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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.

The Editorial Board warmly welcomes both the readers of and the authors for the journal, all those who are enthusiastic in searching new and more effective ways of solving numerous medicine problems. We hope that those who want to make their contribution to the science of medicine will find our journal helpful and encouraging.

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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 effects, 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


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 extrapulmonary 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 MTR/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 Mycobacterium 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: phthisiogenic 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-a blockers or oral steroids, antineoplastic agents), diabetes, cancer, silicosis, chronic respiratory diseases, gastrointestinal diseases, underweight, phthisiogenic 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 Pneumology of Chişinău between 2010 and 2014. The investigations were performed 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 Rifampicin 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 first group there were 61 men (71.7%) and 24 women (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 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 36 (25.5%) compared with the first and second groups, 37 (43.5%) and 36 (25.5%) patients. Married patients predominated in the second group 43 (50.6%) and 92 (65.2%) patients. Comparing the groups of patients was established with the first and second groups, 43 (55.1%) compared with the third group compared with the first group and third group 40 (51.3%) compared with 17 (20.1%) and 48 (61.5%) patients. Comparing the rate of patients with a low school education which included professional and superior studies predominated in the third group compared with the first and second groups. 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 high level of the school education which included professional and superior studies predominated in the third group compared with the first and second groups.

### Table 1

<table>
<thead>
<tr>
<th>Distribution of patients in risk groups</th>
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<tbody>
<tr>
<td>Risk groups</td>
</tr>
<tr>
<td>TB contact</td>
</tr>
<tr>
<td>Migrants</td>
</tr>
<tr>
<td>Former detainees</td>
</tr>
<tr>
<td>Smokers</td>
</tr>
<tr>
<td>Alcohol abuse</td>
</tr>
<tr>
<td>Chronic alcoholism</td>
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<tr>
<td>DIU</td>
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</table>

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 encountered 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. 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 begining 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 antituberculosis treatment assessed by molecular 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>
<thead>
<tr>
<th>Table 3</th>
<th>1st group</th>
<th>2nd group</th>
<th>P</th>
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<tbody>
<tr>
<td>Sputum conversion</td>
<td>n=85 (P%)</td>
<td>n=141 (P%)</td>
<td></td>
</tr>
<tr>
<td>Till 3 months</td>
<td>55 (64.8)</td>
<td>30 (21.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>3-6 months</td>
<td>39 (45.5)</td>
<td>64 (54.7)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>6-12 months</td>
<td>2 (2.4)</td>
<td>13 (4.7)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>More than 12 months</td>
<td>0</td>
<td>1 (0.7)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Non-assessed patients</td>
<td>9 (10.6)</td>
<td>45 (31.9)</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

Obtained data confirmed the impact of molecular genetic test for early diagnosis, intersection 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 beginning 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.
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 4

**Anti-tuberculosis treatment duration in patients from the study sample**

<table>
<thead>
<tr>
<th>Duration</th>
<th>1st group n=85 (P%)</th>
<th>2nd group n=141 (P%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 months</td>
<td>7 (8.2)</td>
<td>14 (9.9)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>18 months</td>
<td>16 (18.8)</td>
<td>21 (14.9)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>24 months</td>
<td>54 (63.5)</td>
<td>37 (26.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>24-30 months</td>
<td>2 (2.3)</td>
<td>74 (52.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&gt;30 months</td>
<td>0</td>
<td>2 (1.4)</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

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


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).

![Normal occlusion](image)

**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 sphenoopticical 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. Sphenoopticical 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 sphenoopticical 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>
<thead>
<tr>
<th>Patients with class II malocclusion</th>
<th>Study group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complex treatment</td>
<td>20% (6 patients)</td>
<td>30% (9 patients)</td>
</tr>
<tr>
<td>Orthodontic treatment</td>
<td>20% (6 patients)</td>
<td>30% (9 patients)</td>
</tr>
</tbody>
</table>

Fig. 3. Patient A., 10 years, with class II malocclusion due to distal position of the mandibular, overjet – 5mm.
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: sphenoccipital 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.
drainage; decompression technique of sphenoooccipital 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 craniocerebral 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.

Results and discussion

The testing osteopathy revealed the following dysfunctions:

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

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, 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**

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/AIDS, 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 underlie 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 patients aged between 45-55 years old predominated in 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. Repartition of the patients into age groups, according to the biological characteristics it was argued 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 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.

Distribution of patients by sex, age and demographic data

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


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; study group IVDU-TB compared to the 2nd control group: ● – p<0.05; ●● – p<0.01; ●●● – p<0.001.

