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# The Treatment of Chronic Obstructive Pulmonary Disease

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**Abstract**

In this study, new aspects of treatment of exacerbations of chronic obstructive pulmonary disease are presented based on vast clinical material and our own studies. According to the obtained data it can be concluded that the treatment of chronic obstructive pulmonary disease exacerbations is a complex process, oriented towards a multitude of pathogenic mechanisms of the disease. One of the pathogenic mechanisms of chronic obstructive pulmonary disease exacerbations is respiratory infection which makes the administration of the antibacterial drugs is an important component in the complex treatment of the disease. The administration of antibacterial therapy diminishes the hospitalization period of these patients and, as a result, reduces the total economical impact of the health management.

**Key words:** chronic obstructive pulmonary disease, complex treatment, antibacterial therapy.

**Лечение обострений хронической обструктивной болезни легких**

В работе представлены современные аспекты лечения обострений хронической обструктивной болезни лёгких, которые основаны на обширном клиническом материале и на основании собственных клинических исследований. На основании полученных результатов можно сделать вывод, что лечение обострений хронической обструктивной болезни лёгких представляет комплексный процесс, обусловленный воздействием множеством патогенетических механизмов данного заболевания. Один из патогенетических механизмов заболевания – респираторная инфекция, что обуславливает назначение антибактериальных препаратов в лечении обострений хронической обструктивной болезни лёгких. Дифференцированный подход к назначению антибактериальных препаратов приводит к уменьшению периода госпитализации пациентов и как следствие этого – к уменьшению экономических затрат при оказании медицинской помощи.

**Ключевые слова:** хроническая обструктивная болезнь лёгких, комплексное лечение, антибактериальное терапия.

Chronic obstructive pulmonary disease (COPD) is a disease which is characterized by a progressive, partial reversible bronchial obstruction, which results from airway inflammation in response to unfavorable external factors (smoking, occupational hazard, pollutants and others). It is established that in cases of COPD, morphological changes are observed in the central and peripheral branches as well as in the lung parenchyma. The result of epidemiological research shows that in Europe and North America 4 – 15% of the adult population suffers from COPD [1, 2]. Official data show that in the Russian Federation 2.4 millions patients with COPD are registered. But the data from epidemiological research leads us to believe that this number could be as high as 16 millions people [3]. The morbidity and the mortality of the patients with COPD increases all over the world, which is primarily related to a high smoking rate. It is shown that this disease affects 4 – 6% of males and 1 – 3% of females older than 40 years [4]. In Europe this is for the cause of death of 200-300 thousands people per year [1, 3].

Exacerbation is a stage of COPD course. It negatively affects the quality of a patient's life, leads to the progression of bronchial obstruction, and is often a cause of hospitalization that considerable increase to the cost of treatment. COPD exacerbations can also be a cause of death. Exacerbations take place approximately 1 – 4 times a year [3].

Exacerbation is an acute increase of symptoms, in comparison with the otherwise stable state of patients. The most frequent exacerbation symptoms are difficult breathing, cough intensification, increase in the production of expectoration and the changes of its characteristics. These symptoms often demand a modification of the pharmaceutical treatment [2, 3, 4]. Their mechanisms are shown in tab. 1.

The exacerbations of COPD are often associated with acute respiratory infections of the upper respiratory tract. We observed intensification of wheezing, subjective reports of feeling pressure in the throat, peripheral edema (appearance of peripheral edemas, weight's increasing etc.), increasing of general weakness and disturbances of conscience. Throat pain and fever normally do not appear in the exacerbations of COPD, but if present, other diseases have to be excluded (pneumonia, pneumothorax, thromboemboli of pulmonary arteries etc.).

**The Reasons of COPD – exacerbations are:**

1. Infection: *H. influenzae, St. pneumoniae, M. catharralis* which correspond to 13 – 46%, 7 – 26% and 9 – 20%, respectively. *Enterobacteriaceae fam.* should be considered as a cause of COPD exacerbation if patients are older than 65 years, have concomitant chronic pathologies or if the peak expiratory flow (PEF) during the first second is less than 50%. In cases of bronchiectasis with a permanent production of purulent expectoration *P. aeruginosa* should be considered [5].
2. Pollutant (NO2, Sulfuric dioxide, Ozone, hard particles).
3. Drugs (beta-blockers, sedatives, barbiturates etc.).
4. Cardiac insufficiency and heart rhythm disorders.

Table 1

**Possible mechanisms for the development of symptoms of exacerbations of COPD**

| Symptoms | Mechanisms of Development | Comments |
|---|---|---|
| 1. Progression of shortness of breath | • Increased catabolism<br>• Bronchial obstruction<br>a) mucosal damage, increased bronchial hyperreactivity/bronchospasm<br>b) the infiltration of inflammatory cells of the respiratory tract<br>c) edema of the bronchial mucosa<br>d) mucous hypersecretion with increase in viscosity leading to formation of mucous plugs, reducing mucociliary clearance<br>e) the progressive worsening of the diffusion-perfusion gradient | Consequence of systemic inflammation and acidosis in COPD.<br>Associated with increased production of neutrophils' proteinases, bronchial epithelium endothelin-1, and colonization with bacteria (*H. influenzae*) and viruses.<br>Increase in the number of CD8+ lymphocytes, neutrophils, eosinophils; associated with increased production of «proinflammatory» cytokines (IL-6, TNFα, RANTES, etc.), neurotransmitters (LTV-4) and increased expression of adhesion molecules (ICAM-1, E-selectin).<br>It can occur by the bacteria (*H. influenzae, S. pneumoniae, Ps. aeruginosa*), viruses, pollutants .<br>Enhancing blood flow on poorly ventilated areas can easily lead to a deterioration of gas exchange and growth of hypoxemia. |
| 2. Increased sputum production | • Hypertrophy of the mucous glands<br>• Hyperplasia of goblet cells<br>• Degranulation of goblet cells | Arises as a result of neutrophils' inflammation and the action of proteases under various pollutants and microorganisms. |
| 3. Appearance of purulent sputum | • The accumulation of eosinophils and neutrophils | Associated with increased production of proinflammatory cytokines and increased expression of adhesion molecules (see above), all of which can occur secondary to bacterial infection. |
| **Note**: IL - interleukin (s); TNFα-tumor necrosis factor α; RANTES - regulated upon activation, novel T-cell expressed and presumably secreted (a molecule that is possibly secreted by activated T-lymphocytes); ICAM-1 - intercellular adhesion molecule - 1, LT-Leukotrienes. | | |

5. Thromboemboli of a. pulmonalis.
6. Pneumothorax.
7. Undetermined reason (approx. in 30% of all cases).

Respiratory infection is the reason for approximately 80% of COPD – exacerbations with a determined etiology. Often you can find *St. pneumoniae* in early and moderate stages of COPD (PEF1 > 50% from basic mean). In cases of severe and very severe course of the disease (PEF1 < 50% from basic mean) – gram-negative microflora (*H. influenzae, M. catharralis*, *P. aeruginosa* etc.) are common.

It is important to know that you can also find bacteria in expectorations of patients with a stable course. Investigations of protected bronchial biopsies of the lower respiratory tract show a colonization by microorganisms in approx. 30% of patients.

The mechanism through which microorganisms cause COPD exacerbations has not been well studied and remains unclear. It is well known that bacteria intensify the inflammation process in the respiratory tract in COPD exacerbations. This inflammation changes the local environment

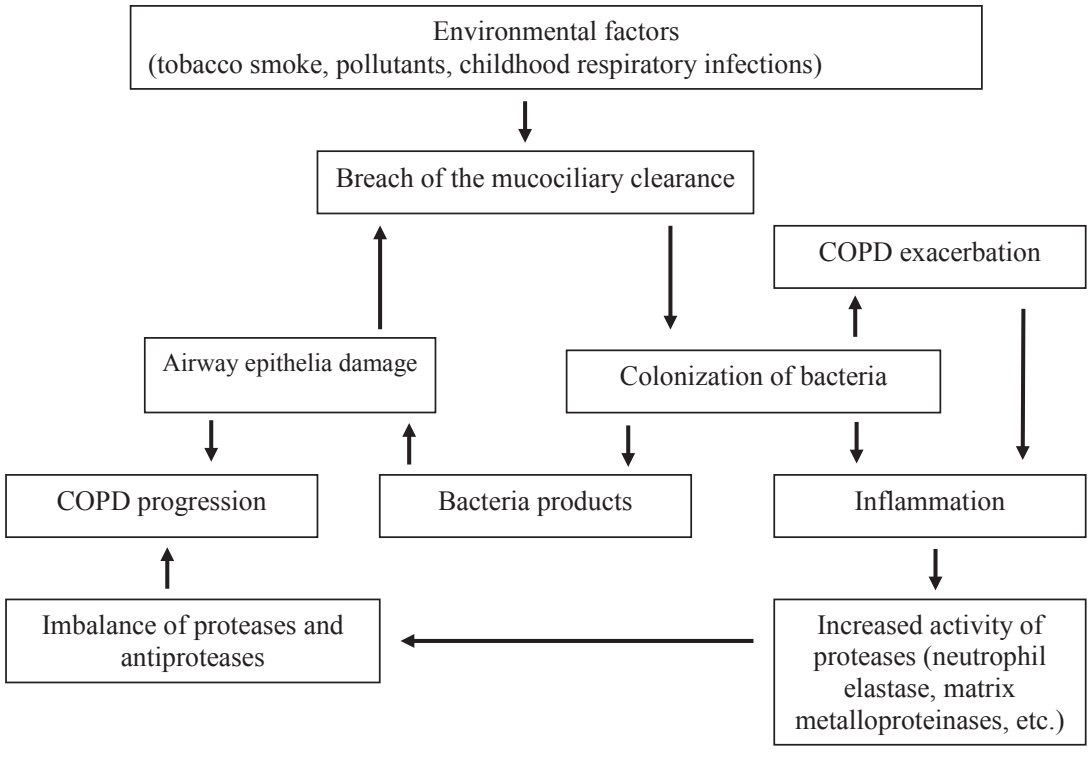



**Fig. 1. The role of microorganisms in evolution of COPD ("vicious circle" conception).**

**Table 2**

**Characteristics of exacerbations of COPD**

| Indicators | The severity of exacerbation | | |
|---|---|---|---|
| | Mild (Grade I) | Moderate (level II) | Severe (Grade III) |
| *History* | | | |
| Associated diseases | + | +++ | +++ |
| Frequent exacerbations | + | +++ | +++ |
| Severity of COPD | Easy / moderate | Moderately severe / severe | Severe / very severe |
| *Physical data* | | | |
| Hemodynamics | Stable | Stable | Stable / unstable |
| Involvement of respiratory muscles, tachypnea | No | ++ | +++ |
| Persistence of symptoms after treatment | No | ++ | +++ |
| *Diagnostic tests* | | | |
| Evaluation of blood oxygen saturation | Yes | Yes | Yes |
| The study of blood gases * | No | Yes | Yes |
| Chest X-ray | No | Yes | Yes |
| Clinical and biochemical blood tests ** | No | Yes | Yes |
| Gram-stained smears and bacteriological analysis of sputum | No**** | Yes | Yes |
| ECG | No | Yes | Yes |
| Measurement of drug concentration in blood serum | If possible | If possible | If possible |

**Note**: + is unlikely: + +probably, + + + highly likely; # diseases and syndromes associated with poor prognosis in exacerbations of COPD: Congestive heart failure, ischemic heart disease, diabetes, renal and hepatic failure, * including the values of PaO2, pH and PaSO2; **Biochemical analysis of blood include the determination of electrolytes and indicators of liver and kidney function; *** if the patient receives theophylline, warfarin, carbamezapine, digoxin; **** if the patient had recently received antibiotics.

in the airways and increases susceptibility to formation of new colonies of microorganisms that colonize the bronchial mucosa (*H. influenzae*) [6, 7, 8]. The role of microorganisms in the evolution of COPD is described by the hypothesis of "vicious circle", which says that the damage of airways is the result of chronic infection. Meanwhile, chronic presence of pro-inflammatory mediators (interleukin 6, 8, TNF, leucotryen B4) decreases the efficiency of bronchial transit (fig.1).

Based on the recommendations of the experts of European and American Thorax Society, we distinguish mild, moderate and severe stages in the courses of COPD (tab. 2).

The treatment of exacerbations is as follows: broncholytics, systemic corticoids, antibiotics.

Antibacterial treatment is indicated in cases of clinical manifestations of exacerbation [5, 9]. Sputum analysis for the etiological factor isn't specific because the respiratory tract of such patients is often colonized by bacteria. Sputum analysis is recommended in cases of frequent exacerbations or in the presence of purulent expectoration when organisms resistant to traditional treatment are suspected.

The most common criteria for indication of antibacterial therapy were evaluated by Anthonisen et al. (1987) which describes three types of COPD exacerbations. The first type is characterized by intensification of dyspnea, increase in produced sputum volume and a change in the sputum characteristic, such as a purulent appearance. The second type includes two of these symptoms, the third – one. It is established that the antibacterial treatment is indicated in the first and the second types of COPD exacerbations.

**The characteristics of COPD exacerbations**

Scientifically proven advantages of antibacterial therapy have been described in the research literature:

-Decrease in the duration of an exacerbation episode

-Avoidance of hospitalization.

-Decrease in duration of temporary disability.

-Prophylaxis for pneumonia.

-Avoid progression of damage to the respiratory tract.

-Increase of remission duration.

The aim of antibacterial therapy is eradication of microorganisms, which provoke COPD exacerbations, thereby decreasing symptoms manifestation and increasing the stable course of the disease. The choice of drugs is made empirically, based on the particular course of a patient's disease as well as on the known resistance profiles of specific organisms. Different options of antibacterial therapy are showed in tab. 3.

In case of non-severe COPD exacerbations it is necessary to consider macrolides (Clarithromycin and Azythromycin) and beta-lactam antibiotics (Amoxycillin and Cephalosporines of the second and the third generation). Macrolides are very active towards *H. influenzae, Str. Pneumonia* and intracellular microorganisms *(C. pneumoniae, M. pneumoniae)*. They have a high biological accessibility, deep penetration into lung tissue and into individual cells where they reach a high intracellular concentration. Clinical and bacterial efficacy of macrolides in COPD exacerbation is about 78-98% [9]. We previously obtained data which prove a high efficacy of azithromycin in treatment of COPD exacerbation. The results of our studies showed that therapy with azithromycin contributes to decrease in respiratory symptoms: cough decreased by 3 times ($p < 0.001$), quantity of expectoration – by 1.5 times ($p < 0.001$), and dyspnea – by 2 times ($p < 0.001$), while night-time symptoms disappeared almost completely. The regression of basic clinical manifestations is the common cumulative index which decreased by 3.2 times ($p < 0.001$). In patients who took standard therapy, the respiratory symptoms also decreased, but less significantly than in the experimental group. After a course of azithromycin therapy, cytosis indicators in induced expectoration decreased by 1.7 times, alveolar macrophages

Table 3

**Variants of exacerbations of COPD and the choice of antibiotic therapy**

| Variants of exacerbations | Patient Characteristics | Current microorganism | A series Antibacterials | Alternative treatment |
|---|---|---|---|---|
| 1. "Simple" exacerbations (without risk factors for microbial resistance to antibiotics) | Worsening of dyspnea and cough, increased sputum production with a purulent aspect. Any age, less than 4 exacerbations / year, the absence of concomitant diseases, FEV1 > 50% predicted | *Haemophilus influenzae, Haemophilus parainfluenzas, Moraxella catarrhalis, Streptococcus pneumoniae* | „New» macrolides (clarithromycin, azithromycin), 2nd and 3rd generations of cephalosporins, amoxicillin | Amoxicillin / clavulanic acid Respiratory fluoroquinolones |
| 2.»Complicated» exacerbation (in the presence of risk factors for microbial resistance to antibiotics) | Symptoms of acute infection and one of the following criteria: age ≥ 65, more than 4 exacerbations / year, concomitant diseases of the cardiovascular system, FEF1 35-50% of predicted, the use of «home» oxygen therapy, antibiotics in the last 3 months. | *Haemophilus influenzae, Haemophilus parainfluenzas, Moraxella catarrhalis, Streptococcus pneumoniae, Klebsiella spp*, other bacteria gram-negative. High probability of microbial resistance to β-lactams | Respiratory fluoroquinolones (levofloxacin, moxifloxacin, and others), amoxicillin / clavulanic acid | Possible parenteral antibiotics and hospitalization of patients |
| 3. Chronic purulent bronchitis (in the presence of risk factors for infection (*Ps. aeruginosae*) | More than 4 exacerbations / year, FEV1< 35% predicted, possible bronchiectasis | Same microorganisms that are in group 2, *Pseudomonas aeruginosae*, multidrug resistant *Enterobacteriaceae* | Fluoroquinolones (ciprofloxacin), β-lactams (ceftazidime, and other means piperacillin/tazobactam with activity against Ps. aeruginosae) | - |

decreased by 1.2 times. The level of IL-1α decreased on the 14-th day of treatment by 4.8 times, TNFα - by 4.5 times. IL-8 in blood decreased by 2.5 times.

Patients who received standard treament without antibiotics also had a decrease in systemic inflammatory markers, however this was less significant as compared to the group that received antibiotics.

Probably the treatment effect with macrolides is determined by it's non-antimicrobial activity. It is known these can modulate the activity of lymphocytes, modify the properties of trachea-bronchial secretions and decrease the intensity of systemic and local respiratory tract inflammation by changing the functional activity of neutrophils.

In recent years, fluoroquinolones with their antipneumococcus activity (levofloxacin, moxifloxacin etc.) have been found to be comparable with macrolides in the treatment of respiratory infections. As proof of the advantages of fluoroquinolones are used arguments such as: a) a high resistance level of *S. pneumoniae* and *H. influenzae* towards the macrolides which respectively corresponds to 30-50% and 35% improvement in different countries; b) a better response in clinical symptoms and bacteriological eradication, a lower relapse rate of COPD exacerbations and decreased long-term need for antibacterial treatment, although the letter has not been consistently supported in all studies.

Analyzing the mentioned results it is important to keep in mind that the local resistance of macrolides towards S. *pneumoniae* amounts to 2 - 6%. The resistance level of macrolides to *H. Influenza* is probably also low. This fact has been proven by a high clinical efficacy of clarithromycin observed in patients with COPD [1, 2, 4, 7]. Most of the research shows the efficacy of respiratory fluoroquinolones, regardless of the stage of COPD. It is also important to note that higher results in treating severe courses of the disease were obtained when macrolides were not used as first line treatment (tab. 3).

Respiratory fluoroquinolones and protected penicillins are indicated in "complicated" exacerbations of COPD. These are indicated when there is presence of risk factors such as resistance of the microorganisms towards amoxicillin or macrolides. If there exists a high risk for *P. Aeruginosae* we recommend use of ciprofloxacin and beta-lactams given their activity against nosocomial pathogens (tab. 3). In most cases antibiotics are taken orally. Duration of antibacterial therapy in "non – complicated" exacerbations is 5 – 7 days, in "complicated" cases – 10 – 14 days until the complete disappearance of clinical symptoms of exacerbation [5, 9].

The dosage regimen of broncholytics is given in tables 3 and 4. In case of mild and severe exacerbations of COPD, especially in older patients, it is necessary to administer nebulizer therapy.

Because of the difficulties in dosage and the many side effects of theophylline, we suggest using these drugs as secondary treatment when the inhaled broncholytics aren't effective enough. However, no everyone agrees with this point of view. Probably the use of these drugs is possible only by respecting the indication rules and monitoring the concentration of theophylline in the blood. The best known of this class is Euphyllin which contains theophylline (80%) dissolved in ethylenediamine (20%). The schedule of its dosage is shown in tab. 4. It is important to mention that theophyllin has to be introduced only intravenously. Theophylline administration over a long period of ime is contraindicated because there is a danger of overdose.

Systemic glucocorticoids are effective in treatment of complications of COPD. These drugs decrease the convalescence time and contribute to a more rapid regeneration of lung functions. They are indicated together with broncho-

lytics when FEV1 is < 50% from normal values. Normally it is recommended 30 – 40 mg prednisone by mouth or the equivalent intravenous methylprendisolone dose for 10 – 14 days. Treatment for a longer period of time doesn't lead to a higher effectiveness but increases the risk of development of side effects. In the last few years' data appeared about the possibility of using inhalational glucocorticoids (Budesonide, introduced with nebulizers, as an alternative to systemic glucocorticoids in the treatment of COPD exacerbations.

**Table 4**

**Dosage of Euphyllin by intravenous introduction**

| Particularities of introduction | Doses |
|---|---|
| Loading dose (*intravenously* infused over 20 min):<br>For patients who didn't get theophylline<br>For patients who got theophylline | 240-250 mg<br>Introduction is contraindicated |
| Maintenance dose<br>(*intravenously infused over* 3 – 5 h)<br>Smokers<br>Nonsmokers<br>Patients with a low theophylline clearance | <br><br>0.9 mg/kg/h<br>0.6 mg/kg/h<br>0.25 mg/kg/h |
| Daily doses of theophylline | 0.75-1.5 g |

Patients with light exacerbations can be treated in an ambulatory setting. Patients with moderate or severe levels of COPD have to be hospitalized. Indications for directing the patients to specialized departments are as follows:

1. Significant increase in symptom intensity (e.g. appearance of dyspnea in rest).
2. Conventional treatment is not effective.
3. Appearance of new symptoms (eg, cyanosis, peripheral edema).
4. Severe concomitant diseases (pneumonia, cardiac arrhythmia, congestive heart failure, diabetes, renal and kidney insufficiency).
5. New onset of abnormal heart rhythm.
6. Older age.
7. Inability to provide adequate medical care in an outpatient setting.
8. Difficulty of diagnosis.

Algorithms for treatment of exacerbations of COPD are shown in figures 2-4.

In severe exacerbations of COPD patients have to be hospitalized in an intensive care unit, the indication for which are:

1. Severe shortness of breath, not releaved by bronchodilators.
2. Impaired consciousness, coma.
3. Progressive hypoxemia ($PaO_2 < 50$ mm Hg), hypercapnia ($PaCO_2 > 60$ mm Hg) and/or respiratory acidosis (pH < 7.25), despite the use of oxygen-therapy and noninvasive ventilation.

Criteria for patient discharge from hospital after a COPD exacerbation:

a) requirement for inhaled short-acting adreno-agonists does not exceed more than every 4 hours.
b) the patient can ambulate on their own, eat and sleep without frequent nighttime awakenings from shortness of breath;
c) stable state for 12-24 hours;
d) stable blood gas analysis for 12-24 hours;
e) patient and responsible family members have a complete understanding of proper medication use;
f) there are arrangements for further observation and treatment at home.

In the next 4-6 weeks the patient should be re-examined by a doctor to evaluate adaptation to life and correct inhalation technique, as well as analysis of pulmonary function tests (PFT), blood gas or oxygen saturation (to decide if a long-term oxygen therapy is necessary). Further treatment may be indicated at this time.

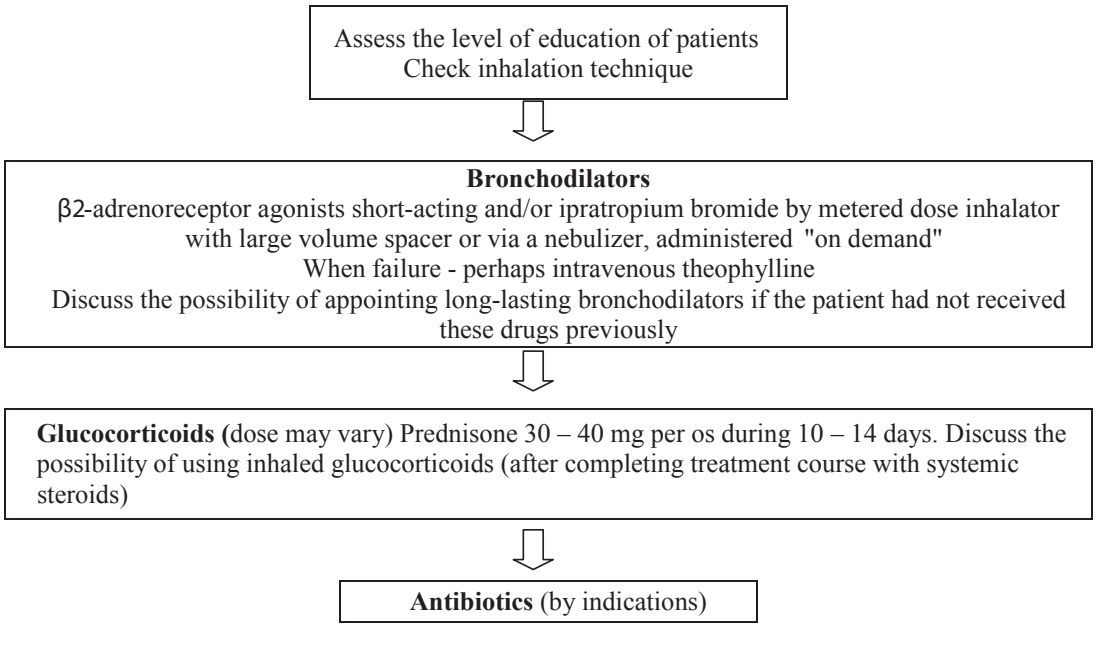
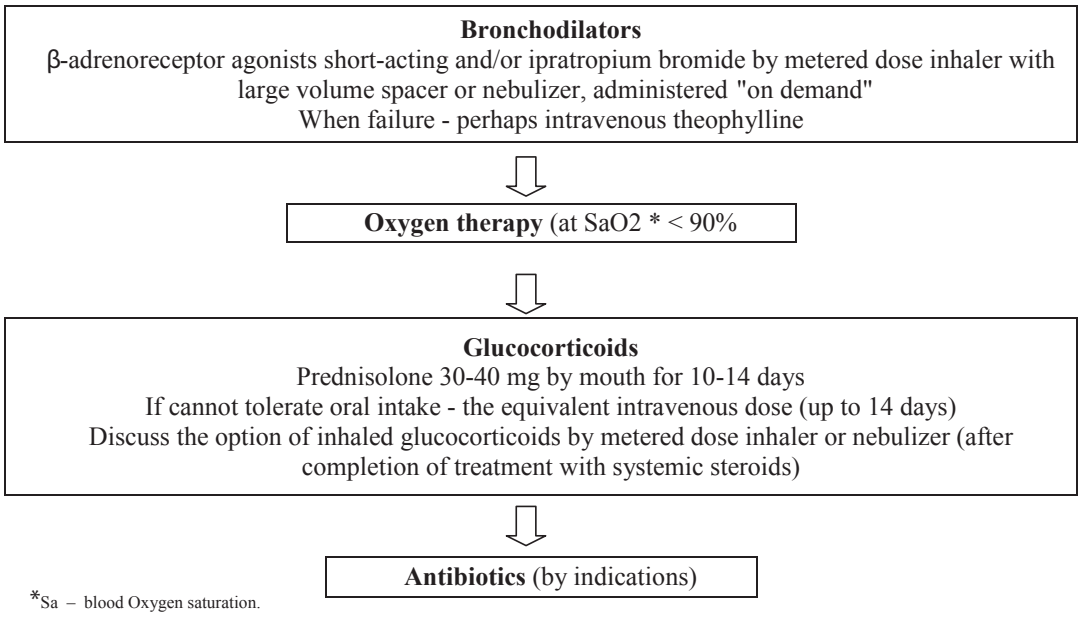



**Fig. 2. Ambulatory treatment of patients with mild exacerbations.**




*Sa – blood Oxygen saturation.

**Fig. 3. Treatment of moderate exacerbations of COPD in hospitalized patients.**

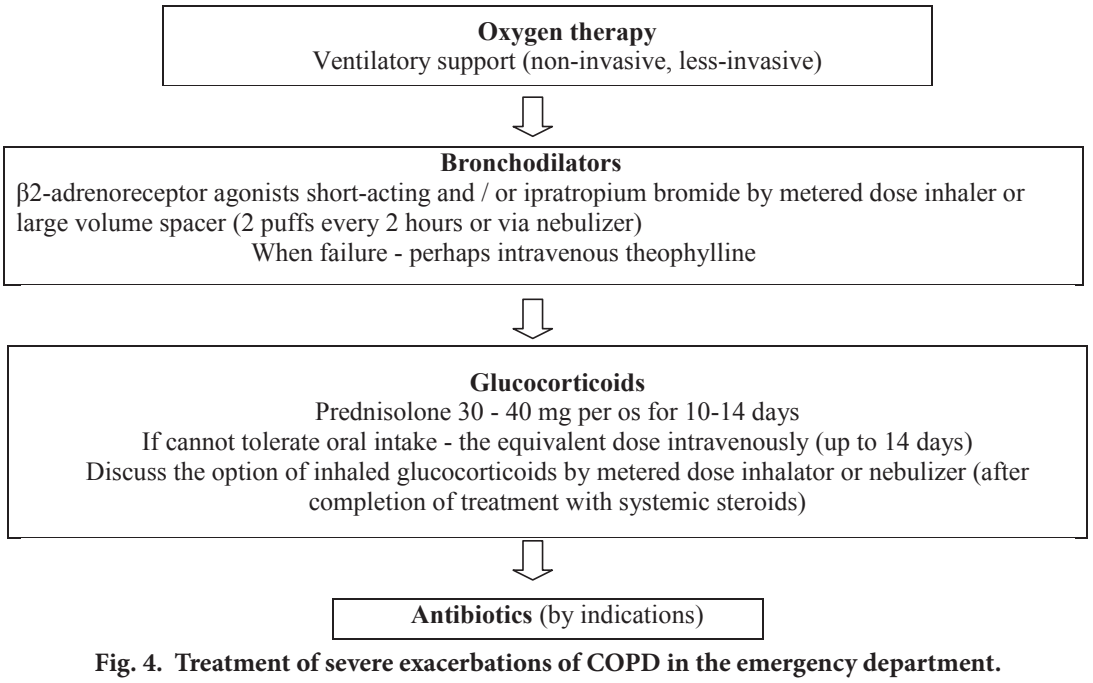



**Fig. 4. Treatment of severe exacerbations of COPD in the emergency department.**

**Table 5**

**Inhalative broncholytics used for COPD exacerbation treatment**

| Drug | Release form | Doses |
|---|---|---|
| Salbutamol (Ventholyn nebulets, Salgym, Steri-neb-Salamolol etc.) | Solution for nebulizers 2.5 and 5 mg/ml<br>Dosed aerosol with spacer (100 mcg/doses) | 2.5-5 mg every 4-6 h in regime «on demand»<br>2-4 inhalations every 4-6 h in regime «on demand» |
| Fenoterol (Berotec and Berotec H) | Solution for nebulizers 1 mg/ml<br>Dosed inhaler with spacer (100 mcg/doses) | 0.5-1.0 mg every 4-6 h in regime «on demand»<br>2-4 inhalations every 4-6 h in regime «on demand» |
| Ipratropium bromide (Atrovent, Atrovent H) | Solution for nebulizers 0.25 mg/ml<br>Dosed inhaler with spacer (40 mcg/doses) | 0.25-0.5 mg every 6-8 h in regime «on demand»<br>2-4 inhalations every 6-8h in regime «on demand» |
| Ipratropium bromide and Fenoterol (Berodual and Berodual H) | Solution for nebulizers (in 1ml 0.25 mg ipratropium bromide and 0.5 mg fenoterol)<br>Dosed inhaler (in 1 inhaler. 20 mcg ipratropium bromide and 50mcg fenoterol) with spacer | 2-4 mg every 6-8 h in regime «on demand»<br>2-4 inhalations every 6-8 h in regime «on demand» |

For the prevention of exacerbations bronchodilators and inhaled glucocorticoids are used in combination with long-acting β2-adrenomimetics (severe and very severe COPD). Yearly influenza vaccination is highly recommended.

In conclusion, it should be noted that if the antibiotic therapy is chosen correctly for the individual, it reduces the duration of hospital stay and the costs associated with medical care.

### References


1. Aaron SD. Vandemheen KL, Fergusson D, et al. Tiotropium in combination with placebo, salmeterol, or fluticasone- salmeterol for treatment of chronic obstructive pulmonary disease a randomized trial. *Ann. Intern. Med*. 2007;146:545-555.
2. Anzueto AR. Clinical course of chronic obstructive pulmonary disease: review of therapeutic interventions. *Am. J. Med.* 2006;119(10)Suppl 1:46-53.
3. Balanag VM, Yunus F, Yang PC, et al. Efficacy and safety of budesonide/formoterol compared with salbutamol in the treatment of acute asthma. *Pulm. Pharmacol. Ther*. 2006;19(2):139–147.
4. Barr RG, Bourbeau J, Camargo CA, et al. Tiotropium for stable chronic obstructive pulmonary disease: A meta- analysis. *Thorax*. 2006;61:854-862.
5. Blasi F, Tarsia P, Aliberti S, et al. Highlights on the appropriate use of fluoroquinolones in respiratory tract infections. *Pulm. Pharmacol. Ther.* 2006; 19(suppl.1):11-19.
6. Buist AS, Mcburnie MA, Vollmes WM, et al. International variation in the prevalence of COPD (the BOLD Study): a population – based prevalence study. *Lancet*. 2007;370:741-750.
7. Хроническая обструктивная болезнь легких. Федеральная программа (издание второе, переработанное и дополненное) / Под ред. акад. РАМН, профессора А. Г. Чучалина. М, 2004;61С.


# The Modification of the Serum Ascites Lymphatic Albumin Gradient in Liver Cirrhotic Decompensated Patients with Ascitic Syndrome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### Abstract

The study involved 23 patients with decompensated liver cirrhosis and ascitic syndrome. This trial was designed to determine the value of serum-ascites and lymphatic albumin gradients. We established diminution of the albumin ascites-lymphatic gradient in relationship with the evolution of the ascitic syndrome. We introduce the notion of serum/ascites/lymphatic albumin gradient and its significance on parameters of the evolution of the ascitic syndrome. It is required to validate the clinical application of the gradient in following studies.


**Key words:** ascitic syndrome, serum ascitic limphatic albumin gradient.


### Изменения альбуминового градиента (асцит, плазма, лимфа) у декомпенсированных больных циррозом печени и асцитическим синдромом

В настоящую работу включены 23 больных с декомпенсированным циррозом печени и асцитическим синдромом. Исследование было посвящено изучению и оценке альбуминового градиента (асцит, плазма, лимфа). Установлено уменьшение альбуминового градиента асцит-лимфа в соотношении с развитием асцитического синдрома. Впервые предложена терминология альбуминового градиента (асцит, плазма, лимфа), однако его значение, как критерия развития асцитического синдрома, требует уточнения в дальнейших исследованиях.


