavla flap. Seventeen (30%) ears were treated by revision surgery. Approximately 82% of the ears had a very small, dry, healthy mastoid cavity, 13% of ears – occasional otorrhea, 5% of ears – granulation tissue, 12.5% of ears – residual cholesteatoma and 7% of ears – wound infection [13].

Ramsey and co-authors presented a retrospective clinical study of 60 consecutive surgical interventions at 59 patients with active OMC aged between 4-84 years (mean age of 39 years old), including 53 (88%) ears with cholesteatoma and 7 (12%) ears with granulation tissue without cholesteatoma. All patients underwent CWD mastoidectomy with simultaneous thympoplasty. In order to obliterate the mastoid cavity, the lower pediculated postauricular periosteal-pericraniam flap was used in combination with autologous bone paste. Anterior and lower canalplasty and extended meatoplasty were performed. The follow-up period constituted of 12-80 months (on average 32 months). Forty (67%) of the procedures were primary and 20 (33%) were revision procedures. During all procedures, 54 (90%) patients passed successfully the infection control, 6 (10%) patients had repeated otorrhea. There were no cases of residual or recurrent cholesteatoma [15].

Roberson and co-authors reviewed 62 ears of 57 patients (25 women and 32 males) after CWD mastoidectomy or revision of the problematic mastoid cavity. At the same time, 33 patients underwent secondary surgery for ossicular reconstruction of 36 ears. Patients were subjected to mastoid and epithelium obliteration with autogenous cranial bone paste reconstruction, covered with temporal fascia. Transplantation of autogenous cranial bones led to osteoneogenesis causing complete obliteration of epitympanic and mastoid areas with the maintenance of the mesothympanic space. Evaluation of long-term outcomes (mean period 18.5 months) found: complete bone obliteration in 95.2% of cases, complete resolution of symptoms in 90% of cases, significant reduction in the frequency and severity of symptoms in 10% of cases, and assessment of air-bone gap ≤20 dB in 60% of cases. In 95% of patients Eustachio tube functioned properly. None of the patients required a revision surgery [7].

Beutner and co-authors presented a number of cases with 18 patients with the mean age – 46.3-years. These patients previously underwent CWD mastoidectomyand revision CWD mastoidectomy by obliteration with autologous bone paste, covered with cartilage plates, meatoplasty and MT reconstruction with thin cartilage slices. The average follow-up period was 6 years. None of the patients had residual or recurrent cholesteatoma, did not report symptoms similar to preoperative symptoms (otorrhea, dizziness), and all patients had dry ears with complete epithelization [12].

Thus, in order to overcome the problem of oversized mastoid cavity, reconstruction of the EAC posterior wall with immediate mastoid obliteration or revision intervention is performed. Several autologous materials (cartilage, bone paste, local pediculated flaps of soft tissue) or synthetic biocompatible (bone cement, HA granules, bioactive glass) have been used to reduce the size of the cavity. The use of the autologous bone contributes to the creation of the new bone, reduces the risk of infection, resorption and subsequent degradation. It contributes to bone graft survival and a lower risk of subsequent repair of the reconstruction compared to heterologous materials. The use of the autologous cortical bone to reconstruct the posterior wall of the EAC has advantages: it is autologous, immediately available and economical, does not develop an inflammatory reaction to a foreign body and improves the life quality.

Conclusions

1. Dynamic assessment found a more significant improvement of complaints and general condition of patients after ETMT and reconstruction by the elaborated method, in comparison to patients with revision surgery of the evidation and reconstruction cavity by the elaborated method.

2. Improvement of anatomical outcomes (low, clean and dry cavity) and functional outcomes (auditory function, vestibular function and life quality) was statistically significantly earlier, more stable and with higher rate in patients from the control group.

3. Use of the autologous bone for the reconstruction of the EAC posterior wall has advantages. It is an autologist;

is immediately available in both primary surgery and revision surgery; is economical; does not develop inflammatory response to the body; contributes to new bone creation; reduces the risk of infections, resorption and subsequent degradation; contributes to the survival of the graft, restores the almost normal anatomy of EAC; improves the life quality.

References

1. Alves R, Cabral Junior F, Fonseca A, et al. Mastoid obliteration with autologous bone in mastoidectomy canal wall down surgery: a literature overview. Int Arch Otorhinolaryngol. 2016;20(1):76-83.
2. Virk J, Patel N. Primary mastoid reconstruction with Sofradex®-soaked autologous bone pâté: is there a sensorineural hearing effect? Clin Otolaryngol. 2017;42(3):758-61.
3. Nadol JB. Revision mastoidectomy. Otolaryngol Clin North Am. 2006;39(4):723-40.
4. Black B. Mastoidectomy elimination. Laryngoscope. 1995;105(12 Pt 2 Suppl 76):1-30.
5. Roux A, Bakhos D, Lescanne E, et al. Canal wall reconstruction in cholesteatoma surgeries: rate of residual. Eur Arch Otorhinolaryngol. 2015;272(10):2791-7.
6. Mobashir MK, Basha WM, Mohamed AE, et al. Posterior canal wall reposition for management of cholesteatoma: Technique and results. Auris Nasus Larynx. 2018;45(2):254-60.
7. Roberson JB, Mason TP, Stidham KR. Mastoid obliteration: autogenous cranial bone pate reconstruction. Otol Neurotol. 2003;24(2):132-40.
8. Leatherman BD, Dornhoffer JL. The use of demineralized bone matrix for mastoid cavity obliteration. Otol Neurotol. 2004;25(1):22-6.
9. Sayed RH. One-stage reconstruction in management of extensive cholesteatoma. IFOS Int Congr Series. 2003;1240:121-31.
10. Mokbel KM, Khafagy YW. Single flap with three pedicles, bone paté and split-thickness skin graft for immediate mastoid obliteration after canal wall down mastoidectomy. Eur Arch Otorhinolaryngol. 2012;269(9):2037-41.
11. Kronenberg J, Shapira Y, Migirov L. Mastoidectomy reconstruction of the posterior wall and obliteration (MAPRO): preliminary results. Acta Otolaryngol. 2012;132(4):400-3.
12. Beutner D, Helmstaedter V, Stumpf R, et al. Impact of partial mastoid obliteration on caloric vestibular function in canal wall down mastoidectomy. Otol Neurotol. 2010;31(9):1399-403.
13. Ghiasi S. Mastoid cavity obliteration with combined palva flap and bone pâté. Iran J Otorhinol. 2015;27(78):23-8.
14. Kurien G, Greeff K, Gomaa N, et al. Mastoidectomy and mastoid obliteration with autologous bone graft: a quality of life study. J Otolaryngol Head Neck Surg. 2013;42:49.
15. Ramsey MJ, Merchant SN, McKenna MJ. Postauricular periosteal-pericranial flap for mastoid obliteration and canal wall down tympanomastoidectomy. Otol Neurotol. 2004;25(6):873-8.

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Limited internal fixation in the distal metaepiphyseal shin fractures

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Abstract

Background: The scientific papers deal with it reporting different treatment technologies for distal tibia metaepiphysis fractures; meanwhile the complication and failure rates remain high.

Material and methods: 265 patients were examined. Their average age was 45.15±15.98 years (from 18 to 86). The patients were distributed into clinical groups according to the type of surgery performed. Clinical group I included patients who underwent mini-invasive osteosynthesis with K-wires and screws (113 patients, 42.64% from general group); Clinical group II included patients with external fixation osteosynthesis (36 patients, 13.58%); and Clinical group III included patients who underwent osteosynthesis with plates (116 patients, 43.77%).

Results: The technology of mini-invasive osteosynthesis of distal metaepiphysis fractures of the lower leg is improved. Reposition is performed under general or conduction anaesthesia under C-arm control. Ligamentotaxis principle is applied pulling fragments by means of distractor. In case separate bone fragments are not repositioned a pricker, single-toothed tenaculum are used through separate pricks of the skin. Every step is controlled by imagic intensifier. After subluxation is eliminated and reposition completed, trans-articular fixation of fragments is performed inserting 2-3 K-wires 2 mm in diameter from the sole in the direction of the tibia middle part.

Conclusions: The analysis of implementation of the improved mini-invasive osteosynthesis technology for fractures of the distal lower limb compared with the traditional methods of surgical treatment of these injuries in 265 patients from the three clinical groups demonstrated that the use of closed reposition and improved technique results in improved results and considerable reduction of costs for treatment in comparison with traditional osteosynthesis.

Key words: mini-invasive osteosynthesis, distal shin fractures.

Introduction

A number of scientific papers deal with the issues of surgical treatment of distal shin fractures (43 and 44 segments by AO), reporting about different aspects of treatment of distal tibia metaepiphysis (so-called pilon) fractures [2, 5, 6, 7, 9]. The main mechanisms of these fractures are injuries of drivers or passengers during road accidents or falling from a height. As a rule, similar fractures occur in persons of young and middle age. These injuries are characterized by bone fragmentation with considerable dislocation of fragments and formation its defects in the metaepiphysis zone with various injuries of the articular surface, soft tissues and neurovascular bundle. Pilon fractures are known to be a frequent component of multiple traumatic injuries.

The volume and character of injury depends on power properties of the osseous tissue, position of the foot during injury, volume and point of traumatic force application. Pilon fractures, especially in case of multiple traumatic injuries, result in great variety, and their treatment remains one of the most complicated problems [2, 6, 9].

Modern traumatology contains great arsenal of osteosynthesis technologies to perform stable fixation of distal tibia fractures [3, 4, 5, 10, 12, 13, 14]. Unfortunately, fractures of this localization are the leaders by the number of complications (up to 30%) and unfavourable consequences. Certain authors report about development of deforming arthrosis transformed into fibrous ankylosis in 16% of cases. The causes of this condition are first of all circulatory disorders in the area of fracture and technical problems while performing surgery [1, 8, 11, 15].

For recent fifty years the philosophy of AO surgical treatment of fractures has changed. Surgical AO principles were first defined in 1958 in the following way:

1. Restoration of anatomy ("fragment-to-fragment" reposition was recommended);
2. Stable fracture fixation, compression between fragments;
3. Preservation of blood supply;
4. Early active mobilization.

The AO principles have evolved and changed periodically. In recent 10-15 years they are guided by more comprehensive realization of biological principles as an important factor of proper fracture healing and functional restoration. Traumatology has gradually transformed into X-ray-associated surgery. Major access was changed into minor cuts, step-by-step X-ray inspection, minor access surgery, mini-invasive plate osteosynthesis (splinting), arthroscope-associated surgery. This technological modernization has resulted in transformation of priorities, and today the AO principles are as follows:

1. Preservation of blood supply;
2. Functional (indirect) reposition;
3. Stable fixation;
4. Early active mobilization.

Placing the care of blood supply of the bone and soft tissues in the first position the role of biology as a prior factor of proper healing is emphasized again.

Objective of the work is to improve the results of surgical treatment of distal metaepiphysis fractures of the tibial and fibular bones by means of development, substantiation and implementation of the improved technology of mini-invasive osteosynthesis.

Material and methods

In the process of investigation the AO classification and Rüedi-Allgöver (Ruedi-Allgower) classification of pilon fractures were used (tab. 1).

In case of impression fracture a trepanation opening is made 5-6 cm proximally from the joint, and through this opening by means of a special instrument articular surface is restored, and autograft is inserted into the opening (fig. 2).

Table 1

Classification of the distal tibia fractures

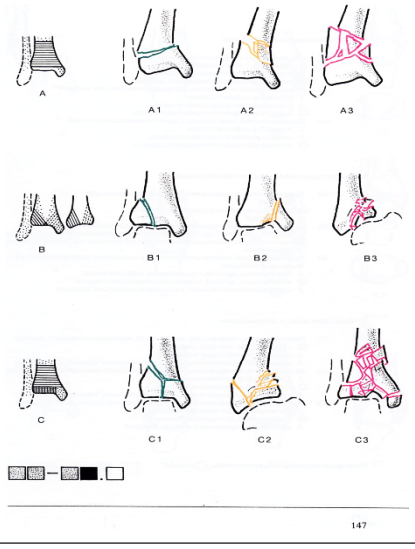
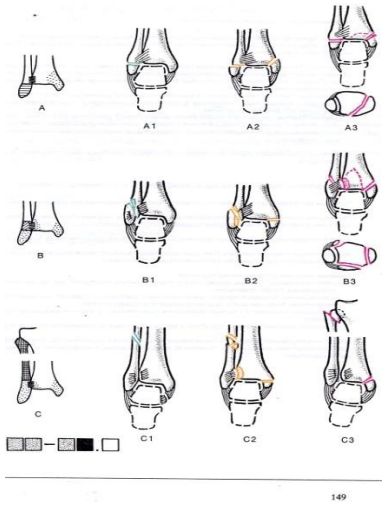
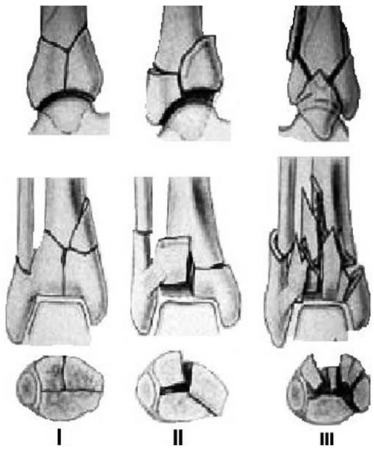
A	B	C
		
AO Classification. 43 – tibia/fibula, distal segment.	**AO Classification. 44 – tibia/fibula, malleolar segment.**	**Rüedi-Allgöver classification of pilon fractures**

Fig. 1. Trans-articular fixation after luxation elimination and closed intramedullary osteosynthesis of the fibula.

We have improved the technology of mini-invasive osteosynthesis of distal tibia metaepiphysis fractures. Reposition is performed under general or regional anaesthesia controlled by C-arm. Ligamentotaxis principle is applied pulling fragments by means of distractor. In case separate bone fragments are not repositioned a pricker, single-toothed tenaculum are used through separate pricks of the skin. Every step is controlled by imagic intensifier. After subluxation is eliminated and reposition completed, trans-articular fixation of fragments is performed inserting 2-3 K-wires 2 mm in diameter from the sole in the direction of the tibia middle part (fig. 1).

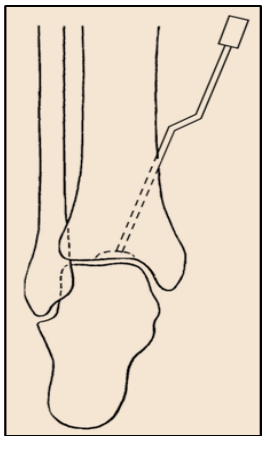


Fig. 2. Figure of impression fracture reposition of the articular surface of the distal tibia epiphysis.

Fig. 3. Mini-invasive osteosynthesis of the distal tibia with screws inserted in sagittal plane.

Fig. 4. Mini-invasive osteosynthesis of the internal and external malleolus. K-wires are bent in the shape of a hook and inserted under the skin.

After that, K-wires are inserted through separate pricks in the skin in the sagittal plane 3-4 mm proximally from the ankle joint, and fragments forming the articular surface are fixed. In some cases K-wires are changed into cannula or common screws (fig. 3).

In case of external malleolus dislocation, it is reduced and closed intramedullary osteosynthesis with one or two K-wires is performed. The internal malleolus is fixed in the same way (fig. 4). In some cases K-wires are bent in the shape of a hook and inserted under the skin. It prevents possible infection of tissues round the K-wires.

In case of fractures with partial contact between fragments (43 A and 43 B by the AO classification, or type 1 and type 2 by Rüedi-Allgöver classification of pilon fractures) the bone is immobilized with orthosis or plaster splint which is changed into scotch-cast 7 days later. In case of unstable fractures with no contact between the main fragments (43 C or type 3 by Rüedi-Allgöver classification of pilon fractures) the bone was immobilized by means of an improved variant of external fixation device (EFD) (fig. 5). K-wires were removed 6 weeks later. The period of immobilization lasted for 10-12 weeks.

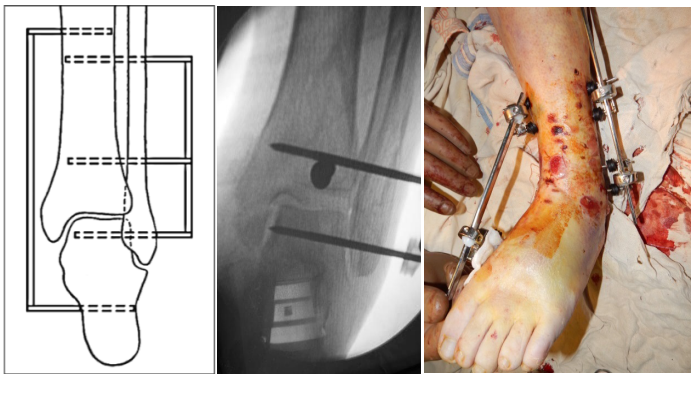


Fig. 5. Drawing and photo of the device for external osteosynthesis of distal tibia fractures.

At the beginning of application of this technique indications for mini-invasive osteosynthesis were: multiple traumatic injuries, diabetes mellitus, old age, severe comorbid pathology, "economic factors". In the course of time, accumulated experience enabled to apply this method wider.

In the clinic of the Department of Traumatology and Orthopedics of Bukovinian State Medical University on the base of Chernivtsi Emergency Hospital during the period from 2007 to 2017, 342 patients – 206 men (60.23%), and 136 women (39.77%) – with fractures of distal segments of the lower leg were operated, including 105 patients with fractures of the distal metaepiphysis of the tibia and fibula (segment 43 by the AO classification, table 1A), and 237 individuals with fractures of the crura (bone segment 44 by the AO classification, table 1B).

265 patients were examined in the process of performing the study. Their average age was 45.15±15.98 years (from 18 to 86), including patients under 30 – 52 (19.62%), from 30

to 40–56 individuals (21.13%), from 40 to 50– 52 individuals (19.62%), from 50 to 60– 47 (17.74%), older than 60– 58 (21.8%) (fig. 6).

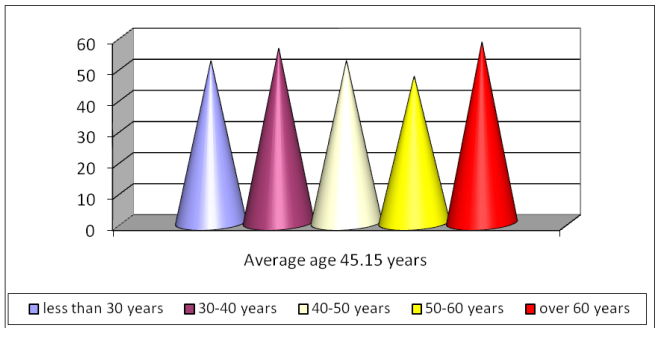


Fig. 6. Distribution of patients by age.

Males constituted the greater part of all the patients examined – 152 individuals (57.36%), females – 113 (42.64%) respectively. Open fractures were registered in 72 (27.17%) patients, closed – 184 (72.83%).

Osteosynthesis was performed on 265 patients, including plates ("clover leaf") – 63 patients (23.77%), LCP (with angle stability) – 21 patients (7.92%), K-wires – 57 patients (21.51%), external fixation – 19 patients (7.17%), external fixation + K-wires – 14 patients (5.28%), external fixation + plates – 23 patients (8.68%), external fixation for minor segments – 68 patients (25.66%).

Kinds of injuries were evidenced in 265 patients including 7 cases of occupational injuries (2.64%), 21 – road accidents (7.92%), 235 cases of off-the-job injuries (88.68%), 1 case of sport injury and 1 criminal (0.38% each). Thus, off-the-job injuries prevailed (fig. 7). It should be noted that real percentage of off-the-job injuries was not so high, since they included not only home accidents, but also concealed injuries in the street, occupational ones, sport injuries, due to road accidents, etc.

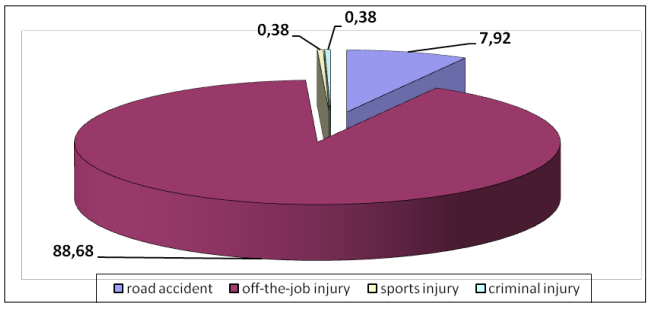


Fig. 7. Distribution of patients by the types of injuries, %.

One of the considerable parameters indicative of the efficacy of the given medical manipulations is the length of hospital stay, including preoperative and postoperative hospital stay. This index is substantially influenced by severity of injury, availability or lack of complications.

In general clinical group the preoperative hospital stay averaged 4.95 days (from one day to a month). The patients were distributed accordingly: less than 10 days before surgery – 210 patients (79.25 %), from 10 to 20 days before surgery – 47 patients (17.73%), from 20 to 30 days – 8 patients (3.02%), (fig. 8).

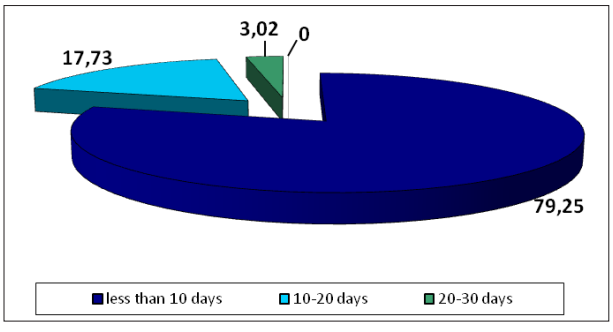


Fig. 8. Distribution of patients by preoperative hospital stay, %.

Postoperative period in general group was on an average 18.69 days (from 1 day, when surgery was performed on the day of admission, to155 days). Less than 10 days – 69 patients (26.04%), 10 to 20 days – 131 patients (49.43%), 20 days to 1 month – 35 patients (13.21%), and over a month – 30 patients (11.32%), (fig. 9). Although the number of patients with multiple traumatic injuries, infectious complications and protracted traumatic disease was not big, it made an average overall hospital stay considerably longer. We did not think it to be reasonable to exclude those patients from sampling (150,180 bed days). At the same time, in spite of severity of injuries, 75.47% of patients stayed in the hospital less than 20 days.

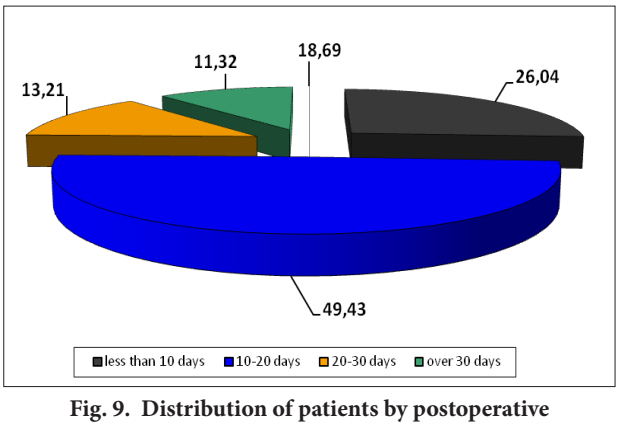


Fig. 9. Distribution of patients by postoperative hospital stay, %.

Distribution of patients into clinical groups depending on surgery performed

The patients were distributed into clinical groups depending on the type of surgery performed.

Clinical group I included patients, who underwent mini-invasive osteosynthesis with K-wires and screws (113 patients, 42.64% from general group); **clinical group II** included patients with external fixation osteosynthesis (36 patients, 13.58%); and **clinical group III** included patients who underwent osteosynthesis with plates (116 patients, 43.77% from general group, fig. 10).

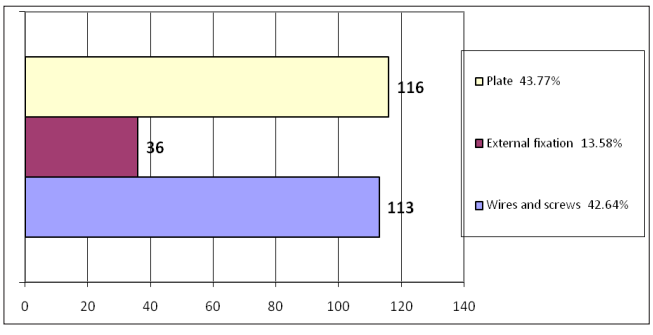


Fig. 10. Distribution of patients by osteosynthesis types into groups.

In **clinical group I** where mini-invasive osteosynthesis was performed, patients older than 60 prevailed – 33 individuals (29.20%). The following age groups included those aged from 30 to 40 (22 patients, 19.47%), 50-60 years (21 patients, 18.58%), less patients in the age group from 40 to 50 (20 individuals, 17.70%), and the least number – under 30 years (17 individuals, 15.04%). There were 51 women among patients (45.13%) and 62 men (54.87%).

This group included 77 patients (68.14%) with closed fractures and 36 patients (31.86%) with open ones. Distribution of patients by diagnosis according to AO code is given in table 2.

Table 2

Distribution of patients by diagnosis (according to AO code)

AO code	Individuals	%
43.A1	3	2,65
43.A2	11	9,73
43.A3	5	4,42
43.B1	2	1,77
43.B2	2	1,77
43.B3	3	2,65
44.A1	1	0,88
44.A2	17	15,04
44.A3	13	11,50
44.B1	2	1,77
44.B2	8	7,08
44.B3	43	38,05
44.C1	1	0,88
44.C2	2	1,77
43.C3	3	2,65
Total	113	100%

Osteosynthesis was performed in 113 patients including 30 patients with primary wound debridement and closure (26.55%), closed osteosynthesis – 13 patients (11.50%), open osteosynthesis – 9 patients (7.96%), and 61 patients with mini-invasive osteosynthesis (53.98%), (fig. 11).

In this clinical group the patients were distributed in the following way by the types of traumas: off-the-job injuries – 98 patients (86.72%), occupational injury – 8 patients (7.09%), and 7 patients after road accidents (6.19%).

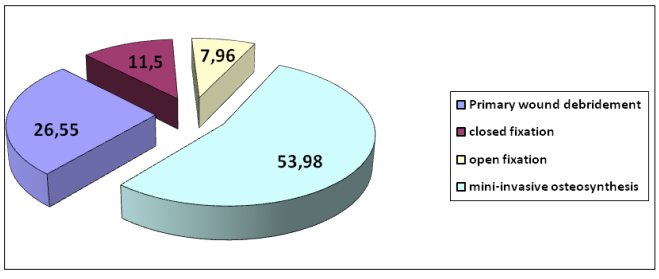


Fig. 11. Distribution of patients from clinical group I by osteosynthesis techniques, %.

Preoperative hospital stay in patients of this clinical group was in an average 4.5 days (from 1 day to 4.5 months). The patients were distributed in the following way: less than 10 days before surgery – 89 (84.08%); from 10 to 20 days before surgery – 17 patients (15.04%); from 20 days to one month – 1 patient (0.88%); more than 1 month – 6 patients (15.04%).

Postoperative hospital stay in this clinical group was on an average 17.32 days (from 1 day to 3 months) – to 10 days – 35 patients (30.97%), from 10 to 20 days – 51 patients (45.13%), from 20 days to 1 month – 17 patients (15.04%) and more than one month – 10 patients (8.85%), (fig. 12).

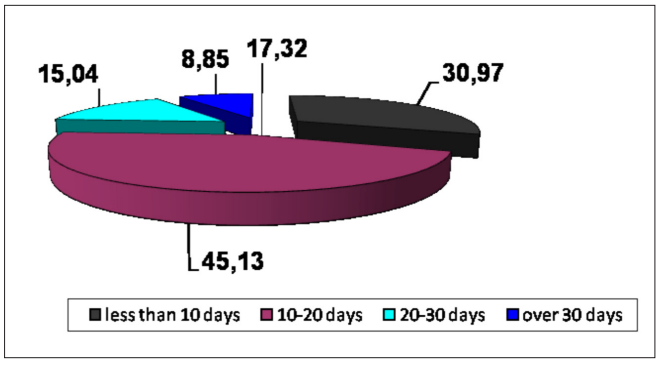


Fig. 12. Postoperative hospital stay in patients from mini-invasive synthesis group, %.

Overall hospital stay was on an average 2.5 weeks (from 2 weeks to 3.5 months).