Distribution according to the socioeconomic data

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


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; study group IVDU-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 1st CG compared with the SG. History of imprisonment statistically predominated in the 1st CG compared with the 2nd 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**

<table>
<thead>
<tr>
<th>Education</th>
<th>Study group</th>
<th>1st control group</th>
<th>2nd control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=48 (P%)</td>
<td>N=185 (P%)</td>
<td>N=34 (P %)</td>
<td></td>
</tr>
<tr>
<td>Primary &amp; low secondary education</td>
<td>10 (20.8)</td>
<td>46 (24.8)</td>
<td>22 (64.7)</td>
</tr>
<tr>
<td>Upper secondary education</td>
<td>27 (56.2)</td>
<td>91 (49.2)</td>
<td>1 (2.9)</td>
</tr>
<tr>
<td>Secondary technical vocational education</td>
<td>7 (14.6)</td>
<td>36 (19.5)</td>
<td>3 (8.8)</td>
</tr>
<tr>
<td>Bachelor studies</td>
<td>4 (8.3)</td>
<td>12 (6.5)</td>
<td>4 (11.7)</td>
</tr>
</tbody>
</table>

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**

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

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;
Comorbid patients statistically predominated in the SG and the 2nd 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.

Table 5
Case-management characteristics of tuberculosis patients

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

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.
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 sensitivity 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.

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

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 7

<table>
<thead>
<tr>
<th>Microbiological characteristics</th>
<th>Study group</th>
<th>1st control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mo</td>
<td>N=48 (P%)</td>
<td>N=185 (P%)</td>
</tr>
<tr>
<td>Masculine positive</td>
<td>20 (41.7)</td>
<td>101 (54.5)</td>
</tr>
<tr>
<td>Culture positive</td>
<td>24 (50)</td>
<td>99 (53.51)</td>
</tr>
<tr>
<td>GeneXpert performed</td>
<td>25 (52.1)</td>
<td>185 (100)</td>
</tr>
<tr>
<td>GeneXpert MTB/Rif positive</td>
<td>13 (27.1)</td>
<td>185 (100)</td>
</tr>
<tr>
<td>GeneXpert MTB/Rif positive resistant</td>
<td>4 (8.3)</td>
<td>147 (79.46)</td>
</tr>
<tr>
<td>GeneXpert MTB/Rif positive resistant</td>
<td>9 (18.7)</td>
<td>38 (20.54)</td>
</tr>
</tbody>
</table>

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.

Table 8

<table>
<thead>
<tr>
<th>Treatment outcome of tuberculosis patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=48 (P%)</td>
</tr>
<tr>
<td>N=185 (P%)</td>
</tr>
<tr>
<td>Available final outcomes</td>
</tr>
<tr>
<td></td>
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<tr>
<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td>Non available final outcomes</td>
</tr>
</tbody>
</table>

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.
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 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 addicts 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).
References


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, 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 suppurrative 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 cavi- ties. 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 otor- rhea 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:
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 meato tympanoplasty 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.

The second variant is used in patients with ETMT antecedents and with MUO. Until the revision of the evidation...
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 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, ootalgia, cephalalgia) and previous surgical procedures, (2) otomicroscopic and/or otoendoscopic examination, (3) complete audiological evaluation (audiometry, instrumental audiometry with diapason, Limbal 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 X² 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 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 whispering (1.95 ± 0.08 m and 1.18 ± 0.04 m, p < 0.001), mean voice perception during speech (4.24 ± 0.19 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 patients from LB group.

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 (67.24 ± 1.2%, p < 0.05) 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 evagination cavity was closed without pathologies in all patients from both study groups.

Hearing test revealed: mean value of voice perception on whispering (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 evagination 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, hypacusis 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 hypacusis (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: 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 patients from LB group.

Thus, 12 months postoperatively, hearing loss was present in all patients in both subgroups of study. Insufficient 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 evagination 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 (tymanoplasty), 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 (HC cement, ionomer 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 meato tympanoplasty 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 evagination 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 examination of the evagination cavity found, 1 month postoperatively, a closed cavity in all patients from both study groups. 1 month postoperatively almost all pathologies of evagination 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 conduction. 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, meato tympanoplasty 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 meato plasty, anterior and infe-
or canalplasty, tympanoplasty, obliteration with bone paste covered by musculoperiosteal 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 tympanectomy, 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, otorrhea, 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 cholesteactomy. 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 tympanoplasty. In order to obliterate the mastoid cavity, the lower pediculated postauricular periosteopericanian flap was used in combination with autologous bone paste. Anterior and lower canalplasty and extended meatooplasty 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 osseicular 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 mesotympanic 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 mastoidectomy and revision CWD mastoidectomy by obliteration with autologous bone paste, covered with cartilage plates, meatooplasty 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 evisdation 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

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 metaphyseal 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.
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 metaphyseal 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 arthroplasty 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-Allgöwer (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).

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, transarticular 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).