**Ключевые слова:** асцитический синдром, градиент концентрации альбумина сыворотка-асцит.

### Introduction

According to literature data, 4-5% of the global population shows disturbances of liver functions, of which about 10-20% are caused by viral hepatitis, toxic hepatitis or ethanol alcohol, that within 10-20 years lead to the evolution of cirrhosis liver [1], which inevitably leads to a high rate of complications such as variceal hemorrhage either cirrhogenous hypersplenism with coagulopathy in progress or ascitic syndrome. Thus, N. Fisher et al. notes that in Great Britain alone, during the years 1993-2000,

the mortality from complications of liver cirrhosis doubled from 6 x 105 in 1993 to 12.7 x 105 in 2000 [3].

In this context the problem of diagnosis, prophylaxis, and treatment of complications of cirrhotic portal hypertension is current and remains the focus of the medical community.

Ascitic syndrome, and especially in its advanced forms (with lack of response to diuretic therapy) is one of the most serious complications of liver cirrhosis, which according to different authors evolves in 50 percent of cases within 10 years of diagnosis of hepatic cirrhosis. More than that, statistics of randomized trials denote a rate of mortality within the limits of 60-70% of cases of these patients in terms of 24-36 months from the onset of resistant and refractory ascites (called in some publications as intractable ascites) [3, 4], which in our view is debatable.

Besides a reduced quality of life, in cases of medically or surgically uncontrolled evolution of ascitic syndrome, patients are at high risk of appearance of hepato-renal failure, due to the fact that in general, hepato-renal failure has 5-year incidence rate of 18-39% in cirrhotic patients primarily diagnosed, the prognosis being extremely poor [5-8], and as a "golden" therapeutic option only liver transplantation is being considered [9], which currently can not fully solve the problem because of the small number of organ donations.

Meanwhile, another major complication in advanced cirrhogenous ascites is ascites fluid infection. The development of spontaneous bacterial peritonitis by intestinal flora translocation and bacteremia in the lymph nodes in turn contributes to deterioration of liver and kidney functions [6].

Thus, future research on the assessment of prognostic factors triggering spontaneous bacterial ascites-peritonitis, without the need for sophisticated laboratory tests, is reasonable and also useful to medical practitioners.

Over the past decades in order to differentiate and diagnose the etiology of ascites (portal hypertension, peritoneal carcinomatosis, tuberculous peritonitis, secondary bacterial or neoplastic), using concepts of exudative vs transudative properties, we studied the total protein concentration in ascitic fluid and serum. Conventionally, it was established that neoplastic ascites ("exudative theory") have a characteristic protein index of > 25 g/l, while in portal hypertension ("transsudative conception") the value of protein concentration is < 25 g/l.

Later, a new biochemical criterion was proposed and namely - assessing the difference in serum albumin concentration and ascitic fluid, or the so-called gradient album serum/ascites (GASA), which proved to be a higher sensitivity compared to determination of the total concentration of proteins in ascitic and serum fluid [12]. Simultaneously, investigations indicate that the value (GASA) > 1.1 g/dl denotes ascitic syndrome in portal hypertension with an accuracy of 97% [12] while the value of the named gradient below 1.1 g/dl, is characteristic to "non-portal hypertension etiology" [13, 14, 15].

Also, no research literature exists regarding the role of the serum-ascites-lymph albumin gradient in evaluating the severity of cirrhotic syndrome.

Aim of the study - the analysis of albumin concentration, serum and lymph in decompensated cirrhotic patient with ascitic syndrome, the serum-ascitic albumin gradient analysis, lymph and its variations depending on the stage of ascites and hepatic functional reserve.

## Material and methods

This study included a prospective analysis of changes in the serum-ascitic-lymphatic albumin gradient in 23 cirrhotic patients with advanced cirrhosis with ascites (13 - resistant, 10 - refractory), surgically treated in the Surgery Clinic "Sfinta Treime" ("Holy Trinity") in the period 2008 - 2010.

The study group included 11 men and 12 women ranging in age limits of 45-58 years, etiology of liver cirrhosis was caused by viral hepatitis (HBV, HBV + HBV-HDV and HCV in 7, 6 and 10 cases respectively, confirmed by immunoserological investigations. Average Child-Pugh score was 10.4 ± 1.28 points. Patients were separated into previously described stages of ascites syndrome in accordance to criteria for classification of Ascites International Club (International Club of Ascites). We mention the fact that the patients with activation of cirrhotic process were not included in the research group. Additionally, perfusion conservative treatment with administration of albumin, plasma, etc. in terms of the previous three months was considered as a criterion of exclusion from the study.

The surgical treatment included the cervical decompression of the thoracic lymph duct, which in 6 cases was associated with paracentesis decompression, performed in patients with tense ascites and cardiopulmonary disturbances.

In order to standardize the research the biological substrate was investigated (serum, lymph and ascitic fluid) and was collected only intraoperative under sterile conditions, being collected 5.0 ml of serum and ascitic fluid obtained by paracentesis. Intraoperatively the thoracic lymph duct was punctured with SECALON catheter (Becton Dickinson Critical Care Systems, USA) and the lymph fluid was collected.

The collected biological fluids were subjected to laboratory research with clinical and general bacteriological proteinogram assessment, and especially the measurement of albumin concentration. A general characteristic of patients included in the study is summarized in tab. 1.

**Table 1**

**Structure of the study group (n = 23)**

| Ascites | Men / Women | Average age | Child Average Score |
|---|---|---|---|
| Resistant | 7/6 | 42.6 ± 2.3 | 9.8 ± 0.35 |
| Refractory | 7/3 | 48.1 ± 2.5 | 10.6 ± 0.45 |
| Total | 14/9 | 45.8 ± 2.1 | 10.2 ± 0.15 |

## Obtained results

The results of research conducted on a group of 23 patients with hepatic decompensate cirrhosis were estimated and analyzed according to the severity of the ascitic syndrome (resistant / refractory). It was found that the albumin concentration in serum, lymph and ascites did not differ statistically

or significantly in those groups. Serum albumin concentration in resistant as compared to refractory ascites was 2.65 ± 0.78 (gm/dl) and 2.45 ± 0.89 respectively. The corresponding lymph and ascites albumin concentrations in resistant ascites were 2.15 ± 0.88 and 1.01 ± 0.98 respectively, while in refractory ascites they were 1.87 ± 0.76 and 0.93 ± 0.66, p > 0.05 (tab. 2).

**Table 2**

**Profile of serum albumin, ascites and lymph to patients included in the study group (n = 23)**

| Index | Resistant ascites | Refractory ascites | p |
|---|---|---|---|
| Serum albumin concentration (gm/dl) | 2.65 ± 0.78 | 2.45 ± 0.89 | > 0.05 |
| Lymph albumin concentration (gm/dl) | 2.15 ± 0.88 | 1.87 ± 0.76 | > 0.05 |
| Ascites albumin concentration (gm/dl) | 1.01 ± 0.98 | 0.93 ± 0.66 | > 0.05 |

We conclude that in this study the quantitative assessment of albumin concentration in serum, ascitic fluid and lymph was not predictive of the development of complicated cirrhogenous ascites in patients with decompensated cirrhosis.

Simultaneously, in both study groups, the albumin gradient of serum-ascites included higher values of 1.1 (gm/dl) in 100% cases confirming the value of this parameter as a characteristic sign of portal hypertension, justifying in this sense, the "transsudative " hypothesis of differential diagnosis of ascites [12, 17, 20].

The analysis of albumin gradients in resistant and refractory ascites was, respectively 0.48 ± 0.09 and 0.51 ± 0.11 gm/dl for serum-lymph gradient, and 1.05 ± 0.20 and 0.89 ± 0.07, for lymph-ascites gradient. Also we found, that ascites-lymph albumin gradient decreases during the evolution of lymph-ascitic syndrome, with a tendency to decrease with the progression of advanced forms, although these results were non-significant (p > 0.05)

**Table 3**

**Changes in albumin gradient in the study group (n = 23)**

| Index | Resistant ascites | Refractory ascites | p |
|---|---|---|---|
| Albumin gradient Ser - ascites (gm/dl) | 1.56 ± 0.64 | 1.49 ± 0.75 | > 0.05 |
| Albumin gradient Ser - Lymph (gm/dl) | 0.48 ± 0.09 | 0.51 ± 0.11 | > 0.05 |
| Albumin gradient Lymph-ascites (gm/dl) | 1.05 ± 0.20 | 0.89 ± 0.07 | > 0.05 |
| Albumin gradient Ser/ascites/Lymph * | -0.40 ± 0.05 | -0.90 ± 0.10 | < 0.01 |

* Negative values of the gradient

On further analysis of our date, we found that the albumin gradient in ser/ascites/lymph proved to be more sensitive in differentiating resistant vs refractory ascites as compared to the analysis of albumin concentration in each of the biological fluids analyzed separately or in pairs. This can be explained by the multiplicity and complexity of peritoneal absorption mechanisms, the phenomenon of "washing" of albumin in ascitic fluid, described by JH Henriksen [16]. In this study we noted a statistically significant difference in the groups investigated for this index, namely a serum/ascites/lymph albumin gradient of - 0.40 ± 0.05 in the resistant ascites group vs - 0.90 ± 0.10 in the refractory ascites group (p < 0.01).

Table 3 summarizes research data of albumin gradients.

## Discussions

The evolution of ascitic syndrome with advancing complications represents a difficult clinical problem, both medically and surgically [17]. Also, many randomized trials have evaluated various laboratory parameters of ascitic fluid, which would allow the differentiation of etiology, such as: proteinogram, cytology, albumin gradient serum/ascites, measurement of lactate hydrogenase concentration, amylase, adenosine deaminase, glucose, fibronectin level [18, 19].

It is known that, in peritoneal fluid of healthy individuals the physiological concentration of proteins exceeds the value of 4 g/dl, while in ascites, serum protein concentrations decrease to below 2.5 g/dl. This traditional concept is also debatable because patients with ascites are usually treated conservatively with diuretics and infusions of plasma or albumin, leading to increased protein concentration in ascitic fluid and thus decreasing the sensitivity of a low serum protein measurement [20].

Therefore a new criterion-gradient of album serum/ascites was proposed, which proved to have a higher sensitivity compared to determination of total protein concentration in ascitic fluid and serum [12]. This gradient is based not on a simple assessment of albumin concentration in the above-mentioned fluids, but serves as a reflection of portal pressure, being derived on the basis of oncotic and hydrostatic balance.

Thus, the research shows a direct connection between the serum/ascites albumin gradient and the degree of portal hypertension. This ratio is clearly superior compared to the overall concentration of proteins in serum and ascites [22, 23]. More than that, some authors recorded a direct connection between the value of portal pressure gradient GASA, as well as its complications, gastro-esophageal varices and ascitic syndrome [24, 26]. However, in this study we were unable to confirm a significant serum-ascites albumin gradient difference between patients with resistant and refractory ascites.

Investigations of lymph fluid components were started in the 60s, with achievements in clinical and experimental studies by several of the well-known men of science who were also the founders of this field, A. Dumont, 1960, H. Mayerson, 1963, M. Orloff, 1966, C. Witte, 1968 [27-29].

Meanwhile, in spite of a long period of time, available literature does not show any scientific works towards protein variations, especially albumin in serum, ascites and lymph appreciation of these gradients. This can be explained by deficiencies in obtaining lymph from lymph central collector (thoracic duct), as well as a relatively small number of men of science specialized in the field.

### Conclusions

Quantitative assessment of albumin concentration in serum, ascitic fluid and lymph is not an index revealing the prognosis in terms of evolution of ascites to patients with cirrhogenous decompensated cirrhosis. This study found that serum-ascites albumin gradient does not reveal a significant difference in the case of resistant and refractory ascites. Ascites albumin gradient decreases during the evolution of lymph and ascitic syndrome and has a tendency to decrease with the progression of advanced forms of ascitic syndrome. Implementation of the notion of serum-ascites-lymph albumin gradient and its significance as a criterion of evolution for ascitic syndrome requires further study and validation.

### References

1. Fisher NC, Hanson J, Phillips A, et al. Mortality from liver disease in the West Midlands, 1993–2000: observational study. *BMJ.* 2002;325:312–313.
2. Ginès P, Fernández-Esparrach G. Prognosis of cirrhosis with ascites. In: Arroyo V, Ginès P, Rodés J, Schrier RW, eds. Ascites and renal dysfunction in liver disease: pathogenesis, diagnosis, and treatment. Malden:Mass.: Blackwell Science, 1999:431-441.
3. Anderson RN. Deaths: leading causes for 2000. *National vital statistics reports.* 2002;50(16):1120-1127.
4. Menon K, Kamath P. Managing the complications of cirrhosis. *May Clin. Proc*. 2000;75(5):501-509.
5. Bosch J, Abraldes JG, Groszmann R. Current management of portal hypertension. *J Hepatol.* 2003;38(Suppl 1):S54-S68.
6. Rimola A, Garcia-Tsao G, Navasa M, et al. Diagnosis, treatment and prophylaxis of spontaneous bacterial peritonitis: a consensus document. *J Hepatol.* 2000;32:142-153.
7. Runyon BA, Montano AA, Akriviadis EA, et al. The serum-ascites albumin gradient is superior to the exudate-transudate concept in the differential diagnosis of ascites. *Ann Intern Med.* 1992;117(3):215-220.
8. Akriviadis EA, Kapnias D, Hadjigavriel M, et al. Serum/ascites albumin gradient: its value as a rational approach to the differential diagnosis of ascites. *Scand J Gastroenterol.* 1996;31(8):814-817.
9. Zhu XH, Liu B, Cheng ZY. Diagnostic value of serum-ascites albumin gradient. *Hunan Yi Ke Da Xue Xue Bao.* 2003;28(3):278-280.
10. Henriksen JH, Parving HH, Christiansen HH, et al. The effect of ascitic fluid hydrostatic pressure on albumin extravasation rate in patients with cirrhosis of the liver. *Scand J Clin Lab*. 1981;41:601-609.
11. Glickman Robert M, Casper D, Braunwald E, et al. In: Harrison's Principles of Internal Medicine. NewYork. Abdominal swelling and ascites., 2005;1:243-5.
12. Gupta R, Mishra SP, Dwivedi M, et al. Diagnosing ascites: Value of ascitic fluid total protein, albumin, cholesterol, their ratios,serum ascites albumin and cholesterol gradient. *Gastroenterol and Hepatol* 1995;10:295-9.
13. Podolsky DK, Isselbacher KJ. Cirrhosis and its complications. In: NewYork Mc Graw Hill publication. 2005;2:1858-68.
14. Sampliner RF, Iber FL. High protein ascites in patients with uncomplicated hepatic cirrhosis. *Am J Med Sci.* 1974;267:275-279.
15. Beg M, Husain S Ahmad, Akhtar N. Serum/Ascites Albumin Gradient in Differential Diagnosis of Ascites. *J Ind Ac Clin Med.* 2001;2(1):511-514.
16. Torres E, Barros P,Calmet F. Correlation between serum-ascites albumin concentration gradient and endoscopic parameters of portal hypertension. *Am J Gastroenter*. 1998;93:2172–2178.
17. Dumont AE, Mulholland JH. Flow rate and composition of thoracic duct lymph in patients with cirrhosis. *N Engl J Med*. 1960;263:471-478.
18. Mayerson HS. The physiologic importance of lymph. In: Handbook of Physiology, Sect.2: Circulation Vol.2, Edited by WF Hamilton. Am. Physiol. Soc., 1963;1035-1073.
19. Orloff MJ, Weight PW, DeBenedetti MJ. Experimental ascites. *Arch Surg.* 1966;93:119-129.
20. Witte CL, Witte MH, Dumont AE, et al. Lymph protein in hepatic cirrhosis and experimental hepatic and portal venous hypertension. *Trans Am Surg Assoc*. 1968;86:256-274.

# The Impact of Long Term Medication Ramipril Versus Eprosartane on Renal Function and on Microalbuminuria in Patients with Essential Arterial Hypertension

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### Abstract

This research presents the experience of the Arterial Hypertension Department in the treatment of patients with essential hypertension and microalbuminuria. The study focused on the analysis of clinical observation materials according to the protocol, established in a group of 100 patients, of whom 50 were treated with angiotensin II converting enzyme inhibitors Ramipril and 50 were treated with angiotensin II receptor antagonist Eprosartane. Both drugs have proven beneficial effect on renal function parameters, especially in microalbuminuria at all stages of control with a peak at the end of the follow-up period. However, the treatment with AT1-receptor antagonist Eprosartan has proven to be superior to angiotensin II in converting enzyme inhibitor Ramipril.

**Key words**: arterial hypertension, microalbuminuria, angiotensin II converting enzyme inhibitors, angiotensin II receptor antagonist.

## Влияние рамиприла и эпросартана на функциональное состояние почек у пациентов с гипертонической болезнью и микроальбуминурией при длительном лечении

В статье излагается собственный опыт лечения 100 пациентов с эссенциальной гипертонией и микроальбуминурией на протяжении 12 месяцев. В I-й группе (50 пациентов) принимали ингибитор ангиотензин-превращающего фермента Рамиприл. Во II-й группе (50 пациентов) принимали блокатор рецепторов ангиотензина II Эпросартан. Результаты исследования свидетельствуют о нефропротективной эффективности не только хорошо знакомого ингибитора ангиотензин-превращающего фермента Рамиприла, но и нового блокатора рецепторов ангиотензина II Эпросартан.

**Ключевые слова:** гипертоническая болезнь, микроальбуминурия, ингибитор ангиотензин-превращающего фермента, блокатор рецепторов ангиотензина II.

### Introduction

Arterial hypertension contributes to prognostic worsening depending on the level of subclinical organ damage and on the associated cardiovascular risk factors.

The most recent guideline on hypertension management, elaborated by the European Society of Hypertension in 2009, suggests that "based on recent trial evidence it is recommended to aggressively lower systolic and diastolic blood pressure to values of at least 140/90 mm Hg and lower if tolerated in all patients, and to values less than 130/80 mm Hg in diabetic patients considering the fact that frequently, especially in the elderly it could be difficult to reach values of systolic BP lower than 140 mm Hg" [1].

In general, lowering blood pressure treatment reduces the risk of stroke by 35-40%, for acute myocardial infarction (AMI) by 20-25%, chronic heart failure by 50% and chronic renal failure by 16-26% [2].

Arterial hypertension continues to be a serious issue in modern medicine and clinical trials have proven that through good control of BP values, the rate of cardiovascular events can be significantly reduced. The National Programs for detection and treatment of arterial hypertension have effectively led to the lowering of BP values, in the same time reducing the cardiovascular risk [3].

Chronic renal disease is a frequent complication of arterial hypertension and it favors the elevation of BP values through mechanisms due to renal dysfunction. Arterial hypertension associated with renal dysfunction usually presents great difficulties in treatment.

Kidney damage, even minor (microalbuminuria), has been confirmed as a major negative predictive factor in many diseases. In the mid '80s the first observations were made which associated increased urinary albumine excretion with amplification of cardiovascular morbidity and mortality, both in diabetic patients and hypertensive ones.

Since the first findings, microalbuminuria (MA) was frequently evaluated in big cardiovascular epidemiological trials, becoming also an essential parameter in modern clinical evaluation.

Microalbuminuria in patients with essential arterial hypertension represents a serious problem, associated either with the alteration of vascular hemodynamics or glomerular selectivity, or with the process of initial nephroangiosclerosis or activation of the sympatic nervous system.

Different authors report a microalbuminuria prevalence of 5-10% in the general population, 4.1-40% in patients with essential arterial hypertension and 16-40% in patients with diabetes mellitus. Generally, the presence of MA depends on age, race, body weight and values of blood pressure. It is detected more frequently in black individuals – 14.3%; in those aged < 60 years 6.2%; smokers -1.4%; hypertensive - 35%; obese – 3.6% [4].

Microalbuminuria represents a urinary albumine excretion of 30-300 mg/24 hours or nocturne of 20-200 μg/min. The diagnosis of renal damage induced by arterial hypertension is based on the proof of reduced renal function and /or on the detection of increased urinary albumine excretion.

In the mid '80s, the first trials showed a close relationship between increased urinary albumine excretion, cardiovascular morbidity and mortality both in diabetic and hypertensive patients.

Therefore, considering the importance of the problem, limited data in the specialty literature that would reflect the renal protection effects of a new angiotensin II receptor antagonist – Eprosartane, we have proposed the initiation of a prospective, randomized trial which would compare this drug to a well-studied angiotensin II - converting enzyme inhibitor – Ramipril.

### The aim of the study

The evaluation of the action of long-term antihypertensive medication of angiotensin II – converting enzyme inhibitor Ramipril versus angiotensin II receptor antagonist Eprosartane on the renal function and microalbuminuria.

### Material and methods

One hundred subjects were included in the trial (48 men, 52 women), mean age 51.1 ± 0.86 years with essential arterial hypertension of II-III grade and microalbuminuria, without associated clinical conditions. After registration and the primary visit the patients signed an informed consent form in order to participate in the trial. All antihypertensive drugs administered before have been suspended for a three week period. After the end of this period the patients came for the second visit to measure BP and to confirm the presence of BP values ≥ 160/90 mm Hg.

According to the study protocol the patients were divided randomly in two groups:

I group (50 patients) administered angiotensin II - converting enzyme inhibitor – Ramipril in the mean dose (15.3 ± 1.2 mg/day) + indapamide 2.5 mg/day.

II group (50 patients) administered angiotensin II receptor antagonist – Eprosartane in the mean dose (850 ± 12.4 mg/day) + indapamide 2.5 mg/day.

The renal excretory function has been evaluated through plasmatic urea, serum creatinine, glomerular filtration rate and microalbuminuria.

The patients were examined and treated in the clinic of Institute of Cardiology during 2007-2010. The observation period lasted 12 months with evaluations in dynamics at 3, 6, 9 and 12 months.

## Results

The determination of renal excretory function is essential for the diagnosis of renal failure. Also, exact knowledge of renal excretory capacity is indispensable for the determination of doses of drugs, in order to avoid potential accidents related to overdoses.

In initial stage the groups were comparable. In this way, serum urea varied in the range 2.7 - 19 mmol/l (mean 7.0 ± 0.45 mmol/l) in group I and 3.8 – 14.9 mmol/l (mean 5.8 ± 0.29 mmol/l) in group II, plasmatic creatinine varied in the range 50.1 – 296.7 mmol/l (mean 98.3 ± 5.34 mmol/l) in group I and, respectively, 50.1-271.4 mmol/l (mean 88.5 ± 4.11 mmol/l) in group II.

The glomerular filtration rate was in the range 67.3 – 115.3 ml/min (mean 87.2 ± 4.17ml/min) in group I and, respectively, 69.3 – 111.3 ml/min (mean 94.5 ± 3.34ml/min) in group II ($p < 0.05$).

**Table 1**

**Parameters of renal function in the initial stage, (M ± m)**

| Groups | PU (mmol/l) | SC (mmol/l) | GFR (ml/min) | MA (µg/min) | p |
|---|---|---|---|---|---|
| I - Ramipril | 7.0±0.45 | 98.3±5.34 | 87.2±4.17 | 73.1±5.52 | < 0.05 |
| II - Eprosartane | 6.8±0.29 | 88.5±4.11 | 94.5±3.39 | 68.1±4.16 | < 0.05 |

**Abbreviations**: PU – plasmatic urea; SC – serum creatinine; GFR – glomerular filtration rate; MA – microalbuminuria.

The microalbuminuria grade, which shows the presence of significant endothelial dysfunction, was also comparable in initial stage in both groups: in the range 35.7 – 98.6 µg/min (mean 73.1 ± 5.52 µg/min) in group I and, respectively, 40.3 – 93.1 µg/min (mean 68.1 ± 4.16 µg/min in group II $p < 0.05$) (tab. 1).

After 3 months of medication, plasmatic urea (mmol/l) was reduced by 11.4% (from 7.0 ± 0.45 to 6.2 ± 0.41) ($p > 0.05$) in group I and 20.5% (from 6.8 ± 0.29 to 5.4.± 0.18) ($p < 0.05$) in group II; after 6 months in group I by 14.2% ( from 7.0 ± 0.45 to 6.0 ± 0.27) ($p < 0.05$) versus 19.1% (from 6.8 ± 0.29 to 5.5 ± 0.16) ($p < 0.05$) in group II; after 9 months by 21.4% (from 7.0 ± 0.45 to 5.5 ± 0.2) ($p > 0.05$) in group I and 23.5% (from 6.8 ± 0.29 to 5.2 ± 2.2) ($p > 0.05$) in group II and after 12 months of observation by 15.7% ($p < 0.05$) and 26.4% ($p < 0.05$) respectively (fig. 1).

Serum creatinine (mmol/l) after 3 months of medication reduced nonsignificantly by 2% (from 98.3 ± 5.3 to 96.3 ± 6.5) in patients receiving Ramipril and statisticaly authentic – by 15.1% (from 96.5 ± 4.1 to 81.9 ± 2.2) ($p < 0.05$) using Eprosartane.

This trend became statistically authentic from 6 months of medication on, but more evident with the administration of Eprosartane, the peak being reached to the end of 12 months of medication – 9.5% group I versus 18.5% group II, respectively (fig. 2).

The glomerular filtration rate decreased nonsignificantly in both therapeutic schemes after 3 months of medication – about 2%. This became statistically authentic from 6 months of medication on, but more evident with the administration of Eprosartane, the peak being reached at the end of 12 months of medication – 31.4% group I versus 22.1% group II. The reduction of renal flow is a normal connotation of systemic blood pressure, however, the absolute values of glomerular filtration rate didn't pass above normal after medication (fig. 3).

The values of microalbuminuria had an impressive evolution. The 3 month-long treatment with Ramipril or Eprosartane resulted in a comparable reduction, statistically authentic, of microalbuminuria – by 67% and 65% ($p < 0.05$), respectively. This was revealed at 6, 9 and 12 months of ob-

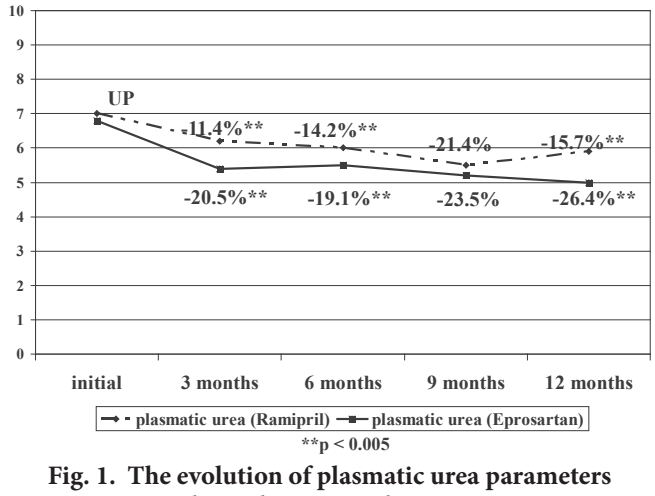


**Fig. 1. The evolution of plasmatic urea parameters depending on medication.**

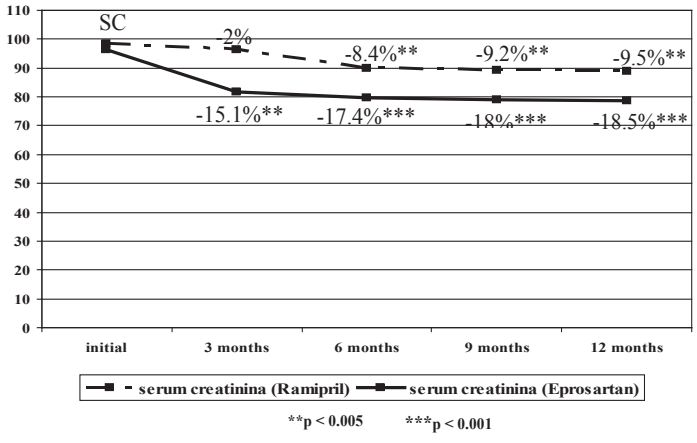


**Fig. 2. The evolution of serum creatinine parameters depending on medication.**

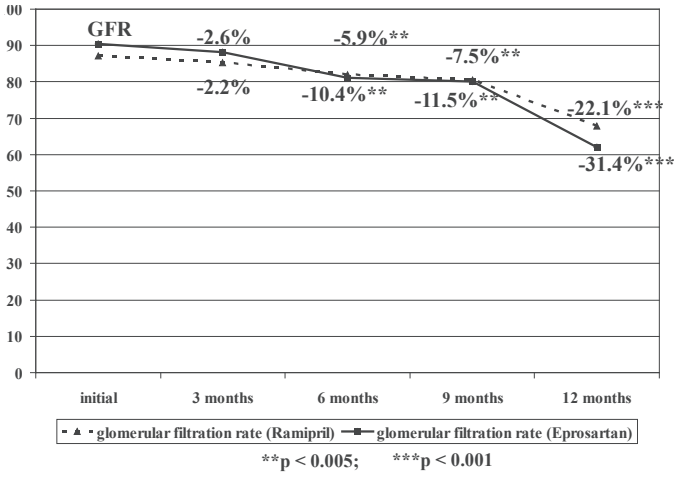


**Fig. 3. The evolution of glomerular filtration rate parameters depending on medication.**

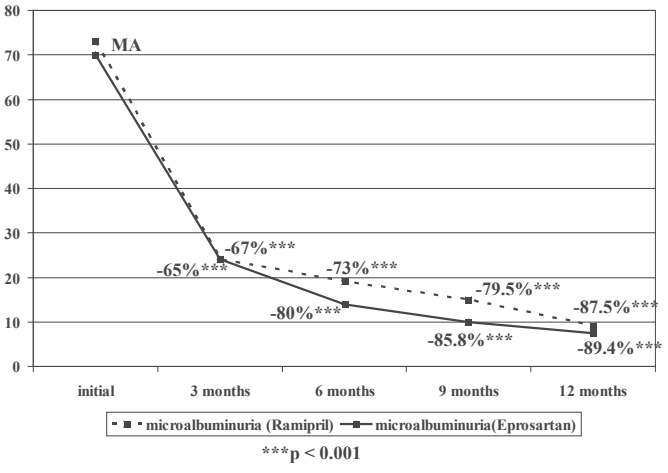


**Fig. 4. The evolution of microalbuminuria parameters depending on medication.**

servation. At the end of the observation period the values of microalbuminuria reached normality (< 20μg/min) in all subjects treated with Eprosartane, having average values of 7.4±1.2 which represents a difference of 89.4% compared to initial ($p < 0.001$) (fig. 4).

Ramipril has also been efficient, but a little more modestly, the average reduction compared to initial being of 87.5% (from 73.1 ± 5.5 μg/min to 9.1 ± 1.4 μg/min). Concomitantly in 5 patients from group I the values of MA passed nonsignificantly the normal target value.

Generally, the presence of proteinuria in the daytime at initial stage was detected in 43 (86%) patients from group I and 40 (80%) from group II. Long-term treatment with Ramipril or Eprosartane resulted in an important reduction of the number of patients with proteinuria.

Therefore, after 3 months of treatment the number of these patients decreased more than 2-fold in both groups, and after 6 months 4.3-fold at Ramipril administration and "sic" 8-fold using Eprosartane. To the end of the trial the percent of patients with proteinuria reduced from 43% to 4% in the group treated with Ramipril and from 40% to 2% in those treated with Eprosartane (tab. 2).

**Table 2**

**The prevalence of patients with proteinuria (Nr; %)**

| Group | Initial | 3 months | 6 months | 9 months | 12 months |
|---|---|---|---|---|---|
| I group (Ramipril) | 43 (86%) | 19 (38%) | 10 (20%) | 4 (8%) | 2 (4%) |
| II group (Eprosartane) | 40 (80%) | 18 (36%) | 5 (10%) | 3 (6%) | 1 (2%) |

Recapitulating, the administration of angiotensin II - converting enzyme inhibitor Ramipril, as well of angiotensin II receptor antagonist Eprosartane did not have a negative impact on renal function. It can be mentioned even a reduction of blood nitrogen evaluated through ureea and serum creatinine, despite of the reduction of glomerular filtration. The decrease, while nonsignificant of the renal flow, can be explained by efficient reduction of systolic blood pressure.

The greatest connotation is due to the normalization in all patients of the values of microalbuminuria at the administration of Eprosartane and reduction to normality in 90% of the ones treated with Ramipril.

## Discussion

Almost 50 years have passed since arterial hypertension has been defined as cardiovascular risk factor for the systolic and diastolic values. The rate of cardiovascular events raises concomitantly with systolic BP at any age, but the correlation of diastolic BP and cardiovascular mortality is directly proportional only until the age of 50, and inversely proportional after the age of 60 [5].

Numerous randomized placebo-controlled trials have investigated the benefit of the lowering of blood pressure using different groups of antihypertensive drugs.

A similar approach was used in order to study new ARB II drugs. In SCOPE trial, in elderly patients (of more than 70 years), candesartane which was often associated with a diuretic, reduced BP versus placebo with 3.2/1.6 mm Hg, noting a significant reduction of the non-fatal stroke incidence. In RENAAL and IDNT trials in hypertensive patients with diabetes mellitus and diabetic nephropathy, the adding of ARB II losartane or irbesartane led to a significant reduction of cardiovascular morbidity [6, 7].

In MOSES trial (hypertensive patients with supported anterior cerebrovascular event) a comparison was performed between medications with eprosartane vs calcium blocker nitrendipine. During 2.5 years of observation, significantly less strokes (31%) were noted in patients treated with eprosartane in conditions of a similar decrease in BP values [8].

Present data confirm the impact of proteinuria, which is a marker of endothelial dysfunction, on general and cardiovascular morbidity.

In this way, Culleton and al. have examined the elderly population included in Framingham trial in the perspective of analysis of the association between proteinuria and the incidence of coronary artery disease, cardiovascular and general mortality. According to the data on other cardiovascular risk

factors, including elevation of serum creatinine, after 17 years of clinical studies proteinuria represented a risk factor for general mortality among the male and female population, the death risk being ampified 1.3 to 2.6 fold because of increased urinary protein excretion [9].

The importance of proteinuria as a risk factor has been studied in another even greater trial MRFIT. After 6 years of observation the presence of proteinuria has been significantly and independently associated with general, cardiovascular and coronary artery disease mortality, the risk raising with the level of proteinuria [10].