Clinical group II of patients by the type of surgery – osteosynthesis with external fixation – included 36 patients (13.58%) from the general group.

In the group of patients with external fixation the patients aged from 40 to 50 prevailed – 11 individuals (30.56%) with an average index in the group was 48.20±16.59 years. The following age groups were 30-40 years – 7 patients (19.44%), 50-60 years – 6 patients (16.67%) and older than 60 years – 7 patients, (19.44%). The patients under 30 were in minority – 5 patients (13.89%), (fig. 13).

An average age was 46.05 years including 14 women (38.89%) and 22 men (61.11%).

In this clinical group there were 7 patients with closed fractures (19.44%), and open ones – 29 (80.56%).

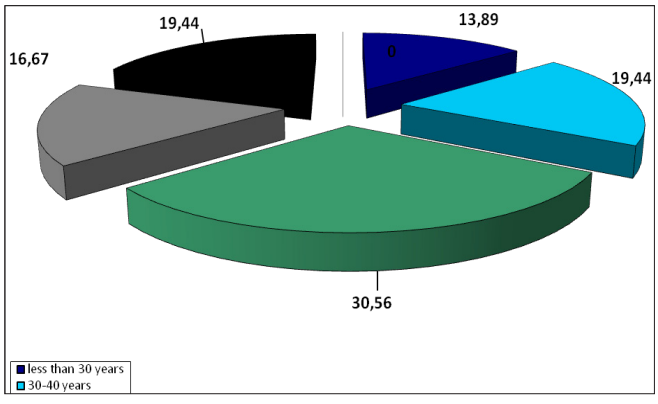


Fig. 13. Distribution of patients by age in the group with external fixation, %.

In this clinical group osteosynthesis was performed in 36 patients, including 18 patients with primary surgical treatment of the wound (50.0%), closed osteosynthesis – 3 patients (8.33 %), open osteosynthesis – 10 patients (27.78%), 1 patient with changed method of treatment of the right tibia to external fixation (2.78%), with Ilizarov's external fixator – 4 patients (11.11%), (fig. 14).

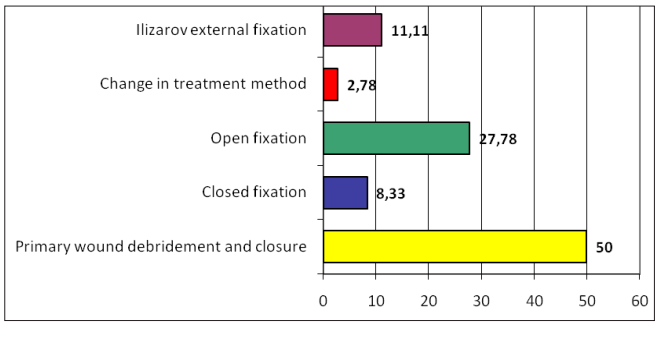


Fig. 14. Distribution of patients from the group of external fixation by the types of osteosynthesis, %.

Types of injuries treated by means of surgical aid among the patients from this clinical group were distributed in the following way: off-the-job injury – 26 patients (72.22%), occupational injury – 6 patients (16.67%) and 4 patients after road accidents (11.11%).

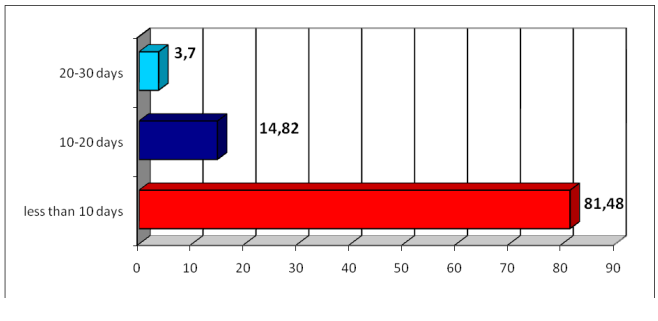


Fig. 15. Distribution of patients from the group of external fixation by preoperative hospital stay, %.

Preoperative hospital stay in this clinical group was on an average 5.19 days (from 1 day to one month). The patients were distributed in the following way: less than 10 days before surgery – 23 (81.48%), from 10 to 20 days before surgery – 4 patients (14.82%) and more than 1 month before surgery – 1 patient (3.70%), (fig. 15).

Postoperative hospital stay in this clinical group was on an average 4 days (from 1 day to 36 days): to 10 days – 31 patients (86.11%), from 10 to 20 days – 4 patients (11.11%), from 20 days to 1 month – 1 patient (2.78%).

Clinical group III. Patients by the type of surgery were distributed in the following way: plate osteosynthesis – 116 patients (43.77%) from the general group.

The age in the group of patients after plate osteosynthesis averaged 41.76±15.04 years. Patients aged from 18 to 30 years prevailed – 30 patients (25.86%). The following age groups were: 30-40 years – 28 patients (24.15%), 40-50 years – (21 patients (18.10%), and from 50 to 60 years – 21 patients (18.10%). The group of patients older than 60 included 16 individuals (13.79%), (fig. 16).

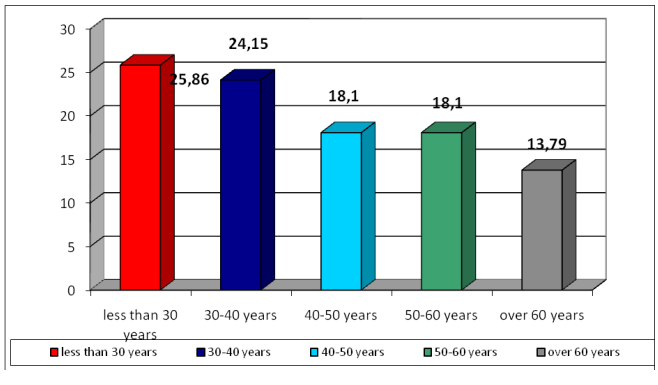


Fig. 16. Distribution of patients by age in the group of plate osteosynthesis, %.

There were 49 women (42.24%) and 67 men (57.76%).

In this clinical group there were 109 patients (93.97%) with closed fractures, and open ones – 7 (6.03%).

The following index of distribution of patients in this clinical group was the type of osteosynthesis performed. Osteosynthesis was performed on 116 patients including 2 patients with primary surgical treatment of the wound (1.72%), closed osteosynthesis – 86 patients (74.14%), open osteosynthesis – 28 patients (24.14%, fig. 17).

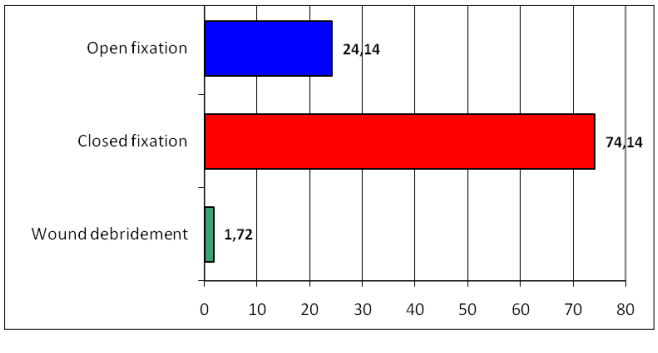


Fig. 17. Distribution of patients from the third clinical group by the type of osteosynthesis, %.

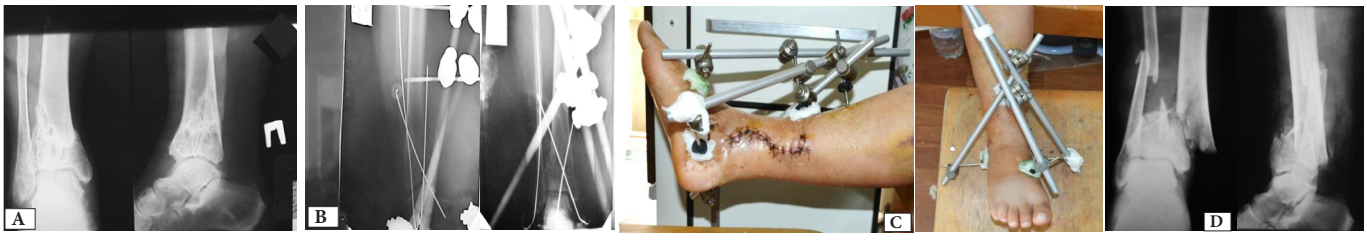


Fig. 18. X-ray and photo of the patient M. A – on the day of getting injury; B – after osteosynthesis with K-wires and pivotal device; C – external view of the limb in the device; D – remote result.

The types of injuries in this group of patients treated by means of surgery were distributed in the following way: off-the-job injury – 84 patients (72.41%), occupational injury – 9 patients (7.76%) and 21 patients after road accidents (18.10%), 1 case of sport injury and 1 of a hooligan attack (0.86% respectively).

Preoperative hospital stay was on an average 6.05 days (from 1 day to month). The patients were distributed appropriately: less than 10 days before surgery – 84 (72.41%), from 10 to 20 days before surgery – 26 patients (22.41%) and more than 20 days of preoperative period – 6 patients (5.17%).

Postoperative hospital stay in this clinical group was on an average 16.75 days (from 1 day to 2 months) – to 10 days – 29 patients (25.00 %), from 10 to 20 days – 60 patients (51.72%), from 20 days to 1 month – 14 patients (12.07%) and more than one month – 13 patients (11.21%).

Overall hospital stay was on average 2.5 weeks (from 1.5 weeks to 2 months).

Results and discussion

Remote results were examined in 46 patients.

Clinical group I. Out of 26 patients of this group who were treated by means of mini-invasive osteosynthesis of fractures with K-wires, screws and external fixation or plaster bandage, 21 of them (80.76%) demonstrated good results, secondary dislocation of fragments occurred in 2 (7.69%) patients, and adduction-flexion contracture of the ankle joint occurred in 3 (11.54%) patients.

Clinical group II. Remote results were examined in 20 patients who underwent external fixation osteosynthesis. All of them had open fractures. Good results were found in 9 (45.0%) patients who completed their treatment in the external fixation device, their limb was supporting, volume of movements deficiency was less than 25%. In 7 patients the treatment with external fixation was the first stage of two-stage protocol. Their method of treatment was changed into mini-invasive osteosynthesis with plates. 2 (7.69%) patients developed complication of chronic osteomyelitis, and therefore sequesternecrectomy and arthrodesis of the ankle joint were performed. 2 (7.69%) patients developed stable adduction-flexion contracture of the ankle joint.

Example. Patient M., born in 1964, was admitted into the Traumatology Department for Adults on 25.08.2015 with the diagnosis: open spiral comminuted fracture of the distal metaepihysis of both bones of the right lower limb (tibia and fibula) with dislocation of 43-A3.2 fragments (fig. 18).

The patient was operated on: primary surgical treatment of the wound; osteosynthesis of the right lower limb with external fixation and Ilizarov's apparatus. Bandages were applied. Postoperative wound healed with primary intention. The results were controlled a year after the construction was removed. Positive good result was evidenced clinically and by X-ray.

Clinical group III. Remote results were examined in 26 patients after osteosynthesis with plates. The specific feature of this group was that osteosynthesis with plates was performed for patients with the best condition of the soft tissues, and blood supply of fragments respectively, compared with the patients from clinical group I and especially clinical group II.

In the days of empirical approach to traumatology as a science a well-known postulate existed saying that «the ankle joint does not like a great number of metallic grafts» (similar to that one «the elbow joint does not like thermal procedures»). This statement was added by the facts that the bone in the distal third of the lower limb is surrounded by the tendons, fasciae and skin, and due to this fact blood supply of the bone is not sufficient.

Having compared the remote results of mini-invasive and traditional open bone osteosynthesis, we were convinced in certain advantages of mini-invasive variant of surgical treatment that corresponds to the latest version of the AO principles.

A certain experience has been accumulated concerning indirect reposition of the majority of fractures under step-by-step X-ray control using a traction table, distractor, joysticks, percutaneous pricker, and sharp hook.

Similar manipulations in our clinic have been performed since 1994. While performing surgery more and more often a natural question arose: since fragments are repositioned, anatomical structure of the bone is restored, is open access reasonable in this case, if it produces additional disorders to the periosteal blood supply? (fig. 19).

Is it reasonable to insert from 4 to 6 screws into the distal epiphysis and 3-4 screws through the diaphysis additionally disturbing intraosseous blood supply? Isn't it possible to do without these manipulations harmful for blood supply? In search of an answer to these questions we have chosen the advanced technique of mini-invasive osteosynthesis apply-

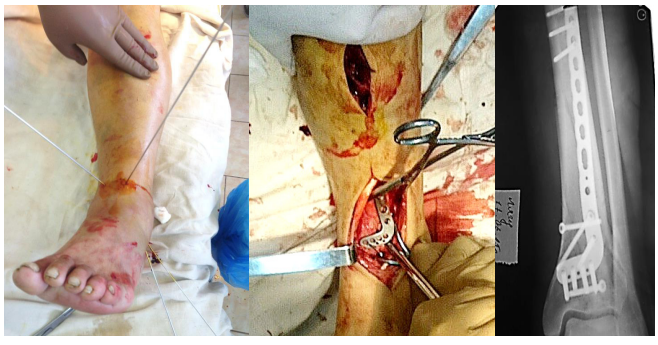


Fig. 19. Visual comparison of closed mini-invasive osteosynthesis and up-to-date osteosynthesis technique with anatomically adjacent plates.

ing K-wires 2 mm in diameter in combination with external fixation or plaster bandage. Immobilization of the limb to some extent produces a positive effect on healing damaged tissues in the place of fracture, and thus promotes restoration of blood supply to fragments. These methods are indispensable when the condition of the soft tissues in the place of fracture is poor, for example, scratches, phlyctena, exfoliation of the soft tissues, necrotic changes, infected wounds, etc. All these factors prevent from performing internal osteosynthesis. Application of mini-invasive methods of treatment of distal tibial fractures to some extent contradicts with the fourth AO principle concerning early function of the operated limb. And what about weight loading? It's not allowed in case of osteosynthesis with plates till consolidation occurs. Meanwhile it is allowed in case of external fixation. In general pathogenesis of fragments consolidation and the influence of such important factors as preservation of blood supply, functions, loading, stable fixation, immobilization of the limb and their interrelations are not completely studied and require considerable scientific investigation.

Economic issues of treatment for injured persons in Ukraine have become of a substantial value, since insurance medicine is not introduced yet, and grafting for patients is not financed from the state budget. Even without making calculations it is clear, that treatment in the first and second clinical groups costs much less than that in the third one. We are planning to determine economic efficacy indices of mini-invasive osteosynthesis. Sometimes a certain conflict arises in the existing system, since both providers of medical equipment and medical staff of hospitals are more interested in application of expensive implants for osteosynthesis than patients themselves.

The results of our study do not mean that we suggest changing the AO protocol concerning treatment of fractures of the distal tibia. All the improvements do not go beyond the scope of the approved AO technologies.

Conclusions

The analysis of implementation of the improved mini-invasive osteosynthesis technology for fractures of the distal lower limb compared with the traditional methods of surgical treatment of these injuries in 265 patients from the three clinical groups demonstrated that the use of closed reposition and improved technique leads to improved results and considerable reduction of costs for treatment in comparison with traditional osteosynthesis.

References

1. Bets G, Stoiko I, Bets I. Predoperatsionnoe planirovanie i primenenie malotravmatichnoi khirurgicheskoi tekhniki pri perelomakh pilon [Preoperative planning and application of minimally traumatic surgical technique in pilon fractures]. Ortop Travmatol Protez (Ukraine). 2013;(4):30-4. Russian.
2. Korzh NA, Romanenko KK, Goridova LD, et al. Perelomy kostei goleni na urovne distal'nogo epimetafiza (perelomy pilon'a) i ikh posledstviia, diagnostika i lechenie [Fractures of distal epimetaphysis of tibia (pilon fracture) and their consequences, diagnosis and treatment]. Travma (Ukraine). 2011;12(2):47-54. Russian.
3. Loskutov O, Panchenko S, Krasovskii V. Biomekhanicheskoe obosnovanie nekotorykh variantov maloinvazivnogo osteosinteza pri nadsindesmoznykh perelomakh narujnoi lodyzhki [Biomechanical grounds for some variants of low-invasive osteosynthesis in suprasyndesmotic fractures of the lateral malleolus]. Ortop Travmatol Protez (Ukraine). 2010;(3):64-71. Russian.
4. L'vov SE, Dzhavad Ali, Artem'ev AA, et al. Algoritm osteosinteza vnutrisustavnykh oskol'chatykh perelomov distal'nogo metaepifiza bol'shebertsovoi kosti [Algorithm of osteosynthesis of intraarticular comminuted fractures of distal tibial meta-epiphysis]. Genii Ortop (Russia). 2011;(3):30-4. Russian.
5. Pobel AN, Trufanov II, Gatsak VS. Osobennosti perelomov pilon i vybor ratsional'noi metodiki lecheniia [Features of pilon fractures and the selection of a rational method of treatment]. Travma (Ukraine). 2011;12(4):234-8. Russian.
6. Romanenko KK. Perelomy kostei goleni na urovne distal'nogo epimetafiza (perelomy pilon'a) i ikh posledstviia, diagnostika i lechenie [Fractures of distal epimetaphysis of tibia (pilon fracture) and their consequences, diagnosis and treatment]. Ukr Med Alm. 2011;14(2):155-8. Russian.
7. Calori GM, Tagliabuea L, Mazza E, et al. Tibial pilon fractures: Which method of treatment? Injury. 2010;41(11):1183-90.
8. Cannada LK. The no-touch approach for operative treatment of pilon fractures to minimize soft tissue complications. Orthopedics. 2010;33(10):734-8.
9. Gaulke R, Krettek C. Tibial pilon fractures: Advoidance and therapy of complications. Unfallchirurg. 2017 Aug;120(8):658-66.
10. Hong J, Zeng R, Lin D, et al. Posteromedial anatomical plate for the treatment of distal tibial fractures with anterior soft tissue injury. Orthopedics. 2011;34(6):161-69.
11. Meena UK, Bansal MC, Behera P, Upadhyay RJ, et al. Evaluation of functional outcome of pilon fractures managed with limited internal fixation and external fixation: A prospective clinical study. J Clin Orthop Trauma. 2017 Nov;8(Suppl 2):S16-S20.
12. Müller FJ, Nerlich M. Tibial pilon fractures. Acta Chir Orthop Traumatol Cech. 2010;77(4):266-76.
13. Sohn OJ, Kang DH. Staged protocol in treatment of open distal tibia fracture: using lateral MIPO. Clin Orthop Surg. 2011;3(1):69-76.
14. White TO, Guy P, Cooke CJ, et al. The results of early primary open reduction and internal fixation for treatment of OTA 43.C-type tibial pilon fractures: a cohort study. J Orthop Trauma. 2010;24(12):757- 63.
15. Zhang ZD, Ye XY, Shang LY, et al. Case-control study on minimally invasive percutaneous locking compression plate internal fixation for the treatment of type II and III pilon fractures. Zhongguo Gu Shang. 2011;24(12):1010-12.

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New approach to heart rate variability analysis based on cardiophysiological biomarkers

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Abstract

Background: The heart rate variability (HRV) analysis is a well-known method demonstrating its value over the years in different medical fields. However, it still has its known limitations.
Material and methods: The new approach to HRV analysis is based on a complementary HRV standard analysis with new cardiophysiological biomarkers. The biomarkers are assessed on cardiorhythmograms obtained by a 5-minute ECG recording using a specialized hardware (Polyspectrum-HRV-device, Neurosoft).
Results: A possible applicative value of the biomarkers is shown on examples of how a prognosis for recurrence of atrial fibrillation (AFib) could be made. When in a rest-state cardiorhythmogram are observed LF drops and are followed by a pathological counterregulation, prognostically, recurrence of atrial fibrillation is expected. When in a cardiorhythmogram LF drops are observed and are followed by a physiological counterregulation, prognostically, sinus rhythm is expected. Physiological background of the biomarkers: increased central modulation of the heart in rest state of a patient, a sympathetic overflow of the heart in calm state and insufficiency of compensatory parasymphatetic counteractivation. Limitations of the paper: this is a methodological paper without description of patients. This paper will be followed by a clinical paper in which we are going to describe the validation of these cardiophysiological biomarkers on patients with AFib.
Conclusions: Complementary to the standard HRV analysis, cardiophysiological biomarkers should be assessed: LF drops and HF counterregulation could be used for prognosis construction in cardiology.
Key words: cardiophysiological biomarkers, heart rate variability.

Introduction

The new methodological approach described in this paper is based on heart rate variability (HRV) analysis. The HRV analysis is a well-known method which has been demonstrating its value over the years in different medical fields [1, 27]. However, it still has its known limitations [2]. Although from a physiological point of view it could offer much more information than offer classical statistical programs for an automatic HRV analysis. One of the limitations in use of HRV in the cardiologic field is the problem of steady-state cardiorhythmograms. Steady-state cardiorhythmogram means, that during the 5-minute measurement no extra waves are triggered from outside or even inside, i.e. the person should demonstrate a constant breathing pattern without hyperventilation, no extrasystoles should occur nor any other arrhythmias and all other standard conditions [3] to avoid the appearance of extra waves, should be respected. As far as additional waves occur in the cardiorhythmogram, i.e. triggered by a change of respiratory pattern, extrasystoles etc., it cannot be analysed using classical methods of HRV analysis. Sometimes, such additional waves are cut out to make the classical analysis possible at all. However, cutting out, the biosignal loses some of its quality and reliability [4]. That is the main reason, why such cardiorhythmograms are mostly just ignored or just minimal information is extracted from such a HRV analysis. For instance, to determine whether the HRV is high or low – from a physiological point of view – there is only a minimum of information that could be extracted from a HRV analysis when applying also physiological skills for a HRV analysis. In cardiology, there are very common cardiorhythmograms which do not at all correspond to a steady-state cardiorhythmogram. That is why in this field there are a lot of limitations in the application of HRV analysis. Mathematicians are trying to solve this problem by using an analysis of non-steady-state cardiorhythmograms with non-linear methods of HRV analysis [5, 27, 31]. Another possible way to solve this problem is described in this paper. It is proposed to assess the standard HRV analysis using additionally cardiophysiological biomarkers for a more advanced physiological HRV interpretation.

Material and methods

The biomarkers are assessed on cardiorhythmograms obtained by a 5-minute ECG recording using a specialized hardware (Polyspectrum-HRV-device, Neurosoft). The biosignal was obtained from a one-channeled ECG, the first derivative was applied. It is important to mention, that the biosignal for further HRV analysis was obtained not from a Holter ECG. In order to obtain a reliable biosignal and to ensure the reproducibility of the data, all standard conditions during measurement were regarded [3].

Standard operating procedure for heart rate variability recording

Resting state probe includes a 5-minute ECG recording which is done in supine position. During the recording the person lies quietly but is alert with free spontaneous breathing. The person has to be in sinus rhythm. Before starting with the recording itself, a steady-state was achieved. Therefore, the person lies with connected electrodes and is checked on the monitor until the moment when a steady-state signal is reached. Only then starts the recording of the ECG signal which will be used for further HRV analysis. The length of the transition phase needed for achieving a steady-state signal is very individual and usually takes from 5 to 20 minutes [6]. This is to avoid false positive reactions of an increased level of the sympathetic or parasympathetic part of the vegetative nervous system. In the rest state probe, there should be an assessment of the functional activity of the regulatory systems of the heart including the medullar level and the central one. It can be concluded that a reliable and qualitative assessment can only be effected, if all additional influences, not belonging to the rest state condition, are excluded.

Results and discussion

The data were analysed by the use of „Neuro-Soft"-software which is working with biosignals and is specialized on HRV analysis. Additionally the data were analysed by using cardiophysiological biomarkers. Standard HRV analysis methods are not described in this paper as these are well known [3, 12]. The new physiological approach to HRV analysis by using cardiophysiological biomarkers will be described. These are applied when evaluating cardiorhythmograms and spectrograms. Were applied several cardiophysiological biomarkers but in this paper are described only the most informative, most important and most convenient ones for the data analysis: Low frequency (LF) drops, high frequency (HF) counterregulation and increased central activity in rest state.

HRV is applied for risk determination in different cardiovascular diseases [5, 28]. In order to illustrate a possible way of applying cardiophysiological biomarkers, there are given examples of how a prognosis for atrial fibrillation and sinus rhythm can be made. Hence, we analysed different cardiorhythmograms and spectrograms using the cardiophysiological biomarkers.

Below are presented cardiorhythmograms and spectrograms, where prognostically a sinus rhythm (SR) is expected by applying the biomarkers' analysis:

1.A. On the rhythmogram no LF drops are recognized and the HRV is predominantly modulated by HF waves (fig. 1).

1.B. On the spectrogram dominates the HF spectrum area (blue) with a physiological peaks' distribution (fig. 2).

2.A. On a rhythmogram LF drops are present but are followed by a physiological counterbalancing via HF waves (fig. 3).

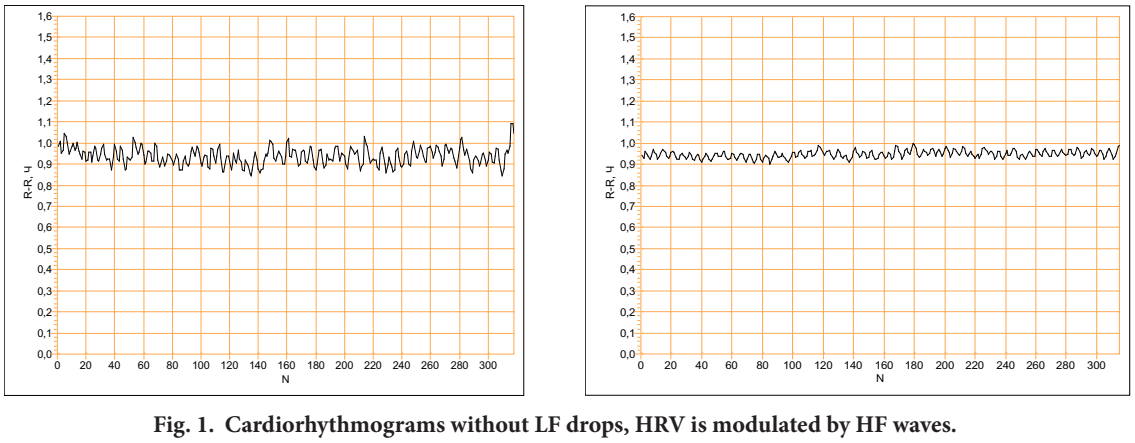

Fig. 1. Cardiorhythmograms without LF drops, HRV is modulated by HF waves. On the left side – rhythmogram of a younger person, on the right side – of an older person.

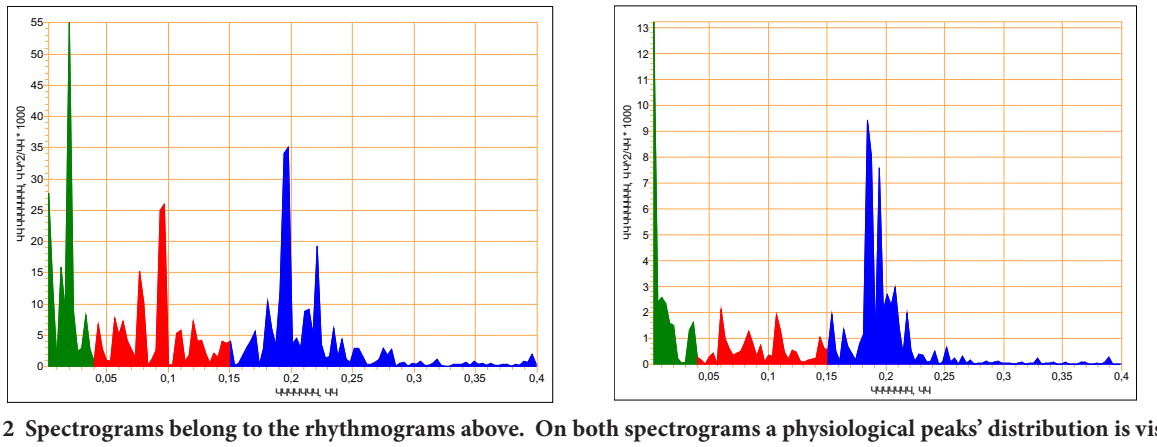

Fig. 2 Spectrograms belong to the rhythmograms above. On both spectrograms a physiological peaks' distribution is visible.