Table 1

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>AO Classification. 43 – tibia/fibula, distal segment.</td>
<td>AO Classification. 44 – tibia/fibula, malleolar segment.</td>
<td>Rüedi-Allgöwer classification of pilon fractures</td>
</tr>
</tbody>
</table>

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

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

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, to 155 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).
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>
<thead>
<tr>
<th>AO code</th>
<th>Individuals</th>
<th>%</th>
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<tbody>
<tr>
<td>43.A1</td>
<td>3</td>
<td>2.65</td>
</tr>
<tr>
<td>43.A2</td>
<td>11</td>
<td>9.73</td>
</tr>
<tr>
<td>43.A3</td>
<td>5</td>
<td>4.42</td>
</tr>
<tr>
<td>43.B1</td>
<td>2</td>
<td>1.77</td>
</tr>
<tr>
<td>43.B2</td>
<td>2</td>
<td>1.77</td>
</tr>
<tr>
<td>43.B3</td>
<td>3</td>
<td>2.65</td>
</tr>
<tr>
<td>44.A1</td>
<td>1</td>
<td>0.88</td>
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<tr>
<td>44.A2</td>
<td>17</td>
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<td>13</td>
<td>11.50</td>
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<td>44.B1</td>
<td>2</td>
<td>1.77</td>
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<tr>
<td>44.B2</td>
<td>8</td>
<td>7.08</td>
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<td>44.B3</td>
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<td>38.05</td>
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<td>44.C1</td>
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<tr>
<td>44.C2</td>
<td>2</td>
<td>1.77</td>
</tr>
<tr>
<td>43.C3</td>
<td>3</td>
<td>2.65</td>
</tr>
<tr>
<td>Total</td>
<td>113</td>
<td>100%</td>
</tr>
</tbody>
</table>

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%).

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 one 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).

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%).
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).

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%).

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).
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 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 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 metaepiphysis 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, joy-sticks, 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-
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

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 applicable 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 etc., it cannot be analysed using classical methods 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 in the cardiologic field is the problem of steady-state cardiorhythmograms. Steady-state cardiorhythmograms 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 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 cardiorhythmograms 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].
**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 cardiorhythograms and spectrograms. We 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 cardiorhythograms and spectrograms using the cardiophysiological biomarkers.

Below are presented cardiorhythograms 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. Cardiorhythograms 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.
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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).

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

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.

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 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).

2.A. On the spectrogram VLF component dominates in the total power spectrum. Pathological peaks’ structure in the HF spectrum area, representing parasympathetic break-down.

2.B. On the cardiorhythmogram LF drops are present followed by a pathological counterregulation: predominated by LF waves.

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

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.

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.

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

Fig. 8 Spectrogram which belongs to the cardiorhythmogram above. 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.
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.

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 between.
Research Studies


Between, 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 recurrence.

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 parasympathetic modulation of the heart. In case of a pathological counterregulation, LF waves will be seen. A pathological counterregulation is 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 parasympathetic 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. 10. Cardiorhythmogram. HRV is modulated predominantly by VLF and LF waves. There are no LF drops.

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.

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 parasympathetic 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 parasympathetic 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.

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 record-ings under steady-state conditions [8, 17]. In case of HRV analysis from a Holter ECG, the LF 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).

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.

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 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].

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 parasympathetic 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
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 helices, 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-kB), 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-
including 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 κB (NF-κB), 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 metalloproteinase 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 Willebrand factor [29]. In 2013 Zamani P. et al. [30] 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 sIL-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-6/IL-6R/gp130) [33], and the tetrameric (IL-6/IL-6R/gp130) 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 proteolytically 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-
vollving 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, Zn2+ 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 (TNFa) [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 1 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 Bouglanger 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 irresponsible 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 (NIA SF) 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 sIL-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 transducer and activator of transcription) factor. STAT1 or STAT3 monomers attach to the distal phosphotyrosine 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 promoter 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


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 activity of the concerned institutions.

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-
distributed 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).

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 Testemitanu 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

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

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**Background:** Gallstone disease and chronic calculous cholecystitis are the most prevalent gastro-entérological diseases requiring a surgical treatment. This disease occupies a special place in the pathology of the hepatobiliary-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 etiopathogenesis 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 duodenal 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.
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 $\geq 70\%$), mixed (30% $\geq 70\%$ cholesterol) and pigments (30% $\geq$ 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 dispersal 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 westernization 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 polymerized 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 hyper-secretion produced by the gallbladder epithelial cells into obstruction of the cystic duct.

Mixed calculi, cholesterololo-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 protic 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 cholecdocholithiasis [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 cholesterolic/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 microlasers 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, 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], Šalimov 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 antrum 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 antral-duodenal 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 antral-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-α 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 neuro-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...
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.

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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 ground LVD is associated with a certain increase in 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.

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
Between drug therapy and surgical revascularization [6].

Obtained of the role of viability assessment for choosing being convincingly proven, so far no direct evidence has yet been determined treatment priorities. Although the best survival dyspnea is often equivalent to angina, making it difficult to determine above-mentioned clinical and instrumental characteristics, 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 STITCH 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 intervention [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


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, othorea, 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 cholesteatome 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 chronic 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, tympanoplasty 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 reconstractive surgery of the ear. After the experimental research the author concludes that the autogenic graft “Osteomatrix forte” is a biocompatible material which shows the osteoconductive and osteoinductive capacity, it is moderately sorbed 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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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 Kishinev 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 Testemitanu State University of Medicine and Pharmacy, in good mood, with a bright face with noble intentions and spiritual uplifting.

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