The importance of proteinuria in cardiovascular morbidity and mortality is deducted indirectly in the HOPE trial. Treatment with ramipril decreased the risk for acute myocardial infarction by 22%, for stroke by 33%, cardiovascular mortality by 37% and general mortality by 24%. The risk for occurrence of clinically manifest diabetic nephropathy reduced by 24%. These effects were independent from the antihypertensive effect of ramipril. The authors concluded that ramipril has an important vascular and renal protective effect [11].

Normaly, a minimum quantity of proteins with a mean of 80 mg/day is being excreted with urine, 15% of which are albumines. Therefore, proteinuria is defined as an urinary protein excretion of more than 0.3 g in urine in 24 hours. In case of febrile disease, urinary infection or excessive effort, proteinuria may become periodicaly significant, without a particular long term importance.

The proteins in normal and pathological urine are generated from three major sources:

- plasmatic proteins filtrated physiologicaly or pathologicaly by glomerular capilars and that avoids reabsorbtion at the level of proximal renal tubes;
- proteins secreted physiologicaly by tubular cells (for example, Tamm-Horsfall protein) or lost in the tubular lumenum because of tubular damage;
- proteins secreted by cells or glands in inferior urinary tract or proteines resulted from inflamation of the urinary tract.

In our study the presence of mild proteinuria (< 1g/24h) in initial stage has been observed in 86% patients in the group treated with Ramipril and 80% with eprosartane. The long-term medication resulted in important reduction of the number of patients with proteinuria.

Therefore, after 3 months of treatment the number of these patients diminished more than 2-fold in both groups, and after 6 months 4.3-fold during Ramipril administration and "sic" 8-fold using Eprosartane. At the end of the trial, the percent of patients with proteinuria reduced from 43% to 4% in the group treated with Ramipril and from 40% to 2% in those treated with Eprosartane. In this way, we can conclude that ARB II Eprosartane has a renoprotective effect through reduction of proteinuria.

It is known the fact that microalbuminuria represents a high cardiovascular and renal risk factor compared to subjects with "normal" urinary excretion (< 30 mg/24h). In MONICA trial it was demonstrated that microalbuminuria in hypertension represents an important and independent cardiovascular risk factor. The presence of microalbuminuria in these patients correlates with a greater prevalence and severity of left ventricular hypertrophy, hypertensive retinopathy, "non-dipping" hypertension and carotid arteriosclerosis [12].

In Groningen trial, a doubling of urinary albumine concentration was associated with an increase by 29% of cardiovascular mortality and by 12% of noncardiovascular mortality. There is evidence that microalbuminuria is a marker not only for endothelial dysfunction in glomerules, but also in the whole vascular system[13].

In those 100 patients with moderate-to-severe hypertension included in our trial, the presence of microalbuminuria was mandatory and it constituted: 73.1 ± 5.52 μg/min in the group treated with Ramipril and 68.1 ± 4.15 μg/min with Eprosartane ($p < 0.05$). The mean values of glomerular filtration rate were within normal limits 87.2 ± 4.17 ml/min in the group treated with Ramipril and 94.5 ± 3.34 ml/min in the group treated with Eprosartane ($p < 0.05$). Therefore, in the case of our trial it can be also firmly said that the presence of microalbuminuria confirms the fact that there were patients included with important vascular damage.

On antihypertensive treatment Ramipril versus Eprosartane in our trial the values of microalbuminuria had an impressive evolution. Only 3 months of medication resulted in a statisticaly significant reduction from initial microalbuminuria 67% on Ramipril and 65% on Eprosartane ($p < 0.05$). At the end of the observation period (12 months) the level of microalbuminuria reached the norm limit (< 20 μg/min) in all subjects treated with Eprosartane, forming a difference of 89.4% compared to the initial ($p < 0.001$). Ramipril was a little more modest, the decrease of microalbuminuria being of 87.5% compared to the initial, concomitantly in 10% of patients the values MA passed nonsignificantly the normal target value.

Despite of the absence of survival benefits, these data prove the necesity of use of ACE II or ARB II in hypertensive patients with high risk and chronic renal disease.

**Conclusions**

1. Microalbuminuria represents an independent and important cardiovascular risk factor in general population, diabetic and hypertensive, being a marker of generalized vascular dysfunction.

2. The presence of proteinuria has to lead not only to detailed kidney investigations for the detection of the etiology of renal damage, but also to cardiologic exploration, evaluation of cardiovascular risk, as well as aggressive treatment.

3. Serious renoprotective effect (important reduction of microalbuminuria) is installed in 6 months from initiation of medication with Ramipril or Eprosartane. Continuation of medication induces progressive reduction of microalbuminuria, superior efficiency being found at the administration of angiotensin II receptor antagonist Eprosartane.

4. The administration of angiotensin II receptor antagonist

Eprosartane, in the presence of contraindications for angiotensin II - converting enzyme inhibitor Ramipril, is absolutely opportune in the presence of hypertensive microalbuminuria.

### References


1. Mancia G, De Backer G, Dominiczak A, et al. 2009 ESH-ESC Guidelines Managment of arterial Hypertension. *J. Hypertens*. 2009;25:1105-1188.
2. Stevens LA, Caresh J, Greene T, et al. Assessing kidney function-measured and estimated glomerular filtration rate. *N Engl J Med.* 2006;354:2473-2483.
3. Chobanian AV, Bakris GL, Black HR, et al. The Seventh Report of Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC report. *JAMA*. 2007;289:2560-2572.
4. Stevens LA, Caresh J, Greene T, et al. Assessing kidney function-measured and estimated glomerular filtration rate. *N Engl J Med*. 2006;354:2473-2483.
5. Franklin SS, Larson MG, Khan SA, et al. Does the relation of blood pressure to coronary heart risk change with aging? *Circulation*. 2001;103:1245-1249.
6. Zeeuw de D, Remuzzi G, Parving HH, et al. Proteinuria, a target for renoprotection in patients with type 2 diabetic nephropathy: lessons from RENAAL. *Kidney Int*. 2004;65:2309-2320.
7. American Diabetes Association: Diabetic nephropathy. *Diabetes Care*. 2002;25(Suppl 1):585-589.
8. Schrader J, Luders S, Kulschewski A, et al. MOSES Study Group. Morbidity and Mortality After Stroke, Eprosartan Compared with Nitrendipine for Secondary Prevention: principal results of a prospective randomized controlled study (MOSES). *Stroke*. 2005;36(6):1218-26.
9. Culleton BF, Larson MG, Parfrey PS, et al. Proteinuria as a risk factor for cardiovascular disease and mortality in older people: a prospective study. *Am J Med*. 2000;109:1-8.
10. Grimm RH, Svendsen KH, Kasiske B, et al. Proteinuria is a risk factor for mortality over 10 years of follow-up. MRFIT Reserch Group. Multiple Risk Factor Intervention Trial. *Kidney Int Suppl*. 1997;63:S10-4.
11. HOPE investigators. Effects of angiotensin-converting-enzime, ramipril, on cardiovascular events in hight-risk patients. *N Engl J Med*. 2000;342:145-53.
12. Ruilope LM, Rodicio JL. Clinical relevance of proteinuria and microalbuminuria. *Curr Opin Nephrol Hypertens*. 1993;2:962-7.
13. Jong de PE, Hillege HL, Pinto-Sietsma SJ, et al. Screening for microalbuminuria in the general population: a tool to detect subjects at risk for progressive renal failure in an early phase? *Nephrol Dial Transplant*. 2003;18:10-13.


# Cathepsin D Activity in Experimental Liver Chirrosis and After the Administration of Copper Coordination Compounds and Bacterian Remedy BioR


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### Abstract

This paper investigates the influence of the copper coordination compounds CMT-28, CMT-67 and of the bacterial remedy BioR on the cathepsin D activity in liver in experimental cirrhosis. The activity of cathepsin D was also detected electron-histochemicaly in the liver during the regression of experimental hepatic cirrhosis. The result suggests that the coordinative compound CMT-67 used in combination with the bacterial remedy BioR has a pronounced stimulating effect on the enzymatic hydrolysis of the extracellular matrix under the action of cathepsin D and contributes to a more efficient breakdown of the excessive fibrous tissue in liver. It was determined that the active cathepsin D is localized intracellularly in the lysosomes of hepatocytes, macrophages, fibroblasts and endothelial cells, as well as extracellularly on the collagen fibrils near the parenchymal and mezenchymal cells. In addition to its participation in the intracellular proteolysis, cathepsin D is secreted by the hepatocytes and connective tissue cells to the extra-cellular space and participates in the extracellular breakdown of the fibrous tissue.


**Key words:** cathepsin D, liver cirrhosis, coordinative compounds of cuprum, bacterial remedy BioR.

### Активность катепсина D при экспериментальном циррозе печени и при введении координационных соединений меди и препарата бактериального происхождения BioR


Было изучено влияние координационных соединений меди CMT-28, CMT-67 и препарата бактериального происхождения BioR на активность катепсина D в печени при экспериментальном циррозе. Активность катепсина D была также выявлена электронно-гистохимически в печени при регрессии экспериментального цирроза. Результаты свидетельствуют о том, что координационное соединение меди CMT-67, введенное в комбинации с препаратом бактериального происхождения BioR, имеет выраженное стимулирующее влияние на ферментативный гидролиз внеклеточного матрикса под влиянием катепсина D и способствует более эффективному распаду фиброзной ткани в печени.

Было выявлено, что активный катепсин D локализуется внутриклеточно в лизосомах гепатоцитов, макрофагов и эндотелиальных клеток, а также внеклеточно – на коллагеновых волокнах вблизи паренхимальных и мезенхимальных клеток. Таким образом, помимо участия во внутриклеточном протеолизе, катепсин D секретируется гепатоцитами и клетками соединительной ткани во внеклеточное пространство и участвует во внеклеточном распаде фиброзной ткани.

**Ключевые слова**: катепсин D, цирроз печени, координационные соединения меди, препарат бактериального происхождения BioR.

## Introduction

Hepatitis and cirrhosis are regional pathologies of Moldova. Morbidity and mortality caused by these diseases are constantly growing. Liver cirrhosis is a chronic diffuse liver disease with diverse etiology, characterized by inflammatory, degenerative, necrotic and concomitant regenerative processes, accompanied by progressive disturbances of the organ structure and function [1]. The etiologic factor causes dystrophy and necrosis of the liver cells, infiltration of the portal ducts, cholestasis and connective tissue development. Hepatocytes necrosis is followed by the regeneration of some of the remaining liver cells that leads to the formation of the pseudolobules, which substantially disrupt the normal structure of the organ, create circulatory problems that results in severe deficiencies in oxygen and nutrients supply to the hepatocytes and their continuous damage [2]. Fibrosis – the deposition of fibrous connective tissue in excess, which replaces necrotizing parenchyma elements, is the consequence of most liver diseases arising from chronic aggression carried out by various agents (viral, toxic, immunologic, metabolic) [3]. Fibrosis is characterized by a 3-6 fold increase in the amount of all extracellular matrix components, some of which will increase disproportionately and induce subtle changes of the microstructure at the molecular level [4, 5]. It is believed that the essential cause of the excessive accumulation of connective tissue in the liver is the impaired balance between synthesis and degradation of liver extracellular matrix components, in particular, collagen, occurring as a result of parenchyma damage, blood circulation deficiencies (including, resulting hypoxia) caused by the products of the unpaired metabolism. Ultimately the self-regulation of the connective tissue is affected and the biosynthesis of the compounds of the extracellular matrix begins to outweigh their catabolism, which provides further progression of fibrosis [6]. It is well known that fibrogenesis insured by the mezenchimal component is balanced by fibrolysis that is controlled by the parenchyma cells. Thus the continuing damage of the parenchyma can unbalance the fibrogenesis and fibrolysis and evolve to fibrosis [7].

When the cell degeneration starts, the reparative processes also begin. Destructive and reparative processes run simultaneously for a long time, thus the necrotized liver tissue is replaced by functionally active one. Mechanisms, which provide structural and functional restoration of the liver in such conditions, particular reparative metabolic processes in the damaged liver tissue are still unknown.

Lysosomal apparatus of the cell with its powerful complex of hydrolases, in particular, cathepsins B, D, G, L, H, possesses a particularly important role in adaptive changes of the disturbed metabolic processes and structure of organs and systems due to the action of exogenous chemicals [8, 9]. Protective function of lysosomes is manifested by their involvement in the intracellular digestion of phagocytized macromolecular and supramolecular structures that a sensed as foreign compounds as well as degraded intracellular structures. Frequently extreme factors increase the activity of intracellular proteolytic enzymes, which lead to the formation of biologically active compounds that influence the biosynthesis of proteins and nucleic acids [10].

Cathepsin D, considered to be a marker of the lysosomal enzymes as acidic phosphatase, is one of the most important lysosomal aspartic proteinase and is capable to degrade the main components of the intercellular matrix: collagen, proteoglycans and glycoproteins (fibronectin). Cathepsin D attacks the non-helical terminal regions of the collagen molecules or the α-helical chains, digesting the solubile colagen and solubilizing about 10% of the unsoluble colagen at pH 3.3-4.0.

Cathepsin D is involved in the degradation of proteoglycans at pH 5.0. The efficacy of the proteolysis, producer by cathepsin D is highest at acidic pH values (2.8 to 5.0), although some authors record *in vitro* high enzymatic activity and a pH of 7.2. This allows supposing that cathepsin D participates not only in the intracellular degradation of the proteins, but also in their extracellular catabolism, but the later is not proven.

Since the cellular lysosomal system is an important part in the body's enzymatic protection against the aggression of foreign substances, the study of the mechanisms of action of coordination compounds of transition metals and cyanobacterial remedies on lysosomal hydrolyses in the regression of experimental liver cirrhosis, is of particular interest especially on purpose to use them as versatile medical preparations for the morpho-functional recovery of organs affected by fibrosis and sclerosis.

The objectives of the study were:

1) The assessment of the changes in cathepsin D activity in experimental liver cirrhosis (CH) and after the administration of copper coordination compounds (CC) and their combinations with BioR.

2) Electron-histochemical detection of cathepsin D activity in the liver in the process of regression of experimental liver cirrhosis.

## Material and methods

The biological activity of copper coordination compounds CMT-28 and CMT-67 and of their combinations with cyanobacterial remedy BioR were evaluated in the experiment on a group of animals consisting of 80 male white rats weighing 200-220g, divided into 8 groups of 10 animals each. The first group-control, consisted of 10 animals, maintained on a normal vivarium diet and treated with normal saline that was injected intramuscularly daily. Group No. 2-8 consisted

of experimental animals that were injected intramuscularly 50% sol. of carbon tetrachloride ($CCl_4$), 1 mg/kg twice a week, over 60 days to induce experimental liver cirrhosis. Carbon tetrachloride ($CCl_4$) enters the body and reacts with amines and proteins resulting in the formation of free radicals. Lipid peroxidation by free radicals disturbs the function of the cell membranes, including the lysosomal membrane. This will increase the permeability that is considered a universal mechanism of cellular damage at membrane level [11, 12].

Animals in group 3 were treated with CMT-28, while those in group 4 - the CMT-67. Animals from group No. 5 were subjected to treatment with CMT-28 in combination with BioR, and group 6 - CMT-67 in combination with BioR. All those preparations were administered intramuscularly for 14 and 28 days, the daily dose being of 1.0 g/kg body weight.

Animals in groups 1-6 were sacrificed under light narcosis with sulfuric ether 24 hours after the last injection. Animals in groups 7 and 8 were sacrificed 7 and 14 days, respectively, after the suspension injections of $CCl_4$. Biological material - the liver, was collected, washed with 0.85% sol. of NaCl and dried with filter paper. Further liver homogenate was prepared in 0.25 M sucrose buffer, containing 1 mM EDTA, pH 7.4, so the final dilution of homogenate to be 1:10.

Cathepsin D activity was determined in liver homogenate according to the procedure described by Barrett A., 1977 [13], modified by Gudumac V. [14]. The principle of this method is based on the enzyme's ability to make an intense hydrolysis with the formation of hemoglobin macromolecule acid-soluble derivatives, which can be estimated spectrophotometrically. Statistical evaluation of biochemical indices was made by parametric t-Student criterion with reliability less than 0.05 ($p < 0.05$).

For electron-histochemical determination of cathepsin D activity in animal liver at ultra structural level the liver of the rats from groups 7 and 8 was processed in accordance with the procedure described by Smith and Van Frank [15]. Tissue samples with dimensions 0.5×0.5×1.0 mm were fixed for 3 hours in 0.05 M cacodilat buffer, pH 7.2 with 1.5% glutaraldehyde. Then during three days the samples were washed in 0.05 M cacodilat buffer, pH 7.2, containing 7% sucrose. The substrate for incubation was BZ-Arg-Gly-Phe-Phe-Pro-4MβNA (Bachem). Incubation lasted 30 min at 37˚C in the medium, which contained 24 mg substrate dissolved in 1 ml dimethylformamide and 25 ml glycine-HCl buffer, pH 3.1. The reaction is stopped by the addition to the reaction medium of 10% KOH with subsequent washing with HEPES buffer, pH 7.0 and was followed by the incubation in pH 5.4 cacodilate buffer with dipeptidil-aminopeptidase II and para-roseaniline at 37˚C for 15 min. After the incubation the material was fixed with 1.5% sol. osmium tetraoxide in cacodilat in buffer (pH 7.2) for 90 min. In the usual manner for electron microscopy examination, the exploratory material was dehydrated in ethyl alcohol solutions with increasing concentrations (50%, 70%, 96%, and 100%) and then incubated for 20 min in absolute acetone. Samples were included in the eponymous and left in the thermostat at 60˚C for 24 hours. As reference, material was used the tissue incubated without substrate.

## Results and discussions

The results of the research, illustrated in figure 1, have shown that in animals with experimental liver cirrhosis induced by $CCl_4$, activity of cathepsin D increase statistically significant by 27% compared to the control group. Knowing the properties of this proteolytic enzyme and its ability to initiate protein degradation, in particular collagen, we can assume that the increased activity of cathepsin D at the stage of maximal cirrhosis development is a manifestation of the fact that in the liver, parallel with fibrogenesis process runs fibrolysis for the degradation of the excessive connective tissue.

Administration of the bioremedy BioR in dose of 1 mg/kg did not affect the enzyme activity, maintaining it practical at the control levels. At the same time, administration of BioR in the dose 2 mg/kg maintains the enzyme activity at levels

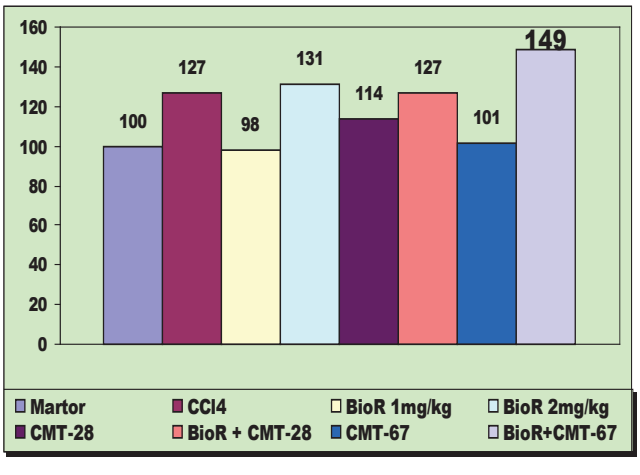


**Fig. 1. Changes in cathepsin D activity (%) in liver cirrhosis and administration of CC and their combinations BioR.**

similar to those assessed in the group of animals with LC and is 31% higher then the control levels. After the treatment with copper coordination compound CMT-28 the activity of cathepsin D showed a tendency to decrease by 10% in animals intoxicated with $CCl_4$. At the same time, the combined use of CMT-28 and BioR does not change the functional level of the enzyme in the liver of the animals with cirrhosis, which was by 27% higher comparative with the original level. The copper coordination compound CMT-67 reduces the degree of cathepsin D activity induction, triggered by cirrhosis, maintaining it at normal values. At the same time combined medication with CMT-67 and BioR increases statistically significant the enzyme activity by 49% ($p < 0.05$) compared with healthy animals and by 17% compared with rats with cirrhosis.

The enhancement of the activity of cathepsin D under the combined influence of CMT-67 and BioR can be considered as a compensatory adaptation reaction of the body, which tends to amplify the biodegradation of defective molecules, resulting from harmful action of $CCl_4$ on liver tissue. The combined administration of copper coordination compound CMT-

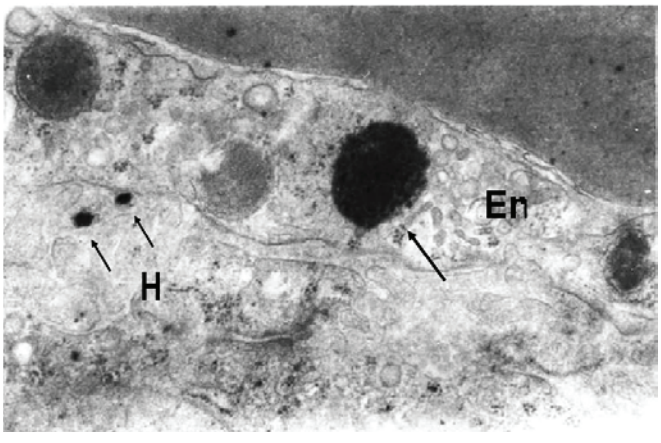


**Fig. 2. 14-th day of experimental cirrhosis regression. A pronounced reaction on cathepsin D (arrows) in the lysosome of endothelial cell (En), and extracellular on hepatocytes microvilli (H). x 20000.**

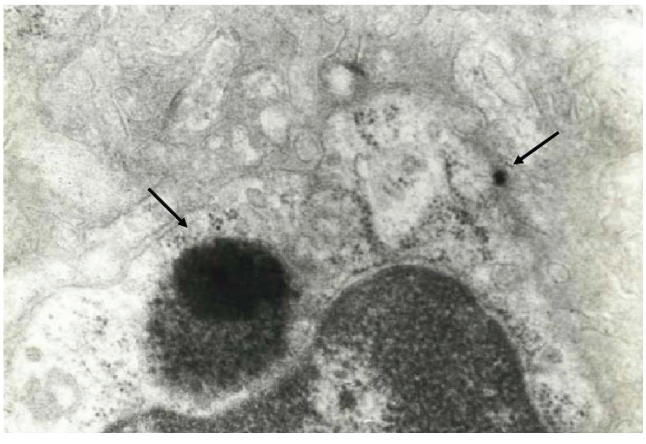

**Fig. 3. 14-th day of experimental cirrhosis regression. Reaction on cathepsin D (arrows) in the lysosome of Kupffer cell, and extracellular on the cytolemma. x 20000.**

67 with cyanobacterial remedy BioR exercise pronounced stimulatory effect on the process of enzymatic hydrolysis of liver extracellular matrix compounds, contributing to a more efficient degradation of fibrous tissue, which is demonstrated by inducing the expression of cathepsin D. The results of the study showed that the investigated remedies have selective action on cathepsin D activity.

*Electron-histochemical detection of cathepsin D activity in the liver in the process of experimental liver cirrhosis regression.* In order to elucidate the participation of cathepsin D in the resorption of the connective tissue in the liver, we performed electron-histochemical detection of cathepsin D. We studied the distribution of the enzyme activity in the liver at the ultrastructural level within two weeks after cessation of intoxication.

The cathepsin D reaction product was detected in the lysosomes of the hepatocytes, macrophages and fibroblasts 7 and 14 day after cessation of $CCl_4$ administration by electron-histochemical investigation. A pronounced reaction was detected in the lysosomes of endothelial cells, too (fig. 2). It was noted a marked heterogeneity in the distribution of reaction product within different cell types and between lysosomes belonging to the same cell. A maximal expressed activity was seen in macrophages (fig. 3) and fibroblasts in all periods of investigation. The activity of cathepsin D was often detected in the myelin-type structures and in the autophagy vacuoles of the hepatocytes after 7 days of regression of cirrhosis.

A major result of our study was the electron-histochemical detection of extracellular activity of cathepsin D at both studied stages of cirrhosis regression. The reaction product is preferentially located on the collagen fibrils near the hepatocytes and cellular elements of connective tissue and, also, on the hepatocytes microvilli and on the external surface of the Kupffer cells cytolemma (fig. 2, 3). Therefore, both parenchymal cells and connective tissue cellular elements are sources of extracellular cathepsin D.

The electron-histochemical investigations of samples of liver tissue in the process of regression of cirrhosis have revealed the intracellular localization of cathepsin D - in the lysosomes of the hepatocytes, macrophages, fibroblasts and endothelial cells, and the extracellular localization - on the collagen fibrils near the parenchymal and mesenchyme cells. The heterogeneity of distribution and intensity of the reaction product, observed in our study reveals different functional status of the lysosomal system, belonging to different cell types.

Extracellular cathepsin D activity detected in the liver damaged by cirrhosis reveals that besides being involved in intracellular proteolysis, cathepsin D is secreted by hepatocytes and cellular elements of connective tissue in the intercellular space and participates in the catabolism of hepatic extracellular matrix and extracellular resorption of fibrous tissue. The important role of cathepsin D was established in liver cell division process during liver regeneration [10].

Therefore, increased activity of the studied proteinase during the regression of the liver pathology could be required for both degradation of connective tissue formed in excess and/or damaged cell structures, and to provide processes of cell division.

## Conclusions

1. Administration of the combination of copper coordination compound CMT-67 with cyanobacterial remedy BioR exhibited a pronounced stimulatory effect on the enzymatic hydrolysis processes of liver extracellular matrix under the action of cathepsin D, contributing to the more efficient degradation of the excessive fibrous tissue.

2. Active cathepsin D is localized intracellular in the lysosomes of the hepatocytes, macrophages, fibroblasts and endothelial cells and extracellular – on the collagen fibrils near the parenchymal and mesenchyme cells.

3. In addition to its involvement in the intracellular proteolysis, cathepsin D is secreted by the hepatocytes and connective tissue cellular elements into the intercellular space and participates in the extracellular resorption of the fibrous tissue.

## References

1. Общая патология человека. Руководство для врачей. В 2-х томах. Под ред. Струкова А.И., Серова В.В., Саркисова Д.С. М.: Медицина, 1990;2.
2. Гальперин ЭИ, Семиндяева МИ, Неклюдова ЕА. Недостаточность печени. М.: Медицина, 1978.
3. Alcolado R, Arthur M, Iredate J. Pathogenesis of liver fibrosis. *Clinical Science*. 1997;92(2;):103-112.
4. Gabrielli G, Corrocher R. Hepatic fibrosis and its serum markers: a review. *Digestive Diseases.* 1991;9(5):303-316.
5. Schuppan D. Structure of the extracellular matrix in normal and fibrotic liver: collagens and glycoproteins. *Sem. Liver Dis.* 1990;10:1-10.
6. Rojkind M. Fibrogenesis in cirrhosis. *Pharmacology & Therapeutics.* 1992;53(1):81-104.
7. Маянский ДН, Шварц ЯЩ, Цырендоржиев ДД, и др. Функциональные перестройки системы мононуклеарных фагоцитов при экспериментальном циррозе печени. *Бюл. эксп. биол. и мед.* 1988;2:214-216.
8. Ivanova S, Repnik U, Bojic L, et al. Lysosomes in apoptosis. *Methods Enzymol.* 2008;442:183-99.
9. Герасимова АМ, Борзова НЮ, Керимкулова НВ, и др. Катепсин D – его физиологическая роль и использование в медицине. *Клиническая лабораторная диагностика.* 2009;3:3-5.
10. Bhaumik SR, Malik S. Diverse regulatory mechanisms of eukaryotic transcriptional activation by the proteasome complex. *Crit Rev Biochem Mol Biol.* 2008;43(6):419-33.
11. Olinescu Radu. Radicali liberi în fiziopatologia umană. Bucureşti: Editura Tehnică, 1994;215.
12. Гонский ЯИ, Корда ММ, Клищ ИН. Антиокислительное действие диметилсульфоксида при остром поражении печени тетрахлорметаном. *Вопр. мед. химии.* 1992;38(2): 43-44.
13. Barrett AJ. Cathepsin D and other carboxyl proteinases Proteinases in Mammalian Cell and Tissues. Amsterdam; New-York; Oxford, 1977;209-248.
14. Gudumac V, Baciu E, Marin V, et al. Investigaţii enzimologice. Elaborare metodică. Chişinău, 2000;56.
15. Smith R, Van Frank R. The use of amino acid derivaties of 4-methoxy-β-naphthylamine for the assay and subcellular localization of tissue proteinases. Lysosomes in Biology and Pathology. New York, 1975;123-249.


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# Macrophage Density Correlates with Severity of Uterine Cervix Neoplasia

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## Abstract

Despite all recent efforts, cancer of the uterine cervix still remains one of the most frequent malignancies among women. Lymphatic vessels represent the primary route of tumor cells dissemination in cervical cancer. It has been demonstrated that cervical neoplasia actively participates in the recruitment of new blood and lymphatic vessels. Macrophages are extremely versatile cells which have a significant contribution to tumor progression. **The aim:** 1) To establish the correlation between tumor-associated macrophages (TAM) and the grade of the uterine cervix neoplasia; 2) To evaluate the distribution of TAM within both intratumoral and peritumoral areas. **Material and Methods:** Ninety-six cases were studied. The specimens were fixed in buffered formalin and paraffin embedded. Step sections, 5μm thick, were performed for each case. Initial sections were stained with haematoxylin-eosin, for the pathological diagnosis and grading of the tumor. Lesions were classified as follows: squamous cell metaplasia (n = 12), CIN I (n = 8), CIN II (n = 6), CIN III (n = 24), microinvasive carcinoma (n = 16) and invasive squamous cell carcinoma (n = 26). Additional sections for each case were stained for CD68 antibody, in order to highlight the macrophages. Quantification of macrophage population has been made based on hot-spot technique. The arithmetic media of 3 (× 200) fields represented the final result. **Results:** We found a statistical correlation between peritumoral macrophages (PTM) and intratumoral macrophages in all stages of cervical neoplasia, macrophage density and tumor stage ($p = 0.01$). In 16 cases we found vascular invasion. Almost in all these cases (87.5%) intravascular tumor emboli were embedded with CD68+ cells. **Conclusions:** based on these findings, we consider that macrophages are key regulators of cervical cancer progression. TAM targeted management could be an essential therapeutic strategy, not only in order to suppress the progression of cervical neoplasia, but also to inhibit macrophage-mediated vascular invasion.


**Key words:** uterine cervix cancer, macrophages, cellular density, tumor progression, CD68.


## Корреляция плотности макрофагов с тяжестью неоплазии шейки матки

Рак шейки матки остается одной из самых часто встречающихся злокачественных заболеваний женского населения. Лимфатические сосуды являются первичным путем метастазирования при данном заболевании. Было доказано, что клетки цервикальной неоплазии активно участвуют в образовании новых лимфатических сосудов. Макрофаги – многофункциональные клетки, оказывающие большое влияние

на прогрессирование опухоли. **Цель:** 1). Выявление корреляции между макрофагами и стадией прогрессии неоплазии шейки матки; 2). Определение особенностей распределения макрофагов внутри опухолевой массы и вокруг нее. **Материал и методы**. Было изучено 96 случаев. Материал фиксировали в формалине с последующим заключением в парафин. Для каждого случая производили срезы, толщиной в 5 мкм. Изначально, срезы окрашивали гематоксилин-эозином для определения гистопатологического диагноза. Были получены следующие группы поражений: плоскоклеточная метаплазия (n = 12), CIN I (n = 8), CIN II (n = 6), CIN III (n = 24), микрокарцинома (n = 16), инвазивный рак (n = 26). Для выявления макрофагов, производили иммуногистохимическое исследование с использованием маркера CD68. Подсчет популяции макрофагов производили по методике hot-spot. **Результаты.** Мы получили статистически значимую корреляцию между внутритуморальными и перитуморальными макрофагами во всех стадиях прогрессии неоплазии шейки матки, между плотностью макрофагов и стадией опухоли (p = 0,01). В 16 случаях выявили сосудистые эмболы. Почти во всех случаях (87,5%) внутрисосудистые эмболы содержали в себе CD68+ клетки. **Выводы**: Основываясь на полученных результатах, мы считаем, что макрофаги вовлечены в прогрессию рака шейки матки.

**Ключевые слова**: рак шейки матки, макрофаги, клеточная плотность, опухолевая прогрессия, CD68.

## Introduction

The crucial importance of the HPV infection in the development of cervical cancer was well established in the middle of the '90s. Based on this evidence, strategies were developed, mainly focused on the prevention of this disease, which led to the dramatic decreasing of its incidence. Despite on the facts named above, cervical cancer still remains one of the most frequent neoplasia for women.

Cancer progression is a complex biological phenomenon, characterized by a multitude of intrinsic and extrinsic events, such as: the blocking of negative signals, the enhancing of positive signals, the over-expression of membrane receptors for pro-tumor growth factors, the promotion of cellular motility, and the recruitment of new blood vessels and new LV.

The ability of cancer cells to migrate from the primary tumor and to give rise to new cellular colonies at the distant sites influences tumor grading, therapeutic management, patient's survival. The lymphatic way of metastasizing involves the regional lymph nodes (RLN), and represents an important criterion of the poor prognosis.

Tumor progression can not be supported only by the tumor cells' related molecular factors. A great importance in cancer development is played by the cells from tumor microenvironment (fibroblasts, myofibroblasts, mast cells, macrophages) [1]. Nowadays the mutual inducing mechanisms between tumor cells and stromal cells are well known. The fact that tumor inflammatory infiltrate (TII) correlates with cancer's progression is widely accepted [2]. Macrophages are extremely versatile and are one the most numerous cell populations in the TII. In addition to a large number of pro-tumor factors, synthesized by the tumor-associated macrophages (TAM), it has been established that these cells produce significant amounts of VEGF-C, which is one important lymphangiogenic factor, as well as VEGF-D.

The pro-tumoral role of TAM in human cancers is supported by many clinical studies that found a correlation between high macrophage density and poor patient prognosis. Many macrophage products released in the tumor stroma can directly stimulate the growth and promote the tumor cell migration and metastasis. Among the molecular factors that mediate these effects are epidermal growth factor (EGF), transforming growth factor $\beta$ (TGF $\beta$), vascular endothelial growth factor (VEGF), cytokines, chemokines.

**The aim was:** 1) To establish the role of TAM in lymphangiogenesis; 2) To evaluate correlation between TAM density and the stage of cervical neoplasia progression; 3) To assess the involvement of TAM in vascular invasion.