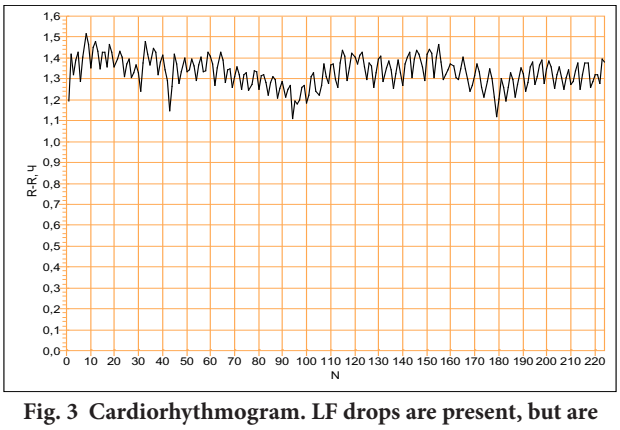

Fig. 3 Cardiorhythmogram. LF drops are present, but are followed by a physiological counterbalancing via HF waves.

2.B. The VLF component is increased on the spectrogram but the HF spectrum has a physiological HF peaks' distribution, sufficient to compensate the increased VLF component (fig. 4).

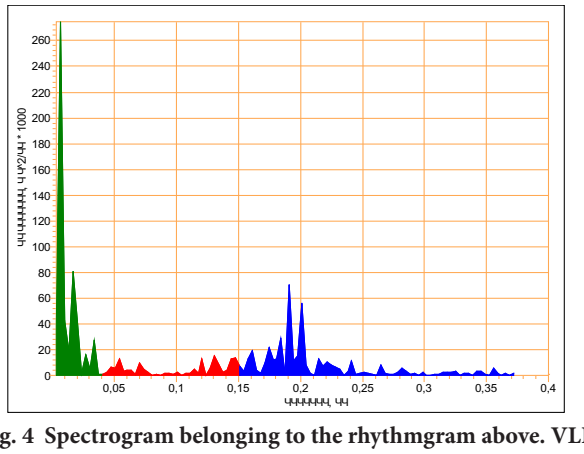

Fig. 4 Spectrogram belonging to the rhythmgram above. VLF (green colour) is increased but the HF spectrum (blue colour) has a physiological HF peaks' distribution.

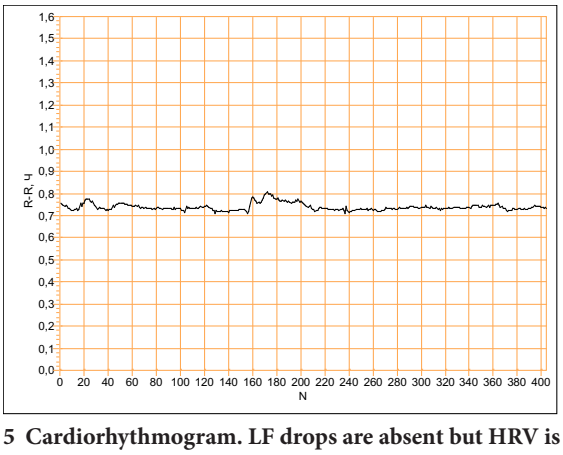

Fig. 5 Cardiorhythmogram. LF drops are absent but HRV is low and is in its modulation dominated by VLF and LF waves.

Below are presented cardiorhythmograms and spectrograms where prognostically AFib is expected by applying the biomarkers' analysis:

1.A. On a rhythmogram, even without LF drops, the HRV is low and is in its modulation dominated by VLF and LF waves (fig. 5).

1.B. On a spectrogram the VLF component dominates in the total power spectrum. Pathological peaks' structure in the HF spectrum area, representing parasympathetic break-down (fig. 6).

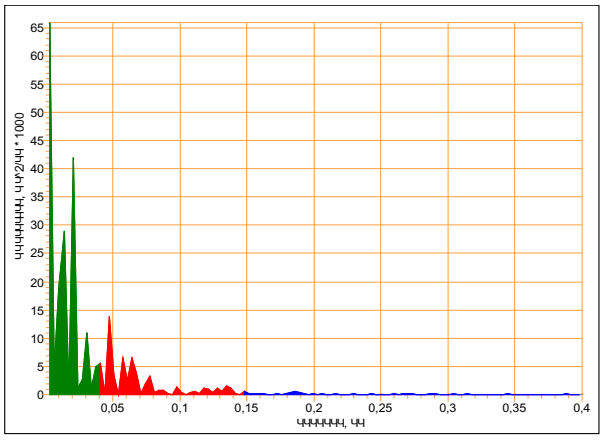

Fig. 6 Spectrogram which belongs to the cardiorhythmogram above. VLF component dominates in the total power spectrum. Pathological peaks' structure in the HF spectrum area, representing parasympathetic break-down.

2.A. On the rhythmogram LF drops are present, followed by a pathological counterregulation: predominated by LF waves (fig. 7).

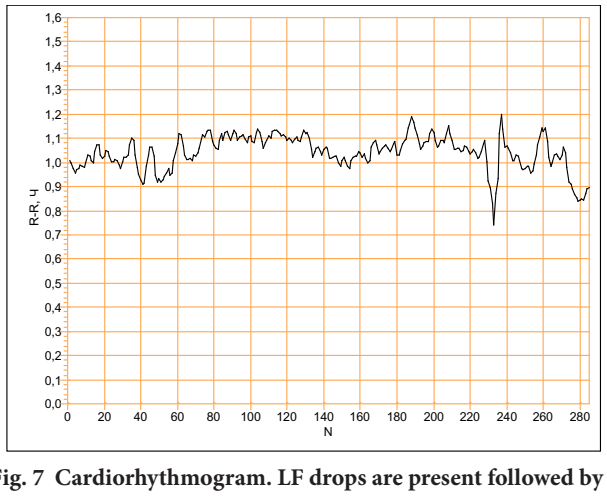

Fig. 7 Cardiorhythmogram. LF drops are present followed by a pathological counterregulation: predominated by LF waves.

2.B. On the spectrogram (fig. 8) the VLF and LF components are dominating in the total power spectrum and at the same time, there is evidence of a pathological peaks' structure in the HF spectrum area, it means a parasympathetic insufficiency, which is unable to compensate for the dominant VLF (central) component and increased LF component (sympathetic overflow in calm state).

The HRV analysis with an additional application of biomarkers, made on the figures above, requires a certain algorithm. Below is presented a simplified algorithm, which is proposed for the use by physicians in the daily practice but still should be validated clinically afterwards. It is based on cardiophysiological biomarkers described in this paper. The algorithm is used for the prognosis regarding sinus rhythm maintenance or AFib recurrence.

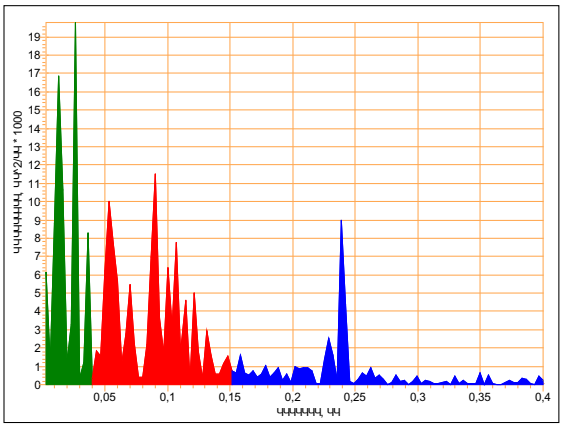

Fig. 8 Spectrogram which belongs to the cardiorhythmogram above. VLF and the LF components are dominating in the total power spectrum. Pathological peaks' structure in the HF spectrum area.

1. In the rhythmogram:
– Are LF drops present or not?
– If there are no LF drops, it is necessary to assess: is the HRV high or low?
– If LF drops are present, it is necessary to assess: is it parasympathetic or sympathetic counterregulation?
2. In the spectrogram:
– Do the peaks in the HF spectrum have a physiological structure?
– Is the VLF component pathologically high in the whole spectrum or in the physiological normal range?
– Is there evidence of a VLF + LF coupling in the transition?

Risk stratification for AFib using the questions described above:

1. In the rhythmogram:
– If LF drops are absent and the HRV is high –the risk is low
– If LF drops are absent, but the HRV is low – the risk is high
– LF drops are present – it is a risk factor. Parasympathetic counterregulation after LF drops – the risk is low, sympathetic counterregulation after LF drops – the risk is high.
2. In the spectrogram:

– Peaks in the HF spectrum – in case of a physiological structure: a main peak with two or three side peaks – the risk is low. Pathological peaks' distribution in the HF spectrum: several main peaks / no major peak/ only low-amplitude peaks scattered in the spectrum – the risk is high.

– VLF component: in case of a physiological VLF proportion in the entire spectrum (not dominating over the HF and the LF components of the total spectrum) – low risk; if pathological (VLF component proportion in the total spectrum is increased and dominates over the HF and LF spectra) – high risk.

– VLF + LF coupling in transition not present – low risk; VLF + LF coupling present in transition – high risk.

Below are given explanations on how to assess the cardiorhythmograms and spectrograms illustrated in the text above. Also, it is described how the cardiophysiological biomarkers are identified, by assessing a cardiorhythmogram or spectrogram and the physiological background of the cardiophysiological biomarkers.

Assessment of cardiorhythmogram

For a more convenient understanding of all cardiorhythmograms in the paper, is given a brief explanation: on the abscissa there is the number of the R peaks deriving from ECG, marked by the letter "N". On the ordinate is shown the beat-to-beat interval measured in seconds.

Now the approach to the cardiorhythmogram analysis proposed in this paper: first of all, it is important to recognize whether any LF drops in the cardiorhythmogram are present (what exactly is considered to be LF drops will be explained in detail later in the text). If there are no LF drops present, it means this is a steady-state cardiorhythmogram (fig. 9 and fig. 10), it can be analysed according to standard HRV analysis procedure [7]. According to the risk stratification, there is a difference between the figure 9 and 10. The cardiorhythmogram represented on figure 9 reflects a low risk for developing AFib. For the risk stratification and prognosis it is important to recognize whether the HRV is modulated by the medullar or by the central level of heart regulation. This physiological background is seen on a cardiorhythmogram recorded in calm state: when it is predominantly modulated by HF waves (fig. 9), then it can be assumed, that the parasympathetic nervous system works physiologically sound and the heart is regulated mainly by the medullar level [8]. Thus this supports a prognosis for SR.

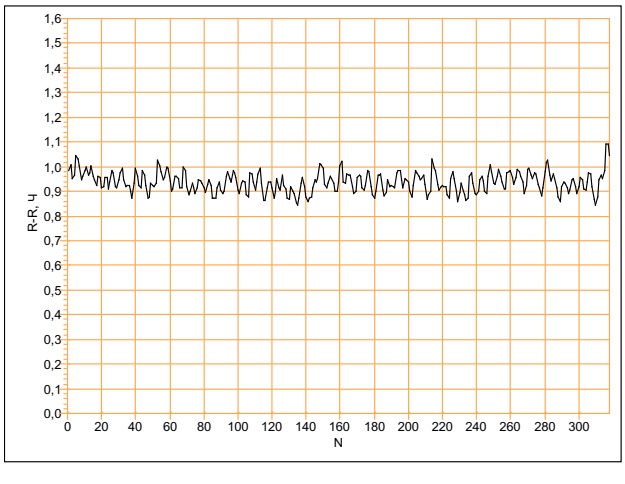

Fig. 9. Cardiorhythmogram. In this cardiorhythmogram the HF waves dominate. There are no LF drops.

On the next figure (fig. 10) there is another extreme. There are still no LF drops present, but the HRV is modulated predominantly by VLF and LF waves. From a physiological point of view, this is a dangerous situation, because the heart is modulated in rest state not by the medullar level but mainly by the central level [8, 9, 24]. This supports that prognostically AFib is expected.

However, examples presented on figure 9 and 10 represent two extreme cases, but there are fluent phases in be-

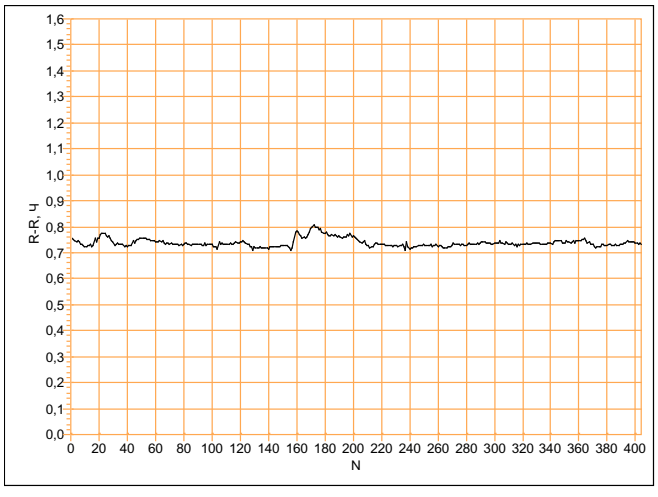

Fig. 10. Cardiorhythmogram. HRV is modulated predominantly by VLF and LF waves. There are no LF drops.

tween, when a prognosis cannot be made so obviously. That is why the use of cardiophysiological biomarkers, in order to apply them in addition to the standard HRV analysis, are proposed. On the next example (fig. 11) you can see a cardiorhythmogram with LF drops. LF drops represent non-steady-state events. As far as these are recognized, the HRV cannot be assessed by standard HRV analysis [3, 25]. How can it be analysed then? Assessing the LF drops as being one cardiophysiological biomarker and the HF counterregulation as another one. What are the LF drops? LF drops are waves on a cardiorhythmogram, occurring suddenly at the end or in the middle of a VLF wave (fig. 11 LF drops are marked by red arrows). They drop down towards the beat-to-beat interval shortening, that is why we called them LF drops, it means low frequency drops. Low frequency (LF) waves on a cardiorhythmogram are normally physiologically driven by sympathetic inputs [10]. But the difference between physiological LF waves and LF drops consists in a sudden appearance of sympathetic overflow represented by LF waves of high intensity on a rhythmogram. That is why we called them LF drops. Taking into account that these are rest-state cardiorhythmograms, it is a pathological condition. A sympathetic overflow of the heart in rest state it is observed, when the medullar heart modulation is working insufficiently, thus the central modulation of the heart increases compensatory [8, 11]. Such a state destabilizes the heart rhythm [10, 26]. As a result, the appearance of LF drops on a cardiorhythmogram in rest state is connected with an increased risk for AFib.

As a second step of analysis of cardiorhythmograms with LF drops, the HF counterregulation should be assessed. The HF counterregulation is represented by the waves following the LF drops (fig. 11 encircled blue) in order to counterbalance them [12]. Under physiological conditions it is expected that the counterbalancing reaction is to be ensured by a parasympathetic compensatory reaction [12]. In this case on cardiorhythmograms HF (high frequency) waves will be observed, which correspond to parasymphatetic modulation of the heart. In case of a pathological counterregulation, LF waves will be seen. A pathological counterregulation is

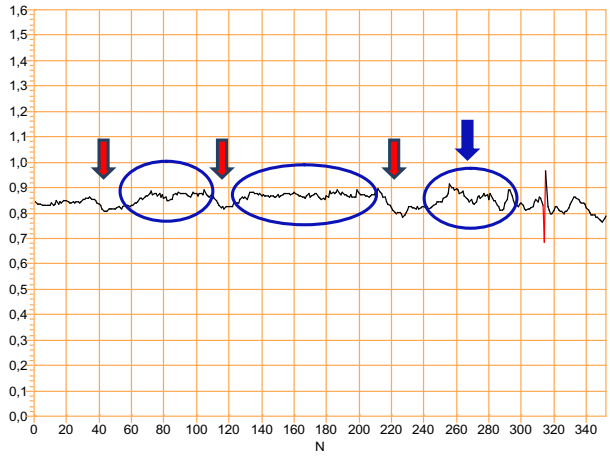

Fig. 11 Cardiorhythmogram. The heart regulation is predominantly ensured by the central level: LF drops are marked with red arrows. The counterbalancing waves (encircled blue) are present predominantly by LF waves instead of HF waves. A parasympathetic break-down (blue arrow) reaction, when counterbalancing the LF drop, is visible.

connected with a high risk for AFib recurrence. Figure 11 represents an example of a pathological counterregulation. It is classified pathological, because it is ensured mainly by LF waves instead of HF waves. It takes place when the counterregulation after LF drops is exerted not by the medullar level of heart regulation, but predominantly, by the central level [8, 14, 32]. In other words, the parasymphatetic activity is responding not effectively enough in reacting to sympathetic activations driven by the increased central regulatory activity in calm state. It is a parasympathetic break-down reaction during answering to LF drops (fig. 11 blue arrow). This is connected with a high risk for AFib recurrence.

LF drops followed by a pathological counterregulation can occur not only on cardiorhythmograms with a low HRV (fig. 11) but they also occur often on cardiorhythmograms with a high HRV (fig. 12).

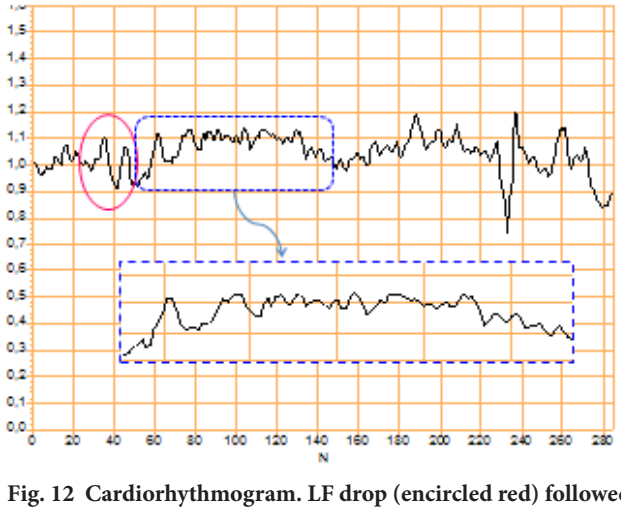

Fig. 12 Cardiorhythmogram. LF drop (encircled red) followed by a pathological counterregulation (blue frame): predominantly modulated by LF waves, instead of HF waves and with a drop-down of waves during counterbalancing.

On figure 12 you can see the LF drop (encircled red) followed by a pathological counterregulation (blue frame). On this cardiorhythmogram the HRV is high, but if you look at the counterregulation, you can recognize, that it is ensured mainly by LF waves. It means, that the parasymphatetic counteractivity is functionally not sufficient to compensate for sympathetic central activity in calm state [15, 32]. This is connected with a high risk for AFib recurrence.

Below, there is an example of a cardiorhythmogram, where the LF drops are present, meaning an increased central modulation, but the counterregulation is modulated by HF waves (fig. 13). That means, the parasymphatetic counterbalancing activity is sufficient to compensate for an increased central modulation of the heart in calm state. In such a situation, a sinus rhythm is expected.

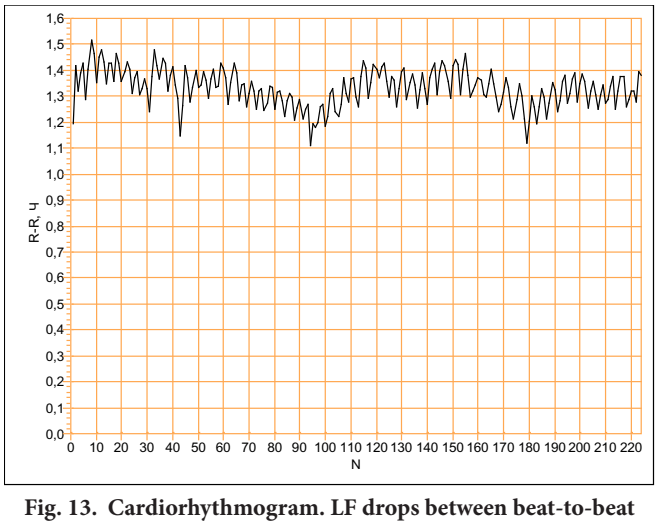

Fig. 13. Cardiorhythmogram. LF drops between beat-to-beat interval 40 – 50, 90 – 100, 170 – 180 (abscissa), followed by a counterregulation by HF waves.

In addition to the rhythmograms, the corresponding spectrograms can also be assessed. On the spectrograms it is also possible to apply the physiological background of the cardiophysiological biomarkers. The influence of the central heart modulation can be analyzed by the VLF component and the vagal activity can be analyzed by the HF spectrum (more in detail in the following text).

Assessment of the spectrogram

For a more convenient assessment of spectrograms it should be briefly noted what is shown on the abscissa and ordinate. On the abscissa you can see three groups of frequencies (obtained from cardiorhythmograms) in Hertz [16]. Marked by blue colour is the HF – high frequency spectrum area, which represents the parasympathetic part of the vegetative nervous system. By the red colour the low frequency (LF) spectrum area is marked, which represents the influence of the sympathetic part of the vegetative nervous system, acting by noradrenaline. The green colour in the spectrogram represents the VLF component of the spectrum. It is the very low frequency component. It represents the central modulation of the heart (cortex, limbic system, hypothalamus) [8, 9, 32]. Important to mention as well is that the VLF component represents the central heart modulation in case of a rest-state probe in 5-minute ECG recordings under steady-state conditions [8, 17]. In case of HRV analysis from a Holter ECG, the VLF component has other characteristics and origin, it does not represent the central modulation of the heart. These three groups of frequencies are measured in Hertz in the spectrogram. All these three spectra make up the total power spectrum in amplitudes, which is represented on the y-axis.

When assessing the spectrogram, it is important to note what the spectral distribution looks like and what proportion of each spectrum is in the overall spectrum. First, the peaks' distribution in the HF area is considered: if a main peak is observed and accompanied side by side by one or two smaller secondary peaks (they should be half-sized or one quarter of the size of the main peak), then it is assumed that the parasympathetic heart modulation physiologically works well and a sinus rhythm is most probably predicted (fig. 14).

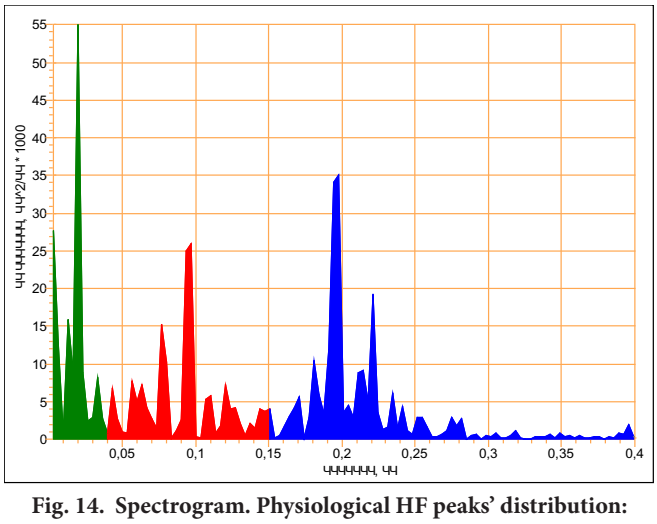

Fig. 14. Spectrogram. Physiological HF peaks' distribution: there is the main peak on frequency about 0.2 Hz (abscissa) and laterally smaller side peaks are seen.

It should be noted that the distribution of the peaks on the frequencies depends on the individual respiratory rate [18, 30]. The closer the main peak is to 0.4 Hz (see abscissa fig. 14), the higher the respiratory rate. The closer the main peak shifts in the opposite direction, closer to the green spectrum area, the slower the person's breathing. As can be seen (fig. 14), the main peak is at the frequency value 0.2. Thus, the respiratory rate is about 10 breath cycles per minute.

In spectral analysis it is also important to estimate the VLF component. This gives information about the central input of the VNS in the cardiac regulation. [17]. Under physiological conditions predominantly the medullar level modulates the heart at rest [8, 9] (fig. 14), opposite to the central level (VLF area). In the spectrogram you can see it well: the majority is made up of the blue and the red spectra. However, the green portion does not dominate over the blue and the red spectra. Under these circumstances we expect a SR.

In pathological situations, when a dominant green spec-

trum area on a spectrogram (fig. 15) during rest-state probe is observed, it is considered that the central level of heart modulation is more involved than the medullar one. This is a dangerous state from a physiological point of view [19, 29], as it represents risk factors for the development of AFib.

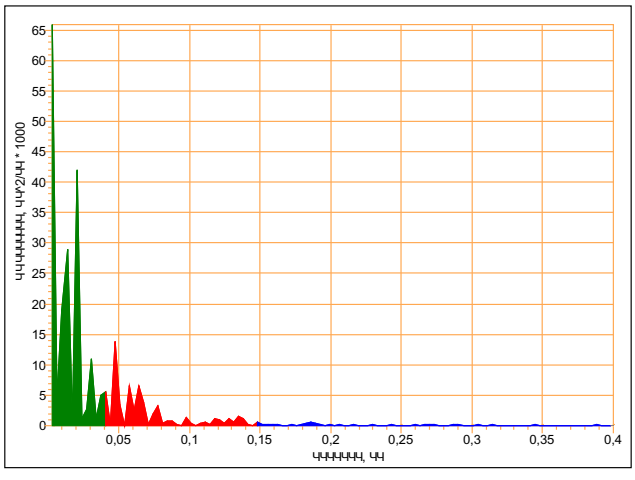

Fig. 15. Spectrogram. Pathological regulation of the heart: an increased central modulation – VLF (green) component in the total power spectrum and a pathological – HF spectrum area (blue) – a parasympathetic break-down.

On the next spectrogram (fig. 16) you can see an increased VLF spectrum (green) followed by a marked increased LF spectrum (red). That means, an increased central modulation of the heart parallel with an increased sympathetic overflow [20, 21, 32] of the heart. It is important to keep in mind, that it was a rest-state ECG registration, so it is an increased sympathicotonia in calm state. In such conditions, it is important to have an effective vagal compensation [8, 31]. But if you look at (fig. 16) the HF spectrum (blue) corresponding to the parasympathetic functional activity, you recognize a lack of functional parasympathetic activity. In the blue spectrum there is a pathological wave distribution: only one peak without side peaks, instead there

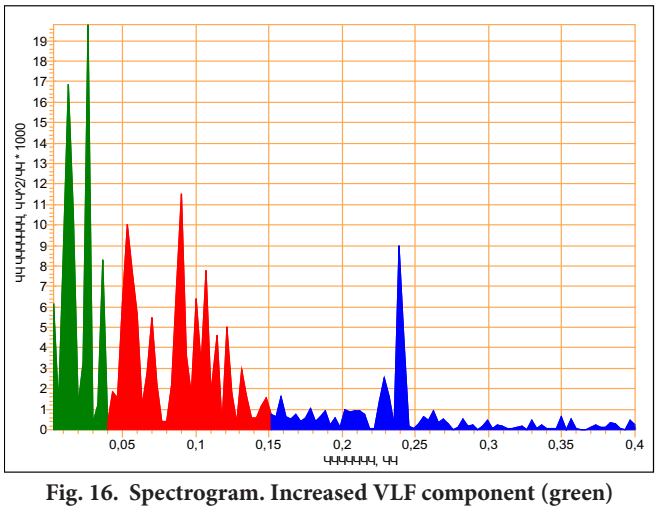

Fig. 16. Spectrogram. Increased VLF component (green) in the total power spectrum and a marked high LF component (red). HF spectrum area: only one main peak without side peaks, just low-amplitude distributed peaks through the whole spectrum area.

are very low amplitude peaks distributed through the whole HF spectrum area. It is obvious, that such a vagal counterbalance is not able to compensate effectively enough such a high central modulation and sympathetic overactivity of the heart [23, 31]. This represents a parasympathetic insufficiency, so the vagus is able to counterbalance only for short-term reactions but is not able to fulfill effectively enough its counterregulatory function against sympathetic central overflow. Hence, prognostically, AFib is expected.

It is important to note, that in case the VLF component is increased in the total power spectrum, the HF component should also be assessed in order to make a correct qualitative prognosis. When VLF is increased, but the HF spectrum has a physiological peaks' distribution, it can be concluded that the parasympathetic functional activity is sufficient in order to compensate for an increased central input to the heart in rest state (fig. 17) [32].