## Material and methods

Targeted biopsies taken from conization were investigated, at Institute of Oncology from Republic of Moldova between June 2008 and May 2009, in patients with macroscopically detectable cervical lesions. The specimens were fixed in buffered formalin and paraffin embedded. Step sections, 5μm thick, were performed for each case. Initial sections were stained with haematoxylin-eosin, for the pathological diagnosis and grading of the tumor. Lesions were classified as follows: squamous cell metaplasia (n = 12), CIN I (n = 8), CIN II (n = 6), CIN III (n = 24) 17 CIN III and 7 CIS microinvasive carcinoma (n = 16) and invasive carcinoma (n = 26). Additional sections for each case were stained for CD68, with LSAB+ technique, using Avidin-Biotin working system. Antigen retrieval was done by microwave oven heating in retrieval solution pH6 (Dako Cytomation). Incubation of primary antibody was for 30 minutes. Identification of primary antibody we performed with DAB chromogen (Dako Denmark). Quantification of macrophage population has been made in accordance with hot-spot technique. The arithmetic media of 3 (×200) fields represented the final result. For nuclei counterstaining, we used Lille's modified Hematoxylin. Images were taken using a Nikon Eclipse (E600) camera. The entire immunohistochemical analysis was performed on autostainer (Dako Cytomation).

## Results

**Squamous metaplasia**

Morphologically, macrophages were small with cytoplasmatic pattern of immunostaining. The density of PTM ranged between 73 and 90, with an average of 85.2. The values of ITM ranged between 16 and 23, with an average of 21.2.

**CIN I and CIN II.**

PTM were placed mainly in lamina propria. ITM were bigger, placed in all layers of the epithelium, with higher density in the basal, parabasal and intermediate layers. Statistically significant correlation was found between stromal macrophage and intraepithelial macrophage densities (p = 0.044).

**CIN III.**

PTM ranged between 149 and 312, with an average of 124.4. Intratumoral macrophages were distributed in all

layers, with a slight increased density in the basal $^{1}/_{3}$ of the squamous epithelium (fig. 1).

The lowest density of ITM was 51, the highest – 144, the mean – 103.7. A statistically significant correlation was found between peritumoral macrophages and intratumoral macrophages (0.015).

**Microinvasive and invasive cancer**

PTM ranged between 165 and 416, with an average of 298.6. ITM were big with an evident tendency to form clusters and with less intensity of the CD68 expression in comparison with PTM. They were ranged between 109 and 310, with an average of 200. Correlation between PTM and ITM was significant (p = 0.001).

In invasive carcinoma, PTM were placed diffuse in peritumoral stroma, mainly around vessels and were organized in groups (fig. 2).

PTM ranged between 219 and 617, with an average of 413.6. ITM were also diffused inside the whole tumor mass (fig. 3).

We observed a tendency of macrophages to fuse and form big multinucleated cells. This phenomenon was found by us only in invasive carcinoma within epithelial tumor mass (fig.3).

Cells were significantly bigger than PTM, while the intensity of expression often was lower. The highest ITM density was 522, the lowest -189, the mean – 322.8. Significant correlation was found between PTM and ITM (p = 0.012). We have found no correlation between both PTM and ITM densities and vascular invasion.

**Vascular invasion**

We have found vascular invasion in 16 of 26 cases of invasive carcinoma. Almost all emboli from invaded vessels contained CD68+ macrophages (fig. 5).

In 22 cases (84.61%) we detected intercalated CD68+ cells among the endothelial cells of vessels (fig. 6).

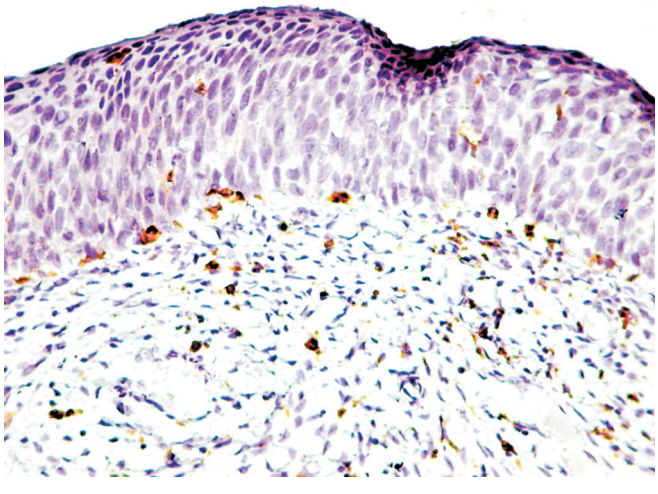

**Fig. 1. Cervical Intraepithelial Neoplasia III (x100). Macrophages are located in all layers of stratified squamous epithelium, with a slight increasing of their density in basal and parabasal layers.**

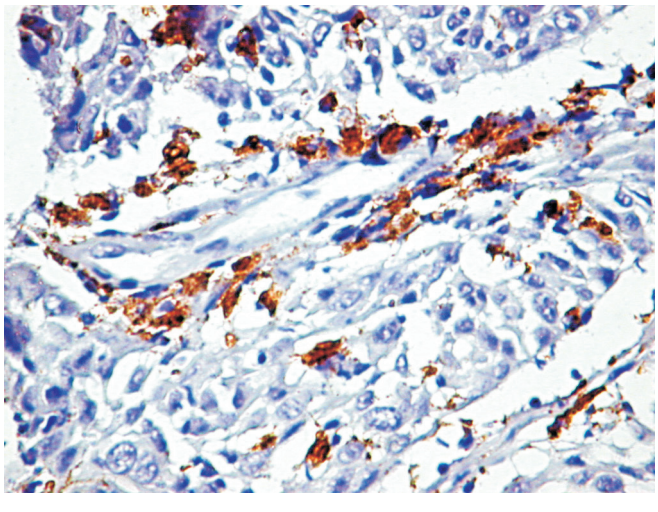

**Fig. 2. Invasive carcinoma (x200). Peritumoral macrophages placed around vascular structure.**

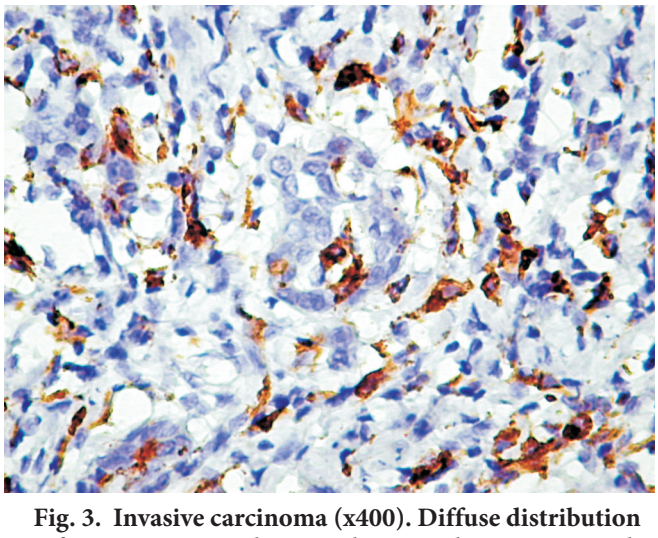

**Fig. 3. Invasive carcinoma (x400). Diffuse distribution of tumor associated macrophages within peritumoral stroma.**

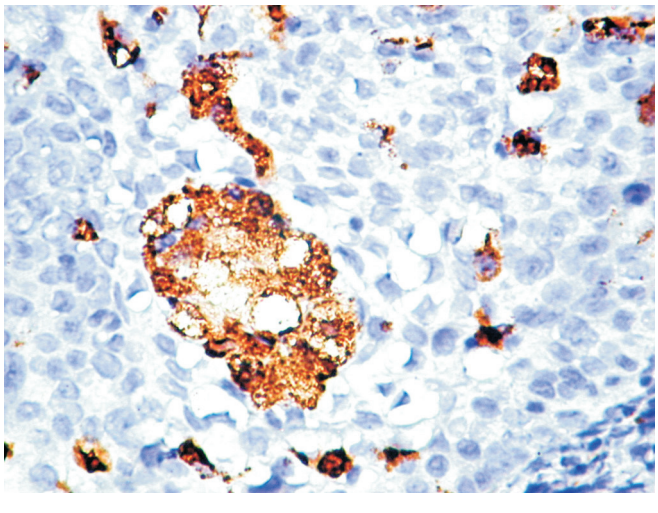

**Fig. 4. Invasive carcinoma (x400). Multinucleated CD68+ cluster inside the tumor mass.**

Table 1

| Type of cervical lesion | MS (n = 12) | CIN I (n = 8) | CIN II (n = 6) | CIN III (n = 24) | Microinvasive Carcinoma (n = 16) | Invasive Carcinoma (n = 26) |
|---|---|---|---|---|---|---|
| PT M | 85.2 | 106.38 | 118.14 | 124.4 | 298.6 | 413.6 |
| IT M | 21.2 | 56.88 | 84 | 103.7 | 200 | 322.8 |

## Discussions

It is well known that macrophages are one the most versatile cells [3]. There is an increasing body of evidence that proves that macrophages represent a key regulator in progression of different human solid tumors. Linear increasing of both, ITM and PTM densities, from pre-invasive to invasive cervical lesions, detected in our study, strongly indicates on catalytic function of these cells in cervical carcinogenesis. The same results have been reported before [4]. It is well known that macrophages have the ability to proliferate. This phenomenon was described in detail in wound healing and glomerulonephritis [5], while, in recent literature there are few data about macrophage proliferation in tumors. It seems to be clear about the origin of PTM. Tumor cells produce a broad spectrum of cytokines and growth factors which are chemoatractants for macrophage precursor cells and lead to their accumulation into the stroma of peritumoral area, and further differentiation into adult macrophages. It is supposed that ITM have a dual origin: intratumoral migration of periepithelial macrophages and their local proliferation. Based on the fact that in all groups of lesions PTM density was higher than ITM, and on the statistical correlation found between them, we suggest that ITM population is mainly provided by the PTM invasion into the tumor mass.

There is a big amount of evidences (experimental and clinical) that proved without any doubts the TAM's role in cancer-cell spreading. This role is mediated by a variety of pathogenic chains, orchestrated by TAM. On one hand, macrophages determine the cancer cells mobility, through EGF secretion, and stromal invasion, by extracellular matrix remodeling. On the other hand, macrophages are actively involved in tumor-derived angiogenesis and lymphangiogenesis. As a result, detaching of neoplastic cell, from its primary locus, and vessel penetrating is much easier. Presence of CD68+ cells almost in all intravascular emboli, obtained by us, underpin these statements.

There is an increasing body of evidence that macrophages are actively involved in LAG [6]. There is a dual mechanism of LAG promoted by macrophages: macrophage transdifferentiation into the LEC, and synthesis of VEGF-C.

Cervical carcinogenesis consists of several well-distinguished stages: CIN, carcinoma in situ, microcarcinoma, invasive carcinoma. It has been reported that potent lymphangiogenic switch occurs in high-grade CIN stage [7]. LAG is dependent on LEC proliferation. VEGF-C (vascular endothelial growth factor C) is the main mitogenic factor which controls this proliferation. It has been demonstrated that macrophages represent an important source of VEGF-C. Macrophage

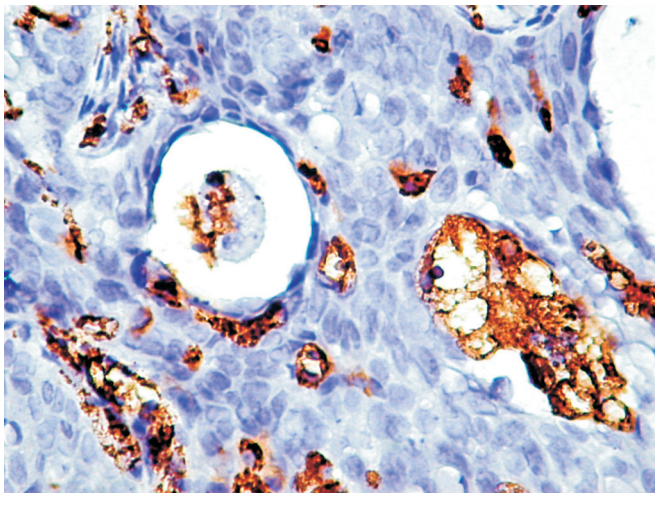

**Fig. 5. Invasive carcinoma (x200). Intravascular tumor embolus embedded with macrophages.**

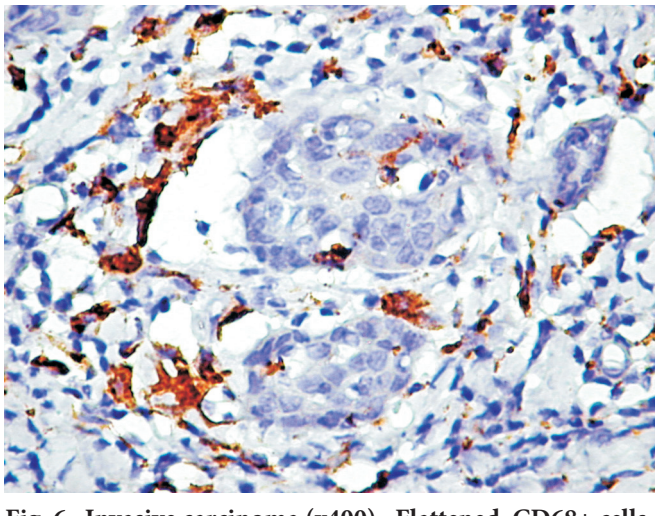

**Fig. 6. Invasive carcinoma (x400). Flattened, CD68+ cells, intercalated between endothelial cells of invaded vessel.**

precursors express VEGFR-3, transmembrane receptor which transduce VEGF-C signals. It has been shown that VEGF-C, produced by tumor cells, interacts with VEGFR-3, from membrane of macrophage precursors, and determines their recruitment [8]. Once these cells reach to the peritumoral area, they begin to secrete own VEGF-C, which enhance the macrophage recruitment and also interact with VEGFR-3 on LEC. On the other hand, macrophages are able to transdifferentiate into LEC and to integrate into sprouting LV [9]. In our study, we detected clusters of macrophages, located around small LV, in CIN III, microcarcinoma and invasive carcinoma. Moreover, in all stages named above, we observed CD68+ cells

intercalated between LEC. This macrophage intercalation was observed, predominantly, in small and flattened LV (hallmarks for young vascular structures) from peritumoral stroma, and also in large but invaded vessels. Our results are consistent with data presented by other authors, and support the hypothesis according which integration of macrophage precursors, transdifferentiated into LEC, occurs in newly-formed LV. We consider this mechanism crucial in the formation of new LV because proliferating LV not only enlarge their size and become able to support the migration of tumor emboli, but also increase the area for targeted action of VEGF-C, secreted both by the tumor and stromal cells, via increasing number of VEGFR-3 expressing cells in newly-formed LV.

## Concluding remarks

Cancer progression represents an extremely sophisticate mutual interaction between a variety of molecular agents related both to tumor mass and tumor microenvironment. From this point of view, macrophages are one of the most important sources of a wide spectrum of biologically active substances that mediate the tumor progression.

Linear increasing of TAM density during the cervical neoplasia progression, their predominant location around vascular structures, integration of CD68+ cells into the endothelium of the vessels, demonstrate their crucial importance in uterine cervix neoplasia progression.

Based on these findings, we consider that macrophages are key regulators of cervical cancer progression. TAM targeted management could be an essential therapeutic strategy, not only in order to suppress the progression of cervical neoplasia, but also to inhibit macrophage-mediated vascular invasion.

## References


1. Lewis EL, Pollard JW. Distinct role of macrophages in different tumor microenvironments. *Cancer Res.* 2006;66(2):605-612.
2. Pollard JW. Trophic macrophages in development and disease. *Nature Reviews Immunology.* 2009;9:259-270.
3. Sica A, Allavena P, Mantovani A. Cancer related inflammation: The macrophage connection. *Cancer Letters* 2008;264:204-215.
4. Hammes LS, Tekmal RR, Naud P, et al. Macrophages, inflammation and risk of cervical intraepithelial neoplasia (CIN) progression – Clinicopathological correlation. *Gynecologic Oncology.* 2007;105:157-165.
5. Schimizzi AL, Massie JB, Murphy M, et al. High-molecular-weigh hyaluronan inhibits macrophage proliferation and cytokine release in the early wound of a preclinical postlaminectomy rat model. *Spine J.* 2006;6(5):550-6.
6. Kerjaschki D. The crucial role of macrophages in lymphangiogenesis. *The Journal of Clinical Investigations.* 2005;115(9):2316-19.
7. VanTrappen PO, Steele D, Lowe DG, et al. Expression of vascular endothelial growth factor (VEGF)-C and VEGF-D, their receptorVEGFR-3, during different stages of cervical carcinogenesis. *J Pathol.* 2003;201:544-54.
8. Schoppmann SF, Birner P, Stockl J, et al. Tumour-associated macrophages express lymphatic endothelial growth factors and are related to peritumoral lymphangiogenesis. *Am. J. Pathol.* 2002;161:947-956.
9. Maruyama K, Ii M, Cursiefen C, et al. Inflammation-induced lymphangiogenesis in the cornea arises from CD11b-positive macrophages. *The Journal of Clinical Investigations.* 2005;115(9):2363-72.

# Antithrombotic Treatment of Patients with Atrial Fibrillation and Ischemic Stroke

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**Abstract**

**Objective:** Assessment of the use of oral anticoagulants in patients with ischemic stroke and atrial fibrillation in the Republic of Moldova. **Methods:** A retrospective study of all patients hospitalized with stroke during one-year period in a municipal hospital. **Results**: Out of 735 patients with ischemic stroke, atrial fibrillation was noted in 28.4% (206/189). The majority were female, 57.8% (119/206) with the mean age of 70.1 ± 0.65 years. Ninety two (92.5%) of all patients had a high thromboembolic risk, 5.8% - medium and 1% - low risks. In the medium risk group the rate of antithrombotic treatment represented 88.9%. Even if about 92% were considered to have a high risk for thromboembolic complications, 60% were eligible for anticoagulation, but only 7.1% received it prior to the stroke. After the cerebrovascular accident all patients were considered to be at high risk, but only 14.8% were anticoagulated and only 26.6% (4/15) had the therapeutic International Normalized Ratio (INR) in the range 2.0-3.0. Physical disability and non-compliance were the most frequent reasons for noncompliance to anticoagulation. At the next visit 65.2% were receiving aspirin, and 20% were not receiving any antithrombotic medication. No hemorrhagic complications were reported after 14 months of follow up. **Conclusions:** A significant proportion of patients (92.9%) with atrial fibrillation and high antithrombotic risk were not anticoagulated before ischemic stroke for various reasons: underestimation of antithrombotic benefit and fear of hemorrhagic complications from physician's behalf and difficulties in systematic INR monitoring from patient's behalf.

**Key words**: atrial fibrillation, ischemic stroke, *antithrombotic* treatment.

**Антитромботическое лечение больных с мерцанием предсердий, перенесших ишемический инсульт**

**Цель**: определить процент применения непрямых антикоагулянтов (НАК) у больных с мерцанием предсердий (МП) и ишемическим инсультом. **Методы**: ретроспективное исследование всех больных с острым ишемическим инсультом, поступивших в одну городскую больницу, в течение одного года. **Результаты**: из 735 больных с ишемическим инсультом, МП было выявлено у 28,4% (206/735) больных среднего возраста (70,1 ± 0,65 лет), из которых большинство были женщины – 57.8% (119/206). Из всех больных 92,5% были с высоким тромбоэмболическим риском, 5,8% - средним и 1% - с низким. Процент назначения антитромботического лечения в группе больных со средним риском составил 88,9%. Из больных с высоким риском, в среднем 60% было показано назначение антикоагулянтов, но лишь 7,1% получили их до развития инсульта. После инсульта все пациенты считались с высоким тромбоэмболическим риском, но лишь 14,8% получали НАК, из которых 26,6% (4/15) поддерживали INR (2,0-3,0), тогда как реальные возможности получения антикоагулянтов было у 40,6% больных. Физическая нетрудоспособность и неспособность соблюдать рекомендации врача были самыми частыми причинами не назначения НАК. На повторном визите 65,2% больных получали аспирин, а остальные 20% не получали никакого антитромботического лечения. За 14 месяцев наблюдения не было зарегистрировано ни одного случая кровотечения. **Заключение**: значительная часть (92,9%) больных с МП и высоким тромбоэмболическим риском не принимали НАК до развития инсульта по разным причинам: недооценка преимуществ антикоагулянтной терапии, опасность геморрагических осложнений, необходимость регулярной проверки уровня антикоагуляции.

**Ключевые слова**: мерцание предсердий, ишемический инсульт, антитромбическое лечение.

## Introduction

Atrial fibrillation (AF) is a common rhythm disturbance in the daily medical practice in Republic of Moldova. The most serious complication associated with AF is cerebral or systemic (non - cerebral) thromboembolism. This rhythm disturbance increases stroke risk 4-5 fold in any age group. Out of the total ischemic strokes, about 20-25% are cardioembolic and almost 60% of them due to AF, therefore, nearly 15% of the total number of ischemic strokes are due to AF. The annual rate of strokes in nonvalvular AF, without anticoagulant treatment, is 4.5% for the first thromboembolic event and 12% per year for recurrent events [1]. Thromboembolic risk is associated with an annual rate of systemic embolisms of about 0.3-0.8%, their frequency being substantially elevated in AF. Significant and independent influence of AF on stroke risk is confirmed by the data of numerous randomized trials. Meta-analysis of multiple studies shows a reduction of approximately 68% in thromboembolic events on treatment with warfarin, in patients with AF [2]. Strokes due to AF have a more severe evolution than other forms of ischemic strokes, being associated with a higher mortality (nearly 30%) and a high rate of irrecoverable and invalidating neurological consequences [3].

Despite clinical evidence, several trials in USA and Europe showed that only 25-50% of patients with AF receive antithrombotic therapy corresponding to the risk [4]. The causes of this discrepancy between clinical practice and clinical trials are not easy to explain and it is not known whether the same situation is in RM.

In this work we have proposed as an aim to examine the use of anticoagulants and of the factors that influence it in patients with AF and ischemic stroke in a medical institution from the Republic of Moldova.

## Material and methods

All patients with stroke, hospitalized in the hospital "SF. TREIME" from Chisinau have been studied retrospectively during the year of 2004. For inclusion in the study the patients have been identified in the data based on stroke from that hospital. At the first step of the research, the details on patients were obtained from the medical cards. The diagnosis of ischemic stroke was established beforehand by neurologists, and the ECG was evaluated by a cardiologist. The patients have been observed for 14.2 ± 0.74 months. The following information, during observation, was obtained through active examination in Institute of Cardiology; for immobile patients – at their homes, being completed by discussions with family doctors and relatives, also by the study of ambulatory medical cards.

## Results

Seven hundred thirty-five patients with acute ischemic stroke were hospitalized during the study, 206 of the patients (28.4%) had AF confirmed through ECG. The characteristics of these patients at hospitalization are listed in tab. 1.

**Table 1**

**Characteristics of patients with stroke and AF at trial inclusion**

| Parameters | Patients with stroke and AF, n = 206 |
|---|---|
| Age, years | 70.1 ± 0.65 |
| Women | 57.8% (119) |
| Chronic atrial fibrillation | 70.4% (145) |
| Arterial hypertension | 87.4% (180) |
| Vascular pathology | 82% (169) |
| Rheumatic valvulopathies | 9.2% (19) |
| Congestive heart failure | 96.1% (198) |
| Diabetes mellitus | 29.1% (60) |
| Previous thromboembolic event | 30.1% (62) |
| ≥ 2 strokes | 4.4% (9) |
| Period between strokes, months | 27.2 ± 5.5 |
| Mortality in the hospital | 30.6% (63) |

The mean age of subjects with AF was 70.1 ± 0.65 years (age range 34-91 years), the third part of them (29.1%) being aged ≥ 75 years. Among subjects with rhythm disturbances and stroke, more than a half was of female gender 57.8% (119). The great majority of patients with AF (84%) have suffered an established ischemic stroke.

The prevalence of AF in patients with stroke was 28.4%, and it varied depending on the type of cerebral ischemia, followed by transient ischemic attack (TIA) with 25%, lacunar stroke – 15.4% and minor stroke – 13.2%. Out of 206 patients with AF, chronic and persistent forms were present in 145 individuals (70.4%), paroxystic AF in 50 (24.3%) and atrial flutter in 11 patients (5.3%). It is notable, that 8 patients (3.9%) had AF, determined for the first time at hospitalization.

Cardiovascular pathology associated with atrial fibrillation increases the risk for stroke and aggravates its evolution, leading to the appearance of serious consequences. The detailed analysis of patients with ischemic stroke, in this trial, revealed the presence of associated diseases, which included associated cardiovascular pathology and diabetes mellitus, in most patients with AF (99.5%), with the exception of a man. Arterial hypertension was found in 87.4% of patients; vascular diseases, presented as stable angina pectoris, AMI, previous MI or diseases of peripheral vessels, were found in 88% of patients; cardiac failure of different grades - in 96.1% of patients; diabetes mellitus was found in 29.1% of patients, and 9.2% of subjects were diagnosed with rheumatic valvulopathy. A significant part of patients (30.1%) had previous thromboembolic events, in 4.4% of cases being multiple (≥ 2). The mean period between thromboembolic events lasted for 26.8 ± 2.6 months; 14.5% of patients have suffered a repeated stroke in less than a month from the anterior thromboembolic episode, and 61.3% individuals-in a year.

The patients were considered to be at high risk if at least 2 risk factors were identified by investigators for AF, in a meta-analysis of 5 randomized trials: (age > 75 years, hypertension, HF, diabetes mellitus) or ischemic stroke or previous TIA – $CHADS_2$ score, tab. 2.

**Table 2**

**The distribution of $CHADS_2$ score and the evaluation of thromboembolic risk**

| Points | Risk | AF + stroke n = 206 | Deceased n = 63 | Survivors n = 143 |
|---|---|---|---|---|
| 0 | Low | 2 (1.1%) | 0% | 2 (1.6%) |
| 1 | Moderate | 12 (6.4%) | 1 (1.7%) | 11 (8.5%) |
| ≥ 2 | High | 173 (92.5%) | 57 (98.3%) | 116 (89.9%) |
| RV | High | 19 (9.2%) | 5 (7.9%) | 14 (9.8%) |

RV- rheumatic valvulopathy.

The data included in the table show that most of the patients – 92.5%, have scored more than ≥ 2 points from the proposed score, which corresponds to a high thromboembolic risk with need for chronic therapy with oral anticoagulants, 5.8% of patients with AF presented a moderate risk, having the necessity of chronic treatment with antiaggregants or optional with warfarin and only about 1% of patients with AF did not have any risk factors for major thromboembolic events, being classified in the low risk group with aspirin considered sufficient for an adequate thromboembolic protection. A separate group was formed by 19 patients with rheumatic valvulopathy (9.2%) – pathology which implies a high thromboembolic risk and which constitutes an absolute indication for the administration of indirect anticoagulants, independently of the gained points according to CHADS2 score. Studying CHADS2 score in patients with AF it was noted that out of 12 patients with moderate risk, (8.3%) patients deceased, and out of patients of high risk 62 (32.3%) deceased, 5 (26.3%) of them suffering from rheumatic valvulopathies.

The examination of ambulatory medical cards at the 2nd research step (repeated examination) made possible the determination of the rate of prescribing antithrombotic treatment

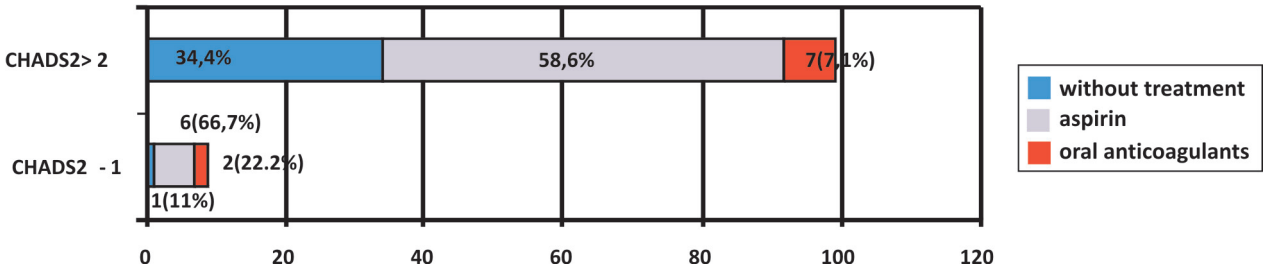


**Fig. 1. The rate of antithrombotic medication in patients with different degrees of thromboembolic risk.**

in the period before the certain thromboembolic event and gave the possibility to appreciate the extent of correspondence to guideline recommendations regarding this medication. The analysis of correlation between the risk evaluated by CHADS2 score and the applied antithrombotic treatment was possible in 108 patients examined repeatedly. As a consequence, it was concluded that none of these patients had a low thromboembolic risk, moderate risk was present in 9 patients (8.33%), and high risk for stroke had 99 patients (91.7%).

The rate of patients with moderate risk, administrating antithrombotic treatment corresponding to risk gradation constituted 88.9% (6 patients (66.7%) received aspirin and 2 patients (22.2%) – thrombostop). About 58 patients (58.6%), with CHADS2 score ≥ 2, administered antiplatelet drugs and only 7 patients (7.1%) had the benefit, before the stroke, of treatment with oral anticoagulants that corresponds to an adequate antithrombotic protection for this category of patients. The rate of antithrombotic treatment appreciated in dependence of the estimated risk for major thromboembolic events in patients with AF is represented in fig. 1.

Therefore, in the period before stroke, 99 (91.7%) patients had a high risk and absolute indications for the prevention with oral anticoagulants and only 9 (8.3%) patients had relative indications for anticoagulants use, presenting moderate thromboembolic risk. In this period, in ambulatory conditions, only 9 (8.3%) patients (7 of which had high risk, 2 of them moderate risk) administered preventive treatment with oral anticoagulants. Only 2 (1.8%) patients had PI in the recommended therapeutic range (40-60%), assuring an optimal antithrombotic protection, the other 7 patients presented values of PI greater than 60%. INR index was not determined in any patient. In the group of patients with valvular AF only 4 patients received indirect anticoagulants. The other 10 patients with AF and mitral valve stenosis remained unprotected, the antithrombotic treatment lacking in the ambulatory medical card.

After discussions with the family doctors and the study of medical documentation it was appreciated that only 64 (59.3%) patients had real possibilities to receive indirect anticoagulants, and regarding the rest of them: 17.6% were incompliant, 7.4% did not have the possibility to monitor coagulation parameters having an advanced grade of disability, 11.1% had uncontrolled hypertension, 3.7% - gastrointestinal pathology with high bleeding potential, and a patient (0.9%) had previously a major hemorrhagic episode, tab. 3.

**Table 3**

**Contraindications for administration of oral anticoagulants**

| Contraindications | Before stroke, n = 108 | After stroke, n = 101 |
|---|---|---|
| Lack of compliance | 17.6% (19) | 27.7% (28) |
| Great disability | 7.4% (8) | 15.8% (16) |
| Uncontrolled hypertension | 11.1% (12) | 4.9% (5) |
| Gastrointestinal pathology | 3.7% (4) | 5.9% (6) |
| Previous bleedings | 0.9% (1) | 1% (1) |
| Recent stroke | - | 2% (2) |
| Oncological pathology | - | 2% (2) |
| Real possibilities to receive oral anticoagulants | 59.3% (64) | 40.6% (41) |

Treatment with aspirin was received by 64 (59.3%) patients (6 with moderate risk and 58 with high risk). Absolute contraindications had only a patient with gastrointestinal acute pathology associated with recidivating bleedings. Six (5.6%) patients presented relative contraindications for aspirin, having gastrointestinal pathologies, and a patient presented allergic reaction to non-steroid anti-inflammatory drugs. After the preventive treatment, 2 patients presented exacerbations of gastrointestinal pathology, and a woman had a hemorrhagic episode.

In this way, based on the received results it can be stated that out of 108 patients who needed antithrombotic treatment, only 14% administered antithrombotic medication in accordance with the risk, in the period before thromboembolic events that is shown in fig. 2.

According to $CHADS_2$ score, all patients evaluated repeatedly were included in the group of patients with high thromboembolic risk and had absolute indications for the administration of oral anticoagulant therapy. Only 15 (14.85%) patients with stroke and AF have been recommended and were administering anticoagulant therapy, only in 4 (3.96%) patients PI reached the target values of (40-60%) and INR 2.0-3.0, in this way being protected by repeated thromboembolic events, the data being graphically presented in fig. 3.