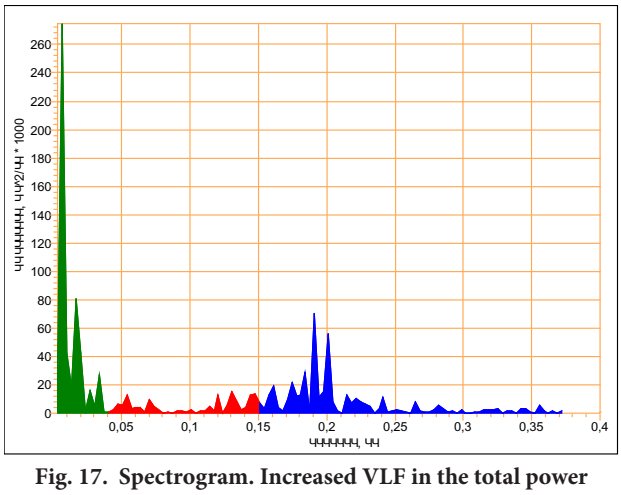

Fig. 17. Spectrogram. Increased VLF in the total power spectrum compensated by a physiological HF spectrum.

Conclusions

1. In addition to the standard HRV analysis, cardiophysiological biomarkers should be assessed: LF drops and HF counterregulation. On the example of prognosis construction for atrial fibrillation based on the biomarkers a possible applicative value of them is explained.

2. When in a steady-state cardiorhythmogram LF drops are observed followed by a pathological counterregulation, prognostically atrial fibrillation recurrence is expected.

3. When in a steady-state cardiorhythmogram LF drops are observed followed by a physiological counterregulation, prognostically, sinus rhythm is expected.

4. Additionally to the cardiorhythmogram the biomarkers can be assessed in a spectrogram using the VLF and the HF components.

5. When the VLF component is pathologically high in rest state and the structure of the HF spectrum is pathological, prognostically the recurrence of atrial fibrillation is expected.

6. When the VLF component is pathologically high in rest state but the structure of the HF spectrum is physiological, prognostically sinus rhythm is expected.

7. Physiological background of the biomarkers: increased

central modulation of the heart in rest state (LF drops present, increased VLF) is a risk factor for atrial fibrillation but if the parasymphatetic modulation is sufficient to compensate for it (HF counterregulation by HF waves, physiological HF spectrum), sinus rhythm is expected. If the parasympathetic modulation is not sufficient to compensate for it (counterregulation by LF waves, pathological HF spectrum), atrial fibrillation is expected.

References

1. Billman GE, Huikuri HV, Sacha J, Trimmel K. An introduction to heart rate variability: methodological considerations and clinical applications. Front Physiol. 2015;6:55. doi: 10.3389/fphys.2015.00055.
2. Rajendra Acharya U, Paul Joseph K, Kannathal N, Lim CM, Suri JS. Heart rate variability: a review. Med Biol Eng Comp. 2006;44(12):1031-51. doi: 10.1007/s11517-006-0119-0.
3. Task force of the European Society of Cardiology the North American Society of Pacing Electrophysiology. Heart rate variability: standards of measurement, physiological interpretation, and clinical use. Circulation. 1996;93(5):1043-65.
4. Cysarz D, Porta A, Montano N, Kurths J, Wessel N, Edelhauser F, Van Leeuwen P. Heart rate dynamics assessed by different strategies of symbolization. In: 8th Conference of the European Study Group on Cardiovascular Oscillations (ESGCO 2014); 2014 May 25-28; Trento, Italy. p. 51-52. doi: 10.1109/ESGCO.2014.6847514.
5. Stein PK, Reddy A. Non-linear heart rate variability and risk stratification in cardiovascular disease. Indian Pacing Electrophysiol J. 2005;5(3):210-20.
6. Sassi R, Cerutti S, Lombardi F, Malik M, Huikuri HV, Peng CK, Schmidt G, Yamamoto Y. Advances in heart rate variability signal analysis: joint position statement by the e-Cardiology ESC Working Group and the European Heart Rhythm Association co-endorsed by the Asia Pacific Heart Rhythm Society. Europace. 2015 Sep;17(9):1341-53. doi: 10.1093/europace/euv015.
7. Akselrod S, Gordon D, Ubel FA, Shannon DC, Barger AC, Cohen RJ. Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat to beat cardiovascular control. Science. 1981;213:220-2.
8. Mikhailov V. Variabel'nost' ritma serdtsa: opyt prakticheskogo primeneniia [Heart rate variability: practical application]. 2-nd ed. Ivanovo (Russia):IGMA; 2002. 288 p. ISBN: 5-89085-096-2. Russian.
9. Sherwood Lauralee. Fundamentals of human physiology. 4th ed. Belmont (USA): Brooks/Cole; 2012. Chapter 9, Cardiac Physiology; p. 228-259. ISBN: 978-0-8400-6225-3.
10. Calkins H, Kuck KH, Cappato R, Brugada J, Camm AJ, Chen SA, Crijns HJ, Damiano RJ Jr, Davies DW, DiMarco J, et al. 2012 HRS/EHRA/ECAS Expert Consensus Statement on Catheter and Surgical Ablation of Atrial Fibrillation: recommendations for patient selection, procedural techniques, patient management and follow-up, definitions, endpoints, and research trial design. Europace. 2012 Apr;14(4):528-606. doi: 10.1093/europace/eus027.
11. Voss A, Schroeder R, Truebner S, Goernig M, Schirdewan A, Figulla HR. Alternans of blood pressure and heart rate in patients with dilated cardiomyopathy. Comput Cardiol (Valencia, Spain). 2006;33:421-4. [cited 2018 Jul 2018]. Available from: http://www.cinc.org/archives/2006/pdf/0421.pdf
12. Hirsh JA, Bishop B. Respiratory sinus arrhythmia in humans: how breathing pattern modulates heart rate. Am J Physiol.1981;241(4):H620-9.
13. Guyton AC, Hall JE. [Textbook of medical physiology]. Moscow: Logosfera; 2008. [Chapter 10, Rhythmical excitation of the heart]; p. 124-131. Translated from English by O. Kositskaia, et al. ISBN: 978-5-98657-013-6. Russian.
14. Rudenko M, Zernov V, Voronova O. Fundamental research on the mechanism of cardiovascular system hemodynamics self-regulation and determination of the norm-pathology boundary for the basic hemodynamic parameters and analysis of the compensation mechanism as a method of revealing the underlying causes of the disease. Heart Rhythm. 2012;9(11):1909-10. doi: https://doi.org/10.1016/j.hrthm.2012.09.091.
15. Katz AM. Physiology of the heart. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2006. 644 p. ISBN: 0781755018.
16. Muller A, Kraemer JF, Penzel T, Bonnemeier H, Kurths J, Wessel N. Causality in physiological signals: Topical review. Physiol Meas. 2016;37(5):R46-72.
17. Malliani A, Lombardi F, Pagani M. Power spectrum analysis of heart rate variability: a tool to explore neural regulatory mechanisms. Br Heart J. 1994;71(1):1-2.
18. Wessel N, Sidorenko L, Kraemer JK, Schoebel C, Baumann G. Assessing cardiac autonomic function via heart rate variability analysis requires monitoring respiration. Europace. 2016;18(8):1280.
19. Penzel T, Kantelhardt JW, Bartsch RP, Riedl M, Kraemer JF, Wessel N, Garcia C, Glos M, Fietze I, Schöbel C. Modulations of heart rate, ECG, and cardio-respiratory coupling observed in polysomnography. Front Physiol. 2016;7:460.
20. Sidorenko L, Kraemer JF, Wessel N. Standard heart rate variability spectral analysis: Does it purely assess cardiac autonomic function? Europace. 2016;18(7):1085. doi:10.1093\europace\euw078.
21. Bauernschmitt R, Wessel N, Malberg H. Risk prognosis of cardiosurgical patients applying biosignal analysis. Biomedizinische Technik / Biomedical Engineering – Proceedings, BMT 2010, October 05-08, 2010, BMT. ISSN 0939-4990. doi: 10.115/BMT.2010.160.
22. Florea VG, Cohn JN. The autonomic nervous system and heart failure. Circ Res. 2014;114(11):1815-26.
23. Runge J, Riedl M, Stepan H, Wessel N, Kurths J. Quantifying the causal strength of multivariate cardiovascular couplings with momentary information transfer. In: 8th Conference of the European Study Group on Cardiovascular Oscillations (ESGCO 2014); 2014 May 25-28; Trento, Italy. p. 149-150.
24. Huikuri HV, Stein PK. Heart rate variability in risk stratification of cardiac patients. Prog Cardiovasc Dis. 2013;56(2);153-9. doi: 10.1016/j.pcad.2013.07.003.
25. Costa MD, Davis RB, Goldberger AL. Heart rate fragmentation: a new approach to the analysis of cardiac interbeat interval dynamics. Front Physiol. 2017;8:255. doi: 10.3389/fphys.2017.00255.
26. Schnabel RB, Larson MG, Yamamoto JF, et al. Relations of biomarkers of distinct pathophysiological pathways and atrial fibrillation incidence in the community. Circulation. 2010;121(2):200-7.
27. Sayers BM. Analysis of heart rate variability. Ergonomics. 1973;16(1):17-32.
28. Riedl M, Suhrbier A, Malberg H, Penzel T, Bretthauer G, Kurths J, Wessel N. Model-based analysis of cardiovascular variability by a non-linear regression approach. In: Beiträge zum Workshop Biosignalverarbeitung 2008, Potsdam, 16.-18. Juli 2008, Braunschweig: PTB, 2008. p. 172-175. ISBN 978-3-9810021-7-1.
29. Penaz J, Roukenz J, Van der Waal HJ. Spectral analysis of some spontaneous rhythms in the circulation. In: Drischel H, Tiedt N, editors. Biokybernetik . Leipzig (Germany): Karl Marx University; 1968. p. 233-241.
30. Pomeranz M, Macaulay RJB, Caudill MA, Kutz I, Adam D, Gordon D, Kilborn KM, Barger AC, Shannon DC, Cohen RJ, Benson M. Assessment of autonomic function in humans by heart rate spectral analysis. Am J Physiol. 1985;248:H151-3.
31. Opie LH. Heart physiology: from cell to circulation. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2004. 648 p. ISBN: 078174278.
32. Vovc V, Moldovanu I, Sidorenko L, Ganenco A. Modificarea variabilității cardiace și a paternului respirator prin stări psihoemoțional evocate [Modifications of heart rhythm variability and respiratory pattern induced by evoked psychoemotional states]. In: Anale Științifice ale USMF "Nicolae Testemițanu". Ed. a 13-a. Chișinău: CEP Medicina, 2012, Vol. 1: Probleme medico-biologice și farmaceutice, pp. 150-157.

REVIEW ARTICLES

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A modern approach to interleukin-6

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Abstract

Background: Interleukin-6 is a multifunctional cytokine with well-defined pro- and anti-inflammatory properties. Binding to the receptor complex composed of specific interleukin-6-receptor (IL-6R) and transmembrane glycoprotein gp130, it stimulates various signalling cascades. Intracellular signal transduction involves both STAT-dependent, and STAT-independent mechanisms. IL-6R exists in the soluble and the membrane-bound forms, thus were described the classical signalling and the trans-signalling pathways. The pro-inflammatory response occurs via trans-signalling, while the anti-inflammatory effects are mediated by classical signalling pathway. IL-6 is produced by cardiovascular components. The high levels of IL-6 are identified in the inflammatory diseases.

Conclusions: IL-6 expresses the well-defined pro- and anti-inflammatory properties. The experimental studies have revealed classic signalling pathway with regenerative and anti-inflammatory effects (via the membrane-bound IL-6R), and trans-signalling responsible for the pro-inflammatory response (via the soluble form sIL-6R). The intracellular signal transduction involves the activation of STATs, MAPK, and PI3K cascades. It has been proved that in the cardiovascular pathologies the serum levels of IL-6 correlate with the disease severity and the degree of myocardial damage, being the indicator of heart disease and a predictive factor of adverse outcomes.

Key words: interleukin-6, cytokine, trans-signalling, cardiac ischemia.

Introduction

Cytokines are glycosylated proteins involved in the intercellular communication. The interleukin-6 (IL-6) type of cytokines participates in the inflammation, regulates the target cells differentiation, proliferation, migration and apoptosis [1], and is a key factor in coordinating innate and acquired immune response [2].

Its biological effect is achieved by homo- or heterodimerisation of glycoprotein 130 (gp130) [3]. The transduction of IL-6 type cytokine signal involves the activation of Janus (Jak) kinases, followed by the phosphorylation of transcription factors STAT [4], as well as triggering MAPK and PI3K signalling pathways [5]. The deregulation of both the IL-6-mediated signal transduction mechanism, and the intercellular communication processes generates neoplasms, severe autoimmune and inflammatory diseases [6].

Interleukin-6 (IL-6) is a small glycoprotein that activates the cells via the heterodimeric signalling complex, consisting of the alpha receptor (IL-6R) and the beta subunit for signal transduction, represented by glycoprotein 130 (gp130) common to all cytokines type IL-6 [7].

IL-6-mRNA encodes the 212 amino acid protein, including the 29 amino acid signalling peptide. The secreted protein contains 184 amino acids (21 kDa) [8], of which 107 are well defined in the final structure, while 18 N-end amino acids and 8 amino acids from AB loop have no visible electron density [9]. There have been described the different 21-28 kDa isoforms, formed due to the various N-linked glycosylation, which determines the stability and half-life of the protein [10]. IL-6 is synthesized and secreted by the most cells, including T cells, fibroblasts, monocytes, and endotheliocytes.

The IL-6 conformation consists of 4 long alpha-helixes (A, B, C, D) [8], with the typical "up-up-down-down" arrangement, that is common to all IL-6 type cytokines [2]. The attachment to the IL-6R / gp130 receptor complex takes place through three well-differentiated contact sites: **site 1* (binds IL-6R), **site 2* (attaches gp130 between the domains 2 and 3), and **site* 3 (contacts the immunoglobulin-like domain 1 of gp130) [2]. The *site 1* is formed by the C-end groups of D-helix and the C-end part of the AB loop, and determines the specificity of binding to IL-6R. The *site 2*, consisting of the middle sequences of the A and C helixes, and the *site 3*, formed by the N-terminal part of the AB loop (3a) and the C-end of the D helix (site 3b), are required for the recruitment of 2 molecules of gp130 [2].

The synthesis of IL-6-mRNA is regulated at both the transcriptional, and the post-transcriptional levels. The key role in IL-6 expression is attributed to nuclear transcription factor kappa B (NF-κB), activated by the bacterial lipopolysaccharides, pro-inflammatory cytokines (TNFα) or viruses [11].

Experimental studies have highlighted fact that a large number of microRNAs (miR) inhibit IL-6 expression, in-

cluding miR-26a [12], miR-142 [13], miR-146a [14], miR-187 [15], miR-200S [16], miR-329 [17].

Very low amounts of IL-6 (about 1-5 μg/ml) there are in the blood of healthy people. The high serum levels of IL-6 are identified in the most of inflammatory and/or autoimmune diseases, reaching the μg/ml values in septic states [18]. IL-6 is the most potent activator of the synthesis of the acute phase proteins in hepatocytes, including the C-reactive protein, and it is an important factor for tumor growth [19]. Kishimoto T. (2010) has suggested that IL-6 is a marker of the continuous inflammation [20].

IL-6 is also produced by cardiovascular components, such as endothelial cells, vascular smooth muscle and ischemic cardiomyocytes [21]. The expression of IL-6 is stimulated by the C-reactive protein via the nuclear factor B (NF-B), and inhibited by the nitric oxide (NO). It was proved the involvement of IL-6 in the cardiac metabolism regulation [22]. Yudkin J. et al. (2000) have shown the role of IL-6 in the pathogenesis and clinical development of atherosclerotic vascular lesions [23].

The high levels of circulating IL-6 are associated with the increased risk of mortality and poor clinical outcome in the patients with unstable angina [24]. Clinically was confirmed that the IL-6 concentrations not only correlate with the severity of the disease, but are also the important predictors of adverse outcomes. It has been shown that the pro-inflammatory cytokines participate in the destabilization and disruption of the atherosclerotic plaque in the coronary arteries, by stimulating the expression of matrix metalloprotease that are responsible for the vascular remodelling and the plaque disorganization [25].

The increased expression of myocardial IL-6 is associated with the progression of heart failure, so IL-6 can be a true indicator of heart damage [26]. The experimental data show evidence that pro-inflammatory cytokines can depress myocardial contractility and are responsible for the cardiovascular disease development and evolution [22]. In the patients with acute coronary syndrome the serum levels of IL-6 correlate with the severity of myocardial lesions [27].

IL-6 is involved in the control of vascular permeability by stimulating the production by the fibroblasts of the vascular endothelial growth factor (VEGF) that acts on the endothelial cells, as well as by enhanced collagen synthesis [28]. There has been identified the association of IL-6 with endothelial cell activation markers, cell adhesion molecules (VCAM1, ICAM1) and von Willebrandt factor [29]. In 2013 Zamani P. et al. (2013) have confirmed that elevated levels of VCAM1 correlate with the increased risk of coronary events in patients with acute coronary syndrome [27].

The published data have proved that IL-6 contributes to the resorption of acute neutrophil infiltration by inducing apoptosis of neutrophils [30]; while the T-cell apoptosis is prevented by activating the STAT3-dependent anti-apoptotic factors (Bcl2, Bclx1) [31].

The *receptor complex* that mediates the biological response of IL-6 consists of the transmembrane glycoprotein type I for IL-6 binding, called alpha-IL-6R (CD126 or gp80), and transmembrane protein type I for signal transduction (beta-subunit, CD130 or gp130) [2]. The expression of gp130 is characteristic of all body cell types, whereas the expression of αIL-6R has a limited character and is characteristic of hepatocytes, megakaryocytes, and some leukocyte subpopulations (monocytes, macrophages, B and T cells) [32].

The experimental functional and structural studies suggest that IL-6 can form both the hexameric, composed of 2 molecules of IL-6, IL-6R and gp130 ($IL\text{-}6_2/IL\text{-}6R_2/gp130_2$) [33], and the tetrameric ($IL\text{-}6/IL\text{-}6R/gp130_2$) signalling complexes [34]. Viswanathan S. et al. (2002) have shown that the low IL-6 concentrations favour the formation of the tetrameric complexes, whereas the high concentrations of IL-6 will lead to the formation of the hexameric complexes [35].

Interleukin-6 receptor (IL-6R) represents the 80 kDa glycosylated membrane protein [36]. The immunoglobulin-like domain of the human IL-6R does not participate in the IL-6 binding, but it is responsible for the receptor stability. The attachment of IL-6 to IL-6R is mediated by the specific sequences located in the domains 2 and 3 of IL-6R [2].

IL-6R exists in both the soluble and the membrane-bound forms, which allow differentiating the classical signalling pathway (via the membrane-bound IL-6R) and the trans-signalling (through soluble (sIL-6R) receptor). In 2012 in his studies, Rose-John S. mentioned that the trans-signalling pathway represents the pro-inflammatory part of the IL-6 biological effect [8], while the classical signalling exhibits the anti-inflammatory and regenerative activity [1]. The experimental studies confirmed that the regenerative, protective and anti-inflammatory effects of IL-6 [37] are mediated through the membrane-bound IL-6R, which is responsible for the differentiation of pro-inflammatory M1 macrophages into anti-inflammatory M2 macrophages [38]. Also via the classical signalling pathway IL-6 is involved in the hepatic regulation of the insulin sensitivity and glucose tolerance [39].

Wilke C. et al. in 2011 highlighted the role of trans-signalling in the immune system adaptation, and the T cells recruitment, activation and apoptosis [40]. The target cells for trans-signalling are the stem cells: hematopoietic [41], nerve [42], smooth muscle [43], and embryonic [44]. The trans-signalling ensures the migration of the lymphocytes in the inflammation area [45]; it induces T cell proliferation and participates in the regulation of the adhesion cells expression in the endothelial cells [46].

IL-6R can be cleaved proteolyticaly from the cell membrane surface and produce the *soluble form of IL-6R (sIL-6R)* [47]. Taga T. et al. (1989) have demonstrated that the cytosolic region and the transmembrane domain of IL-6 do not participate in the signalling mechanisms, so they have hypothesized that the formation of sIL-6R is a tool for in-

volving the cells that possess only gp130 in the signalling pathways mediated by IL-6 [48].

The soluble sIL-6R receptor is generated by two different mechanisms: * the shedding of the membrane-bound receptor (90-99%) [2, 47]; * the alternative splicing (approximately 1-10%) and the transcription of IL-6R-mRNA omitting the exon 10 that encodes the transmembrane and cytosolic domains [47, 49]. The proteolytic cleavage of IL-6R is catalysed by the ADAM-10 and ADAM-17, Zn^{2+} metalloproteases (sheddases) [2, 50], the transmembrane proteins involved in the partial proteolysis of the membrane receptors of the cytokines [51]. The cleavage site for ADAM-17 is located between Gln357 and Asp358 in the region adjacent to cell membrane [52], the complete cleavage of IL-6R from the cell surface occurs within 24 hours [47]. It was supposed that ADAM-10 is responsible for the slow cleavage, whereas ADAM-17 favours the rapid proteolysis of the membrane-bound IL-6R [50]. In addition to the ADAM family of sheddases, the IL-6R also can be cleaved by the cathepsin G [53].

The biological activators of ADAM-17 are the pro-inflammatory cytokines (TNFα) [54], reactive C protein, the bacterial toxins (streptolysin O, hemophilin A) and metalloproteases, as well as the apoptotic pathways [55]. In 2011 Scheller J. et al. have mentioned the decisive role of ADAM-17 in the cancer genesis and inflammation [56]. The published data demonstrated that the apoptosis-induced cleavage of IL-6R is caspase-dependent, and PKC/MAPK/ROS independent [2].

According to McFarland-Mancini M. et al. (2010) the main sources of sIL-6R are the hepatocytes and hematopoietic cells, as well as the immune cells (neutrophils, macrophages) [57]. It has been hypothesized that the soluble forms of the receptor are the important regulators of the inflammatory processes [2].

The recent studies have found that the single base pair polymorphism determines the serum level of sIL-6R in humans. The substitution of adenine with cytosine leads to the replacement of Asp358 with Ala358 in the exon 9 of the IL-6 gene [58]. In some studies it has been mentioned the association of Asp358Ala variant with low risk of coronary events [59].

According to Scheller J. et al. (2011) the *glycoprotein 130 (gp130)* is a membrane type I glycosylated protein with a molecular weight of 130-150 kDa, consisting of 6 extracellular domains, one transmembrane domain and one cytosolic domain. The N-terminus has the immunoglobulin-like domain (D1), followed by 2 cytokine binding domains (CBD, the domains 2 and 3) and 3 fibronectin (FN-III) domains (the domains 4-6) [2].

In 2003 Boulanger M. et al. demonstrated that the immunoglobulin-like domain of gp130 ensures the interaction with IL-6R and the attachment to the IL-6 site 3 [35]. The cytokine binding domains (CBD2 and CBD3) of gp130 associate with IL-6 site 2. CBD2 located in the N-end region contains 2 pairs of Cys, which form inter-chain disulphide bonds, while CBD3 contains the Trp-Ser-X-Trp-Ser sequence [60]. The proximal membrane domains of gp130 are involved in the signal transduction to the cytosolic domain [2]. After the ligand binding, the domains 4 and 5 are rearranged at an angle of 80° with the curve formation, thus the whole gp130 ectodomain acquires the structural conformation similar to a wide open «C» [61].

The receptor gp130 subunit has affinity for neither IL-6 nor IL-6R [62]. Therefore, IL-6 can only fix and stimulate the cells that possess IL-6R, while the cells that have only gp130 are completely irresponsive to the IL-6 cytokine signals [63].

The myocardial ischemia/reperfusion induces the expression of gp130, associated with the stimulation of IL-6 and IL-6R expression, thus confirming the role of the IL-6/IL-6R/gp130 system in acute myocardial infarction [12].

Gp130 serves as the β-subunit of the receptor common for the IL-6 cytokine family. Recently have been described several *soluble molecules (sgp130)* with lower molecular mass [64], generated by: (1) shedding, (2) alternative splicing, (3) the addition of an exon consisting of 85 base pairs that alters the codons reading by forming a STOP-codon prior to the transmembrane domain coding sequence, (4) the deletion of an exon with new C-terminal sequence (NIASF) formation, followed by STOP-codon, or (5) the alternative polyadenylation of the intron 10 resulting in new mRNA [65].

Due to the fact that the secreted IL-6 binds in the plasma with sIL-6R, and with sgp130, it has been experimentally proved that the soluble form of gp130 (sgp130) inhibits the trans-signalling pathway [66]. Jostok T. et al. (2001) have confirmed that sgp130 interacts only with sIL-6R in the presence of IL-6. The balance between the soluble and membrane forms of gp130 plays an important role in regulating the biological effects of cytokines. When the amount of IL-6 exceeds the amount of sIL-6R and sgp130, IL-6 will act systemically [62].

The recent publications have demonstrated that the single base pair polymorphism in the IL-6R gene manifested by the replacement of Gln258 with Ala, results in higher serum concentrations of sIL-6R, and is associated with reduced risk of coronary heart disease [67]. Boekholdt S. and Stroes E. (2012) have determined that the cleavage of membrane-bound IL-6R on the surface of hepatocytes, monocytes and macrophages results in the loss of cell sensitivity to IL-6 mediated signals [68]. Scheller J. and Rose-John S. (2012) have assumed that high serum levels of sIL-6R increase the buffer capacity of the sIL-6R/sgp130 complex in the blood, thereby diminishing the systemic effects of IL-6 [69].

There has been shown that the single base pair polymorphism (G148R) in gp130 deregulates the function and stability of the protein, and it is associated with a low risk of acute myocardial infarction [70]. Elevated serum levels of sgp130 were identified in patients with heart failure, being associated with higher mortality rates. In 2007 Ichiki T. et al.

presented the data that in the patients with acute myocardial infarction the serum levels of sgp130 were the highest at admission, and were followed by the decreasing in the post-infarction period [71]. At the same time, the patients with progressive heart failure have higher levels of sgp130 compared to the patients with stable heart failure, so the sgp130 can be used to identify patients at high risk for the heart failure progression [72].

Both the experimental animal studies, and in vitro modelling, have highlighted the beneficial effect of sgp130 in atherosclerosis as result of the trans-signalling pathway inhibition by the specific binding of the IL-6/sIL-6R heterodimer [62, 73]. It has been assumed that the higher levels of sgp130 represent the compensatory response to increased IL-6 signalling in chronic ischemic disease and vascular remodelling [73]. Moreno Velasquez I. et al. (2015) have shown the association of elevated serum levels of sgp130 with a 30% reduction in acute myocardial infarction incidence [74]. There was confirmed the hypothesis that in acute coronary syndromes the sgp130 changes correlate negatively with the severity of the inflammation [75]. The published results have suggested that sgp130 may be used as the prognostic biomarker in cardiac diseases [76].

The classical *signalling pathway* starts with IL-6 attachment to the membrane receptor αIL-6R, the formation of the signalling complex with the gp130 homodimer, the signal transduction and activation of the intracellular signalling pathways [2]. The cells that express only gp130 can be stimulated by the IL-6/sIL-6R complex via the trans-signalling pathway [77].

Cellular response to IL-6/sIL-6R complex differs substantially from the response to IL-6. This can be explained by: * the cell expresses more gp130 than IL-6; ** IL-6 is internalized faster compared to the IL-6/sIL-6R complex [78]. Experimentally has been demonstrated that the IL-6 affinity for IL-6R is 1nM, whereas the affinity of IL-6/sIL-6R complex for gp130 is 100-fold greater [79].

The gp130 cytokine receptor does not possess intrinsic kinase activity. The first step in the intracellular transduction of the IL-6 signal is the activation of Janus (Jak) kinases (Jak1, Jak2 and Tyk2), that are non-covalently constitutive associated with the cytosolic tail of gp130. FERM domain ensures the interaction of Jak-kinases with gp130 [80]. The IL-6 binding to the specific receptor favours the induction of Jak-kinases by the tyrosine sequence phosphorylation, located on the activation loop of C-terminal JH1 domain. The active Jak-kinases lead to phosphorylation of the distal tyrosine residues from the cytosolic part of gp130, which represent the recruitment sites for STAT (signal transductor and activator of transcription) factor. STAT1 or STAT3 monomers attach to the distal phosphotyrozine residue, the tyrosine phosphorylation of STAT occurs, followed by homo- or heterodimerisation, the translocation of STAT dimers in the nucleus, N- and C-terminal acetylation, CBP/p300 interaction, and the attachment to the promotor region of IL-6 inducible gene [81]. Thus, the cytokines binding will induce the reorganization of the preformed receptor complexes and the successive cross-phosphorylation [2].