Analyzing the indications, contraindications, degree of physical disability of patients, possibility of PI monitoring in policlinics conditions and patient compliance, real possibilities of antithrombotic preventive treatment were appreciated for only 41 (40.6%) of interrogated patients, the rest of them: 28 (27.7%) were incompliant to treatment, 16 (15.8%) had marked physical disabilities, 6 (5.9%) presented exacerbations of gastrointestinal pathology, 5 (4.95%) had uncontrolled hypertension, 1 patient had an acute bleeding, 2 patients had

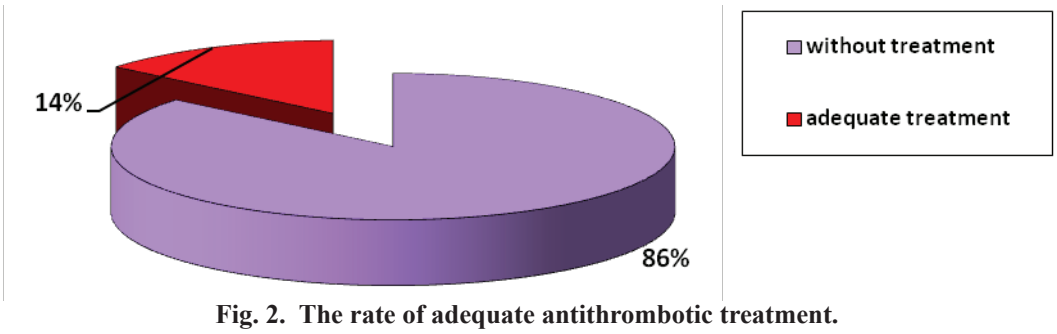


**Fig. 2. The rate of adequate antithrombotic treatment.**

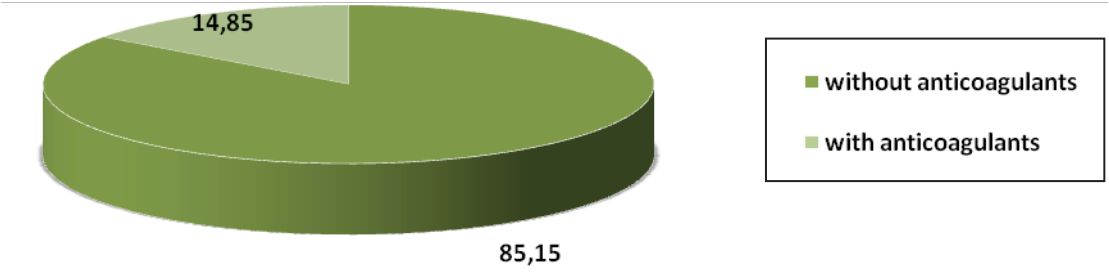


**Fig. 3. The rate of patients who received anticoagulants at repeated examination.**

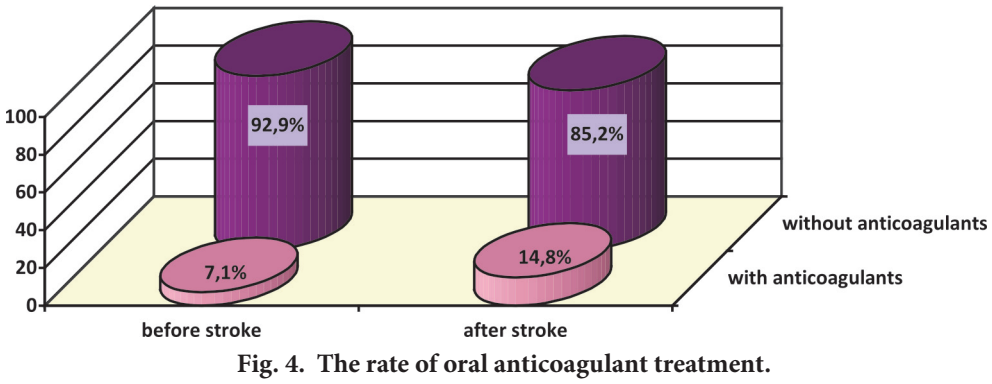


**Fig. 4. The rate of oral anticoagulant treatment.**

repeated stroke during the first month after previous stroke, and in 2 patients oncological pathology was suspected.

Sixty-six (65.3%) patients were administered aspirin, and a patient received clopidogrel. Nine patients did not receive aspirin because of gastrointestinal pathology, a patient presented allergy to aspirin, and 25 patients did not use antiaggregants, in this way ignoring medical indications, or because of the fact that such recommendations were not accepted, or administered irregularly antiplatelet therapy. It is notable that, aspirin was administered to 67.5% of the patients with chronic AF and 15.8% with paroxystic AF, and oral anticoagulants were administrated only to patients with chronic form of AF. On background treatment with antithrombotics 2 patients developed complications of mild and moderate gravity: a patient presented gastrointestinal bleeding, and another one developed acute erosions of gastric mucosa being on aspirin.

Therefore, after repeated stroke the rate of therapy with oral anticoagulants increased 2-fold, but it still remained insufficient in patients with AF and at high thromboembolic risk, fig. 4.

During observation period (14.2 ± 0.74 months) the rate of thromboembolic recurrences was 29.6% (37/125), and of late deaths – 35.2% (44/125). In this period no hemorrhagic complications as a consequence of oral anticoagulant therapy administration were registered.

## Discussions

Atrial fibrillation is the most common rhythm disturbance in clinical medical practice. Annual incidence of stroke is 6-fold higher in patients with AF, comparative to individuals of similar age and sinus rhythm [5]. Many randomized trials have shown the efficacy of anticoagulants in preventing stroke in patients with AF [4]. In a general analysis on AF the investigators have shown that warfarin significantly reduces thromboembolic risk, from 4.5% to 1.45% annually, with relative risk reduction by 68% [6]. The benefic effect of warfarin has been obtained with a minimal risk elevation for

hemorrhagic complications, 1.2% in comparison with 1% for placebo. In absolute terms approximately 90 ischemic strokes are prevented, if 1000 patients with high risk are treated during 1-year period.

Recently guidelines and articles have been published on anticoagulation in AF. In clinical practice the rate of anticoagulant use still remains to be suboptimal. The frequency of oral anticoagulant use in patients without contraindications varies between 15.2 and 78.8%, warfarin being prescribed more often to young patients (< 65 years) [7]. Our study confirms these observations and adds information on circumstances which affect the clinic decision making regarding anticoagulant administration.

Our results show a resistance in use of indirect anticoagulants in patients with AF and high thromboembolic risk, with only 7.1% prescriptions before stroke. The retrospective nature of the study makes the evaluation of reasons difficult, though the registration of contraindications for oral anticoagulants was not evident at the examination of medical cards. These data are lower than the results of Kalro et al. trial in Great Britain. They reported that only 31% of patients with AF, for at least 12 months, with a major risk factor, were anticoagulated at the moment of enrollment in the study [8]. In addition, Jackson et al. have reported similar data in Tansania, with only 34% patients with AF with high risk who received warfarin [9].

Why not is warfarin used enough, despite important arguments which support its benefit in AF? One of the possible reasons is that clinical trials are not considered representative for "the real world", with a greater rate of men and younger patients. Because of monitoring difficulties the patients often refuse the administration of warfarin. These refusals have been documented rarely in the studied medical cards, being found only for 1.2% patients. The physicians can also be reserved in the initiation of treatment with warfarin, because of the time lost in order to make the patient understand why and in which way to administer this drug. Some specialists even doubt the application in their daily practice of the recommendations from guidelines.

The apparent lack of trust of physicians in treatment with warfarin, in patients with AF, can be partially explained through exaggerated care of hemorrhagic risks and underestimation of stroke risk. Another reason is that the guideline for the management of patients with AF, which was valid in 2004, presented relatively other indications for anticoagulation. In fact, the lack of knowledge on recommended antithrombotic therapy can partially explain why the patients treated in neurological departments had fewer chances to receive oral anticoagulation. There is still the possibility of some underestimations regarding the real number of patients with contraindications for anticoagulation.

After discussions with the family doctors and examination of medical documentation it has been appreciated that only 64 (59.3%) patients had real possibilities to receive indirect anticoagulants, and 17.6% were incompliant, 7.4% did not have the possibility to monitor coagulation parameters, having an advanced degree of disability, 11.1% had uncontrolled hypertension, 3.7% - gastrointestinal pathology with high bleeding potential, and a patient (0.9%) had previously a major hemorrhagic episode.

Unified analysis of 5 major randomized trials has shown that advanced age is an independent risk factor for stroke [6]. The risk for stroke in AF starts to increase from the age of 65 years, though, it is clear that the risk for stroke is significant in those ≥ 75 years, who have a greater benefic effect at administration of vitamin K antagonists, comparative to the effect of aspirin [10, 11]. With aging, the relative efficiency of antiplatelet medication in preventing stroke reduces in patients with AF, which does not happen with oral anticoagulants. AF is the most frequent reason of a disabilitating stroke in elderly women [4]. The resistance of physicians in starting oral anticoagulants in elderly patients is generally considered to be related to a higher risk for hemorrhagic complications. The investigators from SPAF II trial have shown that the risk of major bleeding was substantially higher in patients with AF > 75 years, comparative with younger patients, who received anticoagulation therapy at the same extent [4]. Unlike this one, the unified analysis of 5 trials demonstrated only a single intracerebral bleeding among 223 patients of more than 75 years, who received warfarin [6]. But it is known the fact that the incidence of intracerebral bleeding increases with ageing, even in people who do not use oral anticoagulants. It is possible, that the elevated risk for intracerebral bleeding in the elderly could be caused not by warfarin administration, but by physiological changes in the coagulation process happening with ageing [12]. Although our study group was small, there were not reported any hemorrhagic complications in an observational period of 14.2 months.

In the case of initiation of warfarin therapy it is important to obtain the therapeutic level of anticoagulation (INR 2.0-3.0). Recent guidelines recommend a lower intensity of anticoagulation in patients > 75 years, with INR 2.0 [13]. INR level has to be intensely monitored in the elderly patients, in order to minimize not only the risk of oral anticoagulants over dosage, but also of suboptimal therapy, in which the protection for thromboembolic events is lower [14]. In our trial, at the moment of hospitalization, only 2 out of 9 patients on oral anticoagulants had INR within the range 2.0-3.0, and at repeated examination - 4 of 15.

Finally, it is important to mention that a significant proportion of our patients have initiated anticoagulation after stroke. The reason for this change in the management of patients at high risk before the event is not entirely clear, although individual differences can exist in medical practice.

Evaluating the indications, contraindications, physical capacities of patients, as well as technical conditions of policlinics, it has been determined that only 40.6% of reevaluated patients after stroke had real possibilities to administer preventive antithrombotic treatment. Among those who did not receive oral anticoagulation therapy – 27.7% were absolutely incompliant to treatment, 15.8% had marked physical disabilities, 5,9% presented exacerbation of gastrointestinal

pathology, 4,9% had uncontrolled hypertension, 1 woman suffered from frequent metrorrhagies, 2 patients have recently had acute stroke, and 2 patients were suspect for oncologic pathology. In 65.3% patients aspirin was recommended.

In the future, through inclusion of some additional factors it may be possible to determine a more exact rule for appreciation of thromboembolic risk. For example, systolic dysfunction of the left ventricle found at trans-thoracic 2D Echo-CG is an independent risk factor for stroke in AF [15]. It is also probable that hormonal substitutive therapy [16] and smoking [15] increase risk for stroke in AF, while moderate alcohol consumption reduces it [16]. Finally, the next studies will determine whether the biologic markers of inflammation (for ex. C-reactive protein) or endothelial dysfunction (for ex. von Willebrand factor) could help clinicians in risk prediction in the population with AF and ease the decision to initiate oral anticoagulant therapy.

In conclusion, despite of the limitations of a small retrospective single-centered trial, our data show that anticoagulant therapy is insufficiently used in patients with AF and high thromboembolic risk, in Republic of Moldova. The reasons are not clear, but in practical conditions, medical science based on evidence is not always applicable. Recent data confirm the benefits of anticoagulation in conditions outside of trials. Therapy with warfarin needs to be considered in all patients with AF and high thromboembolic risk, and advanced age itself should not be referred to as an absolute contraindication for oral anticoagulation.

### References


1. Singer DE, Albers GW, Dalen JE, et al. Antithrombotic therapy in atrial fibrillation: the Seventh ACCP Conference on antithrombotic and Thrombolytic Therapy. *Chest*. 2004;126(supply):429S- 456S.
2. Hart RG, Pearce LA, Aguilar MI. Meta-analysis: antithrombotic therapy to prevent stroke in patients who have nonvalvular atrial fibrillation. *Ann Intern Med*. 2007;146:857–867.
3. Steger C, Tratter A, Marinek-Bregel M, et al. Stroke patients with atrial fibrillation have a worse prognosis than patients without: data from the Austrian stroke registry. *Europ. Heart J.* 2004;25:1734-40.
4. Jayaraman C, Fisher R, Friedman P, et al. Atrial Fibrillation, Stroke and Anticoagulant Use. *Heart Lung and Circulation*. 2004;13:252-255.
5. Wolf P, Abbot R, Kannel W. Atrilal fibrillation as an independent risk factor for stroke. The Framingham Study. *Stroke.* 1991;22:983-8.
6. Atrial Fibrillation Investigators. Risk factors for stroke and efficacy of antithrombotic therapy in atrial fibrillation. Analysis of pooled data from five randomized controlled trials. *Arch Intern Med.* 1994;154:1449–57.
7. Yoshida M, NakamuraY, Higashikawa M, et al. Predictors of ischemic stroke in non-rheumatic atrial fibrillation. *J. of Cardiology*. 1996;56:61-70.
8. Kalra L, Yu G, Perez I, et al. Prospective cohort study to determine if trial efficacy of anticoagulation for stroke prevention in atrial fibrillation translates into clinical effectiveness. *Br Med J*. 2000;320:1236–9.
9. Jackson S, Peterson G, Vial J, et al. Outcomes in the management of atrial fibrillation: clinical trial results can apply in practice. *Intern Med J*. 2001;31:329-36.
10. Hughes M, Lip GY. Stroke and thromboembolism in atrial fibrillation: a systematic review of stroke risk factors, risk stratification schema and cost effectiveness data. *Thromb Haemost*. 2008;99:295–304.
11. Stroke in AF working group. Independent predictors of stroke in patients with atrial fibrillation: a systematic review. *Neurology*. 2007;69:546–554.
12. Brott T, Thalinger K, Hertzberg V. Hypertension as a risk factor for spontaneous intracerebral hemorrhage. *Stroke*. 1986;17:1078-1083.
13. Fang MC, Go AS, Hylek EM, et al. Age and the risk of warfarin-associated hemorrhage: the anticoagulation and risk factors in atrial fibrillation study. *J Am Geriatr Soc*. 2006;54:1231–1236.
14. Singer DE, Albers GW, Dalen JE, et al. Antithrombotic therapy in atrial fibrillation: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). *Chest*. 2008;133:546S–592S.
15. Gage BF, Walraven C, Pearce L, et al. Selecting patients with atrial fibrillation for anticoagulation. *Circulation*. 2004;110:2287-2292.
16. Hart RG, Pearce LA, McBride R, et al. Stroke Prevention in Atrial Fibrillation (SPAF) Investigators. Factors associated with ischemic stroke during aspirin therapy in atrial fibrillation. Analysis of 2012 participants in the SPAF I-III clinical trials. *Stroke.* 1999;30:1223–9.

# Combined Tamiflu and BioR Treatment in Patients with H1N1 Influenza

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**Abstract**

A comparative study of combined Tamiflu and BioR treatment has been performed in 22 patients with (H1N1) influenza A (the experimental lot) and 17 patients (the control lot) in which only the Tamiflu was administered. The diagnosis of influenza A (H1N1) was confirmed using PCR. The clinical symptomatology varied amongst patients, but the most frequent symptoms were fever, throat pains asthenia, myalgias, hyperemia of the pharyngeal isthmus, dry cough, harsh respiration, chills, and nasal congestion. Tamiflu was administered in a dose of 75 mg orally twice a day, in the morning and in the evening; the average length of treatment was 5 days. BioR was administered to 16 patients as 5.0 mg orally, twice a day, and to 6 patients as1 mg IM, twice a day; average length of treatment was 5 days for all groups. The control group received Tamiflu only, the same dosage as the study group. The average length of treatment was 6.0 days. Comparing the length of the symptoms in the experimental group to that in the control group, we found that duration of symptoms reflecting the influence of the central nervous system in the experimental group was on average 5.0 days as compared to 6.6 days in the control group. Similarly, duration of symptoms affecting the respiratory system in the experimental group was 3.8 days and in the control group 5.3 days. We conclude that the Tamiflu and BioR treatment in patients with influenza A (H1N1) was beneficial and contributed to the decrease of symptom duration as compared to the group of patients treated with Tamiflu alone.

**Key words**: A (H1N1) influenza, Tamiflu, BioR, treatment.

**Комбинированное лечение Тамифлу и БиоР у больных гриппом А (H1N1)**

Было изучено эффективность комбинированного лечения Тамифлу и БиоРом у 22 больных гриппом А (H1N1), экспериментальная группа, и 17 больных, контрольная группа, которые получали только Тамифлу. Диагноз гриппа А (H1N1) был установлен биомолекулярным методом (ПЦР). Клиническая симптоматология была многообразная, но самые частые симптомы, которые встречались у обеих групп, были: лихорадка, боли в горле, слабость, боли в мышцах, гиперемия зева, сухой кашель,ознобы и гиперемия лица. Тамифлу был назначен в дозе 75 мг 2 раза в день утром и вечером, длительность лечения в среднем составляло 5,0 дней. БиоР был назначен в дозе 5 мг 2 раза в день перорально у 16 больных, и по 1,0 мг в/м 2 раза в день у 6 больных. Длительность лечения составила 5 дней. В контрольной группе был назначен только Тамифлу в такой же дозировке как в первой группе. Длительность лечения составила 6 дней. При сравнении длительности симптомов в экспериментальной и контрольной группе было отмечено, что симптомы характерные для поражения нервной системы сохранялись в среднем 5,0 дней, а в контрольной группе 6,6 дней, а симптомы поражения верхних дыхательных путей соотвественно – 3,8 и 5,3 дней. Комбинированное лечение Тамифлу и БиоРом в сравнении с лечением только Тамифлу привело к сокращению длительности клинической симптоматики и к уменьшению периода госпитализации больных гриппом и больных бронхопневмонией.

**Ключевые слова**: Грипп А (H1N1), Тамифлу, БиоР, лечение.

## Introduction

In April 2009 cases of contagious, acute respiratory disease in the USA (South California and Texas) were first registered as an influenza A virus of a new type called H1N1. The new virus appeared suddenly and was simultaneously identified in 2 other countries, Mexico and Canada.

The situation with the infection of a new viral influenza A (H1N1) developed rapidly, affecting in a short period of time a great number of people from all continents. These events forced the WHO to raise on 11 June 2009 the level of pandemic alert from place 5 to 6, which meant the beginning of the first influenza pandemia in the 21st century.

Influenza, as well as other respiratory diseases, generates important economical losses every year, with associated costs of medical care, decreased ability to work throughout the duration of infection – either of an affected adult or one taking care of an affected child. The greatest part of expenditures of medical care constitutes the hospitalization costs. About 14.6 billion dollars are spent every year throughout the world to treat influenza and its complications. A single person, in turn, can spend the equivalent of 30-100 Moldovan den on the influenza infection, covering the cost of treatment post influenza complications – which, depending on severity, can easily amount to a cost of $100 American dollars.

For effective results, influenza treatment should be started as soon as possible within 2 days from the first signs of influenza. Tamiflu (oseltamivir) has been shown to be the most efficient, reducing the complications rate by 55%. It acts as a

neuraminidase inhibitor, preventing cleavage of budding viral progeny and instead fixing the virus to the host cell.

The toxic action of the influenza virus includes inhibition of cellular and humoral immunity, resulting in diminished the resistance. The native preparations such as BioR are of a particular interest which have a large spectrum of action: immunomodulatory, immunostimulatory, antiviral, cytoprotector and regenerant.

Due to these aspects we designed a study with BioR to evaluate its role as a therapeutic agent in influenza treatment.

## Material and Methods

The study included 22 patients (the study group) from 19 - 68 years of age (the average age was 33.6 ± 0.9) comprised of males – 10 (45.5%), females – 12 (54.5%). There was an equal split between patients residing in urban vs rural areas. Twelve patients were addressed to the family doctor and/or emergency personal and ten presented independently to the admission rooms of CHID- "T. Ciorba". Eight patients were admitted in the first 2 days, eleven on days 3- 5, two on days 7- 8 and one on the 13$^{th}$ day. The length of hospitalization was 5 days for 20 patients, 8 days for 1 and 9 days for 1 patient (the average length was 5.09 ± 0.01 days).

17 patients were enrolled into the control group, they were from 19 - 51 years of age (the mean age was 25.6 ± 1.2), comprised of men - 8 (47.1%), women – 9 (52.9%). Eight were from an urban area (47.1%), and 9 (52.9%) patients were from a rural area ($p > 0.05$). Four patients were initially seen by their family doctor, nine by the emergency medical personal, and five patients came independently to the admission room at "T. Ciorba". 12 patients were seen in the first day of the disease, the other five patients were admitted on the 10$^{th}$ day. The hospitalization length was 5 days for 9 patients, 6 days for 2, 7 days for 4, 9 days for 1 and 10 days for 1 patient (the mean length of hospitalization was 6.1 days). The diagnosis of influenza A (H1N1) was confirmed by PCR in all of the patients included in the study. In the control group, PCR confirmed results were available on the first day of the disease in 1 patient, on the 2$^{nd}$ day in 4 patients, on the 3$^{rd}$ day in 4, on the 7$^{th}$ day in 5, on the 6$^{th}$ day in 2 and on the 9$^{th}$ day in 1 patients. In the experimental group, PCR confirmed H1N1 on the 1$^{st}$ day in 1 patient, on the 2$^{nd}$ day in 4, on the 3$^{rd}$ day in 3, on the 4$^{th}$ day in 5, on the 6$^{th}$ day in 5, on the 7$^{th}$ day in 2 and on the 9$^{th}$ day in 1 patient).

**Table 1**

**Clinical symptomatology in patients of A (H1N1) influenza the study lot and control lot**

| Symptoms | The study group 22 patients | | | The control group 17 patients | | | $\chi^2$ | p |
|---|---|---|---|---|---|---|---|---|
| | Abs. | P1±Es1 | The length of symptoms | Abs. | P2±Es2 | The length of symptoms | | |
| Cephalagia | 18 | 81,8±8,2 | 4,5 | 11 | 64,7±11,6 | 6,4 | 4,53 | ** |
| Ocular pains | 6 | 27,3±9,5 | 3,7 | 3 | 17,6±9,2 | 4,2 | 5,25 | ** |
| Myalgias | 13 | 59,1±10,5 | 5,0 | 7 | 41,2±11,9 | 5,6 | 7,79 | *** |
| Arthralgias | 8 | 36,4±10,3 | 4,2 | 4 | 23,5±10,3 | 4,8 | 7,00 | *** |
| Asthenia | 10 | 45,5±10,6 | 6,8 | 14 | 82,4±9,2 | 7,7 | 16,53 | **** |
| Chills | 8 | 36,4±10,3 | 3,4 | 5 | 29,4±11,1 | 3,2 | 1,64 | * |
| Fever < 38°C | 9 | 40,9±10,5 | 7,6 | 6 | 35,3±11,6 | 7,5 | 0,89 | * |
| Fever > 38°C | 13 | 59,1±10,5 | 5,0 | 11 | 64,7±11,6 | 6,2 | 0,49 | * |
| Nasal congestion with choriza | 3 | 13,6±7,3 | 4,3 | 8 | 47,1±12,1 | 5,2 | 23,74 | **** |
| Throat pain | 16 | 72,7±9,5 | 4,2 | 12 | 70,6±11,1 | 4,8 | 0,06 | * |
| Fascies tumefied | 1 | 4,5±4,4 | 1,0 | 5 | 29,4±11,1 | 4,8 | 21,02 | **** |
| Fascies congested | 8 | 36,4±10,3 | 3,2 | 7 | 41,2±11,9 | 4,0 | 0,56 | * |
| Injected scleare | 2 | 9,1±6,1 | 4,5 | 5 | 29,4±11,1 | 4,8 | 14,04 | **** |
| Lacrimations | 2 | 9,1±6,1 | 3,5 | 6 | 35,3±11,6 | 4,6 | 19,45 | **** |
| Hyperemia of the pharynx | 20 | 90,9±6,1 | 5,9 | 17 | 100,0±0,0 | 6,2 | 0,83 | * |
| Chest pains | 10 | 45,5±10,6 | 3,0 | 2 | 11,8±7,8 | 4,0 | 96,48 | **** |
| Dyspnoea | 3 | 13,6±7,3 | 6,2 | 17 | 100,0±0,0 | 8,6 | 74,59 | **** |
| Dry rales | 6 | 27,3±9,5 | 4,2 | 5 | 29,4±11,1 | 6,8 | 0,16 | * |
| Moist rales | 4 | 18,2±8,2 | 8,0 | 2 | 11,8±7,8 | 8,0 | 3,50 | * |
| Crepitant rales | 3 | 13,6±7,3 | 3,3 | 1 | 5,9±5,7 | 6,0 | 10,22 | *** |
| Harsh breathing | 17 | 77,3±8,9 | 5,7 | 10 | 58,8±11,9 | 6,6 | 5,79 | ** |
| Attenuated breathing | 3 | 13,6±7,3 | 3,0 | 1 | 5,9±5,7 | 4,0 | 10,22 | *** |
| Nausea | 4 | 18,2±8,2 | 2,5 | 5 | 29,4±11,1 | 2,6 | 4,29 | ** |
| Vomiting | 5 | 22,7±8,9 | 2,0 | 4 | 23,5±10,3 | 2,7 | 0,03 | * |
| Watery diarrhea | 1 | 4,5±4,4 | 1,0 | 3 | 17,6±9,2 | 1,5 | 9,73 | *** |
| Tachycardia | 5 | 22,7±8,9 | 3,5 | 3 | 17,6±9,2 | 4,3 | 1,46 | * |
| Bronchopneumonia | 5 | 22,7±8,9 | 6,2 | 5 | 29,4±11,1 | 6,6 | 1,52 | * |
| Bronchitis | 18 | 81,8±8,2 | - | 4 | 23,5±10,3 | - | 144,40 | **** |
| Changes of the ECG | 4 | 18,2±8,2 | - | 1 | 5,9±5,7 | - | 25,72 | **** |

$p > 0.05$; **$p < 0.05$; ***$p < 0.01$; ****$p < 0.001$.

### Results and discussions

This study evaluated a wide range of clinical symptomatology in the enrolled patients, presented in tab. 1.

Analysing table 1, we can see multiple clinical symptoms in both groups, affecting the central nervous system, respiratory and cardiovascular systems. The most frequent symptoms were fever(100% in the first lot and 100% in the second one), throat pains (72,7% and 70,6%), asthenia (45,5 % and 82,4%) myalgias (59,1% and 41,2%), hyperemia of the pharyngeal isthmus (90,9 % and 100 %), harsh breathing (77,3 % and 58,8%), chills (36,4 % and 29,4%), fascies congested (36,4% and 41,2%).

When comparing the length of clinical symtoms in the study group and the control group we come to the conclusion that the length of the symptoms reflecting the affected central nervous system in the study group was on average 5,0 days, while in the control the average was of 6,6 days. With respect to the symptoms affecting the respiratory system, duration was 3,8 days in the experimental grouo and 5,3 in the control group.

Bronchopneumonia occurred at the same frequency in both groups in 5 patients. The length of hospitalization with bronhopneumonia in the experimental group was on average 5,2 days and in the control group 7,0 days.

The changes in leukogram are listed in table 2.

According to the data from table 2, leukocytosis is not characteristic for viral infections and leukopenia was revealed only in 5 patients from the experimental group and in one patient from the control group. Normocytosis was revealed more frequently (77.3 % and 94.1 %), left deviation (95.4 % and 76.7%) and monocytosis (45.4 % and 47.1%).

### Treatment

Treatment of patients from the study group was an antiviral, Tamiflu, combined with an immunomodulatory BioR. Treatment for the control group was with Tamiflu and placebo.

Tamiflu was administered in a dose of 75 mg orally twice a day, in the morning and in the evening after meals. The duration of treatment was 5 days in 15 patients, 6 days in 5 patients, 9 in 1 patients and 10 days for 3 patients. The average length of treatment was 5 days.

BioR was administered in a dose of 5 mg orally twice a day in the morning and in the evening in 16 patients and 1 ml. IM twice a day for 5 days in 6 patients. The patients from control group were given the Tamiflu only in a dose of 75 mg orally twice a day in the morning and in the evening. The duration of treatment was 5 days for 9 patients, 6 days for 4 patients, 7 days for 2 and 10 days for 1 patients the average being 6 days.

Taking into account that the toxic action of influenza virus inhibits both cellular and humoral immunity, leading to attenuation of local resistance and increased susceptibility to infection with bacterial foci in trachea, bronchi and lungs, an antibiotic treatment was administrated as shown in tab. 3.

According to the information in table 3, cephalosporins, macrolides, B-lactams and fluoroquinolones were used in the treatment of patients with the influenza virus. Only one patient

**Table 2**

**The changes in leukogram in patients of A (H1N1) influenza, the study lot and control lot**

| The leukogram | The study lot | | | Control lot | | | $\chi^2$ | p |
|---|---|---|---|---|---|---|---|---|
| | Abs. | P1 ± Es1 | Media | Abs. | P2 ± Es2 | Media | | |
| Leukopenia | 5 | 22,7 ± 8,9 | 3,1 | 1 | 5,9 ± 5,7 | 3,9 | 48,24 | **** |
| Leukocytosis | 0 | 0,0 | 0 | 0 | 0,0 | 0 | - | - |
| Normocytosis | 17 | 77,3 ± 8,9 | 5,9 | 16 | 94,1 ± 5,7 | 5,6 | 3,01 | * |
| Left deviation | 21 | 95,5 ± 4,4 | 22,0 | 13 | 76,5 ± 10,3 | 19,6 | 4,71 | ** |
| Lymphocytosis | 5 | 22,7 ± 8,9 | 42,5 | 5 | 29,4 ± 11,1 | 56,8 | 1,52 | * |
| Lymphopenia | 5 | 22,7 ± 8,9 | 11,5 | 0 | 0,0 | 0 | - | - |
| Monocytosis | 10 | 45,5 ± 10,6 | 16,1 | 8 | 47,1 ± 12,1 | 17,3 | 0,05 | * |
| VSH increased | 7 | 31,8 ± 9,9 | 30,4 | 7 | 41,2 ± 11,9 | 24,2 | 2,13 | * |

$p > 0.05$ **$p < 0,05$ **** $p < 0.001$.

**Table 3**

**Antibiotic treatment in the study and control groups for patients with A (H1N1) influenza**

| Antibiotics | The study lot | | | Control lot | | |
|---|---|---|---|---|---|---|
| | No of patients | Dose | Duration of treatment | No of patients | Dose | Duration of treatment |
| Cephalosin | 4 | 1,0x2 td | 3,5 days | 3 | 1,0x3 td | 5,6 zile |
| Ceptriaxin | 4 | 1,0x3 td i.m. | 7,5 days | 1 | 0,25x2 td | 2,0 |
| Cephexim | 1 | 1,0x2 td | 4,0 days | - | - | - |
| Cepin | 2 | 1,0x2 td | 4,5 days | - | - | - |
| Azitromycin | 4 | 500,0x1 td | 3,0 days | - | - | - |
| Ampicillin | 3 | 1,0x3 td | 6,0 days | 1 | 150mgx3 td | 4,0 |
| Amoxicillin | 5 | 1,0x2 td | 4,5 days | 10 | 1,0x3 td | 4,0 |
| Augumentin | 2 | 1,2x2 td | 4,5 days | - | - | - |
| Ciprinol | 1 | 400mgx2 td | 4,0 days | - | - | - |
| Oxacyllin | - | - | - | 1 | 0,4x4 td i.m. | 5.0 |

from the control group was not administered antibiotic treatment. The length of antibiotic treatment in the study group was 4.0 while in the control group it was 4.5 days.

Pathogenic and symptomatic treatment

Both groups received some maintenance therapy. In 9 patients this included glucose 5%, physiological serum 0.9%, haemodesia and arginine in 1 patient, antipyretics in 15 patients, vitamins (ascorutin) in 29 patients, desensitizers in 15 patients, expectorants in 6 patients, broncholytics in 7, antitussives in 8, respiratory analeptics in 6, vasoconstrictive decongestants in 8, diuretics and corticosteroids in one patient for a day.

### Conclusions

Treatment with Tamiflu and BioR in patients with A (H1N1) influenza was beneficial and contributed to:

- Reduction by an average of one day in the length of symptoms that affected the central nervous system, and particularly those reflecting the action of the sympathetic nervous system,
- Reduction of symptoms affecting the respiratory system (3.8 days in the experimental group and 5.3 days in the control one).
- Decreased hospitalization length in patients with influenza A (H1N1) (5.09 days in the experimental group and 6.1 in the control group).
- Decreased hospitalization length of patients with bronchopneumonia in the experimental group (5.2 days vs 7 days)

### Bibliography


1. Spînu C, Scoferța P, Romancenco E, ș. a. Infecția cu virusuri gripale umane: aspecte epidemiologice, clinice, de laborator, tratament și profilaxie: ghid practic: Vol. 1. Chișinău, 2009;99.
2. Spînu C, Scoferța P, Romancenco E, ș. a. Gripa aviară: aspecte epidemiologice, clinice, de laborator, tratament și profilaxie: ghid practic: Vol. 2. Chișinău, 2009;91.
3. Jhung M, Swerdlow D, Olsen S, et al. Epidemiology of 2009 pandemic influenza A (H1N1) in the United States. *Clin Infect Dis.* 2011;52(Suppl 1):S13–S26.
4. CDC. Behavioral Risk Factor Surveillance System operational and user's guide. 2006. Atlanta, GA: US Department of Health and Human Services, CDC; 2006. Available at ftp://ftp.cdc.gov/pub/data/brfss/userguide.pdf. Accessed January 14, 2011.
5. Belongia EA, Irving SA, Waring SC, et al. Clinical characteristics and 30-day outcomes for influenza A 2009 (H1N1), 2008-2009 (H1N1), and 2007-2008 (H3N2) infections. JAMA. 2010;304(10):1091-8.
6. Graitcer SB, Gubareva L, Kamimoto L, et al. Characteristics of Patients with Oseltamivir-Resistant Pandemic (H1N1) 2009, United States. Emerg Infect Dis. 2011;17(2):255-257.
7. Siston AM, Rasmussen SA, Honein MA, et al. Pandemic 2009 influenza A(H1N1) virus illness among pregnant women in the United States. JAMA. 2010;303(15):1517-25.


# Stem Cells in the Future of Dental Care


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### Abstract

Tooth loss compromises human oral health. Although several prosthetic methods (such as artificial denture and dental implants) are clinical therapies to tooth loss problems, they are thought to have safety and usage-time issues. Recently, tooth tissue engineering has attracted more and more attention. Stem cell based tissue engineering is thought to be a promising way to replace the missing tooth. Mesenchymal stem cells (MSC) are multipotent stem cells which can differentiate into a variety of cell types. The potential MSC for tooth regeneration mainly include stem cells from human exfoliated deciduous teeth, adult dental pulp stem cells, stem cells from the apical part of the papilla, stem cells from the dental follicle, periodontal ligament stem cells and bone marrow-derived mesenchymal stem cells. This review outlines the recent progress in mesenchymal stem cell research and its use in tooth regeneration and oral and craniofacial applications.


**Key words:** mesenchymal stem cell, tooth engineering, dental pulp stem cell.

## Стволовые клетки в стоматологии будущего

Потеря зубов ставит под угрозу человеческое здоровье. Ткани зуба у взрослых практически не способны к самостоятельной регенерации и дефект эмали, возникающий в результате действия повреждающих факторов, постепенно приводит к потере зуба. Без сомнения, современные технологии протезирования позволяют произвести реконструкцию даже при полном отсутствии зубов. Однако прогресс современной органотипической регенеративной медицины заставляет исследователей искать новые технологии замещения зубов естественными трансплантатами. Последнее время клеточная инженерия тканей зуба привлекает все больше и больше внимания. Стволовые клетки являются многообещающим способом замены недостающего зуба. Мезенхимальные стволовые клетки способны дифференцироваться в клетки костной ткани, что дает возможность использовать их для восстановления зуба. Этот обзор рассматривает современные исследования стволовых клеток и возможность их использования для стимуляции репаративной регенерации тканей зуба.

**Ключевые слова**: мезенхимальная стволовая клетка, инженерия тканей зуба, стволовая клетка пульпы.

### Introduction

What are stem cells? As dentists, why should we be concerned with stem cells? How would stem cells change dental practice? Is it possible to grow a tooth by tissue engineering using stem cells? What should be the carrier material for stem cells? Probably, development of stem cell research will, over time, transform dental practice in a magnitude far greater than did dental implants. Metallic alloys, composites and even titanium implants are not permanent solutions. In contrast, stem cell technology will generate native tissue analogs that are compatible with that of the patient's. Dental implants are not the perfect solution for replacing missing teeth as the healing process extends for many months and rejection of the implant occurs in about 5 percent of patients. Furthermore, dental implants are expected to last for about 15 years [1]. Despite much advancement in implant technology conventional implants do not provide a truly permanent solution for a missing tooth. But the answer could lie in a highly researched new dental technique – dental implants based on stem cell technologies which could be the future of implant dentistry.

Stem cells in dental pulp were discovered in 2000 by Dr. Songtao Shi, a dental researcher at the National Institute of Health (NIH). After verification that these cells were in fact viable stem cells, the NIH announced the discovery in 2003.

The dentists treat patients because of infections, trauma, congenital anomalies or other diseases, such as orofacial cancer and salivary gland disorders. Caries and periodontal disease remain highly prevalent disorders among humans. Whereas native tissue is missing in congenital anomalies, diseases such as caries or tumor resection result in tissue defects. For centuries, dentistry has been devoted to healing defects with durable materials or the patient's own (autologous) tissue. But we now realize that metallic alloys or synthetic materials are not permanent solutions. Amalgam, composites and even titanium dental implants can fail; and all have limited service time (Rahaman A., Mao J., 2005). Why are stem cells better than durable implants such as titanium dental implants? Stem cells lead to the regeneration of teeth with periodontal ligament that can remodel to the host. Why are stem cells superior to autologous tissue grafts? Autologous tissue grafting is based on the concept that a diseased or damaged tissue must be replaced by like tissue that is healthy. Thus, the key drawback of autologous tissue grafting is donor site trauma and morbidity; the harvest of healthy bone from the patient could be taken from the iliac crest, rib bone, chin or retromolar area for bone grafting needs in cleft palate, ridge augmentation, sinus lifting, and maxillary and mandibular reconstruction. In contrast, stem cell-based therapeutic approaches may circumvent the key deficiencies of autologous bone grafting (Rahaman A., and Mao J., 2005). Stem cells from a tiny amount of tissue, such as the dental pulp, can potentially be multiplied or expanded to sufficient numbers for healing large, clinically relevant defects. Stem cells can differentiate into multiple cells lineages, thus providing the possibility that a common (stem) cell source can heal many tissues in the same patient, as opposed to the principle of harvesting healthy tissue to heal like tissue in association with autologous tissue grafting (Moioli E. K., et al., 2007).

Stem cells can be seeded in biocompatible scaffolds in the shape of the anatomical structure that is to be replaced. Stem cells may elaborate and organize tissues in vivo, especially in the presence of vascularisation. Finally, stem cells may regulate local and systemic immune reactions of the host in ways that favor tissue regeneration. Physicians and scientists have recommended that umbilical cord stem cells, placental and amniotic fluid stem cells could be banked for potential application in the treatment of trauma and pathological disorders [19].

The understanding of mesenchymal stem cells in the tissue engineering of dental, oral and craniofacial structures has advanced tremendously (Marion N., Mao J., 2006). We have witnessed tissue engineering of the tooth, temporomandibular joint condyle, cranial sutures, soft tissue grafts, craniofacial bone and other structures in animal models. With all that we have learned about stem cells and tissue engineering of dental, oral and craniofacial structures, we are in a position to bring awareness to our patients regarding the proper storage of their extracted teeth in conditions that will preserve craniofacial stem cells, including tooth-derived stem cells. These include, but are not limited to, extracted wisdom teeth, deciduous teeth and any teeth extracted for orthodontic purposes and any non-infected teeth extracted. Among postnatal tissues that are sources of stem cells that are obtainable without substantial trauma are extracted wisdom teeth, exfoliating or extracted deciduous teeth, teeth extracted for orthodontic treatment, trauma or periodontal disease. Craniofacial stem

cells, including tooth-derived stem cells, have the potential, as do bone marrow-derived stem cells and adipose-derived stem cells, to cure a number of diseases that are relevant to dentistry as well as for medicine: diabetes, Parkinson's disease, cardiac infarct etc.

Stem cells can be defined as self-replicating cells that are able to differentiate into at least two different cell types. Both conditions must be present for a cell to be considered a stem cell. For example, osteoblasts are not stem cells. Although osteoblasts differentiate into osteocytes, they typically do not differentiate into other cell types except osteocytes. Osteocytes are not stem cells; they are end-lineage cells that typically neither self-replicate and not differentiate in to another cells type [1].

**Mesenchymal stem cells (MSC)**

(MSC) can be isolated from different sources. First described in bone marrow, MSC have been extensively characterized *in vitro* by the expression of markers such as STRO-1, CD146 or CD44. STRO-1 is a cell surface antigen used to identify osteogenic precursors in bone marrow, CD146 a pericyte marker, and CD44 a mesenchymal stem cell marker. MSC possess a high self-renewal capacity and the potential to differentiate into mesoderm lineages thus forming cartilage, bone, adipose tissue, skeletal muscle and the stroma of connective tissues. The potential of dental MSC for tooth regeneration and repair has been extensively studied in the last years. Below, we will present the mesenchymal progenitors that have been assessed for tooth engineering purposes, such as progenitors derived from teeth (adult dental pulp, apical part of papilla, dental follicle, periodontal ligament) (fig. 1) and bone marrow [2].

**Stem cells from human exfoliated deciduous teeth (SHED)**

The isolation of post-natal stem cells from an easily accessible source is indispensable for tissue engineering and clinical applications. Recent findings demonstrated the isolation of mesenchymal progenitors from the pulp of human deciduous incisors. These cells were named SHED (Stem cells from Human Exfoliated Deciduous teeth) and exhibited a high plasticity since they could differentiate into neurons, adipocytes, osteoblasts and odontoblasts. *In vivo* SHED cells can induce bone or dentin formation but, in contrast to dental pulp, DPSC failed to produce a dentin-pulp complex [3].

**Adult dental pulp stem cells (DPSC)**

The possibility that tooth pulp might contain mesenchymal stem cells was first suggested by the observation that severe tooth damage that penetrates both enamel and dentine into the pulp stimulates a limited natural repair process, by which new odontoblasts are formed, which produce new dentine to repair the lesion (Smith A. J., Lesot H., 2001). Putative stem cells from the tooth pulp and several other dental tissues have now been identified. The first stem cells isolated from adult human dental pulp were termed dental pulp stem cells (DPSCs) [1]. They were isolated from permanent third molars, and exhibited high proliferation and high frequency of colony formation that produced sporadic, but densely calcified nodules. Additionally, *in vivo* transplantation into immunocompromised mice demonstrated the ability of DPSCs to generate functional dental tissue in the form of dentine/pulp-like complexes [2]. Further characterization revealed that DPSCs were also capable of differentiating into other mesenchymal cell derivatives *in vitro* such as odontoblasts, adipocytes, chondrocytes and osteoblasts (Koyama N., et al., 2009). DPSCs differentiate into functionally active neurons, and implanted DPSCs induce endogenous axon guidance, suggesting their potential as cellular therapy for neuronal disorders (Arthur A. et al., 2009).

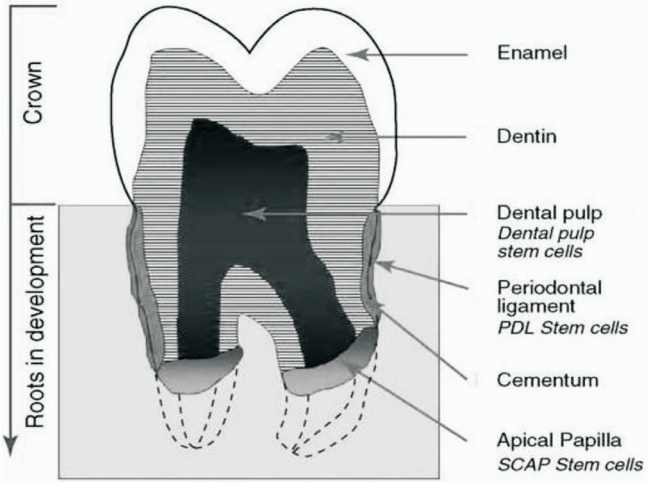


**Fig. 1. Diagram of a human third molar as a source of dental stem cells. Because the tooth was in the process of erupting, root growth is incomplete, and the apical papilla is visible.**

**Stem cells from the apical part of the papilla (SCAP)**

Recently another type of MSCs was discovered in the apical papilla of human immature permanent teeth termed stem cells from apical papilla (SCAP) (Wataru Sonoyama, Yi Liu, Takayoshi Yamaza, 2008). We found that apical papilla is distinctive to pulp in terms of containing less cellular and vascular components than those in pulp. Cells in apical papilla proliferated 2- to 3-fold greater than those in pulp in organ cultures. Both SCAP and DPSCs were as potent in osteo/dentinogenic differentiation as MSCs from bone marrows while weaker in adipogenic potential. The immunophenotype of SCAP is similar to that of DPSCs on the osteo/dentinogenic and growth factor receptor gene profiles. Double staining experiments showed that STRO-1 co-expressed with dentinogenic markers such as bone sialophosphoprotein (BSP), osteocalcin (OCN) and growth factors FGFR1 and TGFβRI in cultured SCAP. Stem cells from the apical part of the human dental papilla (SCAP) have been isolated and their potential to differentiate into odontoblasts was compared to that of the periodontal ligament stem cells (PDLSC). SCAP exhibit a higher proliferative rate and appears more effective than PDLSC for tooth formation. Importantly, SCAP are easily accessible since they can be isolated from human third molars.

**Stem cells from the dental follicle (DFSC)**

DFSC have been isolated from follicle of human third molars and express the stem cell markers Notch1, STRO-1

and nestin. The dental follicle is a loose of ectomesenchyme-derived connective tissue sac surrounding the enamel organ and the dental papilla of the developing tooth germ before eruption (Ten Cate, 1998). It is believed to contain progenitors for cementoblasts, PDL and osteoblasts. Dental follicle cells (DFC) form the PDL by differentiating into PDL fibroblasts that secrete collagen and interact with fibres on the surfaces of adjacent bone and cementum. DFC can form cementoblast-like cells after transplantation into SCID mice (Handa K. et al., 2002). Dental follicle progenitor cells isolated from human third molars are characterized by their rapid attachment in culture, expression of the putative stem cell markers Nestin and Notch-1, and ability to form compact calcifiednodules *in vitro* (Lin N. H. et al., 2008). DFC were transplanted into immunocompromised mice, however, there was little indication of cementum or bone formation (Lin N. H. et al., 2008). DFC, in common with SCAP, represents cells from a developing tissue and might thus exhibit a greater plasticity than other dental stem cells. However, also similar to SCAP, further research needs to be carried out on the properties and potential uses of these cells.

**Periodontal ligament stem cells (PDLSC)**

The PDL is a specialized tissue located between the cementum and the alveolar bone and has the maintenance and support of the teeth as a role. Its continuous regeneration is thought to involve mesenchymal progenitors arising from the dental follicle. PDL contains STRO-1 positive cells that maintain certain plasticity since they can adopt adipogenic, osteogenic and chondrogenic phenotypes *in vitro*. It is thus obvious that PDL itself contains progenitors, which can be activated to self-renew and regenerate other tissues such as cementum and alveolar bone. It was shown that cultured PDLSCs proliferate in higher rate on the rough surface especially at the 75μm $Al_2O_3$ particle treated surface than other surfaces. Also, osteocalcin was highly expressed on the rough surfaces treated with 75μm and 125μm $Al_2O_3$ particles (Heo Y. Y., Um S., Kim S. K., Park J. M., 2011).

**Bone marrow derived mesenchymal stem cells (BMSC)**

BMSC have been tested for their ability to recreate periodontal tissue. These cells are able to form *in vivo* cementum, PDL and alveolar bone after implantation into defective periodontal tissues. Thus, bone marrow provides an alternative source of MSC for the treatment of periodontal diseases (Kawaguchi H., 2004). BMSC share numerous characteristics with DPSC and are both able to form bone-like or tooth-like structures. However, BMSC display a lower odontogenic potential than DPSC (Yu J. et al., 2007), indicating that MSC from different embryonic origins are not equivalent. Indeed, DPSC derive from neural crest cells, whereas BMSC originate from the mesoderm. Furthermore, the comparison of the osteogenic and adipogenic potential of MSC from different origins shows that, even if cells carry common genetic markers, they are not equivalent and are already committed toward a specific differentiation pathway (Musina R. A. et al., 2006). Commitment could arise from conditioning of stem cells by their specific microenvironment or stem cell niche.

**Tissue engineering**

There are several areas of research for which dental stem cells are currently considered to offer potential for tissue regeneration. These include the obvious uses of cells to repair damaged tooth tissues such as dentine, periodontal ligament and dental pulp [16]. Even enamel tissue engineering has been suggested (Honda M. J. et al., 2009), as well as the use of dental stem cells as sources of cells to facilitate repair of non-dental tissues such as bone and nerves (Graziano A. et al., 2008).

The overall goal of tissue engineering is the functional restoration of tissue structures as well as the maintenance of the natural environment, and thus the viability and function of the damaged tissue due to disease or trauma. In this context, dental replacement in clinical applications depends on the use of a potential material which would be anti-inflammatory, antibacterial and can simultaneously enhance the proliferation and induce the differentiation of present DPSC into odontoblast-like cells leading to dentin formation (Nakashima M., Reddi A. H., 2003). Because of the similarities between dentin and bone structures, studies are often performed in dental tissue engineering in dependence on or in comparison to bone formation processes and applied osteoinductive materials. From a tissue engineering point of view it is noteworthy that there are differences between bone formation and a potential dentin formation as well. Different approaches, which are also under investigation for maxillofacial surgery and partly for tooth tissue regeneration, can already be performed for bone reconstruction, such as: 1) An autologous graft from various donor regions comprising bone forming cells and growth factors and therefore being osteogenetic (Kneser U., Schaefer D. J., Polykandriotis E., Horch R. E., 2006); 2) An allograft and xenograft, respectively, i. e. a bone sample from other human beingsor from animals, which is osteoinductive due to certain proteins like growth factors (Richardson C. R., Mellonig J. T., Brunsvold M. A.); 3) Various osteoinductive biomaterials acting as carriers for growth factors inducing bone formation (Spiro R. C., Liu L. S., Heidaran M. A., Thompson A. Y., 2000); 4) Synthetic bone substitutes for bone replacement without or with just partially resorption or for bone repair using osteoconductive porous devices.

The different autogeneic, xenogenetic and alloplastic bone replacement materials can be differentiated according to the functional quality of the new tissue and the dynamics of bone conversion thus induced. Comparing osteoconductive bone substitutes with demineralised, osteoinductive materials and autogenic bone grafts, bone inducing matrices show the largest quantity of new bone formation. In order to extrapolate the findings of bone to dentin repair, it is necessary to understand the dentin-pulp complex in more detail and in particular the challenging situation of the pulp itself especially in case of pulp healing and formation of reparative dentin.

***In vitro* studies, isolation and identification procedures of dental pulp cells**

The proper isolation of cells provides the potential to differentiate into odontoblast-like cells. A lot of experiments have shown that dental pulp cells can be isolated from human

impacted third molars (14-29 years of age), which are extracted for clinical reasons under anaesthesia [5, 6, 7]. Tooth surface were cleaned by covering with 0.3% chlorhexidine gel [8, 9], swabbed with 70% (v/v) alcohol [10] or dipped carefully in 30% hydrogen peroxide for 30 to 120 sec. Pulp was opened by cutting around the cementum enamel junction using sterilized dental fissure burs to reveal the pulp chamber [5]. Other studies describe that teeth were cracked opened, or opened by a dentinal excavator or a Gracey curette [7, 9]. After separation of the pulp tissue, cells can be isolated by various methods. Pulp cells can be either isolated by digestion or the out-grown method [5, 9]. First, the pulp tissue can be digested in a solution of collagenase type I and dispase as reported in details by Gronthos S., et al. 2002 [5, 9]. The cell suspension is then centrifuged and pellets are suspended in Dulbecco's modified Eagle's medium (DMEM). Single-cell suspensions can be obtained by passing the cells through 70 μm strainer and seeding into 6- well plates in DMEM supplemented with 10-20% FCS, 100 μM ascorbic acid 2-phosphates, 2 mM L-glutamine, 100 Units/ml penicillin and 100 μg/ml streptomycin [5, 11, 12]. Secondly, pulp tissue explants (4 mm) were placed in 6-well plates and designated as human pulp cells/out-grown method (HDPC-o). These cells were cultivated to confluence in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS and antibiotics [13]. Further, human pulp primary cultures (HPPc) could be obtained by mincing tissue fragments of extracted pulps into small pieces (< 1 mm), which were then placed in well plates containing RPMI 1640 medium-glutamax supplemented with 100 IU/ml penicillin, 100 μg/ml streptomycin, 10 μg/ml amphotericin-B and 10% FCS [6, 7]. All cultures were maintained in a humidified atmosphere of 95% air and 5% CO2 at 37°C and medium change should be performed every two days.

**Dental pulp cell cultivation**

In order to develop a potential biomaterial for dental pulp regeneration and reconstitution of a complete dentin-pulp-complex, the understanding of the proliferation as well as differentiation processes is indispensable. Hence, studying processes in dental regeneration using an *in vitro* dental pulp cell culture system can provide an insight into biological processes which lead to odontoblast-like cell differentiation and induced dentin matrix mineralization. Just based on a complete knowledge about *in vitro* dental pulp cell (DPC) behaviour and following in vivo experiments, conclusions can be drawn upon the requirements on the development of a highly suitable filling material. The following section demonstrates whether it is possible to isolate a potential cell population comprising DPSC, and furthermore the proliferation and differentiation ability has to be proven.

**Proliferation and differentiation**

Therefore, the first and critical step in order to investigate the proliferation and differentiation ability of these cells is the isolation of a suitable cell population. DPC and DPSC, respectively, have already been isolated from adult human teeth (14-29 years of age) [5, 7, 9, 10, 11, 12, 13, 16], pork [14] and rat dental pulp [15]. A further cell culture system was obtained from human exfoliated deciduous teeth (SHED) (6-10 years of age) [16, 9]. Miura M., et al. 2003 reasoned from his findings that SHED are distinct from DPSC because of a higher proliferation rate, increased cell-population doubling, and stem cell typical formation of spherical cell clusters and osteoinductive potential *in vivo.* However, these cells do not maintain the capacity to reconstitute a dentin-pulp-complex for which reason there remain only mentioned [16].

Currently, two isolation methods are performed in various reports to isolate DPSC by either enzyme digestion, or the out-grown method, as described before Huang et al. investigated whether cell isolation methods yield in the same pool of cell population. Although the out-grown method is more convenient and not as technically extensive as the enzymatic digestion, cells migrate out of the tissue fragments growing slower than human DPC obtained by digestion method until becoming confluent in 2-3 weeks [13]. Even enzymatic digestion may cause a cell damage; it allows different types of cells to form compact and loose types of colonies within 1-2 weeks, which can separately be characterized [5, 13]. All cell cultures display a wide range of cell morphology such as fibroblast-like cells, endothelial-like or epithelial-like cell populations. Gronthos S., 2002 have applied the enzyme digestion method and were able to demonstrate that dental pulp cells differentiated into odontoblast-like cells, which also formed dentin matrix *in vivo* [5, 18]. The out-grown method showed that cells are potentially capable to differentiate into odontoblasts or forming mineralized nodules *in vitro* [10, 11, 17]. Concerning the growth behavior and characterization ability of single cell colonies the digestion method seems to be more reasonable. Both methods demonstrated the ability to isolate cells containing a minor population of odontoblast precursor cells with typical criteria for postnatal somatic stem cells, such as their high rate of proliferation, clonogenic nature [5], and co-expression of specific markers.

Identification studies showed that DSPC express the cell surface antigen STRO-1, which is known to immunoselect osteogenic precursors in bone marrow stromal cells [5, 16, 7]. Alliot-Licht et al. investigated the effect of dexamethasone contained in the differentiation medium resulting in a significant increase of STRO-1 positive cell population in human DPSC [7]. Previous studies have demonstrated that isolated SHED cells proliferated *in vitro* contain approximately 9% of STRO-1 positive cell population [16].

These observations agree to that of Gronthos S., 2002 demonstrating a similar percentage of about 5-6% of the total pulp cell population. Further analysis revealed that DPSC express the perivascular cell marker CD146, but does not react with the hematopoietic markers CD14 (monocyte/macrophage), CD45 (leucocyte) or CD34 (hematopoietic stem cells/endothelium). To date there is no investigation published that demonstrates the effect of the applied isolation method on the yield of precursor cells in DPC. After providing the evidence to isolate stem/progenitor cells out of the dental pulp, proliferation studies have been described in various reports

and exhibit a high proliferation rate. The growth potential was beyond 100 population doublings and cell populations formed clonogenic cell clusters [5].

Studies have also demonstrated that cultures can be maintained after extensive subculturing of up to 20 passages after seeding isolated DPSC [5, 7]. After subculturing they are able to adhere quickly to conventional plastic dishes showing a typical fibroblastic, spindle-shape to polygonal morphology [10].

## Conclusions

It is obvious that our knowledge in dental tissue engineering is expanding rapidly, and existing data confirm a realistic feasibility of dental tissue repair in the near future. In this context it has been demonstrated that present dental pulp stem/progenitor cells have the ability to differentiate *in vitro* as well as *in vivo* into odontoblast-like cells. Furthermore, the application of bioactive glasses incorporated into a biodegradable polymer matrix also seems to be a suitable material as a regenerating dental substitute. The next step has to be the design of a "smart" and appropriate growth factors release system for diffusion through a residues dentin matrix after cavity preparation.

Future experiments should be focused on the design of a highly sophisticated biological based scaffold system, which would greatly improve tooth viability and health maintenance in dentistry including nanotechnologies, in particular, the material would provide stability and a stimulation effect on bone tissue formation.

## References

1. Gronthos S. Postnatal human dental pulp stem cells (DPSCs) *in vitro* and *in vivo*. *Proc. Natl. Acad. Sci. USA*. 2000;97;13625–13630.
2. Gronthos S. Stem cell properties of human dental pulp stem cells. *J. Dent. Res.* 2002;81:531–535.
3. Bluteau G, Luder H-U, De Bari C, et al. Stem cells for tooth engineering. *European cells and Materials*. 2008;16:1-9.
4. Shimonishi M. *In vitro* differentiation of epithelial cells cultured from human periodontal ligament. *J. Periodontal Res.* 2007;42:456–465
5. Gronthos S, Mankani M, Brahim J, et al. Postnatal human dental pulp stem cells (DPSCs) *in vitro* and *in vivo*. *Proc Natl acad Sci USA*. 2000;97:13625-13630.
6. Honda Masaki J, Imaizumi Mari, Tsuchiya Sh, et al. Dental follicle stem cells and tissue engineering. *Journal of Oral Science*. 2010;52;4:541-552.
7. Alliot-Licht B, Bluteau G, Magne D, et al. Dexamethasone stimulates differentiation of odontoblast-like cells in human dental pulp cultures. *Cell Tissue Res*. 2005;321:391–400.
8. Papaccio G, Graziano A, d'Aquino R, et al. Long-term cryopreservation of dental pulp stem cells (SBP-DPSCs) and their differentiated osteoblasts: a cell source for tissue repair. *J Cell Physiol*. 2006;208(2):319-25.
9. Laino G, Graziano A, D´Aquino R, et al. An approachable human adult stem cell source for hard tissue engineering. *J Cell Physiol*. 2006;206:693-701.
10. About I, Bottero MJ, de Denato P, et al. Human dentin production in vitro. *Exp Cell Res*. 2000;258(1):33-41.
11. Shiba H, Fujita T, Doi N, et al. Differential effects of various growth factors and cytokines on the syntheses of DNA, type I collagen, laminin, fibronectin, osteonectin/secreted protein, acidic and rich in cysteine (SPARC), and alkaline phosphatase by human pulp cells in culture. *J Cell Physiol*. 1998;174(2):194-205.
12. Lopez-Cazaux S, Bluteau G, Magne D, et al. Culture medium modulates the behaviour of human dental pulp-derived cells: technical note. *Eur Cell Mater*. 2006;11:35-42.
13. Huang GTJ, Sonoyama W, Chen J, et al. *In vitro* characterization of human dental pulp cells: various isolation methods and culturing environments. *Cell tissue Res*. 2006;324:225-236.
14. Heo YY, Um S, Kim SK, et al. Responses of periodontal ligament stem cells on various titanium surfaces. 2011;17:320-327.
15. Nakao K, Itoh M, Tomita Y, et al. FGF-2 potently induces both proliferation and DSP expression in collagen type I gel cultures of adult incisor immature pulp cells. *Biochem Biophys Res Commun*. 2004;325(3):1052-1059.
16. Miura M, Gronthos S, Zhao M, et al. SHED. Stem cells from human exfoliated deciduous theeth. *Proc Natl acad Sci USA*. 2003;100:5807-5812.
17. Razieh Alipour, Farzaneh Sadeghi, Batool Hashemi-Beni, et al. Phenotypic Characterizations and Comparison of Adult Dental Stem Cells with Adipose-Derived Stem Cells. *Int J Prev Med*. 2010;1(3):164-171.
18. Batouli S, Miura M, Brahim J, et al. Comparison of stem-cell-mediated osteogenesis and dentinogenesis. *J Dent Res*. 2003;82:976-981.
19. Nacu V. Optimizarea regenerării osoase posttraumatice dereglate. Chişinău: Sirius, 2010;188.
20. Yen-Hua Huang, Jen-Chang Yang, Chin-Wei Wang, et al. Dental Stem Cells and Tooth Banking for Regenerative Medicine. *J Exp Clin Med*. 2010;2(3):111–117.

# Риск врожденных пороков развития при родственных браках

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**S. G. Israfilbeyli, P. M. Aliyeva**
**Risk of Congenital Developmental Defects in Interrelated Marriages**

The aim of this study was to estimate the risk of congenital developmental defects in fetuses from interrelated parents. We identified and controlled for various socio-economical factors that may potentially influence or alter the risk of congenital defects

**Key words:** related marriage, congenital developmental defects, factors of the risk.

**Реферат**

В работе поставлена цель – обосновать истинный уровень риска врожденных пороков развития у плодов при близкородственном браке между родителями. С этой целью выявлены ситуационные факторы, потенциально влияющие на риск врожденных пороков. Путем стандартизации нивелирована роль ситуационных факторов и определена истинная роль родственных браков в повышении риска врожденных пороков развития.

**Ключевые слова**: родственный брак, врожденные пороки развития, факторы риска.

## Введение

Врожденные пороки развития (ВПР) являются одной из ведущих причин спонтанных абортов, мертворождаемости и перинатальной смертности [2, 3, 4, 5]. Известно много факторов риска ВПР, среди которых управляемым считается родственный брак [1]. Вероятность повышения риска ВПР при родственных браках соответствует современным представлениям о механизме и патогенезе формирования аномалий у плода. В то же время отсутствуют сведения об истинном уровне риска ВПР при родственных браках. Данная работа посвящена научному обоснованию истинного уровня риска ВПР по материалам репрезентативной совокупности в соответствии с современными требованиями доказательной медицины.

## Материал и методы

Исследование планировалось с применением ретроспективного анализа. Единицей статистического наблюдения был любой исход беременности, не прерванной искусственным абортом. Учитывались все диагнозы ВПР, установленные у мертворожденных (по определению ВОЗ, которое включает все случаи мертворождения без учета срока беременности и массы тела плода) и у новорожденных плодов. Объем общей совокупности составлял 33682 единицы наблюдения, среди которых 7265 были плодами от родственных браков. Определялась частота ВПР по отдельным блокам класса XVII МКБ-10 у плодов от родственных и неродственных браков. Учитывались известные потенциальные факторы риска ВПР (возраст матери, течение беременности, инфекции мочеполовых органов и перенесенные респираторные инфекции во время беременности). Сравнение частоты ВПР в группах от родственных и неродственных браков, неассоциированные и ассоциированные с отмеченными потенциальными факторами риска, проводилось путем расчета «t» критерия Стьюдента. Весовой индекс повышенного риска ВПР определялся путем соотношения максимальной и минимальной частоты в подгруппах, формированных по градациям потенциального фактора риска. Нормированный интенсивный показатель (НИП) вычисляется путем деления частоты ВПР в подгруппах на таковой в общей совокупности (условный стандарт). Стандартизованная частота ВПР у плодов от родственных и неродственных браков определялась в соответствии с методическими подходами, обоснованными в трудах Е. Н. Шиган и Ф. Б. Агаева [1, 6].

## Результаты и обсуждение

Частота ВПР у мертворожденных и живорожденных плодов от родственных браков соответственно составляла 397,8 ± 22,7 и 13,7 ± 1,4‰ (на 1000 плодов и новорожденных 38,3 ± 2,3). Эти показатели в группе от неродственного брака были существенно ниже (соответственно: 245,0 ± 20,2 и 8,3 ± 0,6‰; 8,9 ± 0,6‰). Достоверность различия подтверждается ($p < 0,05$). Относительный риск ВПР (соотношение интенсивных показателей) в подгруппе мертворожденных по сравнению с живорожденными плодами составлял 29,0 в группе плодов рожденных в родственных браках и 29,5 в группе плодов от неродственных браков. Различия отмеченных показателей прослеживались в подгруппах,

дифференцированных по возрасту матери на момент беременности (таб. 1).

Наиболее высокие показатели ВПР у мертворожденных плодов отмечались при возрасте матери в возрасте 35 лет и старше (787 ± 50,3‰ при родственных браках, 600 ± 69,3‰ при неродственных браках в подгруппах мертворожденных плодов). При этом соотношение показателей у мертворожденных и живорожденных плодов соответственно составляла: 33,7 и 78,9. В отмеченном возрасте матери в связи с родственным браком относительный риск ВПР у мертворожденных (1,3) и живорожденных (3,1) друг от друга отличался. Надо отметить, что эти показатели при возрасте матери до 25 лет (1,7 и 2,5), и при возрасте 25-34 лет (1,5 и 2,9) были относительно близкими. В целом риск ВПР в связи с родственным браком составлял 4,3 и колебался в пределах 3,8-5,2 в зависимости от возраста матери.

Таким образом, родственный брак является существенным фактором риска ВПР у плодов. Структура ВПР по блокам класса XVII МКБ-10 приведена в таб. 2.

Доля врожденных аномалий развития нервной системы в группах от родственных и неродственных браков соответственно составляла 24,5 ± 2,6 и 15,4 ± 2,4‰ (p < 0,5). Эта форма ВПР занимает первое ранговое место в группе плодов от родственных браков и второе ранговое место в группе от неродственных браков. По удельному весу в составе ВПР у плодов от родственных браков на втором месте находились так называемые другие врожденные аномалии (Q80 – Q89), которые в основном затрагивают несколько систем (17,9%). Доля этих аномалий у плодов от неродственных браков были существенно выше (25,1 ± 2,8%) и находились на первом месте в структуре ВПР.