IL-6 activates the cascades STATs, MAPK, and PI3K [82]. The balance between activation of STAT3 and MAPK is crucial for the controlled proliferation of cells, and ultimately for the body's homeostasis [83]. This balance is regulated by the cytosolic Tyr759 of gp130; the tyrosine phosphorylation will reduce IL-6-dependent STAT3 activation [84], and induce MAPK activation [85]. At the same time, the phosphorylation of the gp130 Tyr759 residue is essential for the membrane IL-6-dependent recruitment of Gab-1 protein, which acts as the bridge between the cascades MAPK, and PI3K, and the activated receptor complex [86]. Chen R. et al. (1999) have concluded that the survival mechanisms are maintained via the activation of PI3K and Akt [87].

Conclusions

Interleukin-6 expresses the well-defined pro- and anti-inflammatory properties. The experimental studies have revealed classic signalling pathway with regenerative and anti-inflammatory effects (via the membrane-bound IL-6R), and trans-signalling responsible for the pro-inflammatory response (via the soluble form sIL-6R). The intracellular signal transduction involves the activation of STATs, MAPK, and PI3K cascades. It has been proved that in the cardiovascular pathologies the serum levels of IL-6 correlate with the disease severity and the degree of myocardial damage, being the indicator of heart disease and a predictive factor of adverse outcomes.

References

1. Scheller J, et al. Interleukin-6: From basic biology to selective blockade of pro-inflammatory activities. Semin Immunol. 2014 Feb;26(1):2-12.
2. Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S. The pro- and anti-inflammatory properties of the cytokine interleukin-6. Biochem Biophys Acta. 2011;1813:878–88.
3. Heinrich P, Behrmann I, Haan S, Hermanns H, Müller-Newen G, Schaper F. Principles of interleukin (IL)-6-type cytokine signalling and its regulation. Biochem J. 2003;374:1–20.
4. Akira S. IL-6-regulated transcription factors. Int J Biochem Cell Biol. 1997;29:1401-18.
5. Eulenfeld R, Dittrich A, Khouri C, et al. Interleukin-6 signalling: more than Jaks and STATs. Eur J Cell Biol. 2012;91:486-95.
6. Dittrich A, et al. Systems biology of IL-6, IL-12 family cytokines. Cytokine Growth Factor Rev. 2015;26(5):595-602.
7. Wolf J, et al. Interleukin-6 and its receptors: A highly regulated and dynamic system. Cytokine. 2014;70:11-20. doi: 10.1016/j.cyto.2014.05.024
8. Rose-John S. IL-6 Trans-Signalling via the Soluble IL-6 Receptor: Importance for the pro-inflammatory activities of IL-6. Int J Biol Sci. 2012;8(9):1237-47.
9. Somers W, Stahl M, Seehra JS. A crystal structure of interleukin 6: implications for a novel mode of receptor dimerization and signalling. EMBO J, 1997;16(5):989-97.
10. Schiel X, Rose-John S, Dufhues G, Schooltink H, Gross V, Heinrich PC. Microheterogeneity of human interleukin 6 synthesized by transfected NIH/3T3 cells: comparison with human monocytes, fibroblasts and endothelial cells. Eur J Immunol. 1990;20(4):883-7.

11. Mansell A, Jenkins BJ. Dangerous liaisons between interleukin-6 cytokine and toll-like receptor families: a potent combination in inflammation and cancer. Cytokine Growth Factor Rev. 2013;24(3):249-56.
12. Yang X, Liang L, Zhang X-FF, Jia H-LL, Qin Y, Zhu X-CC, et al. MicroRNA-26a suppresses tumor growth and metastasis of human hepatocellular carcinoma by targeting Interleukin-6-Stat3 pathway. Hepatology. 2013;58(1):158-70.
13. Sun Y, Sun J, Tomomi T, Nieves E, Mathewson N, Tamaki H, et al. PU.1-dependent transcriptional regulation of miR-142 contributes to its hematopoietic cell-specific expression and modulation of IL-6. J Immunol. 2013;190(8):4005–13.
14. He Y, Sun X, Huang C, Long XR, Lin X, Zhang L, et al. MiR-146a regulates IL-6 production in lipopolysaccharide-induced RAW264 7 macrophage cells by inhibiting Notch1. Inflammation. 2014;37(1):71–82.
15. Rossato M, Curtale G, Tamassia N, Castellucci M, Mori L, Gasperini S, et al. IL-10-induced microRNA-187 negatively regulates TNF-a, IL-6, and IL-12p40 production in TLR4-stimulated monocytes. Proc Natl Acad Sci USA. 2012;109(45):E3101-10.
16. Dou L, Zhao T, Wang L, Huang X, Jiao J, Gao D, et al. MiR-200s contribute to Interleukin-6 (IL-6)-induced insulin resistance in hepatocytes. J Biol Chem. 2013; 288(31):22596–606.
17. Garg M, Potter JA, Abrahams VM. Identification of microRNAs that regulate TLR2-mediated trophoblast apoptosis and inhibition of IL-6 mRNA. PLoS ONE. 2013;8(10):e77249.
18. Waage A, Brandtzaeg P, Halstensen A, Kierulf P, Espevik T. The complex pattern of cytokines in serum from patients with meningococcal septic shock. J Exp Med. 1989;169(1):333-8.
19. Taniguchi K, Karin M. IL-6 and related cytokines as the critical lynchpins between inflammation and cancer. Semin. Immunol. 2014;26(1):54–74.
20. Kishimoto T. IL-6: from its discovery to clinical applications. Int Immunol. 2010;22(5):347-52.
21. Gwechenberger M, Mendoza LH, Youker KA, et al. Cardiac myocytes produce interleukin-6 in culture and in viable border zone of reperfused infarctions. Circulation. 1999;99(4):546-51.
22. Kanda T, Takahashi T. Interleukin-6 and Cardiovascular Diseases. Jpn Heart J. 2004;45(2):183-93.
23. Yudkin JS, Kumari M, Humphries SE, et al. Inflammation, obesity, stress and coronary heart disease: is interleukin- 6 the link? Atherosclerosis. 2000;148(2):209-14.
24. Biasucci LM, Liuzzo G, Fantuzzi G, et al. Increasing levels of interleukin (IL)-1Ra and IL-6 during the first 2 days of hospitalization in unstable angina are associated with increased risk of in-hospital coronary events. Circulation. 1999;99(16):2079-84.
25. Rajavashisth TB, Xu XP, Jovinge S, et al. Membrane type 1 matrix metalloproteinase expression in human atherosclerotic plaques: evidence for activation by proinflammatory mediators. Circulation. 1999;99(24):3103-9.
26. Plenz G, Song ZF, Reichenberg S, et al. Left-ventricular expression of interleukin-6 messenger-RNA higher in idiopathic dilated than in ischemic cardiomyopathy. Thorac Cardiovasc Surg. 1998;46(4):213-6.
27. Zamani P, Schwartz GG, Olsson AG, et al.; Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering (MIRACL) Study Investigators. Inflammatory biomarkers, death, and recurrent nonfatal coronary events after an acute coronary syndrome in the MIRACL study. J Am Heart Assoc. 2013;2(1):e003103.
28. Nakahara H, et al. Anti-interleukin-6 receptor antibody therapy reduces vascular endothelial growth factor production in rheumatoid arthritis. Arthritis Rheum. 2003;46(8):1521-9.
29. Mulvihill NT, Foley JB, Murphy RT, et al. Risk stratification in unstable angina and non-Q wave myocardial infarction using soluble cell adhesion molecules. Heart. 2001;85(6):623-7.
30. Kaplanski G, Marin V, Montero-Julian F, Mantovani A, Farnarier C. IL-6: a regulator of the transition from neutrophil to monocyte recruitment during inflammation. Trends Immunol. 2003;24(1):25-9.
31. Jones SA. Directing transition from innate to acquired immunity: defining a role for IL-6. J Immunol. 2005;175(6):3463-8.
32. Oberg HH, Wesch D, Grussel S, Rose-John S, Kabelitz D. Differential expression of CD126 and CD130 mediates different STAT-3 phosphorylation in CD4+CD25- and CD25 high regulatory T cells. Int Immunol. 2006;18(4):555-63.
33. Boulanger MJ, Chow DC, Brevnova EE, Garcia KC. Hexameric structure and assembly of the interleukin-6/IL-6 alpha receptor/gp130 complex. Science. 2003;300:2101-4.
34. Grötzinger J, Kernebeck T, Kallen K-J, Rose-John S. IL-6 type cytokine receptor complexes: hexamer or tetramer or both? Biol Chem. 1999;380(7-8):803-13.
35. Viswanathan S, Benatar T, Rose-John S, Lauffenburger DA, Zandstra PW. Ligand/ receptor signalling threshold (LIST) model accounts for gp130-mediated embryonic stem cell self-renewal responses to LIF and HIL-6. Stem Cells. 2002;20(2):119-38.
36. Rincon M. Interleukin-6: from an inflammatory marker to a target for inflammatory diseases. Trends Immunol. 2012;33(11):571-7. doi: 10.1016/j.it.2012.07.003.
37. Luig M, Kluger MA, Goerke B, Meyer M, Nosko A, Yan I, et al. Inflammation-induced IL-6 functions as a natural brake on macrophages and limits GN. J Am Soc Nephrol. 2015;26(7):1597-607.
38. Mauer J, Chaurasia B, Goldau J, Vogt MC, Ruud J, Nguyen KD, et al. Signalling by IL-6 promotes alternative activation of macrophages to limit endotoxemia and obesity-associated resistance to insulin. Nat Immunol. 2014;15(5):423-30.
39. Febbraio MA, Rose-John S, Pedersen BK. Does interleukin-6 receptor blockade the Holy Grail for inflammatory diseases? Clin Pharmacol Ther. 2010;87(4):396-8.
40. Wilke CM, Bishop K, Fox D, Zou W. Deciphering the role of Th17 cells in human disease. Trends Immunol. 2011;32(12):603–11.
41. Audet J, Miller CL, Rose-John S, Piret JM, Eaves CJ. Distinct role of gp130 activation in promoting self-renewal divisions by mitogenically stimulated murine hematopoietic cells. Proc Natl Acad Sci USA. 2001;98(4):1757-62.
42. März P, Heese K, Dimitriades-Schmutz B, Rose-John S, Otten U. Role of interleukin-6 and soluble IL-6 receptor in region specific induction of astrocytic differentiation and neurotrophin expression. Glia. 1999;26(3):191-200.
43. Klouche M, Bhakdi S, Hemmes M, Rose-John S. Novel Path of activation of primary human smooth muscle cells: upregulation of gp130 creates an autocrine activation loop by IL-6 and its soluble receptor. J Immunol. 1999;163(8):4583-9.
44. Humphrey RK, Beattie GM, Lopez AD, Bucay N King CC, Firpo M, et al. Maintenance of pluripotency in human embryonic stem cells is Stat3 independent. Stem cells. 2004;22(4):522-30.
45. Rabe B, Chalaris A, May., Waetzig GH, Seegert D, Williams AS, Jones SA, Rose-John S, Scheller J. Transgenic blockade of interleukin 6 trans-signalling abrogates inflammation. Blood. 2008;111(3):1021-8.
46. Chen Q, Fisher DT, Clancy KA, Gauguet JM, Wang WC, Unger E, Rose-John S, von Andrian UH, Baumann H, Evans S.S. Fever-range thermal stress promotes lymphocyte trafficking across high endothelial venules via an interleukin 6 trans-signalling mechanism. Nat Immunol. 2006;7(12):1299-1308.
47. Müllberg J, Schooltink H, Stoyan T, Gunther M, Graeve L, Buse G, et al. The soluble interleukin-6 receptor is generated by shedding. Eur J Immunol. 1993;23(2):473-80.
48. Taga T, Hibi M, Hirata Y, Yamasaki K, Yasukawa K, Matsuda T, et al. Interleukin-6 triggers the association of its receptor with a possible signal transducer, gp130. Cell. 1989;58(3):573-81.
49. Lust JA, Donovan KA, Kline MP, Greipp PR, Kyle RA, Maihle NJ. Isolation of a mRNA encoding a soluble form of the human interleukin-6 receptor. Cytokine. 1992;4(2):96-100.
50. Matthews V, Schuster B, Schutze S, Bussmeyer I, Ludwig A, Hundhausen C, et al. Cellular cholesterol depletion triggers shedding of the human Interleukin-6 receptor by ADAM10 and ADAM17 (TACE). J Biol Chem. 2003;278(40):38829–39.
51. Black RA. Tumor necrosis factor-alpha converting enzyme. Int J Biochem Cell Biol. 2002;34(1):1–5.
52. Müllberg J, Oberthür W, Lottspeich F, Mehl E, Dittrich E, Graeve L, et al. The soluble human IL-6 receptor. Mutational characterization of the proteolytic cleavage site. J Immunol. 1994;152(10):4958–68.
53. Bank U, Reinhold D, Schneemilch C, Kunz D, Synowitz H, Ansorge S. Selective proteolytic cleavage of IL-2 receptor and IL-6 receptor

ligand binding chains by neutrophil-derived serine proteases at foci of inflammation. J Interferon Cytokine Res. 1999;19(11):1277–87.
54. Franchimont N, Lambert C, Huynen P, Ribbens C, Relic B, Chariot A, Bours V, Piette J, Merville MP, Malaise M. Interleukin-6 receptor shedding is enhanced by interleukin-1beta and tumor necrosis factor alpha and is partially mediated by tumor necrosis factor alpha-converting enzyme in osteoblast-like cells. Arthritis Rheum. 2005;52(1):84–93.
55. Chalaris A, Rabe B, Paliga K, Lange H, Laskay T, Fielding CA, et al. Apoptosis is a natural stimulus of IL6R shedding and contributes to the pro-inflammatory trans-signalling function of neutrophils. Blood. 2007;110(6):1748–55.
56. Scheller J, Chalaris A, Garbers C, Rose-John S. ADAM17: a molecular switch of inflammatory and regenerative responses? Trends Immunol. 2011;32(8):380-7.
57. McFarland-Mancini MM, Funk HM, Paluch AM, Zhou M, Giridhar PV, Mercer CA, Kozma SC, Drew AF. Differences in wound healing in mice with deficiency of IL-6 versus IL-6 receptor. J Immunol. 2010;184(12):7219-28.
58. Galicia J, Tai H, Komatsu Y, Shimada Y, Akazawa K, Yoshie H. Polymorphisms in the IL-6 receptor (IL-6R) gene: strong evidence that serum levels of soluble IL-6R are genetically influenced. Genes Immun. 2004;5(6):513–6.
59. Collaboration IRGCERF, Sarwar N, Butterworth AS, Freitag DF, Gregson J, Willeit P, et al. Interleukin-6 receptor pathways in coronary heart disease: a collaborative meta-analysis of 82 studies. Lancet. 2012;379:1205–13.
60. Bazan JF. Structural design and molecular evolution of a cytokine receptor superfamily. Proc Natl Acad Sci USA. 1990;87(18):6934–8.
61. Lorenzen I, Shang W, Perbandt M, Petoukhov MV, Svergun D, Waetzig GH, Rose-John S, Hilgenfeld R, Grötzinger J. The structure of the unliganded extracellular domain of the interleukin-6 signal transducer gp130 in solution. Eur J Cell Biol. 2011;90(6-7):515-20.
62. Jostock T, Müllberg J, Özbek S, Atreya R, Blinn G, Voltz N, et al. Soluble gp130 is the natural inhibitor of soluble IL-6R trans-signalling responses. Eur J Biochem. 2001;268(1):160-7.
63. Fischer M, Goldschmitt J, Peschel C, Kallen KJ, Brakenhoff JPJ, Wollmer A, et al. A designer cytokine with high activity on human hematopoietic progenitor cells. Nat Biotech. 1997;15(2):142-5.
64. Tanaka M, Kishimura M, Ozaki S, Osakada F, Hashimoto H, Okubo M, Murakami M, Nakao K. Cloning of novel soluble gp130 and detection of its neutralizing autoantibodies in rheumatoid arthritis. J Clin Invest. 2000;106(1):137-44.
65. Diamant M, Rieneck K, Mechti N, Zhang XG, Svenson M, Bendtzen K, et al. Cloning and expression of an alternatively spliced mRNA encoding a soluble form of the human Interleukin-6 signal transducer gp130. FEBS Lett. 1997;412(2):379–84.
66. Richards PJ, Nowell MA, Horiuchi S, McLoughlin RM, Fielding CA, Grau S, et al. Functional characterization of a soluble gp130 isoform and its therapeutic capacity in an experimental model of inflammatory arthritis. Arthritis Rheum. 2006;54(5):1662–72.
67. Hingorani AD, Casas JP. The interleukin-6 receptor as a target for prevention of coronary heart disease: a mendelian randomization analysis. Lancet. 2012;379(9822):1214-24.
68. Boekholdt SM, Stroes ES. The interleukin-6 pathway and atherosclerosis. Lancet. 2012;379(9822):1176-8.
69. Scheller J, Rose-John S. The interleukin 6 pathway and atherosclerosis. Lancet. 2012;380(9839):338.
70. Benrick A, Jirholt P, Wernstedt I, Gustafsson M, Scheller J, Eriksson AL, et al. A non-conservative polymorphism in the IL-6 signal transducer (IL6ST)/gp130 is associated with myocardial infarction in a hypertensive population. Regul Pept. 2008;146(1-3):189–96.
71. Ichiki T, Jougasaki M, Setoguchi M, Shimokawahara H, Nakashima H, Matsuoka T, et al. Plasma levels of soluble glycoprotein 130 in acute myocardial infarction. J Cardiol. 2007;50(2):101–9.
72. Gwechenberger M, Pacher R, Berger R, Zorn G, Moser P, Stanek B, et al. Comparison of soluble glycoprotein 130 and cardiac natriuretic peptides as long-term predictors of heart failure progression. J Heart Lung Transplant. 2005;24(12):2190–5.
73. Morieri ML, Passaro A, Zuliani G. Interleukin-6 (Trans-Signalling) and ischemic vascular disease: the important role of soluble gp130. Mediators Inflamm. 2017;2017:1396398.
74. Moreno Velasquez I, Golabkesh Z, K´allberg H, Leander K, de Faire¨ U, Gigante B. Circulating levels of interleukin 6 soluble receptor and its natural antagonist, sgp130, and the risk of myocardial infarction. Atherosclerosis. 2015;240(2):477-81.
75. Ritschel VN, Seljeflot I, Arnesen H, et al. Circulating levels of IL–6 receptor and gp130 and long-term clinical outcomes in ST–elevation myocardial infarction. J Am Heart Assoc. 2016;5(6). pii: e003014.
76. Askevold ET, Nymo S, Ueland T, Gravning J, Wergeland R, Kjekshus J, et al. Soluble glycoprotein 130 predicts fatal outcomes in chronic heart failure: analysis from the Controlled Rosuvastatin Multinational Trial in Heart Failure (CORONA). Circ Heart Fail. 2013;6(1):91–8.
77. Rose-John S, Waetzig GH, Scheller J, Grotzinger J, Seegert D. The IL-6/sIL-6R complex as a novel target for therapeutic approaches. Expert Opin Ther Targets. 2007;11(5):613-24.
78. Peters M, Blinn G, Solem F, Fischer M, Meyer zum Büschenfelde KH, Rose-John S. In vivo and in vitro activity of the gp130 stimulating designer cytokine Hyper-IL-6. J Immunol. 1998;161(7):3575-81.
79. Rose-John S, Schooltink H, Lenz D, Hipp E, Dufhues G, Schmitz H, et al. Studies on the structure and regulation of the human hepatic interleukin-6 receptor. Eur J Biochem. 1990;190(1):79-83.
80. Haan C, Heinrich PC, Behrmann I. Structural requirements of the interleukin-6 signal transducer gp130 for its interaction with Janus kinase 1: the receptor is crucial for kinase activation. Biochem J. 2002;361:105-11.
81. Ray S, Boldogh I, Brasier AR. STAT3 NH2-terminal acetylation is activated by the hepatic acute-phase response and required for IL-6 induction of angiotensinogen. Gastroenterology. 2005;129(5):1616-32.
82. Eulenfeld R, Dittrich A, Khouri C, Mu¨ller PJ, Mu¨tze B, Wolf A, et al. Interleukin-6 signalling: more than Jaks and STATs. Eur J Cell Biol. 2012;91(6-7):486-95.
83. Fukada T, Ohtani T, Yoshida Y, Shirogane T, Nishida K, Nakajima K, et al. STAT3 orchestrates contradictory signals in cytokine-induced G1 to S cell-cycle transition. EMBO J. 1998;17(22):6670-7.
84. Schaper F, Gendo C, Eck M, Schmitz J, Grimm C, Anhuf D, et al. Activation of the protein tyrosine phophatase SHP2 via the interleukin-6 signal transducing receptor protein gp130 requires JAK1 and limits acute-phase protein expression. Biochem J. 1998;335:557-65.
85. Lai CF, Ripperger J, Wang Y, Kim H, Hawley RB, Baumann H. The STAT3- independent signalling pathway by glycoprotein 130 in hepatic cells. J Biol Chem. 1999;274(12):7793-802.
86. Eulenfeld R, Schaper F. A new mechanism for the regulation of Gab1 recruitment to the plasma membrane. J Cell Sci. 2009;122:55–64.
87. Chen RH, et al. Interleukin-6 inhibits transforming growth factor-beta-induced apoptosis through the phosphatidylinositol 3- kinase/Akt and signal transducers and activators of transcription 3 pathways. J Biol Chem. 1999;274(33):23013-9.

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The role of scientific research in the field of medicines and the development of the pharmaceutical industry

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Abstract

Background: Approximately 91% of drugs are imported, some of which are quite expensive and unaffordable for the majority of the population. The process of drug development, i. e. the pathway from synthesis of active substance to drug, its implementation in the clinic, is quite complicated and long-lasting, of great responsibility and particularly costly, requires enormous investment. The manufacture and implementation of the medicine in the medical practice creates great difficulties in starting it. Pharmaceutical manufacturing companies from the country with modest profitability are unable or unwilling to invest financial resources in scientific research – an important stage in the development of medicines and in ensuring the beneficial activity of the concerned institutions.

Conclusions: National industry must be focused on the modern manufacturing medicine, competitive and effective medicines from local raw material and first of all on the needs of the health system in the country. The situation requires insistence and can only be improved by a joint activity of specialists in many fields (production, distribution and rational use) and strengthening the research-development and coordination forces of the scientific activities in the country for research, development and implementation of new native original drugs, mainly based on local raw materials. Thus, in finalizing the analysis of the role of the pharmaceutical industry in the economic development of the state, it is necessary to mention the important social aspect of it, the close interrelation with other branches of industry, as well as the high innovation and investment capability.

Key words: drug development, implementation, investments, pharmaceutical companies, scientific support.

Introduction

The contemporary pharmaceutical industry is the nucleus of the pharmaceutical branch and one of the strategically important and strategically developed economic segments of each state [1, 2, 3]. Generally, high and stable production growth rates are characteristic of the branch, little dependent on economic ups and downs [4].

It is well known that in the 21st century medicine came in with a vigorous arsenal of medicines and that the world pharmaceutical industry is currently producing tens of thousands of pharmaceutical preparations. More than 5 thousand of them are registered in the Republic of Moldova and authorized for use in medical practice in the country. The nomenclature of medicines used in medical practice has been renewed by 62-75%, so the contemporary physician has an enormous number of drugs for the prophylaxis and treatment of the most diverse conditions [5, 6, 7, 8].

Supplying quality, effective, safe and affordable medicines greatly determines the viability of the healthcare system of the country. The state of the pharmaceutical field has a clear influence on the health, insurance, financing, employment and other spheres, and also contributes to the development of mutual cooperation with other fields of industry (chemical, machine building, agriculture, biotechnology, military industry complex and others) [4].

In addition, the pharmaceutical industry invites contributions to research and development, to the development of innovative potential, trade relations and the creation of technological infrastructure [9].

Domestic pharmaceutical production by 10 authorized manufacturers in 2015 of 804 authorizations, 13% or 104 were indigenous products, in 2016 of 1028 authorizations, only 9% or 92 were autochthonous products [10].

Thus, about 91% of drugs are imported, some of which are quite expensive and unaffordable for the majority of the population. 9% of the pharmaceutical products in the country are generic (reproduced) and only 14 names of drugs proposed by the country's researchers are native, previously registered, with antiviral, anti-inflammatory, antihypotensive, and antiseptic actions, etc., but unfortunately not all are produced.

In this situation, the lack of essential medicines, of course, is compensated by expensive imports, through expensive drugs, less accessible to the population. Today we import medicines from 62 countries (343 companies). In 2013, pharmaceuticals and parapharmaceutical products were imported in the amount of $241.74 million, $271.95 million in 2014, $339.39 million in 2015, and in the Republic of Moldova during this period were manufactured drugs amounting to 12.3-32.7 million dollars (fig. 1).

The volume of sales (external) amounted in 2013 to 13.75 million dollars, in 2014 – 11.95 million dollars, in 2015 – 26.47 million dollars. This has demonstrated the low level of national pharmaceutical industry and directly con-

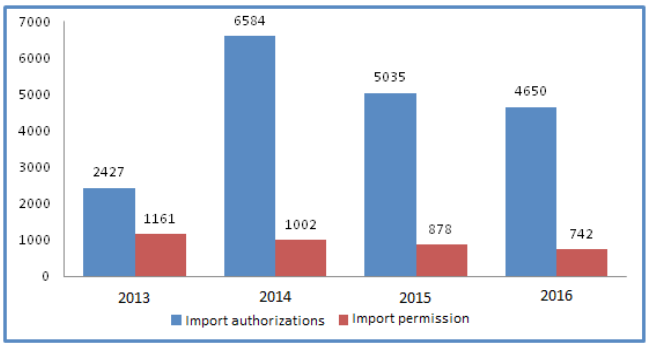

Fig. 1. The dynamics of the import of medicines for 2013-2016.

tributed to the development of other countries' pharmaceutical industries [10].

According to the volume of imports on the names of medicines, the first 10 places are: medicinal coal, sodium chloride, Valeriana officinalis extract, acetylsalicylic acid, citramon, sea buckthorn oil, mucaltin, injectable water, naphthasin, etc., less essential preparations, when certain requirements are advanced to medicinal products presented on the pharmaceutical market: to be effective, safe, quality and affordable. We import a lot and expensive, we produce less, not totally according to GMP and production is not required for export - we produce for us.

The pharmaceutical industry includes science and production enterprises specializing in the synthesis of synthetic drugs, preparations of vegetable raw materials, vitamins, enzymes, antibiotics, endocrine preparations, blood substitutes, galenical preparations and bacterial medicinal products [11]. It is well known that no country in the world produces the whole arsenal of drugs; at the same time any country tends to develop its own pharmaceutical industry, for the following reasons:

- Guaranteeing the population with essential medicines;
- Creating new jobs;
- Improving the economy of the country, because the pharmaceutical industry around the world is one of the most cost-effective.

The pharmaceutical industry is one of the most science-intensive and high-tech sectors of the economy. Worldwide pharmaceutical manufacturers' spending on research and development in this field is 15-20% of the profit [12]. Thus, the USA pharmaceutical research spending represents 25% of the overall costs for research and development, England – 24%, France – 13.8%, Japan – 7.5% [13, 14]. The pharmaceutical industry is characterized by the technical and economical particularities that influence its functionality: high labor productivity, small volume of production, systematic renovation of the nomenclature of manufactured medicinal preparations, lasting production cycle, completeness of technological products, high requirements for quality of production [11].

The drug development process, i.e. the pathway from drug-to-drug synthesis, to its implementation in the clinic, is quite complicated and long-lasting (10-12 years), of great responsibility and costly, requires enormous investment. The total cost of the research project exceeds US $ 350 million (fig. 2).

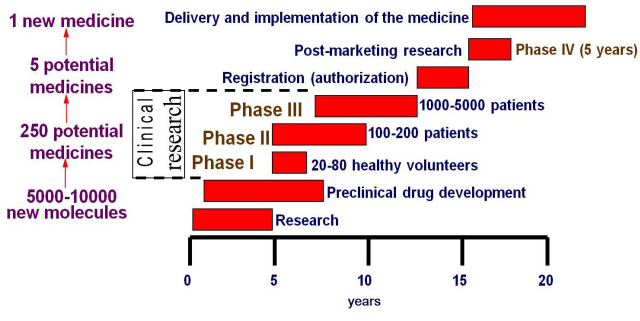

Fig. 2. The process of clinical research of new drugs (typically exceeding 10 years).

This process includes several stages of preclinical and clinical assessments, requires the participation and collaboration of specialists (chemists, pharmacists, pharmacists and clinicians) in many fields, the application of quite sophisticated contemporary methodologies and technologies.

A special role belongs to the pharmacological research, the idea as such and the detection of the actions and the basic effects of the new substances, which subsequently generate the pharmaceutical activities, the clinical research, and the manufacturing and implementation technologies.

Manufacture and implementation in medical practice are two final stages of the complicated drug development process, which creates great difficulties in starting it.

The contemporary pharmaceutical industry relies on a considerable volume of fundamental scientific research, using chemical synthesis, biotechnology, genetic engineering and others in its arsenal. For these reasons, drug companies invest in developing synthesis, performing preclinical and clinical assessments, improving technology, and implementing new quality control methods around 20-25% of revenue.

State-owned pharmaceutical companies, all joint-stock companies, LLCs, manufacturing companies in the country are currently involved, as far as possible, in the reproduction of less important drugs, approved and used many years ago, especially from imported raw material, cheap, suspected to be valid and not local (e. g. hydrogen peroxide, iodine solution, brilliant green, ascorbic acid, analgin, nistatin, novocaine etc). We found that no native enterprise is working with scientific researchers to develop new drugs.

Drug-producing companies in the country with modest profitability are unable or unwilling to invest financial resources in scientific research – an important stage in the development of medicines and ensuring the beneficial activity of the institutions concerned.

The national industry must be geared towards develop-

ing modern, competitive and efficient medicinal products from local raw material and the main one to the needs of the health system of the country.

However, we will try to bring to the attention of specialists trained in drug development, collaborators of institutions interested in and involved in solving the problem of developing and implementing pharmaceutical preparations for ensuring the health system with essential medicines and accessible to the population, finding ways of significantly reducing the import monopoly of medicines and developing the national pharmaceutical market.

During the last few years, in several institutions of the Academy of Sciences of Moldova (ASM) and in the Nicolae Testemitsanu State University of Medicine and Pharmacy were researched various active biological substances, possessing antidepressive, antihypotensive, hepatoprotective, immunomodulating and biostimulating, antimicrobial and antifungal, regenerative and cytoprotective, used in the treatment of cardiovascular, gastrointestinal, infectious, immunodeficiency diseases, etc. But unfortunately these scientific achievements are slowly evolving into the stage of microproduction (experimental pilot) and mass production with subsequent implementation in practice. For our country, the issue of using existing natural sources and the implementation of domestic pharmaceutical products in medical practice is extremely important.

The Republic of Moldova has a considerable potential of scientists in the field of scientific, academic and university institutions, local raw materials necessary for the development and implementation of new medicines. This is confirmed by the results of the scientific researches carried out under the State Program «Elaboration and implementation of new pharmaceutical preparations based on the use of local raw materials for the years 2007-2010» [15] elaborated by the ASM, and continued in subsequent periods, which determined the necessity of:

- Forming the scientific basis of development of the national pharmaceutical industry by implementing in the production of new original medicinal, vegetal, animal, entomological and synthetic medicinal preparations based on local raw materials and creation of new jobs;
- By technologies of synthesis and obtaining biologically active substances from the local vegetal, animal, entomological and synthetic raw material from industrial waste present in enormous quantities in the country (seeds, kernels etc.) to develop and research antibacterial and antifungal drugs of new generations, anti-atherogenic preparations of algal origin, various types of oils (with regenerative and cytoprotective properties), dyes, astringent and adsorbing substances, lipo-and water-soluble antioxidants, antiviral, immunomodulatory and hepatoprotective products: all necessary for the treatment of diseases and pathological conditions (immunodeficiency, etc.);
- The expansion of preclinical and clinical scientific research in the field of medicine with the rational use of the intellectual potential of the country and the endowment of the bases and scientific research centers with modern and efficient equipment;
- Rational alignment to the strategy for the development of scientific research in the field of medicines and the pharmaceutical industry with respect to the requirements and good practice rules (GLP, GCP, GMP etc) established by international bodies in the field and timing of actions using rational and well-grounded human, financial and material resources available;
- Centralizing research and development activity of the pharmaceutical industry by setting up an accreditation center for all (state and private) manufacturing companies, coordinating their work (including state order), implementing GMP, protecting and supporting the manufacturer, accomplishing production inside and outside the country.

All that is mentioned is only one measure – how could science, including scientific research in the field, contribute to the expansion of the domestic drugs problem in our country?

The elaboration and approval of the National Program of Scientific Research in the field of medicine and the continuous development of the pharmaceutical industry in Moldova will contribute to:

- Strengthening the research and development forces and coordinating the scientific activities in the country in order to research, develop and implement new native drugs, mainly based on local raw materials;
- Excluding the unwanted distances between scientists and producers (pharmaceutical industry) – working in the interests of the health system;
- Increasing the range of less expensive (indigenous) medicines and providing medical institutions and population, including socially vulnerable, with first quality, harmless, effective and affordable medicines;
- A significant reduction in the cost of purchased medicines and the cost of treatment – import control, producer support;
- Improving the country's economy, because the pharmaceutical industry is one of the most cost-effective;
- Orientation towards the production of competitive and efficient products at the necessity of the health system in the country [5, 6, 7, 10].

But the healthcare situation in the country is far more difficult (immense, but unstable and irresponsible import of drugs, less accessible to the population, lack of effective native medicines, irrational and ungainly use of medicines, poorly developed national pharmaceutical industry, lack of scientific basis for manufacturing enterprises and other problems).

This situation requires insistence and can only be improved by a joint activity of specialists in many fields (pro-

duction, distribution and rational use), concurrent activity - what today needs to be well organized, essentially restructured and strengthened. In order to help to remedy the situation in the field of domestic medicine and the national pharmaceutical industry, some scientific opportunities for improvement have been resorted to, and have been supported by ASM and P.I. The Expert Advisory Council of the ASM adopted the project for young researchers – «New Perspective Drugs» (2017-2018), the State Program «New Autochthonous Drugs in Optimizing the Treatment» (2018-2021), the technology transfer project, "Autochthonous Antihypotensives – New Emergency Medicines (Production and Implementation)" (2018-2019) and others.

Conclusions

Supplying quality, effective, safe and affordable medicines greatly determines the viability of the healthcare system in the country. The accessibility, quality, efficacy and harmlessness of drugs is the priority policy of the State in the social sphere, contributing to increasing the life of the population and enhancing its well-being. The national industry must be oriented towards the manufacture of modern, competitive and efficient medicines from the local raw material and mainly to the needs of the health system in the country. The situation requires insistence and can only be improved by a joint activity of specialists in many fields (production, distribution and rational use) and strengthening the research-development and coordination forces of the scientific activities in the country for research, development and implementation new native original drugs, mainly based on local raw materials. Thus, in finalizing the analysis of the role of the pharmaceutical industry in the economic development of the state, it is necessary to mention the important social aspect of it, the close interrelation with other branches of industry, as well as the high innovation and investment capability.

References

1. Ivashchenko A, Kravchenko D. Kontseptsiia innovatsionnogo razvitiia otechestvennoi farmatsevticheskoi otrasli [Concept of innovative development of domestic pharmaceutical industry]. In: [Pharma 2020: Problems and perspectives]. Moscow; 2009. p. 19-26. Russian.
2. Petrov VI, Lutsevich AN, Reshet'ko OV. Novye tekhnologii, regulirovanie, standartizatsiia i farmakoekonomika v sfere obrashcheniia lekarstvennykh sredstv [New technologies, regulation, standardization and pharmacoeconomics in the field of drug circulation]. Moscow: Meditsina; 2006. 456 p. Russian.
3. Kholn TA. Regulirovanie farmatsevticheskogo rynka: sushchestvuet li pochva dlia razvitiia innovatsionnykh farmatsevticheskikh preparatov v Rossii? [Regulation of the pharmaceutical market: is there a possibility for the development of innovative pharmaceutical products in Russia?] Problemy ucheta i finansov. 2012;4(8):73-5. Russian.
4. Iurgel' NV, Tel'nova EA. Sostoianie farmatsevticheskogo rynka Rossiiskoi Federatsii i vozdeistvie na nego mirovogo finansovogo i ekonomicheskogo krizisa [The Russian pharmaceutical market in the time of the global financial and economic crisis.]. Vestnik Roszdravnadzora. 2009;(1):46-56. Russian.
5. Ghicavii V, et al. Farmacologie clinică [Clinical pharmacology]. Chisinau: Medicina; 2009. 1068 p. Romanian.
6. Ghicavii V, Bacinschi N, Gusuila G. Farmacologie [Pharmacology]. Chisinau; 2012. 996 p. Romanian.
7. Belousov YuB, Leonova MV, editors. Osnovy klinicheskoi farmakologii i ratsional'noi farmakoterapii [Basics of clinical pharmacology and rational pharmacotherapy]. Moscow: Bionika; 2002. 368 p. Russian.
8. Belousov IuB, Kukes VG, Lepakhin VK, Petrov VI. Klinicheskaia farmakologiia: natsional'noe rukovodstvo [Clinical pharmacology: national guide]. Moscow: Geotar-Media; 2009. 965 p. Russian.
9. DSM Group. Farmatsevticheskii rynok Rossii 2013 [Pharmaceutical market of Russia 2013]. Moscow: DSM Group; 2013 [cited 2018 Jul 9]. Available from: www.dsm.ru/docs/analytics/dsm_report2013.pdf. Russian.
10. Agenția Medicamentului și Dispozitivelor Medicale a Republicii Moldova [Medicines and Medical Devices Agency of the Republic of Moldova]. Raport de activitate 2016 [Annual activity report 2016]. Chisinau: The Agency; 2016 [cited 2018 Jan 15]. Available from: http://amed.md/sites/default/files/Despre%20Agentie/Raport%20%20AMDM%202016%20general.pdf. Romanian.
11. Balashov AI. Formirovanie mekhanizma ustoichivogo razvitiia farmatsevticheskoi otrasli: teoriia i metodologiia [Formation of the mechanism of sustainable development of the pharmaceutical industry: theory and methodology]. St. Petersburg: SPbGUEF; 2012. 161 p. Russian.
12. Tishchenko AN, Dorovskoi AV. Sotsial'no-ekonomicheskoe znachenie i kharakternye cherty farmatsii [Socio-economic significance and characteristic features of pharmacy]. Problemi ekonomiki (Ukraine). 2013;(3):13-20. Russian.
13. International Federation of Pharmaceutical Manufacturers and Associations (IFPMA). Farmatsevticheskaia promyshlennost' i global'noe zdravookhranenie: fakty i tsifry = The pharmaceutical industry and global health: facts and figures]. Moscow: IFPMA; 2013. 208 p. [cited 2018 Jul 19]. Available from: www.ifpma.org/fileadmin/content/Publication/2013/Facts_and_fi gures_2013_Russia__web.pdf. Russian, English.
14. OECD Science, Technology and R&D Statistics. [cited 2018 Jun 5]. Available from: www.oecd-ilibrary. org/ science-and-technology/oecd-science-technology-and-industry-scoreboard_20725345.
15. Academy of Sciences of Moldova; Ghicavii V, coord. Elaborarea și implementarea noilor preparate farmaceutice în baza utilizării materiei prime locale: Program de stat [Elaboration and implementation of new pharmaceutical preparations based on the use of local raw materials: State program] [cited 2018 Jan 15]. Available from: http://www.asm.md/administrator/fisiere/programe/f3.pdf. Romanian.

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The reevaluation of the role of duodenal dysmotility in the etiopathogenesis of vesicular cholelithiasis

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Abstract

Background: Gallstone disease and chronic calculous cholecystitis are the most prevalent gastro-enterological diseases requiring a surgical treatment. This disease occupies a special place in the pathology of the hepato-bilio-pancreatic area, which is important for the etiological diagnosis as well as for the resonance and the complex impact on the function of the adjacent organs. Besides, gallstone disease can result in serious outcomes, such as acute gallstone pancreatitis and gallbladder cancer. This article analyzes the clinico- morphological characteristics of gallbladder stones. At the same time, the role of duodenal dysmotility in the ethiopathogenesis of cholestasis was reevaluated through the contemplation of the contemporary concepts of lithogenesis.
Conclusions: The pathogenesis of gallstone disease is suggested to be multifactorial and probably develops from complex interactions between many genetic and environmental factors and the state of adjacent organs. Based on its anatomical and physiological features, the duodenum is a completely unique crossroads where the digestive pathways of the stomach, liver and pancreas meet. The sealing functionality of these organs allows them to be cataloged as an integral system, and the duodenum due to its specific role exerts "the pituitary function" of the gastrointestinal tract. Therefore, any disruption of the duodenum activity may not be etiopathogenetically reflected on the hepatobiliary-pancreatic disease, and biliary cholelithiasis is no exception in this regard. The achievement in the study of the pathophysiology of bile stones formation and the pathogenesis of gallstone disease can help to improve the complex medico-surgical treatment of this category of patients.

Key words: Cholelithiasis, duodenal dysmotility, gallstone.

Introduction

Vesicular cholelithiasis (VC) is a plurifactorial pathology (with intrinsic and extrinsic mechanisms) characterized by disruption of the properties of gall-particle dispersion associated with agglomeration, aggregation and formation of bile calculi, – a starting point for the evolution and persistence of chronic inflammation of the bladder wall. The disease occupies a special place in the pathology of the hepato-bilio-pancreatic area, which is important for the etiological diagnosis as well as for the resonance and the complex impact on the function of the adjacent organs.

At present, the etiopathogenetic aspects of vesicular cholelithiasis in males are unclear [1-6]. Therefore, it would be logical and legitimate to suppose that the evolution of VC in men requires the presence of mechanisms of another nature, or the accentuation of the already known phenomena, which at the moment are arbitrarily assigned a secondary, complementary role. This assumption is also admitted as possible in the development of vesicular cholelithiasis in children (in the Republic of Moldova with an incidence of 1197 cases per 10000 population, in the USA – 1% in girls, – 0.4% in boys, [7]), where the sex hormone-dependent etiopathogenetic factor is totally excluded [8-10].

In this context, the following question seems reasonable. Why it is the role of common etiopathogenetic links, already known (apparently "less" complex) is underestimated or neglected in the evolution of vesicular cholelithiasis and recognized in clinical practice as secondary factors? In our opinion, this is a consequence of the education of a specific medical mentality, formed over the decades, of a state of conduct that allows a certain degree of simplistic attitude to the canons of human general pathologies, explicitly formulated by academics Sarkisov D.S. and Palițev M.A. in 1995 [11]:

- *if the chronic evolution of the condition is conditioned solely by the change of the «causal-determinant» relationship and is not dependent on the cause, the treatment should be addressed specifically to the causal factor;*
- *since some factor has contributed to the initiation of the disease and has not lost its value over time and continues to maintain it, then it needs to be included in the field of medical action.*

Currently, when it comes to VC risk factors in men, so-called abdominal obesity is considered, which contributes to increased intra-abdominal pressure and motor disorder not only of the gastrointestinal tract, but also of the gall bladder, type I diabetes, insulin resistance diabetes mellitus), metabolic syndrome, biliary sludje of various etiology, liver cirrhosis [12-16].

At the same time, the latest scientific publications pay attention to the role of dysfunctional duodenal motility in the etiopathogenesis of VC, overlooking this important etiopathogenetic link, or simply limiting itself to finding the latter in the cholecystectomized patient [17-20]. We consider this unjustified simplistic approach, having the tendency to bring some perceptions based on the bibliographic sources analyzed and estimated by the contemplation of the con-

temporary concepts of biliary lithogenesis and the reevaluation of the etiopathogenetic role of the gastro-duodenal dismotility in the formation of bile calculi.

Composition of gallstones. Analysis of the literature shows a certain connection between the characteristics of bile stones and sex. It is known that 3 types of base [13, 21-23] of the calculus are specified according to the cholesterol composition. In connection with the detection frequency, overwhelming majority of cholesterol is detected (cholesterol ≥ 70%), mixed (30% ≥70% cholesterol) and pigments (30% ≥ cholesterol). However, the composition of bile calculi did not attract adequate attention in current studies conducted on investigated population cohorts, probably due to the need to use laborious and costly techniques (infrared transformation spectrometry) and clinical practice is limited to visual inspection [24-27]. On the other hand, the study of the compositional structure with the homogeneity analysis is important because it directly reflects the mechanisms of constitution, as follows: a) cholesterinic calculi are characteristic of the dyslipidemia processes [28-31], while the pigments or mixed ones indicate the prevalence gallbladder stasis mechanisms with excessive absorption of bile salts, with significant differences in hydration degree and dispersant level of constituents [30,32-34].

Various researches indicated that pigment calculi possess a constitutive element «microbial center», determined by bacterial colonization [21,38,42] (mainly Helicobacter subspecies, but not Helicobacter pilory proper) - it is yet another «force» reasoning in favor of the gallbladder congestion hypothesis as well as duodenal-biliary reflux on the background of duodenostasis as well as the etiopathogenetic role of the gastrointestinal tract microbe in the evolution of biliary cholelithiasis [35-37].

Some authors denote a high proportion of cholesterol bile calculi in the latest investigations, cholesterol (95%), bilirubin (30%), and calcium (10%) [21, 22, 24, 29]. Rare components include palmitate / stearate, polysaccharides and protein substances. In contrast, research in the 1960s and 1970s showed the prevalence of pigment build-ups within 23-30 percent of observations. These statistical uncertainties can be caused either by the absence of age-related randomization in the investigated cohorts or by westernisation of the society, which also lead to an increase in the prevalence of cholesterol bile calculi among susceptible populations.

Pigmentation calculations are divided in turn into black ones (compact and small) and brown (softer and bigger). Pigmentation stones account for about 20% of vesicular calculi and are more common in the elderly. They are mainly composed of calcium bilirubinates, phosphates and carbonates without any cholesterol impurity [23, 36, 37]. Brown ones are mainly located in the bile duct and amount to about 10% of the total number of calcium bilirubinates less polimerized than black pigments, such as cholesterol and palmitate or calcium stearate. So in the case of pigment stones the over-saturation of the bile with unconjugated bilirubin plays an important role, leading to physico-chemical changes of the bile with the expression of agglomeration and crystallization processes [38,39]. This explains the fact that intrahepatic calculi contain high levels of free bile acids deconjugated by glycine and taurine by intestinal bacterial agents involved in the etiopathogenesis of VC.

Therefore, the evolution of dysfunctional duodenal motility leads directly to the amplification of synthesis processes of bile acids deconjugated with their subsequent absorption and the increase in blood bed concentration. The biochemical investigations of these stones have demonstrated a high level of saturated free fatty acids as well as the involvement of phospholipases that decompose bile phospholipids, particularly phospholipase A1 [29,40,41].

Pure calcined gallstones, exclusively composed of calcium carbonate, are very rare in adults [42-44], whereas they are relatively common in children [45], with a mucin hypersecretion produced by the gallbladder epithelial cells into obstruction of the cystic duct.

Mixed calculi, cholesterolo-pigmented are most freely detected, possess a lamellar structure and are different in shape and size. The causes and factors that induce the alternation of layers and their chemical heterogeneity remain unknown. Data obtained by electronic microscopic scan suggests that the composition and structure of mixed solitary or multiple calculi is different [46,47]: (1) the solitary stones display a protein-cholesterol-nucleus composition; (2) the multiple stones denote nucleic protein-bilirubin composition; and (3) additionally, both contain a protein component disposed along the sectional plane. Whether or not bile glycoproteins are involved in cholesterol formation is currently a subject of discussion. The data of the qualitative and quantitative biochemical research of the mucinous glycoprotein protonic activity is at the moment contradictory and uncertain [48].

Knowledge of the chemical, structural and component composition of gallbladder stones is essential to understanding the VC etiopathogenesis. In order to identify the predisposing factors, X-ray diffraction analysis, atomic absorption spectroscopy and various biochemical estimates were performed [8,48-51]. The elemental analysis records the primary role of calcium as the major constituent element, complemented by iron, magnesium and zinc [2,52].

Patients with VC are exposed to growth of total plasma bilirubin and conjugated bilirubin levels, as well as liver function parameters (glutamic pyruvic transaminases, oxalo-acetic acid transaminases and alkaline phosphatase). Higher concentrations of malondialdehyde are found, significantly escalating the glutathione disulfide / glutathione ratio, essentially decreasing the activity of antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) compared to patients without VC [19,48]. Further studies are needed to determine whether the observed differences are a cause or effect of calculi formation [8,46]. These studies could eventually result in the development of new medical-surgical strategies for the treatment of vesicular cholelithiasis, providing useful information from the aspect of drug prophylaxis of VC relapse [47, 54, 55].

Analyzing the available literature, we mention a very small number of studies, which would refer to the morpho-clinical, etiopathogenetic particularities of VC performed exclusively in men [2,4,5].

At the same time, in the researches, based on mixed samples of the population, it is indicated that male individuals affected by cholelithiasis are characteristic in the vast majority of cases of pigment calculi [47,56,57], as with clinical observations of recurrence or evaluation of the primary choledocolithiasis [58-60].

Thus, Schafmayer C. et al. (2006) [47], analyzing 1025 observations of VC with the study of biliary calculus composition by means of spectro-electron microscopy, showed that pigments (small in size and an average weight of 0.6g) were detected in 58% of the cases, in 38% of the cases were mixed cholesterol-biliary calculi, while women predominated in cholesterol-based calculi with an incidence of 95%. There is not only a direct interrelation between the sex and the calculus structure, but it is also found that men up to the age of 40 have a uniform distribution of the ratio of cholesterinic / pigmentary calculi, whereas in the age group of over 40 the pigmentation predominates totally [4,5].

By making a simple analogy, we can deduce that in men the formation of calculi is largely determined by the processes of stinging the bile (bile congestion with its compositional changes), regardless of the causative-determinant factor of the bile stasis, although the role can not be completely denied disorders of cholesterol metabolism, thus there is a way of «symbiosis» of etiopathogenetic mechanisms, which mutually amplify. This hypothesis in our opinion explains to some extent the following phenomena observed in everyday clinical practice:

– a higher rate of evolution of acute cholecystitis in men, relative to the number of men carrying calculi, with all of these negative repercussions (intraoperative technical difficulties, impossibility of subsequent laparoscopic cholecystectomy resulting in a higher number of conversions, slower postoperative recovery, negative economic effect and so on);

– relatively sudden onset of clinical manifestations in young male subjects, with a rather rapid progress in the clinical picture; the time "addressing- surgical treatment" is broadly explained by the reduced dimensions of the pigment calculi compared to the cholesterol, so a greater "chance" of closing the cystic channel with the triggering of the acute inflammatory process; either by migrating the microcalculators through the extrahepatic bile ducts with their "irritation" → spasm reflector → biliary hypertension → progression of the inflammatory process;

– as a rule, in older males the clinical picture does not differ essentially from that which evolves in women, allowing for pre-operative pre-treatment according to somatic status and compensation of concomitant diseases with the approach of expectative-active tactics, – the fact that with age, appears a deficiency of enzymes responsible for metabolism of cholesterol, as a possible explanation, the constitutive mechanism is similar to the evolution of VC in women; a more thorough anamnesis may well indicate that we have the same period of "illness-addressing-treatment";

Of course, these personal findings are not devoid of subjective factors (health culture of the population, different in men and women, more frequent imaging examinations in women and a higher rate of detection of asymptomatic "silent" calculi, general aging of the population; for our country there is also a "specific" factor, conditioned by the massive migration of people able to work, and thus disproportionality in relation to age groups, etc.) and are questionable, require scientific argumentation and confirmation.

Literature data show that about 2-12% of patients develop acute cholecystitis [61,62], the first case being described by Duncan J. in 1844 [63]. From a histological point of view, the evolution of the inflammatory process in the gallbladder does not differ as compared to acute VC [37,41], which was also confirmed in experimental research [64]. According to the illustrious surgeons and eminent savants Cuzin M.I. [65], Şalimov A.A. [66] the pathological phenomenon initially behaves aseptically in the presence of biliary passage disorders (neurovegetative disorders with dismotility), subsequently associating the infectious factor, the primary role being the obstruction of the bile flow. At the same time, the vast majority of authors attributed the role of primary etiologic factor in acute cholecystitis to the motor disorders of the digestive tract, statistically demonstrating a definite higher rate of male illness compared to women (with a significant prevalence of the male gender of about 3-4 times) [46, 47].

Thus, we can conclude that a potential etiologic factor of vesicular cholelithiasis in males is the state of the neuro-vegetative system, the activation of its parasympathetic component with the dysfunction of the gastrointestinal tract motility [46,67,68]. This finding seems to be reflected in the results of other authors [69-72]. Thus, Rîjcova O.V. [73] denotes the state of activation of the sympathetic system in women with VC, and vice versa, in male vesicular cholelithiasis is associated with vagotonia and an exacerbation of parasympathetic neurovegetative component. Assessed from this point of view, the etiopathogenicity of biliary calculi formation in males suggests the possibility of indirect effects of suprasegmental components of the autonomic nervous system directly on biliary tract motility by modulating the regulation of sympathetic-parasympathetic activity.

An argument in this regard is also the fact that there is a frequent association of vesicular cholelithiasis with ulcerative disease, which ranges from 11-34% [75,78], and according to some authors it exceeds more than half of the total number of cases of combined diseases [74]. In this contingent of patients, biliary dysfunction is reported in about 54%, predominantly in the hypotonic state of the gallbladder (72%) [68,75].

Multiple studies indicate the presence of duodeno-stomachal reflux in patients with vesicular cholelithiasis. The incidence of these symptoms varies between different authors from 2.6% to 80%, and although several hypotheses have been proposed, the cause remains unknown [76,77]. Some attribute to it a defensive pathophysiological func-

tion, aimed at reducing the acidification of the duodenum, while recognizing that the duodeno-stomachal reflux is also characteristic of healthy people [78]. Conversely, others dispute this view by taxing the alkalization of the stomach as a responsible for reducing the gastric motility [70,71], its functional loss in a humoral aspect evolved from processes of either hypotrophic nature or determined by metastatic or dysplastic phenomena of the anthrum mucosa under the action of bile salts [79,80].

At present, the role of duodenal gastric reflux in patients with VC is not defined and requires specification. Finally, the evolution of the duodenal gastric reflux in the VC directly reflects the occurrence of anthro-duoodenal region contraction disturbances, the increase of intraduodenal pressure through fluid accumulation, the duodenal wall distension, and indirectly signals the initiation of a hypoxic and nutritional stress of the duodenal mucosa resulting from disturbances at the level of micro-circulation within the duodenostasis.

It is well known that the gallbladder evacuation motor function is dependent on the gastrointestinal migratory myoelectric complex (MMC) and particularly correlates with the functional state of the duodenum [43]. The integral MMC activity is ensured by neuro-humoral factors, and propulsive pacemakers are Cajal cells, located mainly in the antral part of the stomach, duodenum and ileo-cecal angle. The Cajal cells along with plex neurons Auerbach and Meissner coordinate the synchronization of the motor movements of the anthro-duodenal region, and are directly responsible for the proper passage of the bile duct into the duodenal lumen.

Moreover, there is a disruption of the secretion of humoral factors (especially the cholecystokinin - the «main orchestrant of bladder and bile duct motility», YY peptide, gastrin, secretin, diminishing of the number of specific receptors) [8,55,80], a consequent secretion of proinflammatory cytokines (IL-1, IL-6, TNF-α) and vasoactive remedies (prostaglandins, nitric oxide) [81,82], that mediate dysfunction of intestinal muscle contraction, aggravating in this sense duodenostasis [83,84].

In turn, TNF-a activates the leukocyte chemotaxis, the monocytes accelerate their migration processes into the bladder wall, leading to inflammation, edema, and desquamation of the mucosal epithelium of the gallbladder [80,85]. Processes of atrophy and sclerosis of the bladder wall evaluate gradually, essentially disrupted by its absorption, secretory and motor functions. Moreover, atrophic sclerotic processes have a direct impact on the number of sensitized receptors to cholecistokinin produced by endotheliums of the duodenum, thus further exacerbating the gallbladder hypomotility.