Доля врожденных аномалий развития нервной системы в группах от родственных и неродственных браков соответственно составляла 24,5 ± 2,6 и 15,4 ± 2,4‰ (p < 0,5). Эта форма ВПР занимает первое ранговое место в группе плодов от родственных браков и второе ранговое место в группе от неродственных браков. По удельному весу в составе ВПР у плодов от родственных браков на втором месте находились так называемые другие врожденные аномалии (Q80 – Q89), которые в основном затрагивают несколько систем (17,9%). Доля этих аномалий у плодов от неродственных браков были существенно выше (25,1 ± 2,8%) и находились на первом месте в структуре ВПР. Доля врожденных аномалий системы кровообращения в группах плодов от родственных и неродственных браков была близкой друг к другу (10,8 ± 1,9 и 9,4 ± 1,9%),

**Таблица 1**

**Частота ВПР в зависимости от родственных отношений между родителями (Р – родственный, НР – неродственный брак)**

| Возраст (годы) | Брак | Мертворожденные (определение ВОЗ) | | | Живорожденные | | | Мертворожденные и живорожденные | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Всего | ВПР | P ± m% | Всего | ВПР | P ± m% | Всего | ВПР | P ± m% |
| < 25 | Р | 151 | 63 | 417,2 ± 40,1 | 1638 | 22 | 13,4 ± 2,8 | 1789 | 85 | 47,5 ± 5,0 |
| | НР | 106 | 26 | 245,3 ± 41,8 | 6553 | 35 | 5,3 ± 0,9 | 6659 | 61 | 9,1 ± 1,2 |
| 25-34 | Р | 248 | 70 | 282,3 ± 28,6 | 4263 | 50 | 11,7 ± 1,6 | 4511 | 120 | 26,6 ± 2,4 |
| | НР | 297 | 55 | 185,2 ± 22,5 | 17054 | 70 | 4,1 ± 0,5 | 17351 | 125 | 7,2 ± 0,6 |
| 35 и более | Р | 66 | 52 | 787,9 ± 50,3 | 899 | 21 | 23,4 ± 5,0 | 965 | 73 | 75,6 ± 8,5 |
| | НР | 50 | 30 | 600,0 ± 69,3 | 2357 | 18 | 7,6 ± 1,8 | 2407 | 48 | 19,9 ± 2,8 |
| Итого | Р | 465 | 185 | 397,8 ± 22,7 | 6800 | 93 | 13,7 ± 1,4 | 7265 | 278 | 38,3 ± 2,3 |
| | НР | 453 | 111 | 245,0 ± 20,2 | 25964 | 216 | 8,3 ± 0,6 | 26417 | 234 | 8,9 ± 0,6 |

**Таблица 2**

**Структура ВПР в зависимости от родственных отношений между родителями**

| Наименование блока по МКБ-10 класса XVII (врожденные аномалии) и коды | Родственный брак | | Неродственный брак | |
|---|---|---|---|---|
| | Число ВПР | % к итогу | Число ВПР | % к итогу |
| Врожденные аномалии развития нервной системы (Q00-Q07) | 68 | 24,5 ± 2,6 | 36 | 15,4 ± 2,4 |
| Врожденные аномалии глаза, уха, лица и шеи (Q10-Q18) | 13 | 4,7 ± 1,3 | 11 | 4,7 ± 1,4 |
| Врожденные аномалии системы кровообращения (Q20-Q28) | 30 | 10,8 ± 1,9 | 22 | 9,4 ± 1,9 |
| Врожденные аномалии органов дыхания (Q30-Q34) | 18 | 6,5 ± 1.5 | 14 | 6,0 ± 1,6 |
| Расщелина губы и неба (Q35-Q37) | 15 | 5,4 ± 1,4 | 16 | 6,8 ± 1,6 |
| Другие врожденные аномалии органов пищеварения (Q38-Q45) | 14 | 3,0 ± 1,3 | 17 | 7,3 ± 1.7 |
| Врожденные аномалии половых органов (Q50-Q51) | 15 | 5,4 ± 1,4 | 13 | 5,6 ± 1,5 |
| Врожденные аномалии мочевыделительной системы (Q60-Q64) | 13 | 4,7 ± 1,3 | 11 | 4,7 ± 1,4 |
| Врожденные аномалии и деформации костно-мышечной системы (Q65-Q79) | 18 | 6,5 ± 1,5 | 17 | 7,3 ± 1,7 |
| Другие врожденные аномалии (Q80-Q89) | 50 | 17,9 ± 2,3 | 59 | 25,1± 2,8 |
| Хромосомные нарушения, не классифицированные в других рубриках (Q90-Q99) | 24 | 8,64 ± 1,7 | 18 | 7,7 ± 1,7 |
| Итого | 278 | 100,0 | 234 | 100,0 |

**Таблица 3**

**Частота ВПР в зависимости от потенциальных факторов риска при родственных браках**

| Потенциальные факторы риска | Градация факторов | Родственный брак (РБ) | | | Неродственный брак (НРБ) | | | Весовой индекс (к) | | НИП | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Всего | ВПР | На 1000 P ± m | Всего | ВПР | На 1000 P ± m | РБ | НРБ | РБ | НРБ |
| Возраст матери | < 25 | 1789 | 85 | 47,5 ± 5,0 | 6659 | 61 | 9,1 ± 1,2 | | | 3,15 | 0,60 |
| | 25-34 | 4511 | 120 | 26,6 ± 2,4 | 17351 | 125 | 7,2 ± 0,6 | 2,84 | 2,76 | 1,75 | 0,47 |
| | > 34 | 965 | 73 | 75,6 ± 8,5 | 2407 | 48 | 19,9 ± 2,8 | | | 4,97 | 1,31 |
| Течение беременности | С гестозом | 423 | 29 | 68,6 ± 12,3 | 1615 | 38 | 23,5 ± 3,8 | 1,88 | 2,97 | 2,39 | 0,52 |
| | Без гестоза | 6842 | 249 | 36,4 ± 2,3 | 24802 | 196 | 7,9 ± 0,6 | | | 2,39 | 0,52 |
| Инфекции мочеполовых органов | Имеется | 196 | 14 | 71,4 ± 18,4 | 743 | 10 | 13,5 ± 4,2 | 1,91 | 1,55 | 4,69 | 0,89 |
| | Не имеется | 7069 | 264 | 37,4 ± 2,3 | 25674 | 224 | 8,7 ± 0,6 | | | 2,46 | 0,57 |
| Респираторные инфекции | Отмечены | 2656 | 58 | 21,8 ± 2,8 | 8532 | 102 | 11,9 ± 1,2 | 2,19 | 1,61 | 1,43 | 0,78 |
| | Не отмечены | 4609 | 220 | 47,7 ± 3,1 | 17885 | 132 | 7,4 ± 0,6 | | | 3,14 | 0,49 |

занимая третье место в структуре ВПР. В сравниваемых группах места распределились в следующем порядке: 1 – хромосомные нарушения (8,6 ± 1,7 и 7,7 ± 1,7%); 2 – врожденные аномалии костно-мышечной системы (6,5 ± 1,5 и 7,3 ± 1,7%); 3 – ВПР органов дыхания (6,5 ± 1,5 и 6,0 ± 1,6%); 4 – ВПР половых органов (5,4 ± 1,4 и 5,6 ± 1,6%); 5 – ВПР органов пищеварения (5,0 ± 1,3 и 7,3 ± 1,7%); 6 – расщелина губ и неба (5,4 ± 1,4 и 6,8 ± 1,6%).

Надо отметить, что за исключением двух форм врожденных аномалий (аномалии развития нервной системы и хромосомных нарушений), доля всех остальных форм ВПР в группах плодов от родственных и неродственных браков друг от друга достоверно не отличалась. Ранги выделенных блоков класса XVII (врожденных аномалии) в сравниваемых группах имеют прямую сильную корреляционную связь (δ = 0,91). Эти данные показывают, что родственный брак ассоциируется с высоким риском развития всех врожденных пороков развития. При этом структура ВПР по клиническим формам (в соответствии с блоками класса XVII МКБ-10) друг от друга не отличается.

Таким образом, родственный брак является распространенным явлением, в Азербайджане 21,5%, следствием которого следует считать повышение риска рождения детей с ВПР (4,3 раза). Возраст матери до 25 лет, ассоциированный родственным отношением супружеских пар, относительно больше сопровождается добавочным риском ВПР (5,2 раза).

Однако родственный брак ассоциируется с рядом других факторов риска, которые могут участвовать в формировании повышенного риска. Это хорошо прослеживается из данных таб. 3, где представлена частота ВПР в зависимости от соответствующих потенциальных факторов риска.

У плодов от родственных и неродственных браков внутриутробное развитие произошло соответственно на фоне гестозов в 5,8% и 6,1% случаях, на фоне инфекций мочеполовых органов в 2,7% и 2,8% случаях и на фоне респираторных инфекций в 36,6% и 32,3% случаев. Возраст матерей в этих группах (< 25; 25-34; 35 лет и старше: 24,6%; 62,1% и 13,3% при родственном браке; 25,2%; 65,7% и 9,1% при неродственном браке) существенно не отличается друг от друга. Относительный риск ВПР в группе плодов от родственных браков на фоне потенциальных факторов риска составлял 5,2; 3,7 и 3,8 раз (кратность) при возрасте < 26, 25-34; 35 и старше; 2,9 и 4,6 в зависимости от течения беременности; 5,3 и 4,3 в зависимости от инфекций мочеполовых органов.

Наиболее частой формой ВПР были врожденные аномалии нервной системы, частота которых составляла 9,4‰ у плодов от родственных браков и была в 6,7 раза больше у плодов от неродственных браков (1,4‰). Частота врожденных аномалий системы кровообращения составляла (соответственно 4,1 и 0,8%), органов дыхания (2,5 и 0,5‰), мочеполовой системы (3,9 и 1,1‰), костно-мышечной системы (2,5 и 0,6‰) и других форм (6,9 и 2,2‰), а также хромосомных нарушений (3,2 и 0,7‰) в сравниваемых группах друг от друга отличалась более чем в 3 раза. Очевидно, что родственный брак повышает риск веса форм врожденных аномалий, но степень риска больше всего выражена по аномалиям развития нервной системы. Расчет стандартизованных показателей риска ВПР у плодов от родственных и неродственных браков, имеющий цель нивелирования роли ситуационных факторов показал, что различие между сравниваемыми группами сохраняется. В целом стандартизованная частота в этих группах составляет 34,5 и 8,5‰ (относительный риск 4,1). По всем формам ВПР фактические и стандартизованные показатели риска были сходными, что позволяет подтверждать истинную роль родственных браков в формировании риска пороков развития.

Таким образом, примененная нами методология организации исследования позволяет доказать степень риска ВПР, обусловленной различными факторами риска, ассоциированного с родственными браками. Принимая во внимание отмеченное, была определена чувствительность, специфичность и прогностическая ценность родственного брака как фактора риска ВПР.

Необходимо отметить, что общая вероятность ВПР для Азербайджанской популяции не превышает 2,0%.

Принимая во внимание прогностическую ценность родственного брака, наличие этого признака у беремен-

ной женщины следует считать показателем для применения комплекса мероприятий по ранней пренатальной диагностике ВПР.

### Выводы

1. Родственный брак является распространенным явлением в Азербайджане (21,5%), последствием которого следует считать повышение риска рождения детей с ВПР (4,3 раза). Возраст матери до 25 лет, ассоциированный родственным отношением супружеских пар, относительно больше и сопровождается добавочным риском ВПР (5,2 раза).

2. Чувствительность родственного брака как критерия риска ВПР (вероятность этого признака в группе матерей родивших детей с ВПР) составляла 54,3%. Специфичность (вероятность отсутствия родственного брака в группе матерей родивших детей с нормальным внутриутробным развитием) этот критерий значительно выше (78,9%). Прогностическая ценность родственного брака (вероятность развития ВПР) составляла 19,2%.

### Литература

1.Агаев ФБ. Методические подходы к углубленному изучению акушерской и перинатальной патологии. Москва, 1983;20.

2. Патрушев АВ, Мурашко МА. Инвазивная перинатальная диагностика хромосомной патологии плода у беременных женщин в республике Коми. Проблемы адаптации человека к экологическим и социальным условиям Севера. Материалы международной научно-практической конференции. Сыктывкар, 2005;12.

3. Патрушев АВ, Мурашко МА, Дворянский СА. Пренатальная диагностика врожденных пороков развития плода. Министерство здравоохранения и социального развития Республики Коми. Информационно методическое письмо. Сыктывкар, 2004;62-63.

4. Уншигбаяр Оюунгилег. Частота и факторы риска врожденных пороков развития у новорожденных в г. Улан-Батор: Автореф. дисс. канд. мед. наук. М., 2007;18.

5. Юдина ЕВ, Сынченко ЕВ, Медведьев МВ, и др. Инвазивные методы исследования в акушерской практике. *Перинатальная диагностика*. 2002;2:91-96.

6. Шиган ЕН. Методика социально-гигиенических исследований. В кн. Руководство по социальной гигиене и организация здравоохранения. М.,1987;200-278.

# Potențialul avansat al unui fotopletismograf de construcție autohtonă pentru diagnosticul vascular non-invaziv

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### Advanced Potential of a Local Construction Photopletysmography for Non-invasive Vascular Diagnosis

Advanced sensor device for shape analysis of the tissue-reflected mean single period photoplethysmography (PPG) signals have been designed and clinically tested. The PPG signal shape reveals individual features of the patient's cardiovascular state. Clinical studies of several patient groups (e.g. diabetes mellitus, atherosclerosis obliterans, Raynaud's syndrome) made it possible to specify components of the PPG signal that are sensitive to the corresponding organic or functional pathologies. Comparison of the right and left arm finger PPG signal shapes, for instance, appears to be an efficient tool for early screening of unilateral atherosclerosis obliterans.

**Key words:** photoplethysmography, diabetes, atherosclerosis, Raynaud's syndrome.

### Расширенный потенциал фотоплетизмографа местной конструкции для сосудистой неинвазивной диагностики

Данные, представленные в настоящем документе, подтверждают эффективность фотоплетизмографа-2 (FPG-2), используемого в диагностике неинвазивного анализа кровотока пульсирующей формы волны, которая была разработана и клинически апробирована. FPG формы сигнала выявили индивидуальные особенности сердечно-сосудистой системы пациента. Популяционные исследования (например, сахарный диабет, окклюзирующий атеросклероз, синдром Рейно), позволили определить различные компоненты сигнала фотоплетизмографа.

**Ключевые слова:** фотоплетизмография, диабет сахарный, атеросклероз, синдром Рейно.

## Introducere

Fotopletismografia (FPG) este o metodă neinvazivă care semnifică înregistrarea grafică a modificărilor de volum ale unui segment de corp, strâns legate de modificările fluxului de sânge în timpul excursiei sistolo-diastolice. Fotopletismografia este o tehnică dezvoltată de Blazek şi Wienert în 1981 [1]. Această tehnică, bazată pe diferite principii fizice, a fost aplicată în evaluarea clinică şi comensurarea fluxului sanguin arterial şi venos.

Senzorul fotopletismografic constă dintr-o lumină infraroşie diodă şi una fotodiodă. Lumina emisă penetrează straturile superioare ale dermului în cazul, în care o parte din acestea este absorbită, iar o alta este reflectată şi capturată de fotodiod. Intensitatea luminii reflectate şi, prin urmare, semnalul electric produs de fotodiod, va fi în corespundere cu volumul de sânge din zona de măsurare. Fotopletismografia este folosită ca metodă funcţională complementară, pentru capacitatea sa de a evidenţia precoce o stare de rigiditate sau spasm muscular al arteriolelor şi capilarelor. Compararea semnalelor de la braţul drept şi stâng pare a fi un instrument eficient de depistare precoce a aterosclerozei obliterante unilaterale.

Fotopletismografia este o examinare uşor de executat, dar deseori cu multe artefacte, ce duc la erori de diagnostic. Progresele în microelectronică şi tehnologiile de calculator au deschis noi posibilităţi. Spectrul de analize realizate la FPG furnizează informaţii valoroase asupra funcţiei cardiace, respiraţiei, stării vasculare şi a sistemului nervos [2, 8]. FPG se poate utiliza lejer şi sigur pentru expres-diagnosticul şi depistarea precoce a diverselor patologii cardiovasculare.

Forma de undă FPG, detectată la periferie, poate să difere semnificativ de cea repetată pe „arterele magistrale", dar va depinde de rezistenţa sistemului vascular. În cazul în care rezistenţa este anormală, mai des pe fond de ateroscleroză, prin diabet zaharat sau alte patologii vasculare, care îngustează vasele, viteza fluxului de sânge în arterele mari faţă de capilarele mici scade dramatic. Hipertensiunea arterială duce la pierderea completă a vârfului dicrotic, când se ajunge la periferie. Absenţa vârfurilor secundare ale semnalelor FPG înregistrate pe degetele pacienţilor cu hipertensiune arterială au fost semne clinice [12].

De notat, că semnalele FPG nu sunt strict repetate, periodic existând fluctuaţii uşoare ale amplitudinii semnalului.

Mulţi medici preferă informaţii vizuale (imagini sau curba de diagnosticare). Pentru a depăşi acest lucru, Universitatea Tehnică din Republica Moldova, Catedra Microelectronică şi Dispozitive Semiconductoare a propus un fotopletismograf (FPG-2). Principiul său prevede capacitatea de a detecta şi a acumula o secvenţă de 60 de semnale fiecărui pacient şi posibilitatea de a preciza ulterior formele exacte de semnal pentru analizele clinice ulterioare. Memoria internă a dispozitivului permite de a introduce până la 4 mii de pacienţi în baza de date, conectând dispozitivul la calculatorul personal, astfel colectând şi transmiţând datele în timp real.

Datorită faptului că dispozitivul este mic, compact şi se alimentează de la bateriile acumulatoare proprii, permite auto-monitorizarea stării vasculare la domiciliu sau în timpul exerciţiilor fizice, având grijă ca ambianţa în care se face examinarea să asigure un confort termic (22 – 25°C) şi calm psihic.

În cele ce urmează a fi relatate ne-am propus să demonstrăm informativitatea FPG-2 în diagnosticul neinvaziv al afecţiunilor cardiovasculare şi analiza fluxului de sânge ce pulsează sub formă de undă.

## Material şi metode

Pentru acest obiectiv am selectat un grup de persoane practic sănătoase, care să ne ofere parametrii normali de elasticitate a sistemului vascular. Caracteristicile acestea s-au utilizat la aprecierea parametrilor cantitativi şi calitativi ai semnalelor FPG, evaluate în cadrul studiilor clinice efectuate cu acest aparat.

Interpretarea fotopletismografică se bazează pe evaluarea anumitor *parametri cantitativi şi calitativi* [9, 13]. Parametrii cantitativi sunt: amplitudinea curbei, viteza, timpul până la vârf, timp de undă, crestătura dicrotică şi totală. Parametrii calitativi sunt: morfologia totală de val şi a componentelor sale.

Unele semnale măsurate iniţial la un grup de persoane sunt prezentate în fig. 2. Semnalele au fost luate la aceeaşi locaţie

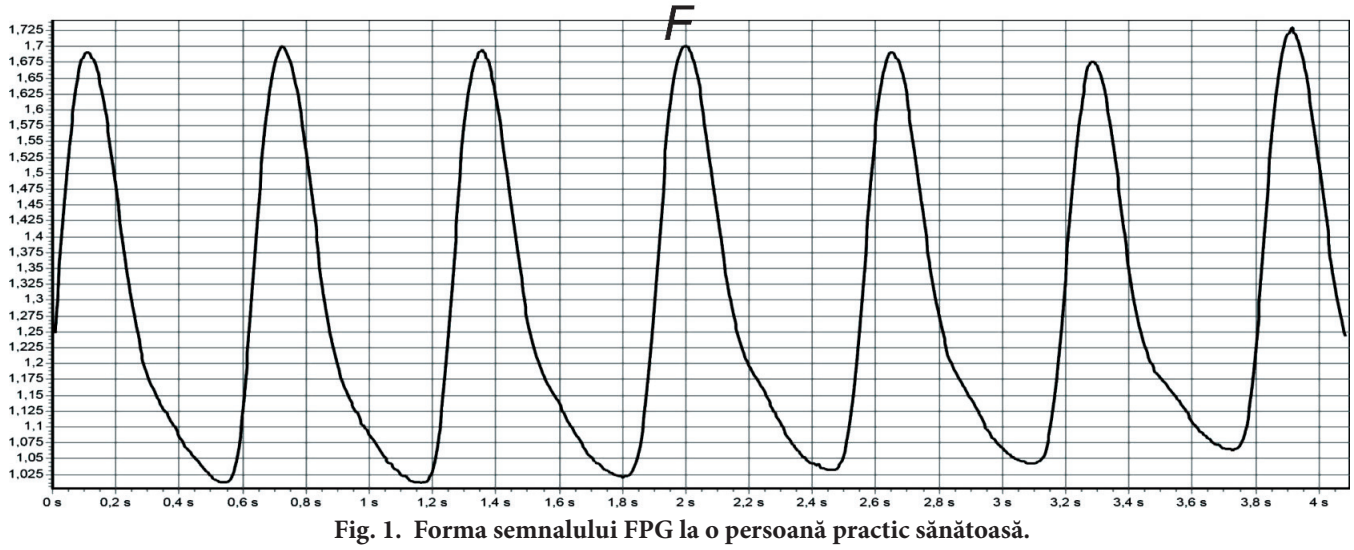


**Fig. 1. Forma semnalului FPG la o persoană practic sănătoasă.**

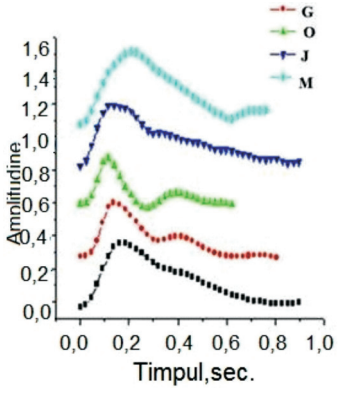


**Fig. 2. Diferite forme de semnale FPG la un grup de pacienți practic sănătoși.**

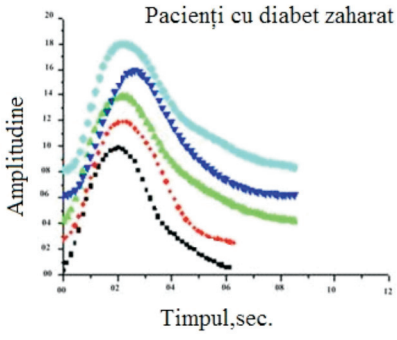


**Fig. 3. Semnale FPG la 5 pacienți cu diabet.**

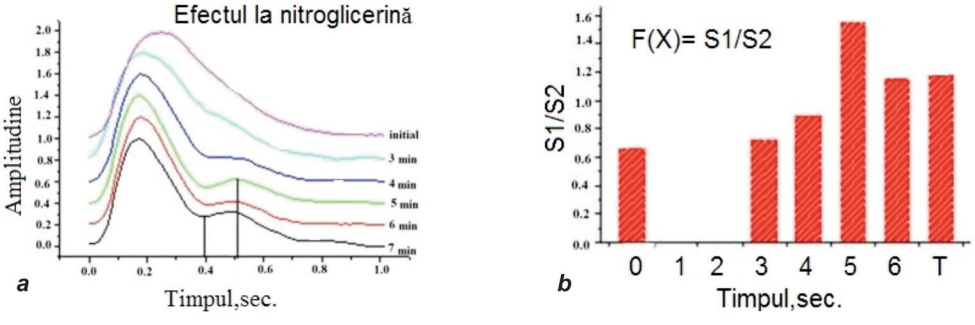


**Fig. 4. A - reprezintă modificări apreciate la pacienți asimptomatici cu modificări ușoare aterosclerotice, după ce au administrat o doză de nitroglicerină; B - timpul de dezvoltare al efectului determinat de nitroglicerină caracterizat prin semnalul T2/T1 s, ce formează vârful secundar la partea catacrotă a semnalului. Este o dovadă clară de creștere a fluxului de sânge.**

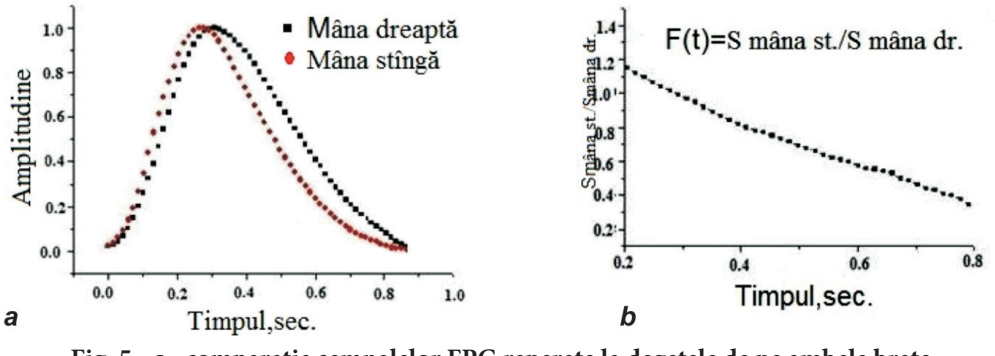


**Fig. 5. *a* - comparația semnalelor FPG reperate la degetele de pe ambele brațe, în caz de ocluzie în artera subclavie, *b* – raportul unghiului pantă în funcție de semnal $S_{stânga}/S_{dreapta}$.**

a corpului (vârful degetului mare). Persoanele monitorizate au fost practic sănătoase. S-au utilizat următoarele abrevieri: bărbați - A, G și O, de vârsta 24-26 de ani, J – un bărbat de 49 de ani, M – o femeie de 56 de ani.

Figura 2 ilustrează diferențele clare de semnale FPG, înregistrate la cinci persoane sănătoase. Crestătura dicrotică este mai pronunțată la pacienții mai tineri [13], ceea ce ar putea fi interpretat ca un semn bun de elasticitate vasculară, comparativ cu pacienții mai în vârstă.

Studiile noastre la 5 pacienți cu diabet zaharat au confirmat pe deplin această ipoteză – toate semnalele FPG preluate din degetele lor au fost în formă de clopot, fără nici un vârf secundar la partea catacrotă (fig. 3).

Studiul clinic la pacienții cu ateroscleroză a realizat forme de semnale similare de FPG. La administrarea unei doze de nitroglicerină, ce reflectă dilatarea farmacologică a vaselor de sânge, s-a observat formarea vârfului secundar. Este un indiciu sigur de creștere a fluxului de sânge. Modificările obținute au fost prezentate în figura 4.

Potențialul FPG aplicat pentru reperarea precoce a patologiei organice în arterele magistrale – de ex.: un segment obliterant cu localizare în a. subclavie a fost confirmat într-un alt studiu clinic. Semnalele FPG pentru acest grup de pacienți au fost luate pe degetele de la ambele brațe (vezi rezultatele prezentate în fig. 5 a).

Astfel se poate observa o întârziere clară de timp și de lărgire a semnalului la brațul drept, în comparație cu brațul stâng,

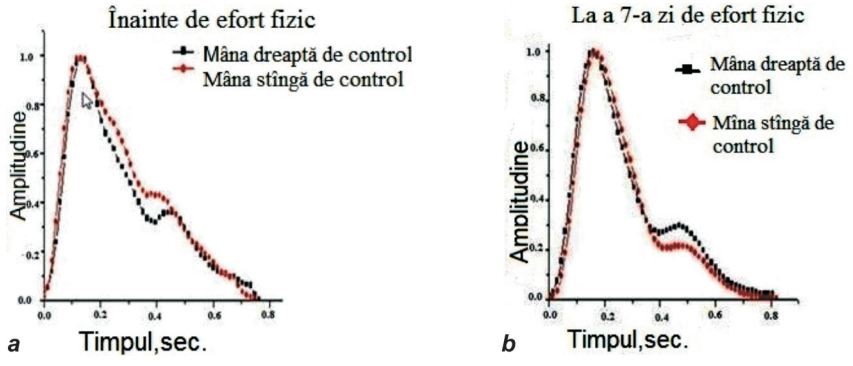


**Fig. 6. Compararea semnalelor FPG luate de la degetele ambelor mâini la pacientul cu sindrom Raynaud, înainte şi după atestarea periodică de circulaţie în palma stângă.**

ceea ce dovedeşte o rezistenţă vasculară crescută şi o viteză mai lentă a fluxului de sânge în braţul drept. Prin urmare raportul unghiului (panta) în funcţie de semnal S stânga/S dreapta ar putea să servească, în cele din urmă, drept criteriu diagnostic pentru evaluarea ocluziei vasului de sânge (fig. 5 b).

Sindromul Raynaud (SR) este o tulburare paroxistică a circulaţiei periferice, localizată, de obicei, la nivelul membrelor superioare, caracterizată prin apariţia intermitentă a unui spasm bilateral şi simetric la nivelul arterelor digitale, apărând la frig sau emoţii, cu stare normală între accese. Este o afecţiune rară, care se întâlneşte, de obicei, la femei tinere (sub 40 de ani), etiologia fiind necunoscută. FPG poate furniza informaţii suplimentare despre această boală [14, 15].

FPG de monitorizare a fost utilizată pentru a urmări schimbările vasculare în timpul unui efort fizic al unei paciente (L., 22 de ani) cu SR. Semnalul FPG a fost comparat înainte şi după efortul fizic. Modificările observate pot servi drept probe ale alimentării braţului, „antrenat" cu sânge îmbunătăţit. Rezultatele obţinute sunt prezentate în figura 6.

**Comentariul nostru.** Rezultatele prezentate şi analiza caracteristicilor funcţionale ale aparatului testat confirmă potenţialul dispozitivului dotat cu senzor FPG-2, aplicat în procedura metodologică de diagnosticare vasculară, precum şi prin testul cu efort în faza preclinică.

Am consemnat mai multe elemente ale semnalelor FPG măsurate la vârful degetului, care pot servi drept criterii de diagnostic şi screening dinamic:

- creşterea duratei fazei anacrotice (DFA) a undei pulsatile caracterizează rezistenţa fluxului de sânge în vase;
- forma generală a semnalelor FPG: clopot, fără semne de DIP dicrotic şi crestătura catacrotă redusă anunţă diferite anormalităţi ale vaselor periferice de sânge (definite de diabet zaharat, ateroscleroză);
- apariţia şi creşterea/scăderea vârfului secundar, apreciate pe fondul unui drog (de ex., nitroglicerina), se pot utiliza pentru monitorizarea timpilor de extindere/îngustare a vaselor de sânge;
- modificările de formă ale semnalului FPG, atinse în urma eforturilor fizice sau fiziologice (fluxul de sânge), reflectă progresele înregistrate de starea fiziologică a celui observat.

## Concluzii

Fotopletismografia cu lumină reflectată s-a dovedit a fi un instrument adecvat pentru testele de anticipare a rezultatului terapeutic (de ex. în hipertensiunea arterială, diabet zaharat, în ateroscleroza obliterantă, la pacienţii cu sindrom Raynaud etc.).

Analizând cercetările care au vizat performanţele aparatului FPG-2, conchidem că acestea oferă clinicienilor posibilitatea unor estimări rapide şi de încredere.

## Bibliografie

1. Hertzman AB. Photoelectric plethysmograph of the finger and toes in man. *Proc. Soc. Exp. Biol. Med.* 1937;37:1633-1637.
2. Ugnell H. Phototplethysmographic Heart and Respiratory Rate Monitoring. Ph. D. Thesis No. 386, Linkoping University, 1995.
3. Nitzan M, de Boer H, Turivnenko S, et al. Power spectrum analysis of spontaneous fluctuations in the photoplethysmographic signal. *J. Bas. Clin. Physiol. Pharmacol*. 1994;5(3-4):269-276.
4. Bernardi L, Radelli A, Solda PL, et al. Autonomic control of skin microvessels: assessment by power spectrum of photoplethysmographic waves. *Clin. Sci.* 1996;90:345-355.
5. Nakajima K, Tamura T, Miike H. Monitoring of heart and respiratory rates by photoplethysmography using a digital filtering technique. *Med. Eng. Phys.* 1996;18:365-372.
6. Larsen PD, Harty M, Thiruchelvam M, et al. Spectral analysis of AC and DC components of the pulse photoplethysmography at rest and during indication of anaesthesia. *Int. J. Clin. Monit. Comput*. 1997;14:89-95.
7. Nitzan M, Babchenko A, Khanokh B, et al. The variability of the photoplethysmographic signal – a potential method for the evaluation of the autonomic nervous system. *Physiol. Meas.* 1998;19:93-102.
8. Perez-Ocon F, Abarca A, Abril J, et al. Optical measurement of cardiac rhythm using a personal computer with telediagnosis possibilities. *J. Biomed. Opt.* 2001;6(1):90-96.
9. Spigulis J, Rubins U. Photoplethysmographic sensor with smoothed output signals. *Proc. SPIE.* 1998;3570:195-199.
10. Venckus G, Spigulis J. Frequency filtering effects on the single-period photoplethysmography signals. *Med. Biol. Eng. Comput.* 1999;37(Suppl.1):218-219.
11. Spigulis J, Venckus G. Single-period photoplethysmography: a potential tool for noninvasive cardiovascular diagnostics. Springer Series "Optics for Life Sciences" OFLS-VI, Berlin (in press).
12. Spigulis J, Venckus G, Ozols M. Optical sensing for early cardiovascular diagnostics. *Proc. SPIE.* 2000;3911:27-31.
13. Ozols M, Spigulis J. Acquisition of biosignals using the PC sound card. Proc. Int. Conf. "Biomedical Engineering" (KTU, Kaunas, LT). 2001;24-27.
14. Wouda AA. Raynaud's phenomenon. Photoelectric plethysmography of the fingers of persons with and without Raynaud's phenomenon during cooling and warming up. *Acta Med. Scand.* 1977;201:519-523.
15. Engelhart M, Nielsen HV, Kristensen JK. The blood supply to fingers during Raynaud's attack: a comparison of laser-Doppler flowmetry with other techniques. *Clin. Physiol.* 1985;5:447-453.

# Aspecte noi în imunopatogeneza hepatitei cronice virale B

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**New Aspects in the Immunopathogenesis of Chronic Hepatites B**

Successful clearance and resolution of infection depends on the age and immune status of the individual with most infections of immunocompetent adults being self-limiting. Persistent or chronic infection is more likely to occur following vertical transmission (from mother to child) or horizontal transmission to children or to immunocompromised adults. The immune determinants of successful clearance of HBV are not fully understood but both cellular and humoral immune responses are important. At the same time, however, liver inflammation and disease are also believed to be largely immune-mediated. Therefore, a comlex interaction exists between HBV and the host in the initial clearance of HBV, the long-term persistence of HBV and the pathogenesis of HBV liver disease. There are some mechanisms of HBV-suppressing effect on immune response parts: exhaustion of virus-specific CTL functional activity as a result of persistence of high concentrations of viral antigens; elevation of activity of regulatory T-cells; ability HBV to exist in the form of sustained endocellular template – *covalently closed circular* DNA (ccc DNA). The role of activated platelets in immunopathological reactions is also considered at chronic viral liver damage.

**Key words**: viral hepatitis B, innate immunodefence, adaptive immunodefence.

**Новые аспекты в иммунопатогенезе хронического гепатита В**

Успех элиминации и разрешения вирусной инфекции гепатита В зависит от возраста и иммунного статуса больного. Персистирование хронической инфекции чаще передаётся вертикальным путём (от матери к ребёнку) или горизонтальной трансмиссией у детей, или у иммуннокомпромиссных взрослых. Иммунные детерминанты успешной элиминации вирусной инфекции гепатита В недостаточно исследованы, но следует отметить важность как гуморального, так и клеточного иммунного ответа. Вместе с тем считается, что воспалительный процесс в печёночной паренхиме в большей мере опосредован иммунологически. Известен факт, что существует комплексное взаимодействие между HBV и хозяином в начале фазы элиминации HBV при длительном персистировании HBV и в патогенезе воспалительного процесса печени. Существует несколько механизмов подавляющего влияния HBV на звенья иммунного ответа: истощение функциональной активности вирусоспецифических CTL в результате персистенции высоких вирусных антигенов; повышение активности регуляторных Т-клеток; способность HBV к существованию в форме устойчивой внутриклеточной матрицы – ковалентной замкнутой циркулярной ДНК (ссс ДНК). Рассматривается также роль активированных тромбоцитов в иммунопатологических реакциях при хроническом повреждении печени.

**Ключевые слова**: вирусный гепатит В, врождённый иммунитет, адаптивный иммунитет.

## Introducere

Virusul hepatitei B (HBV) în organismul uman poate dezvolta hepatita acută sau cronică, ciroza hepatică sau carcinomul hepatocelular. Peste 350 de mln persoane infectate cu HBV prezintă o sursă de răspândire a infecţiei pe cale orizontală şi verticală [1]. Manifestările clinice şi evoluţia infecţiei HBV sunt mediate de interacţiunile complexe dintre virus şi răspunsul imun al gazdei. Virusul HBV nu este direct citopatic asupra hepatocitelor, dar interacţiunea dintre virus şi răspunsul imun joacă un rol central în patogeneza necroinflamaţiei şi fibrozei hepatice [6]. Rolul principal în dezvoltarea răspunsului imun în infecţia HBV le aparţine reacţiilor sistemului imun adaptiv (dobândit), care stau la baza patogenezei afectării hepatice şi clearance-ului HBV [1].