At the same time, one of the factors contributing to the overproduction of cytokines in the pathophysiological aspect is ischemia, both macro- and microcirculatory, as well as that of tissue, so the duodenostasis represents an impulse to modify the immune system (immunosuppression) and especially cytokine secretion pro-inflammatory as first-line mediators. Thus, the duration of the intestinal wall ischemia and subsequent production of TNF-α and IL-6, cytokinemia being favored concurrently by the presence of «out of control» bacterial colonies. Disorders of autonomic neuronal intestinal reflexes in turn reduce the sensory extension of the stomach and duodenum, thereby contributing to aggravation of duodenostasis with tissue ischemic changes [83], as a mirroring activity is induced and a dismotility of the subadditive intestinal segments.

The hypomotor state of the gastrointestinal tract determines the microbial modification and essential growth of microbial flora and its metabolic products in the small intestine, - the phenomenon of enteric colonization with exacerbation of flora activity and microbial hypersensitivity of secondary bile acids (especially deoxycholic) followed by their absorption into the portal bed [1,70,71,82].

As a result, the deterioration of the enterohepatic cycle of biliary metabolism increases with the increase in the hydrophobic bile acid ratio, which in turn is the cause of the lithogenic characteristics of the bile. Viewed as a whole, the triggering of the above-mentioned mechanisms potentiates the unfavorable effects, constituting the creation of a «circle vicious», the elements of which possess a cumulative character and mutual potentiation [21,35,55].

Summarizing the literature data, we conclude on the important role of the duodenum in the evolution of biliary lithiasis, a concept that remains the subject of permanent discussion, with all its arguments and contradictions, but

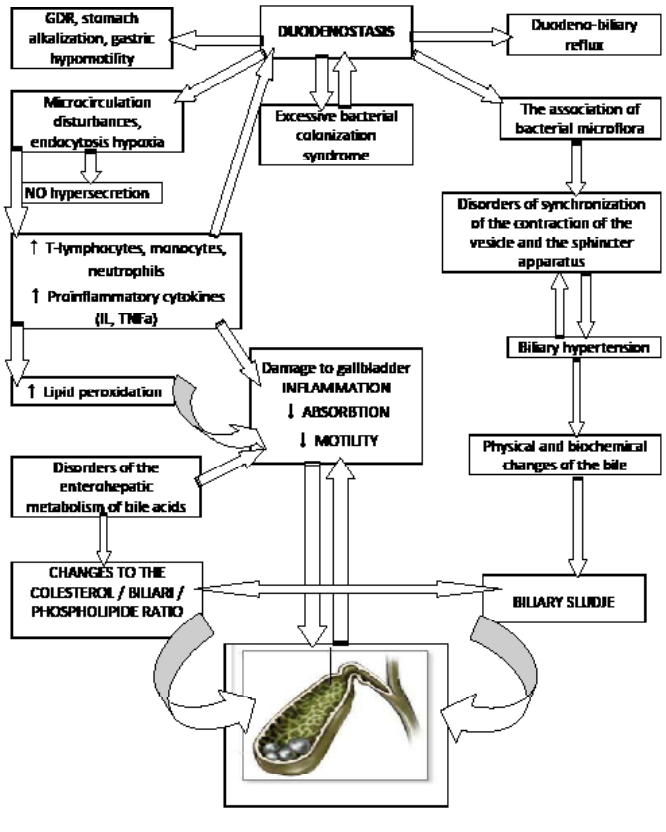

Fig. 1. The scheme of etiopathogenesis of vesicular cholelithiasis evaluated in connection with the functional state of the duodenum.

still completely unresolved at present. In our schematic view, some mechanisms of etiopathogenesis of cholelithiasis evaluated in terms of disorders of duodenal functionality can be ranked in the following scheme.

Conclusions

Based on its anatomical and physiological features, the duodenum is a completely unique crossroads where the digestive pathways of the stomach, liver and pancreas meet. The sealing functionality of these organs allows them to be cataloged as an integral system, and the duodenum due to its specific role exerts the pituitary function of the gastrointestinal tract. Therefore, any disruption of the duodenum activity may not be etiopathogenetically reflected on hepatobiliary-pancreatic disease, and biliary cholelithiasis is no exception in this regard.

References

1. Stinton LM, Shaffer EA. Epidemiology of gallbladder disease: cholelithiasis and cancer. Gut Liver. 2012;6(2):172-87.
2. Sun H, Tang H, Jiang S, et al. Gender and metabolic differences of gallstone diseases. World J Gastroenterol. 2009;15(4):1886-91.
3. Shaffer EA. Epidemiology and risk factors for gallstone disease: has the paradigm changed in the 21st century? Curr Gastroenterol Rep. 2005;7(1):132-40.
4. Tsai CJ, Leitzmann MF, Willett WC, Giovannucci EL. Prospective study of abdominal adiposity and gallstone disease in US men. Am J Clin Nutr. 2004;80(3):38-44.
5. Chang Y, Sung E, Ryu S, et al. Insulin resistance is associated with gallstones even in non-obese, non-diabetic Korean men. J Korean Med Sci. 2008;23(4):644-50.
6. Cirillo DJ, Wallace RB, Rodabough RJ, et al. Effect of estrogen therapy on gallbladder disease. JAMA. 2005;293(3):330-9.
7. Mihu I, Gudumac E, Tighineanu O; [Ministry of Health of the Republic of Moldova]. Litiaza biliară la copil: Protocol clinic național [Gallstone disease in children: National clinical protocol]. Chisinau: [The Ministry]; 2011. 22 p. (PCN-153). Romanian.
8. Hofmann AF. Biliary secretion and excretion in health and disease: current concepts. Ann Hepatol. 2007;6(1):15-27.
9. Conte D, Fraquelli M, Giunta M, Conti CB. Gallstones and liver disease: an overview. J Gastrointestin Liver Dis. 2011;20(5):9-11.
10. Marschall HU, Einarsson C. Gallstone disease. J Intern Med. 2007;261(3):529-42.
11. Sarkisov DS, Pal'tsev MA, Khitrov MA. Obshchaia patologiia cheloveka [General human pathology]. Moscow: Meditsina; 1995. 272 p. Russian.
12. Velichko AV, Dundarov ZA, Lin VV. Kliniko-morfologiceskie predposylki ratsional'noi khirurgicheskoi taktiki lechenia bol'nyh s ostrym kholetsistitom [Clinical and morphological premises for rational surgical treatment of patients with acute cholecystitis]. Problemy zdorov'ia i ekologii. 2009;19(1):14-20. Russian.
13. Grigor'eva IN. Osnovnye factory riska zhelchnokamennoi bolezni [The main risk factors for cholelithiasis]. RZhGGK. 2007;21(6):17-21. Russian.
14. Hansel SL, DiBaise JK. Functional gallbladder disorder: gallbladder dyskinesia. Gastroenterol Clin North Am. 2010;39(2):369-79.
15. Zhang Y, Liu D, Ma Q, et al. Factors influencing the prevalence of gallstones in liver cirrhosis. J Gastroenterol Hepatol. 2006;21(3):1455-8.
16. Déry L, Galambos Z, Kupcsulik P, et al. Cirrhosis and cholelithiasis. Laparoscopic or open cholecystectomy? Orv Hetil. 2008;149(4):2129-34.
17. Janoo SS, Mohandas S, Almond LM. Postcholecystectomy syndrome (PCS). Int J Surg. 2010;8(1):15-7.
18. Sakorafas GH, Milingos D, Peros G. Asymptomatic cholelithiasis: is cholecystectomy really needed? A critical reappraisal 15 years after the introduction of laparoscopic cholecystectomy. Dig Dis Sci. 2007;52(1):1313-25.
19. Thistle JL, Longstreth GF, Romero Y, et al. Factors that predict relief from upper abdominal pain after cholecystectomy. Clin Gastroenterol Hepatol. 2011;9(5):891-6.
20. Legorreta AP, Silber JH, Costantino GN, et al. Increased cholecystectomy rate after the introduction of laparoscopic cholecystectomy. JAMA. 1993;270(8):1429-32.
21. Marakhovskii IuKh. Zhelchnokamennaia bolezn': sovremennoe sostoianie problemy [Gallstone disease: current state of the problem]. RZhGD. 2003;34(1):81-92. Russian.
22. Dederer IuM, Krylova NP, Ustinov GG. Zhelchnokamennaia bolezn' [Gallstone disease]. Moscow: Meditsina; 1983. 176 p. Russian.
23. Cojocaru D-C. Studiul factorilor de risc în litiaza biliară asociată diabetului zaharat și sindromului metabolic [Study of risk factors for gallstone disease associated with diabetes mellitus and metabolic syndrome] [dissertation summary]. Iasi (Romania): Grigore T. Popa University of Medicine and Pharmacy; 2011. 51 p. Romanian.
24. Dowling RH. Review: pathogenesis of gallstones. Aliment Pharmacol Ther. 2000;14(2):39-47.
25. Festi D, Reggiani ML, Attili AF, et al. Natural history of gallstone disease: Expectant management or active treatment? Results from a population-based cohort study. J Gastroenterol Hepatol. 2010;25(2):719-24.
26. Il'chenko AA. Zabolevaniia zhelchnogo puzyria i zhelchnykh putei: Rukovodstvo dlia vrachei. [Diseases of the gallbladder and biliary tract: A guide for doctors]. Moscow: Anaharsis; 2006. 448 p. Russian.
27. Tsimmerman IaS. Khroniceskii kholetsistit i khroniceskii pankreatit [Chronic cholecystitis and chronic pancreatitis]. Perm (Russia): Zvezda; 2002. 251 p. Russian.
28. Corradini SG, Elisei W, Giovanelli L, et al. Impaired human gallbladder lipid absorption in cholesterol gallstone disease and its effect on cholesterol solubility in bile. Gastroenterology. 2000;118(5):912-20.
29. Shcherbinina MB, Zakrevskaia EV. Zhelchnokamennaia bolezn', kholesteroz zhelchnogo puzyria, ksantogranulematoznyi kholetsistit: kliniko-morfologicheskie paralleli [Gallstone disease, gallbladder cholesterosis, xanthogranulomatous cholecystitis: clinical and morphological parallels]. Ter Arkh. 2008;24(2):66-71. Russian.
30. Tiuriumin IaL, Shanturov VA, Tiuriumina EE. Patogenez i lechenie kholesterinovogo kholetsistolitiaza (obzor) [Pathogenesis and treatment of cholesterol cholecystolithiasis (review)]. Biulleten' VSNTs RAMN. 2012;84(2):181-6. Russian.
31. Carey MC. Formation and growth of cholesterol gallstones: the new synthesis. In: Fromm H, Leuschner U, editors. Bile acids-cholestasis-gallstones. Advances in basic and clinical bile acid research. Dordrecht: Kluwer; 1996. p. 147-175.
32. Portincasa P, diCiaula A, Vendemialeet G, et al. Gallbladder motility and cholesterol crystallisation in bile from patients with pigment and cholesterol gallstones. Eur J Clin Invest. 2000;30(4):317-24.
33. Schirmer B, Winters KL, Edlich RF. Cholelithiasis and cholecystitis. J Long Term Eff Med Implants. 2005;15(3):329-38.
34. Carey MC. Pathogenesis of cholesterol and pigment gallstones: some radical new concepts. In: Gerok W, Loginov AS, Pokrowskij VI, editors. New trends in hepatology. Dordrecht: Kluwer; 1996. p. 64-83.
35. Carey MC, Duane WC. Enterohepatic circulation. In: Arias IM, et al., editors. The liver: biology and pathobiology. 3rd ed. New York: Raven Press; 1994. p. 719-67.
36. Bilhartz LE, Horton JD. Gallstone disease and its complications. In: Feldman M, et al., editors. Sleisenger and Fordtran's gastrointestinal and liver disease: pathophysiology, diagnosis, management. 6th ed. Philadelphia: WB Saunders Company; 1998. p. 48–972.
37. Csendes A, Smok G, Burdiles P, et al. Histological findings of gallbladder mucosa in 95 control subjects and 80 patients with asymptomatic gallstones. Dig Dis Sci. 1998;43(6):931-4.
38. Van Erpecum KJ, Portincasa P, Dohlu MH, et al. Biliary pronucleating proteins and apolipoprotein E in cholesterol and pigment stone patients. J Hepatol. 2003;39(1):7-11.
39. Buchner AM. Factors influencing the prevalence of gallstones in liver disease: the beneficial and harmful influences of alcohol. Am J Gastroenterol. 2002;97(4):905-9.
40. Sarvanov IA. Nekotorye formy diskinezii 12-perstnoi kishki kak prichina zhelchnoi gipertenzii [Some forms of dyskinesia of the duodenum as the cause of bile hypertension]. Pacific Med J. 2003;17(4):75-7. Russian.

41. Kuntz E, Kuntz HD. Hepatology and practice: history, morphology, biochemistry, diagnostics, clinic, therapy. Berlin. Heidelberg. New York: Springer; 2000. 825 p.
42. Larin AK, Shcherbakov PL, Kharitonova LA, et al. Opredelenie mikrobnoi flory pigmentnykh zhelchnykh kamnei na osnove analiza 16S ribosomal'noi RNK [Determination of the microbial flora of pigmented gallstones based on the analysis of the 16S gene of ribosomal RNA]. RZhGGK. 2009;36(5):49-54. Russian.
43. Angelescu N. Tratat de patologie chirurgicala. Vol. 2 [Surgical pathology. Vol. 2]. Bucuresti: Editura Medicala; 2003. 2013 p. Romanian.
44. Cooper AD. Plasma lipoprotein metabolism. In: Fromm H, Leuschner U, editors. Bile acids-cholestasis-gallstones. Advances in basic and clinical bile acid research. Dordrecht: Kluwer; 1996. p. 97-126.
45. Russo MW, Wei JT, Thiny MT, et al. Digestive and liver diseases statistics. Gastroenterology. 2004;126(8):1448-53.
46. Kaur T, Kaur S. Pathophysiological conditions in cholelithiasis formation in North Indian population: spectroscopic, biophysical, and biochemical study. Biol Trace Elem Res. 2010;138(4):79-89.
47. Schafmayer C, Hartleb J, Tepel J, et al. Predictors of gallstone composition in 1025 symptomatic gallstones from Northern Germany. BMC Gastroenterol. 2006;6(3):36-45.
48. Tukhtaeva NS. Biokhimia biliarnogo sladzha [Biochemistry of the biliary sludge] [dissertation summary]. Dushanbe: Tajik Academy of Sciences; 2006. 28 p. Russian.
49. Maksimenko VB. Narusheniia kontsentratsionnoi i motorno-evakuatornoi funktsii zhelchnogo puzyria pri kholetsistolitiaze [Disturbances of concentration and motor-evacuation function of the gallbladder in cholecystolithiasis]. RZhGGK. 2006;14(4):24-8. Russian.
50. Dowling RH. Review: pathogenesis of gallstones. Aliment Pharmacol Ther. 2000;14(2):39-47.
51. Bistritz L, Bain VG. Sphincter of Oddi dysfunction: Managing the patient with chronic biliary pain. World J Gastroenterol. 2006;12(24):3793-802.
52. Riyad K, Chalmers CR, Aldouri A. The role of 99mtechnetium-labelled hepato imino diacetic acid (HIDA) scan in the management of biliary pain. HPB. 2007;9(2):219-24.
53. Bear CE, Strasberg SM. Techniques for studying biliary secretion: electrolytes in bile. Hepatology. 1994;4(5 Suppl):25S-30S.
54. Zacks SL, Sandler RS, Rutledge R, Brown RS Jr. A population based cohort study comparing laparoscopic cholecystectomy and open cholecystectomy. Am J Gastroenterol. 2002;97(6):334-40.
55. Reshetnyak VI. Concept of the pathogenesis and treatment of cholelithiasis. World J Hepatol. 2012;4(2):18-34.
56. George J, Baillie J. Biliary and gallbladder dyskinesia. Curr Treat Options Gastroenterol. 2007;10(3):322-7.
57. Vítek L, Carey MC. New pathophysiological concepts underlying pathogenesis of pigment gallstones. Clin Res Hepatol Gastroenterol. 2012;36(2):122-9.
58. Tritapepe R, Piro D, Annoni F, et al. Predictive factors for cholelithiasis complications. Panminerva Med. 1999;41(5):243-6.
59. Cirillo DJ, Wallace RB, Rodabough RJ, et al. Effect of estrogen therapy on gallbladder disease. JAMA. 2005;293(1):330-9.
60. Gurusamy KS, Davidson BR. Surgical treatment of gallstones. Gastroenterol Clin North Am. 2010;39(7):229-44.
61. Iyer SG, Ravishankar KD, Huang E, Masud K. Acute acalculous cholecystitis: challenging the myths. HPB. 2007;9(1):131-4.
62. Savoca PE, Longo WE, Zucker KA, et al. The increasing prevalence of acalculous cholecystitis in outpatients. Results of a 7-year study. Ann Surg. 1990;211(3):433-7.
63. Duncan J. Femoral hernia; gangrene of gallbladder; extravasation of bile; peritonitis; death. North J Med. 1844;2:151-2.
64. van Erpecum KJ, Wang DQ, Moschetta A, et al. Gallbladder histopathology during murine gallstone formation: relation to motility and concentrating function. J Lipid Res. 2006;47(1):32-41.
65. Kuzin MI. Khirurgicheskie bolezni [Surgical diseases]. 3rd ed. Moscow: Meditsina; 2002. 478 p. Russian.
66. Shalimov AA, Shalimov SA, Nechitailo ME, Domanskii BV. Khirurgia pecheni i zhelchevyvodiashchikh putei [Surgery of the liver and bile ducts]. Kiev: Zdorov'ia; 1993. 512 p. Russian.
67. Lavoie B, Nausch B, Zane EA, et al. Disruption of gallbladder smooth muscle function is an early feature in the development of cholesterol gallstone disease. Neurogastroenterol Motil. 2012;24(7):e313-e324.
68. Atroshchenco EA. Khirurgicheskoe lechenie bol'nykh s ostrym kholetsistitom pri nalichii soputstvuiushchikh zabolevanii zheludka i dvenadtsatiperstnoi kishki [Surgical treatment of patients with acute cholecystitis in the presence of concomitant diseases of the stomach and duodenum] [dissertation]. Rostov (Russia): Rostov State Medical University; 2011. 116 p. Russian.
69. Hotineanu V. Megacoledocul secundar [Secondary megacholedochus]. Arta Medica (Republic of Moldova). 2003;(1):4-11. Romanian.
70. Nesterenco IuA, Stupin VA, Fedorov VA, Bogdanov AE. Khronicheskaia duodenal'naia neprokhodimost' [Chronic duodenal obstruction]. Moscow: Meditsina; 1990. 238 p. Russian.
71. Tsimmerman IaS. Khronicheskaia duodenal'naia neprokhodimost' [Chronic duodenal obstruction]. Klin Med (Russia). 1988;6:132-9. Russian.
72. Vitebskii IaD. Khronicheskie narushenia duodenal'noi prokhodimosti i iazvennaia bolezn' zheludka i dvenadtsatiperstnoi kishki [Chronic disorders of duodenal patency and peptic ulcer of the stomach and duodenum]. Chelyabinsk (Russia): Iujno-ural'skoe kn. izdat-vo; 1976. 190 p. Russian.
73. Ryjova OV. Kliniko- patogeneticheskie osobennosti, rasprostranennost' i lechenie zhelchnokamennoi bolezni s pozitsii sistemnogo podkhoda [Clinical and pathogenetic features, prevalence and treatment of cholelithiasis from a position of the system approach] [dissertation summary]. Kazan: Kazan State Medical Academy; 2007. 43 p. Russian.
74. Selezneva EIa, Il'chenko AA. Zhelchnokamennaia bolezn', sochetaiushchaiasia s iazvennoi bolezn'iu dvenadtsatiperstnoi kishki (obzor literatury) [Gallstone disease, combined with duodenal ulcer (review of literature)]. Exp Klin Gastroenterol. 2008;5(6):48-55. Russian.
75. Grafov AK. Sovremennye aspekty diagnostiki i lechenia bol'nykh pri sochetanii kholelitiaza i iazvennoi bolezni [Modern aspects of diagnosis and treatment of patients with a combination of cholelithiasis and peptic ulcer disease] [dissertation summary]. Voronej: Voronej State Medical Academy; 2006. 46 p. Russian.
76. Duca S. Sindromul biliarilor operați: profilaxie, diagnostic, tratament [Postcholecystectomy syndrome: prophylaxis, diagnosis, treatment]. Cluj-Napoca: Genesis; 1992. 204 p. Romanian.
77. Martínez-Augustin O, Sánchez de Medina F. Intestinal bile acid physiology and pathophysiology. World J Gastroenterol. 2008;14(37):5630-40.
78. Korot'ko GF, Shcherbina II, Korochanskaia NV, Demina AO. Evakuatornaia funktsiia gastroduodenal'nogo kompleksa kak sostavliaiushchaia pishchevaritel'nogo protsessa [Evacuation function of the gastroduodenal complex as a component of the digestive process]. Eksp Klin Gastroenterol. 2010;8(1):35-41. Russian.
79. Duncan CB, Riall TS. Evidence-based current surgical practice: calculous gallbladder disease. J Gastrointest Surg. 2012;16(11):2011-25.
80. Arai K, Lee F, Miyajima A. Cytokines: coordinators of immune and inflammatory responses. Annu Rev Biochem. 1990;59(2):783-836.
81. Beckman JS, Koppenol WH. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and the ugly. Am J Physiol. 1996;171(4):1424-9.
82. Borruel N, Casselas F, Antolin M, et al. Effects on nonpathogenic bacteria on cytokine secretion by human intestinal mucosa. Am J Gastroenterol. 2003;98(2):857-65.
83. Boeckxstaens GE, Rumessen JJ, De With L, et al. Abnormal distribution of the interstitial cell of Cajal in an adult patient with pseudoobstruction and megaduodenum. Am J Gastroenterol. 2002;97(8):2120-6.
84. Camborova P, Hubka P, Sulkova I, et al. The pacemaker activity of interstitial cells of Cajal and gastric electrical activity. Physiol Res. 2003;52:275-84.
85. Ashby B. Co-expression of prostaglandin receptors with opposite effects: a model for homeostatic control of autocrine and paracrine signaling. Biochem Pharmacol. 1998;55(3):239-46.

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Coronary revascularization in patients with ischemic left ventricular dysfunction

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Abstract

Background: Coronary artery disease (CAD) dominates in the contemporary world among the causes of left ventricular dysfunction (LVD). Prognosis of survival and the course of heart failure (HF) are worse in patients with CAD than in many kinds of non-ischemic cardiomyopathy. Development of cardiac surgery technologies and formation of the relevant evidence base have significantly expanded the role of revascularization in patients with CAD and reduced left ventricular ejection fraction (LVEF). The possibility and expediency of coronary artery bypass grafting (CABG) for improving left ventricular ejection fraction (LVEF), clinical course of HF and survival are evidence-based. Stenting is less effective than coronary artery bypass grafting (CABG) regarding the influence on primary endpoints but improves the quality of life of patients with CAD and ischemic LVD.

Conclusions: In patients with ischemic LVD, surgical revascularization can ensure an improvement in the pumping function of the heart, provided there is a sufficient amount of viable myocardium. From the standpoint of evidence-based medicine, the effectiveness of CABG surgery in patients with multivessel lesion of the coronary bed and LVD has been proven for correction LVEF, improvement of the course of the disease and prediction of survival compared with the optimal drug therapy. Percutaneous intervention is inferior to surgical revascularization in terms of its effect on endpoints, but it can make an improvement in the quality of life of patients with ischemic LVD. Evaluation of myocardial viability may be of additional importance for the decision on the feasibility of CABG in the presence of multivessel coronary artery disease combined with a sharp decrease in LVEF.

Key words: coronary artery disease, reduced left ventricular ejection fraction, myocardial revascularization.

Introduction

Heart failure (HF) combined with a reduced left ventricular ejection fraction (LVEF) determines an unfavorable prognosis for survival and is associated with frequent hospitalizations due to decompensation of the blood circulation. According to the Framingham study, the average life expectancy of patients with LVEF is less than 20% and clinical manifestations of HF is 1.7 years in men and 3.2 years in women [22]. Coronary atherosclerosis prevails among the causes of left ventricular systolic dysfunction (LVSD), and the survival prognosis in patients with coronary artery disease (CAD) is worse than in many types of non-ischemic cardiomyopathy [11]. At the same time, unlike many other causes of left ventricular dysfunction (LVD), patients with CAD potentially have a chance of recovery of myocardial contractile function after revascularization.

Multivessel coronary artery disease and concomitant diabetes mellitus are often found in patients with reduced LVEF, presenting strong arguments in favor of surgical myocardial revascularization. On the other hand, background LVD is associated with a certain increase in the risk of "major" cardiac surgeries and was even considered a limitation for their implementation. The first attempts of surgical treatment of patients with CAD and LVD were made almost half a century ago [27]. Initially, coronary artery bypass grafting (CABG) surgery in patients with LVD resulted in high mortality (from 1.4 to 18.6%) and multiple postoperative complications [35]. However, due to the rapid development of cardiac surgery technologies and formation of an appropriate evidence base, the role of revascularization in patients with ischemic LVD has gradually expanded [17, 26, 30]. Despite this, according to a recent review of clinical practice, the incidence of coronaroventriculography (CVG) in patients with newly diagnosed HF is still insufficient [9]. Moreover, a number of questions remain unanswered regarding the selection of patients and monitoring the effectiveness of revascularization, in particular regarding the possibility of LVD correction after surgery. This review summarizes the current understanding of the possibilities and the choice of the optimal method of revascularization in patients with ischemic LVD and signs of HF.

Ischemic cardiomyopathy and myocardial viability

In 1970, G. Burch offered the term "ischemic cardiomyopathy", referring to the course of CAD with multiple lesions of the coronary arteries, increased ventricular heart sizes and clinical symptoms of congestive HF [7]. The cause of myocardial dysfunction in such patients is the decreased blood flow in the subendocardial zones. Progressive decrease in the availability of high-energy compounds and acidosis cause increased myocardial rigidity, while volume overload of the heart causes dilatation of its chambers. An important feature of ischemic cardiomyopathy is the potential reversibility of regional and global abnormalities in LV function.

The term "viable myocardium" refers to ischemic heart muscle with a reduced functional capacity which does not

have, or has a limited amount of scar tissue, which in turn determines the potential possibility of restoring its function after revascularization. With LVD progression myocardial viability is ensured due to a significant limitation of its energy expenditure, that is, the state of hibernation. There should be a distinction between hibernating and "stunned myocardium", the latter being a condition of post-ischemic LVD, which persists after reperfusion, despite the restoration of coronary blood flow [12].

Determining the percentage of viable myocardium using stress echocardiography, perfusion scintigraphy or magnetic resonance imaging with contrast enhancement may be useful in candidates for revascularization or heart transplantation after undergoing common myocardial infarction with left ventricular aneurysm, as well as in patients with CAD and LVEF less than 35% in the absence of a large scar. It should be noted that in many patients with the above-mentioned clinical and instrumental characteristics, dyspnea is often equivalent to angina, making it difficult to determine treatment priorities. Although the best survival prospects for patients with viable myocardium have been convincingly proven, so far no direct evidence has yet been obtained of the role of viability assessment for choosing between drug therapy and surgical revascularization [6].

CABG compared to medical treatment therapy

The results of many studies indicate the possibility of improving the long-term survival of patients with CAD and LVD after surgical revascularization compared with drug therapy [13, 36]. In 1994, Yusuf et al. carried out a cumulative analysis of data from seven largest studies of that time, comparing the effects of CABG and drug therapy on 10-year survival (n = 2650). More than 50% of patients were diagnosed with a three-vascular lesion of the coronary arteries. In CABG group, a positive effect on survival and symptoms was observed compared with drug therapy: mortality over 10 years was 26.4 and 30.5%, respectively (p = 0.03). Ultimate benefit of surgical revascularization was the greatest in patients with LVD, with the mortality rate almost halved [38].

It should be noted that in the first revascularization studies, patients from the comparison group could not receive a number of modern medicines. At that time, statins and blockers of the renin-angiotensin system were not yet available; other agents with proven beneficial prognostic effects, such as antiplatelet agents, beta-blockers and mineralocorticoid receptor antagonists, have not been used routinely. Of course, this feature somewhat limits the possibility of extrapolating the data to the current population of patients with CAD and LVD. However, from the standpoint of evidence-based medicine, new convincing evidence has recently emerged in favor of CABG in patients with LVD who received optimal drug therapy [31, 36].

In a surgical treatment for ischemic heart (STICH) study 1212 patients with CAD and LVEF ≤35% (mean LVEF ratio of 27%) were randomized into CABG groups and optimal drug therapy. After 5 years, the frequency of death cases from all causes did not differ in the compared groups. At the same time, the analysis of a number of secondary efficacy criteria (death from all causes or hospitalization due to HF, death from all causes or hospitalization due to cardiovascular reasons, death from all causes, or the need for revascularization) showed the best results in the CABG group [36]. The results of a 10-year STICHES observation showed an ultimate increase in the life expectancy of patients after CABG by 1.44 years (7.73 versus 6.29 years, respectively). The results obtained present a convincing argument in favor of the implementation of CABG in patients with CAD and LVD [37].

The STICH study revealed a clear dependence of patients' survival on the volume of viable myocardium. Retained viability testified in general to the better prospects for survival both after CABG surgery and in the presence of drug therapy, but was not a specific indicator of the potential benefits of surgical revascularization [6]. Obviously, the inconsistency of the data obtained was due to the relatively small number of cardiovascular events in the compared groups and the lack of a unified methodology for determining the viability of the myocardium. In general, the assessment of viability can be considered in a modern clinic as an additional criterion for predicting the results of CABG in patients with initially reduced LV pump function [4].

CABG in comparison with the stentation of coronary arteries

From the standpoint of evidence-based medicine, the results of surgical revascularization compared with coronary artery stenting in patients with CAD and LVD were compared in a small number of randomized studies, as well as retrospective observations. In a meta-analysis of 19 studies involving 4766 patients with LVEF less than 40% who underwent percutaneous interventions, hospital and annual mortality rates after stenting did not differ from those in CABG studies [21]. In another study, no significant differences were found in the survival of patients with CAD and LVD (LVEF less than 35% in 446 patients) after stenting and CABG with 36-month follow-up (72% vs. 69%, respectively) [32]. In a HEART study, stenting or CABG was performed in patients with ischemic LVD and viable myocardium. After 4 years no difference was found in the compared groups either in mortality from all causes, or in indicators of the quality of patients' life. At the same time, the study of only 138 patients was not powerful enough to assess the differences between groups in terms of their impact on the endpoints. [8]. The advantages of CABG compared with coronary artery stenting in patients with LVD are identified during the long-term follow-up in studies with a large number of patients [5, 18]. At the same time, stenting retains its place as a means of improving the quality of life of patients with the corresponding anatomical indications.

The benefit of CABG is most noticeable in patients with more pronounced and complex stenosing lesions of the coronary arteries. According to the analysis of a subgroup of patients with a "stem" lesion from SYNTAX study, the difference in treatment results in favor of CABG compared with stenting was evident in patients with moderate or severe coronary lesions determined by the SYNTAX scale [16]. In the FREEDOM study, the beneficial effect of CABG on survival was proven in patients with diabetes mellitus, who often have diffuse coronary artery lesion [10]. Therefore, concomitant diabetes mellitus in patients with two or more coronary arteries lesions (including those with CAD and reduced LVEF) is a strong argument in favor of CABG, rather than stenting [25].

Revascularization in consensus guidelines

First of all, in the European guidelines for the diagnosis and treatment of HF, indications are given for CVG in patients with HF [29]. In particular, CVG is recommended for patients with angina pectoris, with myocardial revascularization being potentially possible. In addition, symptomatic ventricular arrhythmias or a postponed episode of cardiac arrest with successful resuscitation are indications for CVG. CVG should also be considered in patients with HF and moderate or high pretest likelihood of CAD and evidence of ischemia when performing non-invasive stress tests.

In patients with stable CAD, the decision to perform revascularization is based on angiographic and clinical criteria, and the goal of revascularization is to improve the prognosis of survival and / or their quality of life [26]. Both of these tasks are undoubtedly relevant for patients with LVD of ischemic etiology. It is important to take into account the absence of LVEF lower threshold when assessing the feasibility of revascularization. Moreover, improvement in the prognosis and the course of disease after CABG is most pronounced in patients with ischemic LVD.

When choosing the optimal method of revascularization, it is necessary to consider not only the severity of atherosclerosis of the subepicardial coronary arteries, but also the state of the distal bed, as well as the collateral blood flow. Ischemic LVD is often accompanied by a multivessel lesion, and LVEF decreases with the increase of the total lesion of the coronary bed [1]. According to the European guidelines [26], CABG surgery is indicated for patients with stenosis of the left coronary arterial trunk, three-vascular lesion or a two-vascular lesion, including the anterior descending artery, taking into account the severity of the lesion of the coronary bed on the SYNTAX scale. Based on the results of the STICH study (which did not include patients with stem lesions and III-IV functional classes angina), CABG was recommended for patients with HF and LVEF ≤35%, lesion of the left anterior descending artery or a multivessel lesion, to reduce mortality and hospitalizations from cardiovascular causes [29]. LVD is a convincing additional argument in favor of performing CABG surgery in the presence of the corresponding changes in the anatomy of the coronary bed and clinical symptoms, since it is in the category of patients with reduced LVEF that the most convincing evidence of the beneficial effect of revascularization on survival is obtained [14, 17, 28, 38].

The American guidelines for CABG also indicate that the presence and severity of LVD is one of the clinical factors influencing the choice of the optimal method of revascularization. According to their authors, the existing evidence base has certain limitations, especially in patients with severe LVD, but the data on the effectiveness of CABG are more convincing compared to stenting. Clinical parameters, such as anatomy of the coronary arteries, the presence of concomitant diabetes mellitus or chronic kidney disease, as well as the opinion of the patient, are important for choosing a management strategy for patients with coronary heart disease and LVD. The final decision is made jointly by an interventional cardiologist and a heart surgeon [17].

In some special cases of a pronounced decrease in LVEF, the final decision on the feasibility of CABG can be made taking into account the assessment of myocardial viability. In particular, the absence of conclusive evidence of recurrent ischemia combined with a small number of viable myocardium is considered an argument against the surgical treatment. In general, the criteria for the selection of patients for revascularization depending on the state of viability of the cardiac muscle are still not clearly defined due to the lack of evidence base and the lack of a consistent research methodology. It should be also noted that in patients with terminal HF, heart transplantation may be considered.

Revascularization effectiveness criteria

Unlike randomized studies, where the effect of revascularization is evaluated by endpoints, in clinical practice the main criteria for the effectiveness of the intervention are changes in the pumping function of the heart and quality of life, which is primarily determined by clinical symptoms. The increase in LVEF, in turn, favorably influences the course and prognosis of the disease [19]. The data of most studies indicate a positive effect of revascularization on the global pumping function of the heart and local contractility in the area of functioning shunts in patients with LVEF less than 40-30% [3, 33, 34]. Greater growth of LVEF was recorded after myocardial revascularization in patients with worse initial indices of LVEF [15]. At the same time, in patients with initially preserved LVEF, a slight decrease in LVEF was observed in the postoperative period [20]. In the original study of the authors (n = 111), the median of LVEF in 6-12 months after CABG surgery increased from 35 (quartile 30-39) to 42 (35-45%), on average – by 18.9% (5.3 -32.4%) [2]. Moreover, in most cases, the growth of the LVEF was not immediately observed, but from the end of the first month after the operation. With an increase in the duration of postoperative follow-up, the increase in LVEF achieved during the first year of observation usually does

not continue increasing, and the LVEF indicator comes to its plateau [23]. Obviously, there remains the need to study the predictors of favorable or negative dynamics of LVEF in patients with CAD after CABG surgery.

Another sensitive indicator of the outcome of revascularization intervention is the reduction in clinical symptoms and improvement in the quality of life associated with health status of patients. This aspect in patients with ISHD and LVD can be determined not only by LVEF and HF functional class according to NYHA classification, but also by age, sex and associated diseases. The correct selection of patients for surgical revascularization allows us to expect an improvement in the quality of life indicators after the intervention in the vast majority of patients, this improvement being more tangible than in the presence of drug therapy [24]. Stenting of the coronary arteries has advantages in influencing the quality of life soon after the intervention, while the advantages of CABG become apparent 6-12 months after the operation.

Conclusions

Coronary atherosclerosis is the most common cause of a decrease in LVEF and the occurrence of HF in the modern world. In patients with ischemic LVD, surgical revascularization can ensure an improvement in the pumping function of the heart, provided there is a sufficient amount of viable myocardium. From the standpoint of evidence-based medicine, the effectiveness of CABG surgery in patients with multivessel lesion of the coronary bed and LVD has been proven for correction LVEF, improvement of the course of the disease and prediction of survival compared with the optimal drug therapy. Percutaneous intervention is inferior to surgical revascularization in terms of its effect on endpoints, but it can make an improvement in the quality of life of patients with ischemic LVD. Evaluation of myocardial viability may be of additional importance for the decision on the feasibility of CABG in the presence of multivessel coronary artery disease combined with a sharp decrease in LVEF.

References

1. Bokeria LA, Rabotnikov VS, Buziashvili IuI, et al. Ishemicheskaia bolezn' serdtsa u bol'nykh s nizkoi sokratitel'noi sposobnost'iu miokarda levogo zheludochka (diagnostika, taktika lecheniia) [Ischemic heart disease in patients with reduced myocardial left ventricular contractility (diagnosis, tactics of treatment)]. Moscow: [A.N. Bakulev National Medical Research Center of Cardiovascular Surgery]; 2001. 195 p. Russian.
2. Ivaniuk NB, Jarinov OI, Mikhalev KA, Epanchintseva OA, Todurov BM. [Changes of left ventricular ejection fraction in patients with ischemic cardiomyopathy after coronary artery bypass grafting]. Ukr Kardiol J. 2016;(4):45-54. Ukrainian.
3. Adachi Y, Sakakura K, Wada H, Funayama H, Umemoto T, Fujita H, Momomura S. Determinants of left ventricular systolic function improvement following coronary artery revascularization in heart failure patients with reduced ejection fraction. Int Heart J. 2016;57(5):565-72.
4. Allman KC, Shaw LJ, Hachamovitch R, Udelson JE. Myocardial viability testing and impact of revascularization on prognosis in patients with coronary artery disease and left ventricular dysfunction: a meta-analysis. J Am Coll Cardiol. 2002;39(7):1151-8.
5. Bangalore S, Guo Y, Samadashvili Z, Blecker S, Hannan EL. Revascularization in patients with multivessel coronary artery disease and severe left ventricular systolic dysfunction: everolimus-eluting stents versus coronary artery bypass graft surgery. Circulation. 2016;133(22): 2132-40.
6. Bonow RO, Maurer G, Lee KL, et al.; STICH Trial investigators. Myocardial viability and survival in ischemic left ventricular dysfunction. N Engl J Med. 2011;364(17):1617-25.
7. Burch GE, Giles TD, Colcolough HL. Ischemic cardiomyopathy. Am Heart J. 1970;79(3):291-2.
8. Cleland JG, Calvert M, Freemantle N, et al. The heart failure revascularization trial (HEART). Eur J Heart Fail. 2011;13(2):227-33.
9. Doshi D, Ben-Yehuda O, Bonafede M, et al. Underutilization of coronary artery disease testing among patients hospitalized with new-onset heart failure. J Am Coll Cardiol. 2016;68:450-8.
10. Farkouh ME, Domanski M, Sleeper LA, Siami FS, Dangas G, Mack M, Yang M, Cohen DJ, Rosenberg Y, Solomon SD, Desai AS, Gersh BJ, Magnuson EA, Lansky A, Boineau R, Weinberger J, Ramanathan K, Sousa JE, Rankin J, Bhargava B, Buse J, Hueb W, Smith CR, Muratov V, Bansilal S, King S 3rd, Bertrand M, Fuster V.; FREEDOM Trial Investigators. Strategies for multivessel revascularization in patients with diabetes. N Engl J Med. 2012;367(25):2375-84.
11. Felker GM, Thompson RE, Hare JM, et al. Underlying causes and long-term survival in patients with initially unexplained cardiomyopathy. N Engl J Med. 2000;342(15):1077-84.
12. Ferrari R, La Canna G, Giubbini R, et al. Hibernating myocardium in patients with coronary artery disease: identification and clinical importance. Cardiovasc Drugs Ther. 1992;6(3):287-93.
13. Filsoufi F, Jouan J, Chilkwe J, et al. Results and predictors of early and late outcome of coronary artery bypass graft surgery in patient with ejection fraction less than 20%. Arch Cardiovasc Dis. 2008;101(9): 547-56.
14. Hawranek M, Zembala MO, Gasior M, Hrapkowicz T, Pyka Ł, Cieśla D, Zembala M. Comparison of coronary artery bypass grafting and percutaneous coronary intervention in patients with heart failure with reduced ejection fraction and multivessel coronary artery disease. Oncotarget. 2018;9(30):21201-10.
15. Haxhibeqiri-Karabdic I, Hasanovic A, Kabil E, Straus S. Improvement of ejection fraction after coronary artery bypass grafting surgery in patients with impaired left ventricular function. Med Arh. 2014;68(5):332-4.
16. Head SJ, Davierwala PM, Serruys PW, Redwood SR, Colombo A, Mack MJ, Morice MC, Holmes DR Jr, Feldman TE, Ståhle E, Underwood P, Dawkins KD, Kappetein AP, Mohr FW. Coronary artery bypass grafting vs. percutaneous coronary intervention for patients with three-vessel disease: final five-year follow-up of the SYNTAX trial. Eur Heart J. 2014;35:2821-30.
17. Hillis LD, Smith PK, Anderson JL, et al. ACCF/AHA Guideline for coronary artery bypass graft surgery. J Am Coll Cardiol. 2011;58(24): e123-210.
18. Iribarne A, DiScipio AW, Leavitt BJ, Baribeau YR, McCullough JN, Weldner PW, Huang YL, Robich MP, Clough RA, Sardella GL, Olmstead EM, Malenka DJ; Northern New England Cardiovascular Disease Study Group. Comparative effectiveness of coronary artery bypass grafting versus percutaneous coronary intervention in a real-world Surgical Treatment for Ischemic Heart Failure trial population. J Thorac Cardiovasc Surg. 2018;156(4):1410-1421.
19. Kalogeropoulos AP, Fonarow GC, Georgiopoulou V. Characteristics and outcomes of adult outpatients with heart failure and improved or recovered ejection fraction. JAMA Cardiol. 2016;1(5):510-8. doi:10.1001/jamacardio.2016.1356.
20. Koene RJ, Kealhofer JV, Adabag S, Vakil K, Florea V. Effect of coronary artery bypass graft surgery on left ventricular systolic function. J Thorac Dis. 2017;9(2):262-70.
21. Kunadian V, Pugh A, Zaman AG, Qui W. Percutaneous coronary intervention among patients with left ventricular systolic dysfunction: a review and meta-analysis of 19 clinical studies. Coron Artery Dis. 2012;23(7):469-79.

22. Lloyd-Jones DM. The risk of congestive heart failure: sobering lessons from the Framingham heart study. Curr Cardiol Rep. 2001;3(3):184-90.
23. Lupon J, Gavidia-Bovadilla G, Ferrer E, et al. Dynamic trahectories of left ventricular ejection fraction in heart failure. J Am Coll Cardiol. 2018;72(6):591-601.
24. Mark DB, Knight JD, Velazquez EJ, et al. Quality-of-life outcomes with coronary artery bypass graft surgery in ischemic left ventricular dysfunction: a randomized trial. Ann Intern Med. 2014;161(6):392-9.
25. Nagendran J, Bozso SJ, Norris CM, et al. Coronary artery bypass surgery improves outcomes in patients with diabetes and left ventricular dysfunction. J Am Coll Cardiol. 2018;71:819-27.
26. Neumann F-J, Sousa-Uva M, Ahlsson A, et al. 2018 ESC/EACTS Guidelines on myocardial revascularization: The Task Force on myocardial revascularization of the European Society of Cardiology (ESC) and European Association for Cardio-Thoracic Surgery (EACTS). Developed with the special contribution of the European Association for Percutaneous Cardiovascular Interventions (EAPCI). Eur Heart J. 2018;00:1-96. doi:10.1093/eurheartj/ehy394
27. Oldham HN, Kong I, Bartel AG, et al. Risk factors in coronary artery bypass surgery. Arch Surg. 1972;105(6):918-23.
28. Peng D, Liu JH. Improvement of LVEF in patients with HFrEF with coronary heart disease after revascularization – A real-world study. J Interv Cardiol. 2018 Sep 5.
29. Ponikowski P, Voors AA, Anker SD, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. Eur Heart J. 2016;37:2129-2200.
30. Pyka Ł, Hawranek M, Gąsior M. Revascularization in ischemic heart failure with reduced left ventricular ejection fraction. The impact of complete revascularization. Kardiochir Torakochirurgia Pol. 2017 Mar;14(1):37-42.
31. Reibis R, Salzwedel A, Bonaventura K, Völler H, Wegscheider K. Improvement of left ventricular ejection fraction in revascularized postmyocardial infarction patients: indication for statistical fallacy. BMC Res Notes. 2017 Jul 5;10(1):244.
32. Sedlis SP, Ramanathan KB, Morrison DA, et al. Outcome of percutaneous coronary intervention versus coronary bypass grafting for patients with low left ventricular ejection fractions, unstable angina pectoris, and risk factors for adverse outcome with bypass (the AWESOME randomized trial and registry). Am J Cardiol. 2004;94:118-20.
33. Sohn GH, Yang JH, Choi SH, Song YB, Hahn JY, Choi JH, Gwon HC, Lee SH. Long-term outcomes of complete versus incomplete revascularization for patients with multivessel coronary artery disease and left ventricular systolic dysfunction in drug-eluting stent era. J Korean Med Sci. 2014;29(11):1501-6.
34. Todurov B, Shevchenko V, Zelenchuk O, et al. The immediate results of coronary artery bypass grafting in patients with low ejection fraction of left ventricle. Pol J Cardiothorac Surg. 2011;8:24-25.
35. Topkara VK, Cheema FH, Kesavaramanujam S. Coronary artery bypass grafting in patients with low ejection fraction. Circulation. 2005;112:344-50.
36. Velazquez EJ, Lee KL, Deja MA, et al. Coronary-artery bypass surgery in patients with left ventricular dysfunction. N Engl J Med. 2011;364:1607-16.
37. Velazquez EJ, Lee KL, Jones RH, et al. Coronary-artery bypass surgery in patients with ischemic cardiomyopathy. N Engl J Med. 2016;374(16):1511-20.
38. Yusuf S, Zucker D, Passamani E, et al. Effect of coronary artery bypass graft surgery on survival: overview of 10-year results from randomized trials by the coronary artery bypass graft surgery trialists collaboration. Lancet. 1994;344(8922):563-70.

The monograph "The operated ear disease"

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The classical radical operation made for the sanitation of the middle ear system and the profilaxy of the otogenic complications is the most used surgical procedure in the world. It has a low rate of recurrent or/and residual lesions, it facilitates the control of the recidivant cholesteatome and of the infection, and it also offers the possibility of treatment in ambulatory conditions, fact that reduces the costs and the waiting lists. Nevertheless this method has an imperfection as the healing is not definitive. The operated ear continues to represent the patient's suffering cause. There are a lot of ENT surgeons that use the radical-conservative method of sanitation in order to get rid of the consequences of the radical intervention. Patients who have experienced an ear intervention often have a condition called "the operated ear disease".

The work is presented on 224 pages; it consists of introduction, 8 chapters, general conclusions, 99 figures, 19 annexes and bibliographic index with 221 references.

In the introduction there are presented the scientific importance and the topicality of the problem, its theoretical and practical value. The general part of the monograph is composed of 4 chapters.

In the first chapter – there is a detailed description of the contemporary data of the embryology, clinical anatomy, physiology and pathophysiology of the vestibular – acoustic system. There is brief and well presented information concerning the morphology and the morphopathology of the temporal bone, the hearing physiology and the balance function.

The second chapter – has the most recent data on the ear semiology. The otological syndromes like hypoacusia, tinnitus, ear pain, othoreea, prurritus and the vestibular periferic syndrome are described with the precision of different clinical variants that permits to correctly diagnose and apply the adequate method of conservative or surgical treatment.

In the third chapter – there are widely described principles and the order of the otological contemporary diagnosis steps. The applicative value of this method of clinical and imagistic research, are also specified. The oto-endoscopy and the video-endoscopy are the new methods in the mastoid surgery and in the middle ear surgery.

The use of the endoscope elevates the safety in the total choleasteatome removal. It allows the surgeon to keep the EAC untouched.

The knowledge of the prevailing species and the antimicrobial sensibility guides the clinicians towards a correct empiric treatment. The patients will benefit of better management, a more specific one and of a lower rate of intracranial and otomastoidian complications of SCOM.

The fourth chapter – is totally dedicated to the chronicle inflammation of the middle ear system. There is presented the pathogenesis and the differentiations of the clinical forms of the SCOM. The diagnosis, surgical treatment and the profilaxy of this disease are given in detail. There are described advantages and disadvantages of different mastoidectomy techniques. There are 3 types of traditional surgical procedures: 1) simple mastoidectomy (cortical, complete), 2) radical mastoidectomy, 3) modified radical mastoidectomy. The fourth procedure is the mastoidectomy with tympanoplasty. The objectives of this method besides the cure of the disease are also the maintenance or the reconstruction of the posterior-superior wall of the EAC and the osicular chain. These procedures can be categorized in 2 types: with the opened cavity CWD or with the closed cavity CWU.

The special part is based on the personal research results and is exposed in 4 chapters.

In the fifth chapter – there is a description of the causes and complications of the operated ear disease. A special part is taken by the methods and the treatment techniques in surgery. There are described the methods of reconstruction of the posterior wall of the CWR, timpanoplasty and the mastoidian obliteration (mastoidoplasty). Special attention is paid to the influence of the reconstructive surgery on the life quality of the patients with the OED.

The sixth chapter – represents the experimental argumentation of the autogenic bone graft with bone inductive properties of the AOMF in the reconstructive surgery of the ear. After the experimental research the author concludes that the autogenic graft "Osteomatrix forte" is a biocompatible material which shows the osteocunductive and osteoinductive capacity, it is moderately resorbed which guides and stimulates the tissue regeneration till the total substitution with the grown and organospecific bone tissue.

The seventh chapter – reflects the details on the revision surgeries on the patients with the OED with the traditional reconstruction and with the reconstruction with the autograft bone having osteoinductive properties of the AOMF, and surgeries of sanitation on patients with medium cronical suppurated otitis with traditional reconstruction and with the use of bone autografts having osteoinductive properties AOMF.

In the eighth chapter – the comparative analysis is done concerning the anatomical and functional results of the primary reconstructive interventions and the revision interventions. It is shown that the results of surgical interventions, made after an elaborate method are better and more stable than the results of surgical interventions made after the known method.

The work is finished by 8 general well formulated conclusions that come from the results of the research done by the author.

The work fully complies with the requirements, is elaborated by a thorough study, with multifactorial analysis, with recommendations addressed to the public health services, offering distinct management solutions for practicing physicians and doctors of different specialties as well as to all those interested in this pathology and may be recommended for publishing.

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Victor Vovc 70-year anniversary

The distinguished professor, doctor habilitated in medical sciences, Emeritus of the Republic of Moldova, Mr. Victor Vovc, is an outstanding distinguished personality of the university environment, remarkable not only by professionalism, but also by good native education, with great intellectual capabilities, honesty, dignity, soul balance and pedagogical tact.

He became a student at the General Medicine Faculty of the Kishinev State Medical Institute in 1966. From the first years of his studies he was interested in scientific research. Subsequently, research continues within the students' scientific circle at the Department of Normal Physiology. Here he is captivated by the atmosphere of scientific research in the field of normal physiology, which today is called medical physiology, created by the renowned scholar and scientist Professor Anatoly Zubcov.

After graduating from the Kishnev State Medical Institute in Chisinau in 1972, he continued his doctoral studies at the Department of Normal Physiology. Work on the PhD thesis, the promotion of advanced physiological methods at the department allowed him to develop his skills as a true experimenter, marked with 8 innovation certificates.

After completing his doctorate he works as a scientific researcher, later as an assistant at the Department of Normal Physiology. Together with Professor Aurel Saulea and Assistant Professor Nicolae Bolocan, they conducted research in a field started by their predecessor, Professor Anatoly Zubcov, many years before – the physiology of the heart. In 1975 he made an internship at the Department of Normal Physiology of the Institute of Medicine, Hygiene and Sanitation in Leningrad under the supervision of Professor Ratmir Orlov, a renowned physiologist.

In 1981, he defended his PhD thesis in Biological Sciences on "The Influence of Hypobaric Hypoxia Adaptation on the Contractile Function of the Papillary Muscles of the Rat Heart".

In the years 1987-1990 he completed a scientific internship in the Laboratory of Heart`s Physiopathology of the Institute of General Pathology and Pathophysiology of the Academy of Medical Sciences of the Russian Federation (Moscow). Under the guidance of the famous scientist in the area of stress and adaptation, Professor Felix Meerson, Victor Vovc conducts a number of important scientific researches, the results of which have been published in prestigious international scientific journals.

In 1993 he defended a thesis of doctor habilitate in medical sciences at the Scientific Council of the Institute of General Pathology and Pathophysiology of the Academy of Sciences of the Russian Federation (Moscow), a thesis with the theme: "Cardioprotective effect of the organism's adaptation to hypobaric hypoxia and stress".

The next scientific project was realized at the "Joseph Fourier" University of Grenoble, France, in the Laboratory of Breath Physiology. The scientific collaboration with the scientists from France and with the Department of Neurology (Professor Ion Moldovanu) materialized in the joint scientific papers, which focus on the analysis of the breathing pattern as a new test in the functional diagnosis, specialized in psychovegetative affections, parkinsonism and others.

In the last period, the scientific interests of Professor Victor Vovc are related to the physiology and pathology of sleep and wakefulness. In this field, he publishes scientific works, presents communications to national and international conferences.

Thanks to the fruitful collaboration with professor Ion Moldovanu, the Somnology Center was established, officially formed by the Institute of Neurology and Neurosurgery due to Order of the Minister of Health of the Republic of Moldova in December 2013. The Center of Somnology became in the short time a reference institution for medical services specialized in sleep and wakefulness disturbances, as well as a teaching center for students and medical doctors. In this context, we note that Professor Victor Vovc is the founder and president of the Sleep Medicine Association of the Republic of Moldova.

The scientific merits of the eminent Professor Victor Vovc are valued by the scientific community, he has been elected to the governing body of the Society of Physiologists of the Republic of Moldova, a member of the European Society of Physiological Sciences, a member of the editorial board of several prestigious editions.

He promotes consistently the involvement of the medical community from the Republic of Moldova, the medical professors and students in the implementation of international programs and projects in medicine. Due to the direct participation of Professor Victor Vovc, 23 international and national projects in medicine and medical education were carried out.

During 1999-2009, tireless Professor Victor Vovc leads the Department of Biophysics, Computer Science and Human Physiology and in 2009 he was elected the Chief of the Department of Human Physiology and Biophysics.

Professor Victor Vovc is the author and co-author of more than 250 scientific papers, 5 textbooks, 12 compendia, 3 guides, 3 methodical instructions. He has prepared 5 doctors in medical sciences and 4 masters in physiology. Currently, the authors of the 5 PhD theses in medical sciences benefit from his advice.

We are always glad to see him in full creative forces, keeping up with the advances in his profession and the institution that formed him. Our sincere desire is to see him at Nicolae Testemitsanu State University of Medicine and Pharmacy, in good mood, with a bright face with noble intentions and spiritual uplifting.

Ion Ababii, MD, PhD, Professor, Academician
Rector of Nicolae Testemitsanu State University
of Medicine and Pharmacy
Chisinau, the Republic of Moldova

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3. The abstract should be written on the title page and limited from 220 to 240 words. The abstract of research articles should have four parts: Background, Material and methods, Results, Conclusions. The abstract of review articles should have two parts: Background and Conclusions. The abstract should end with 3 to 6 key words.

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