Importanţa majoră în afectarea ficatului şi evoluţia HBV-infecţiei le aparţine limfocitelor citotoxice specifice (CTL). Persistenţa HBV reflectă incapacitatea CTL de a asigura un răspuns imun adecvat şi conduce spre dezvoltarea procesului inflamator-necrotic în ficat, cu formarea ulterioară a cirozei hepatice şi/sau carcinomului hepatocelular [1].

Carcinomul hepatocelular în majoritatea cazurilor este diagnosticat la pacienţii cu hepatită cronică B pe fundalul unei inflamaţii, care decurge lent în ficat şi a distrucţiei minimale în hepatocite. O astfel de inflamaţie este menţinută de CTL virusspecifice funcţional deficitare, incapabile de-a asigura clearance-ul virusului HBV din hepatocite. Astfel, afectarea cronică a hepatocitelor de către virusul hepatitei B este privită ca un proces potenţial precanceros în rezultatul dereglării balanţei dintre regenerarea hepatocitelor (sinteza ADN celular) şi inflamaţie (producerea mutagenelor) [1].

Procesul prelungit de regenerare şi inflamaţie în ficat poate conduce la apariţia tulburărilor genetice/cromozomiale, spontane şi multiple, răspunzătoare de dezvoltarea carcinomului hepatocelular [1].

**Imunopatogeneza hepatitei cronice virale B**

Răspunsul gazdei contra virusului se realizează printr-un complex de interacţiuni celulare. Iniţial răspunsul este non-specific şi include sistemul de interferoni, killeri naturali şi activarea non-specifică a celulelor Kupffer. După acest răspuns non-specific, răspunsul imun direcţionat specific împotriva proteinelor virale devine important. Două arme

majore ale sistemului imun sunt: arma umorală, care constă din B-limfocite ce produc anticorpi și arma celulară, care este compusă din variate tipuri celulare, incluzând macrofagii și T-limfocitele (fig.1). Celulele dendritice constituie un grup heterogen de celule antigen-prezentatoare, care constituie puntea de legătură dintre agenții patogeni și sistemul T-celular [9]. APCs (celulele prezentatoare de antigen) numite astfel celulele Kupffer și, în special, DCs (celulele dendritice) sunt implicate în prezentarea și maturizarea celulelor-T HBV-specifice, principalii efectori ai clearance-ului HBV. APCs prezintă antigenul celulelor-T CD4+ și CD8+ și produc citokine, IL-12 și TNF-α, care induc producerea IFN-γ și proliferarea celulelor CD8+. IL-12 induce de asemenea diferențierea celulelor-T CD4+ în celule T-helper de tip 1(Th1) [7].

Există câteva mecanisme de acțiune supresivă a infecției virale B asupra verigilor sistemului imun: epuizarea activității funcționale a limfocitelor citotoxice (CTL) virusspecifice, în rezultatul persistenței concentrațiilor înalte de antigene virale; creșterea activității celulelor-T regulatoare; capacitatea virusului HBV de a exista sub forma unei matrice intracelulare stabile – ADN circular închis covalent (ccc ADN). Se menționează și rolul trombocitelor activate în reacțiile imunopatologice în cadrul afectării virale hepatice [1]. Reieșind din studiile imunopatogenetice recente pe modele de animale și studii *in vitro* prin biopsii hepatice la pacienții cu HBV, s-a demonstrat un potențial important al interacțiunii dintre antigenul hepatic Be și componentele răspunsului imun ereditar, precum *Toll-like receptors*, celulele Kupffer, killerii naturali (*natural killer T-cells*) și celulele dendritice. Aceste date sugerează ideea precum că răspunsul imun ereditar are de asemenea un rol major în influența consecințelor infecției HBV acute și cronice asupra organismului [6].

Inițial recunoașterea infecției HBV poate fi mediată de *Toll-like receptors* (TLRs) [3, 8]. *Toll-like receptors* fac parte din PRRs (*pattern recognition receptors*). PRRs sunt un grup de receptori, care includ TLRs (*Toll-like receptors*), *nucleotide-binding oligomerization domain leucine - rich repeat proteins, peptidoglycan recognition proteins, caspase recruitment domain – helicase proteins, manose-binding lectins* (MBLs) [6].

PRRs sunt expresați pe numeroase celule efectorii ale sistemului imun ereditar. Odată ce PRR identifică pattern-ul molecular patogen-asociat, celulele efectoare inițiază imediat funcția lor [6].

TLRs au fost identificate pe numeroase tipuri de celule, inclusiv intestinale, endoteliale și renale. Stimularea TLRs prin liganzii săi inițiază activarea complexului de rețele de căi de transducere a semnalului intracelular, care coordonează ulterior răspunsul inflamator (fig. 2).

Această rețea include: adaptorul protein MyD88, protein kinases (*IL-1 receptor-associated kinase, p 38 mitogen-activated protein kinase, TNF receptor-associated kinase*) și transcripția factorului NF-kB (*nuclear factor kappa B*). Activarea NF-kB duce la expresia diferitor mediatori proinflamatori precum TNF-α, IL-1, IL-6 și *monocyte chemoattractant protein* [6].

TLRs participă la recunoașterea virală prin intermediul sistemului imun ereditar. Răspunsul gazdei împotriva virusului implică inducerea IFNs de tipul 1 (IFN-α, -β și -γ), care duc la creșterea expresiei și activării genelor IFN-stimulante. Inducerea genelor IFN-stimulante (care includ *protein kinase receptor*, IFNs, *IFN regulatory factors*) realizează răspunsuri antivirale, antiproliferative și imunoregulatoare [6].

În pofida faptului că cea mai caracteristică particularitate imunologică a infecției HBV cronice este diminuarea răspunsului celulelor-T CD8+ și CD4+ HBV-specific, există susținerea conceptului, precum că sistemul imun ereditar (*innate immune system*) este implicat de asemenea în această fază a bolii.

Această dovadă include descreșterea regulării PRRs (*down-regulation*), potențialul rol al celulelor dendritice în inducerea toleranței T-celulare HBV-specifice și rolul NK-celulelor în afectarea hepatocitelor.

În modelul șobolanului HBV-transgenic, administrarea liganzilor specifici pentru TLR4, 5, 7 și 9 rezultă inhibiția semnificativă a replicării virale. Aceasta a avut loc în decurs de 24 de ore într-o manieră IFN-dependentă [6]. Studiile recente demonstrează o scădere a regulării TLR2 de pe hepatocite, celulele Kupffer și monocitele din sângele periferic la pacienții HBeAg pozitivi, în comparație cu pacienții HBeAg-negativi și grupul de control sănătos. La pacienții cu hepatita activă HBeAg-negativi există o creștere a regulării TLR2 și conectarea secreției TNF-α [6]. Efectul de diminuare a regulării TLR2 a fost confirmat prin studii *in vitro,* folosind celulele hepatice și monocitele CD14+. Alte rapoarte demonstrează, că HBsAg inhibă expresia lipopolysaccharid-indusă a cyclooxigenazei-2 și, de asemenea, reduce producerea de IL-12 și IL-18 prin blocarea kinazelor extracelulare semnal-regulatoare (*extracellular signal – regulated kinase*) și a căii NF-kB, de asemenea și regularea producerii IFN [6]. Aceste date sugerează proprietatea antigenelor HBV specifice țintite TLRs să scape de recunoașterea imună.

MBL (*mannose-binding lectin*), o lectină tip C calciu-dependentă cu structură analogică, cu componentul complementului C1q, de asemenea funcționează ca o moleculă PRR a sistemului imun ereditar, unindu-se cu suprafața microbiană. MBL este capabilă să activeze sistemul complementului prin intermediul proteazelor sau să acționeze ca o opsonină, care intensifică fagocitoza. Nivelul seric al MBL, de asemenea, joacă un rol în regularea citokinelor inflamatorii precum IL-6, IL-1 β și TNF-α în răspunsul patogenetic.

Două studii recente au raportat despre rolul MBL și polimorfismul genelor sale (mbl 2) la pacienții cu hepatită cronică B [6]. Ambele studii au stabilit, că pacienții cu genotipuri asociate cu niveluri scăzute de MBL în ser probabil au putut mai bine să demonstreze persistența virală, progresia fibrozei și dezvoltarea carcinomului hepatocelular. Purtătorii HBV fără progresia afecțiunii hepatice și cei ce s-au însănătoșit spontan nu arată diferență în nivelurile MBL sau polimorfisme mbl 2, comparativ cu lotul de control sănătos. Experimentele *in vitro* de asemenea demonstrează, că MBL poate lega HBsAg într-o manieră doză-dependentă, calciu-dependentă, o interacțiune care, de asemenea, crește depozitarea C4 [6].

Clearance-ul viral este mediat prin distrugerea celulelor infectate de către limfocitele T citotoxice antigen-specifice (CTLs).

CTLs posedă potenţial citopatic şi curativ. Autorii relatează, că lezarea hepatică este mediată de celulele inflamatorii nespecifice recrutate (atrase) de CTL, probabil prin eliberarea interferon-γ-mediată a citokinelor hemotactice şi inflamatorii.

CTL activate şi citokinele secretate de ele pot deregla expresia genelor HBV şi replicarea prin mecanisme de inactivare intracelulară non-citotoxică, incluzând degradarea ARN viral şi, probabil, degradarea nucleocapsidelor virale şi DNA replicativ fără distrugerea celulelor [9].

Acest proces este mediat de IFN-γ şi TNFα, secretaţi de către CTL după recunoaşterea antigenului fără calea semnalelor Fas-dependente sau perforin-dependente. Aceste rezultate sugerează că diferite populaţii de celule inflamatorii pot fi responsabile de clearence-ul viral precoce şi patogeneza virală tardivă în infecţia HBV.

Celulele-T prin intermediul receptorilor (TCRs) recunosc antigenul procesat, prezentat fiind cu ajutorul moleculelor MHC. Celulele-T citotoxice CD8+ recunosc antigenul procesat, prezentat prin intermediul moleculelor MHC clasa I şi distrug celulele infectate, prevenind replicarea virală. Într-o oarecare măsură virusurile procesate sunt imediat susceptibile la efectul anticorpilor. Paralel cu distrugerea directă a celulelor infectate, celulele-T CD8+ de asemenea produc câteva citokine, incluzând *tumour necrosis factor-α* (TNF-α) şi limfotoxina. Interferonul-γ, un alt produs al celulelor-T CD8+, intensifică apărarea antivirală cu ajutorul celulelor adiacente rezistente la infecţie. T-limfocitele CD4+ recunosc antigenul procesat, prezentat fiind prin intermediul moleculelor MHC, clasa II. T-celulele helper de tip 1 (Th1) secretă interferonul-γ, IL-2, iar T- helper de tip 2 (Th2) secretă IL-4, 5 şi 6.

Studiile anterioare în infecţia HBV acută simptomatică arată un răspuns viguros policlonal multispecific CTL clasa I, restricţionate către toate proteinele HBV [9].

De asemenea, un viguros CD4+ T-helper răspuns HLA - clasa II restricţionat, către multiplii epitopi din nucleocapsida HBV, HBcAg şi HBeAg, este detectat în sângele periferic al pacienţilor cu hepatite acute de sinestătător limitate, pe când răspunsul HLA clasa II, restricţionat specific către HbsAg, este mai puţin viguros.

Dezvoltarea unui răspuns CD4 MHC clasa II, restricţionat către *core* este temporar asociat cu clearance-ul HBV în ser şi este, probabil, esenţial pentru un control eficient al viremiei. Acest răspuns CD4+ *core* specific exercită efectul său prin producerea Th1-citokinelor (în hepatita acută de sinestătător limitată), dominantă fiind producerea IFN-γ, ceea ce sugerează că efectele Th1-mediate ar contribui la lezarea celulară a ficatului şi la însănătoşire [9].

În timpul infecţiei HBV cronice, răspunsul T-celular HLA, clasa II în sângele periferic către toţi antigenii virali, inclusiv HBcAg şi HBeAg, este mai puţin viguros decât la pacienţii cu hepatite acute [9].

Răspunsul T-celular specific către nucleocapside pare să fie accentuat în timpul exacerbărilor acute ale bolii, care poate deseori să fie precedat de creşterea concentraţiilor DNA HBV şi HBeAg.

Clonele T-celulare din ficat la persoanele cronice HBV, stimulate cu mitogen, produc predominant un răspuns citokinic de tip 2 [9].

Trei forme structurale ale proteinelor virale HBsAg, HBcAg şi HBeAg pot obţine diferite subseturi de celule Th. HBeAg induce răspuns imun celular Th2 la şobolani [5], în timp ce HBcAg induce răspuns imun celular Th1. S-a demonstrat că dozele mici de virus sunt abile să inducă un răspuns citotoxic T-limfocitar Th1-mediat, în timp ce dozele înalte de virus induc răspuns non-protectiv umoral Th2-mediat [5].

În contrast cu infecţia HBV acută de sinestătător limitată, răspunsul CTL periferic la pacienţii cronic infectaţi este dificil de detectat [9]. S-a constatat că pacienţii cronic infectaţi, care au obţinut o remisie spontană sau interferon-indusă dezvoltă un răspuns CTL către HBV, similar pacienţilor, care s-au însănătoşit după hepatita acută [9]. Rezultatele sugerează, că intensificarea imunoterapeutică specifică a răspunsului CTL contra HBV poate fi posibilă la pacienţii cronici infectaţi şi că aceasta poate duce la clearance-ul viral la aceşti pacienţi cu rezoluţia maladiei cronice hepatice.

CTLs HBV-specifice au fost detectate într-o proporţie mică în ficat la pacienţii cronic infectaţi, posibil contribuind la inflamaţia cronică, dar insuficiente pentru eliminarea virală [9].

La pacienţii cronici HBV-infectaţi cu o afectare hepatică mică, dar cu un control al replicării HBV, erau prezente T-celule CD8+ HBV-specifice funcţional active în circulaţie şi în ficat. Din contra, pacienţii cu o rată înaltă a replicării HBV şi prezenţa inflamaţiei vădite hepatice au arătat un pattern diferit al T-celulelor CD8+ virus-specifice [9].

Prezenţa unei cantităţi mari de antigene virale, de regulă, este însoţită de creşterea insuficienţei T-celulelor HBV-specifice (cantitative şi funcţionale), care se manifestă prin reducerea treptată (în decurs de câţiva ani – zeci de ani) a populaţiilor T-celulare CD4+ şi CD8+ practic până la nivel nedetectabil. Insuficienţa imunităţii celulare se observă la pacienţi de rase diferite (asiaţi-europeni), infectaţi cu diverse genotipuri HBV. Se apreciază un spectru larg de modificări ale T-celulelor HBV-specifice – de la insuficienţă funcţională (în urma deprimării potenţialului de proliferare şi scăderii producerii de citokine) până la supraproducerea moleculelor proapoptogene [1]. Până în prezent nu este pe deplin clar, în ce mod în cadrul infecţiei cronice HBV T-celulele virusspecifice, insuficiente cantitativ şi funcţional, sunt capabile de a regula procesele imunologice în ficat. Există o părere, precum că episoadele acutizării hepatitei cronice B reflectă restabilirea răspunsului adecvat al T-celulelor HBV-specifice. Această ipoteză nu are o bază experimentală şi se bazează pe cercetările care indică restabilirea T-celulelor helper HBV-specifice imediat după acutizare. Anterior s-a demonstrat că răspunsul T-celular CD8+ HBV-specific corelează cu capacitatea de a controla nivelul viremiei şi nu corelează cu intensitatea afectării hepatice [1]. Până nu demult lipseau date exacte despre participarea altor componente ale sistemului imun, în particular ale imunităţii congenitale, şi doar în ultimii ani au apărut

date despre posibila implicare a NK-celulelor (la activarea lor prin intermediul citokinelor) în procesul afectării celulare şi acutizării hepatitei cronice B [1].

Înţelegerea cineticii răspunsului imun în acutizarea hepatitei cronice B este posibilă cu ajutorul analizei indicilor imunologici după sistarea tratamentului antiviral (fig. 3). Este cunoscut faptul că preparatele alfa-interferon (IFN-α) şi analogii nucleotidelor/nucleozidelor, ce deprimă activitatea transcriptazei inverse, într-o anumită măsură permit controlul replicării virale, dar eliminarea HBV se întâmplă rar sub acţiunea acestor preparate. Din această cauză întreruperea tratamentului antiviral conduce spre activarea rapidă a replicării HBV cu apariţia ulterioară a manifestărilor clinice ale acutizării hepatitei. Aceste două faze – activarea replicării HBV şi începutul acutizării hepatitei cronice – sunt cert delimitate. În lucrarea lui A. Bertoletti s-a demonstrat, că activarea replicării virale după sistarea tratamentului antiviral nu duce imediat la afectarea hepatică [1]. Răspunsul imun asociat cu apariţia semnelor clinice ale acutizării hepatitei cronice se dezvoltă doar peste 8 - 12 săptămâni după sistarea terapiei. Pe lângă aceasta, mecanismele imunoregulatoare (de exemplu, IL-10 şi T reg) nu participă la reţinerea răspunsului imun, deoarece nivelurile IL-10 şi ale limfocitelor T-reg nu se majorează imediat după sistarea preparatelor antivirale, dar urmează majorarea lor după creşterea activităţii alaninaminotransferazei (ALAT) [1].

Răspunsul imun umoral este cea de-a doua armă majoră, îndreptată împotriva infecţiei HBV. Anticorpii HBV – specifici sunt indicatorii stadiilor maladiei. Ig M specifică către HBcAg este un marker precoce al infecţiei, în timp ce anticorpii către HBeAg şi HBsAg sunt markeri tardivi şi indică o rezolvare favorabilă a infecţiei. Anticorpii specifici HBsAg mediază o imunitate protectivă. Anticorpii IgG specifici HBcAg şi HBsAg persistă pe parcursul vieţii după însănătoşirea clinică [4].

Compoziţiile proteice diferite ale virusului HBV pot evoca generarea diverselor subclase IgG. La persoanele natural infectate anti-HBs IgG constau din IgG3 şi IgG1 [5]. Studiile recente de asemenea sugerează că anti-HBs, subclasa IgG1 au fost predominanţi la pacienţii vindecaţi, la purtătorii cronici şi la cei vaccinaţi. Titrele relative anti-HBs IgG au fost IgG1 > IgG3 = IgG4 la purtătorii cronici şi persoanele vindecate. Pentru anti HBc pattern-ul subclasei anti HBc IgG a fost IgG1 > IgG3 > IgG4 - la purtătorii cronici şi IgG 3 > IgG1 > IgG4 - la persoanele vindecate. Pentru anti HBe pattern-ul subclasei anti HBe IgG a fost IgG1 > IgG4 > IgG3 la purtătorii cronici şi IgG1 > IgG3 > IgG4 - la persoanele vindecate. Nivelul IgG2 al anti HBe, anti HBc şi anti HBs este cel mai mic [5].

Este cunoscut faptul că în deprimarea răspunsului imun viralspecific pot lua parte mecanismele, ce inhibă activitatea T-celulelor autoreactive. Rolul principal în acest proces le aparţine T-celulelor regulatoare (Treg) [3]. Regularea celulelor-T HBV specifice este efectuată prin activitatea celulelor-T CD4+ regulatoare. Celulele-T regulatoare pot limita clearance-ul viral în infecţia cronică prin inhibiţia proliferării şi funcţiei efectoare a celulelor-T şi a altor celule imune. S-a demonstrat că pacienţii cu infecţia cronică HBV au crescut procentajul celulelor-T regulatoare CD4+ CD25+ FoxP3+ în sângele periferic, comparativ cu grupul de control, format din persoane sănătoase şi indivizi care au tratat infecţia HBV. Celulele-T regulatoare izolate din sângele periferic al acestor pacienţi sunt capabile să inhibe răspunsul T-celular CD4+ şi CD8+ HBV specific *in vitro* [2].

Numărul înalt de celule-T regulatoare şi citokine imunosupresive pot limita abilitatea celulelor-T intrahepatice de a eradica HBV.

Pe lângă celulele sistemului imun, în ultimii ani s-a determinat şi rolul major al trombocitelor în afectarea hepatică de către infecţia virală. Astfel, în cercetările M. Jannacone şi coaut. s-a demonstrat, că trombocitele activate participă în patogeneza afectării hepatice şi clearance-ului HBV şi HCV cu implicarea CTL virus-specifice. În rezultatul interacţiunii CTL cu trombocitele în sinusoidele ficatului, CTL pot migra din patul vascular la celula parenchimatoasă (de exemplu, în hepatocit) şi să realizeze efectul patogenetic şi/sau antiviral [1].

Participarea trombocitelor activate în atragerea CTL virusspecifice presupune că acţiunea farmacologică asupra moleculelor, implicate în activarea trombocitelor, poate preîntâmpina migraţia CTL în ficat şi reduce gradul de afectare a hepatocitelor.

Cercetările experimentale au arătat că combinarea dozelor mici de aspirină şi clopidogrel duce la deprimarea exprimată a ambelor procese. Complicaţii hemoragice nu s-au apreciat. Observările efectuate confirmă că pe lângă prevenirea activării exagerate a reacţiilor imune în ficat (de exemplu, în hepatitele fulminante) sau încetinirii clearence-ului virusurilor, administrarea îndelungată a aspirinei şi clopidogrelului este însoţită de "înmuierea" severităţii atingerii hepatice cronice CTL-induse şi preîntâmpină sau încetinesc tempoul dezvoltării carcinomului hepatocelular. La şobolanii HBV-transgenici, care reprezintă modelul portajului "sănătos" la om, se formează toleranţă imunologică la antigenele virale şi nu se observă dezvoltarea hepatitei pe parcursul vieţii. În caz de înlocuire a sistemului imun tolerant al unui astfel de şobolan cu sistemul imun al unui şobolan singen netransgenic, anterior imunizat cu produse proteice HBV, se dezvoltă hepatita cronică CTL-dependentă cu activitate joasă, cu trecerea în carcinom hepatocelular. Aceste date pot servi ca bază pentru alcătuirea principiilor utilizării factorilor antitrombocitari în calitate de măsuri de profilaxie a dezvoltării carcinomului hepatocelular [1].

Infecţia HBV cronică se caracterizează prin persistenţa în organism a concentraţiilor înalte ale virusului (până la $10^{10-12}$ copii ADN/ml) şi particulelor sale, care conţin antigenul de suprafaţă (HBsAg); producerea ultimelor de $10^{4-6}$ ori este mai înaltă decât concentraţia virionilor completi [1].

La baza unor astfel de sisteme efective de reproducere stă capacitatea HBV de a crea o matrice episomală transcripţională foarte stabilă (fig. 4).

Nivelul replicării virale şi formarea ccc ADN corelează cu efectul citopatic în hepatocitele infectate [10].

Preparatele antivirale permit realizarea controlului replicării virale, dar eliminarea HBV se determină foarte rar, drept cauză servind capacitatea virusului de-a exista sub formă ADN circular închis covalent (*covalently closed circular*) (ccc ADN), care poate iniția replicarea HBV imediat după întreruperea tratamentului antiviral. În cazul persistenței cccADN, eliminarea HBV este posibilă doar prin inducerea unui răspuns imun efectiv. Un astfel de răspuns poate fi atins prin "setarea" sistemului imun cu ajutorul preparatelor imunomodulatoare, vaccinării adecvate sau prin intermediul terapiei T-celulare, utilizându-se T-celulele *ex vivo* T-celule activate sau reprogramate [1].

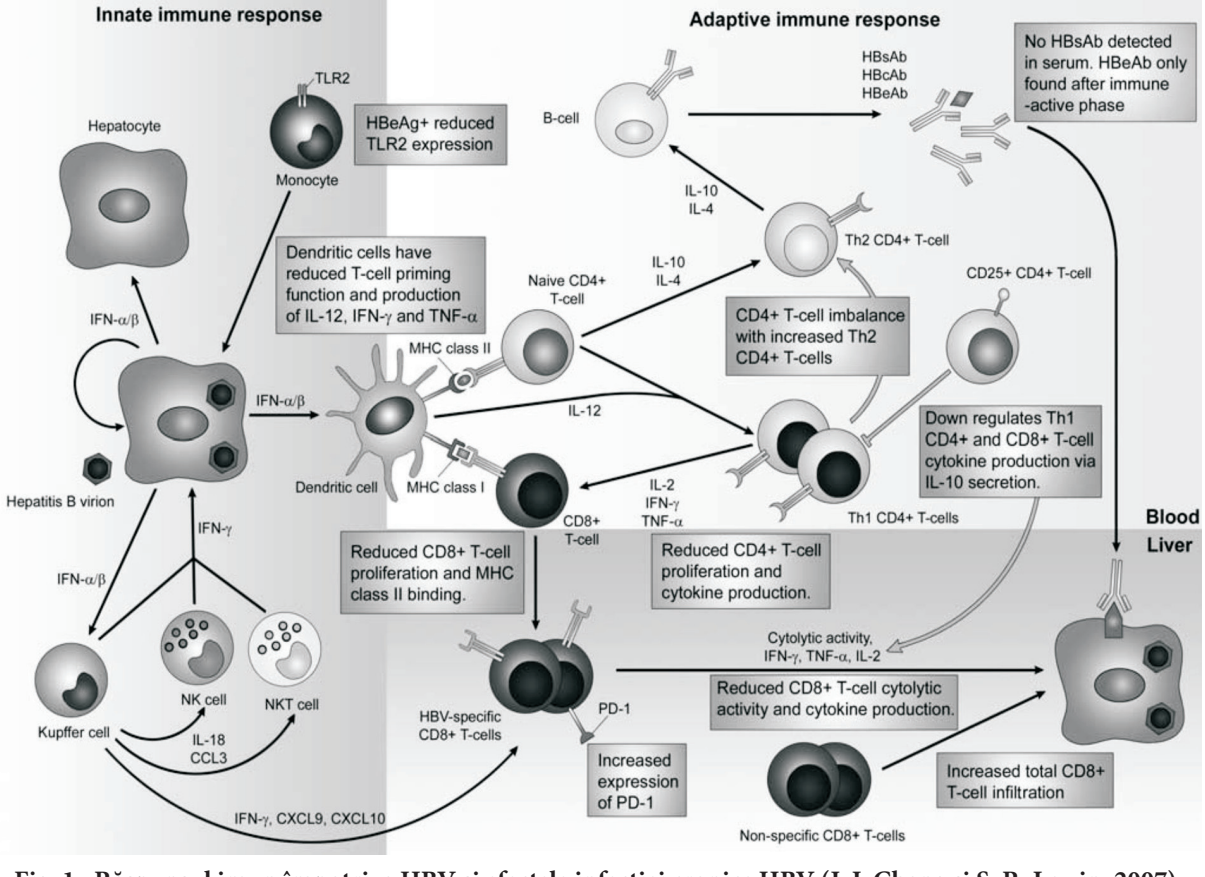


**Fig. 1. Răspunsul imun împotriva HBV și efectele infecției cronice HBV (J. J. Chang și S. R. Lewin, 2007).**

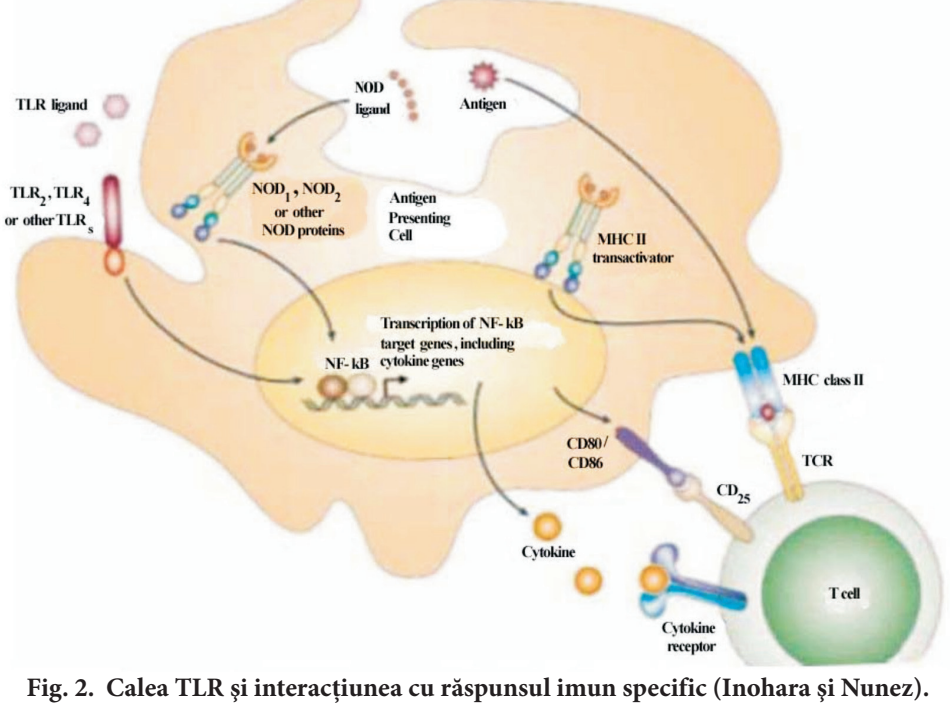


**Fig. 2. Calea TLR și interacțiunea cu răspunsul imun specific (Inohara și Nunez).**

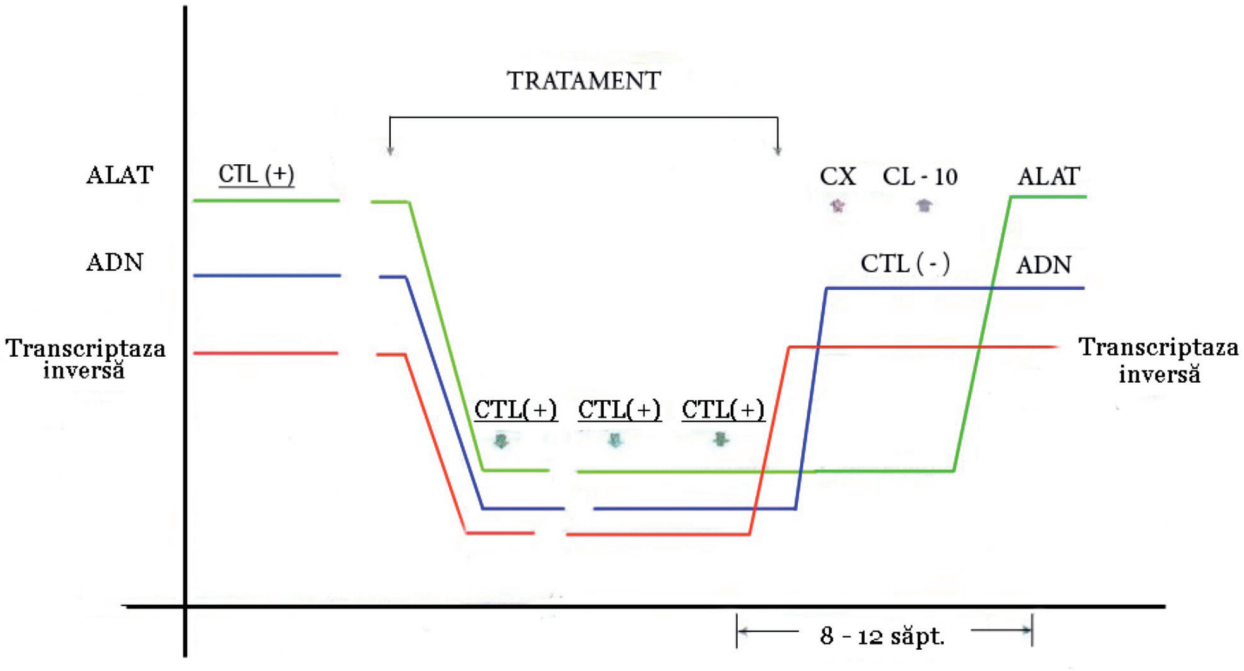


**Fig. 3. Recidiva hepatitei cronice B după sistarea tratamentului (V. T. Ivashkin, 2009).**

Acutizarea hepatitei cronice B sigur se asociază cu elevarea în sângele pacienților a nivelurilor hemotoxinelor CXCL-9 și CXCL-10, induse de interferonul-gamma (IFN-γ) [1].

Comparația cantității NK-celulelor circulante și T-celulelor virusspecifice (producători mult mai probabili de IFN-γ) nu a scos în evidență o corelare evidentă dintre activarea NK-celulelor sau cantitatea T-celulelor HBV-specifice și debutul acutizării hepatitei cronice. Dimpotrivă, celulele-T HBV-specifice se apreciază doar la pacienții fără activarea replicării HBV (păstrându-se controlul replicării) și în lipsa semnelor afectării hepatice. Pe lângă aceasta, recunoașterea hepatocitelor infectate de către limfocitele HBV-citotoxice se efectuează preponderent prin intermediul CXCL-10 și în măsură mai mică CXCL-9. Producerea de către hepatocite a ultimului hemotoxin se realizează prin semnale suplimentare, regulate de IFN-α [1].

IFN-γ produs de NKT, NK și Th1/Tc1 T-celule, induc sinteza hemokinelor CXCL-9 (*monokine induced by* IFN-γ (Mig), CXCL-10 (IP-10), și CXCL-11 (*interferon-inducible Tcell chemoattractant* [ITAC]). Aceste hemokine din familia CXC sunt produse de celulele endoteliale sinusoidale, macrofage și hepatocite și se leagă de CXCR3, un receptor care este expresat la un nivel înalt pe ficatul infiltrat, apoi pe T-celulele din sângele periferic [4].

Astfel IFN-γ, responsabil de diminuarea replicării HBV, este produs nu de celulele inflamatoare recrutate ale gazdei, dar numai de CTLs transferate adoptiv [4].

Lezarea hepatică și distrugerea hepatocitelor poate decurge pe câteva căi. Hepatocitele infectate pot fi ucise de celulele-T CD8 prin FasL, TNFα și/sau calea perforin/granzyme [4].

Organele și țesuturile, în care HBV persistă nu sunt cunoscute. În hepatita cronică B formele replicative ale virusului au fost detectate în epiteliul ducturilor biliare și celulele musculare netede, în pancreas, rinichi și piele, creer, țesutul endocrin, nodulii limfatici și formele non-replicative în celulele sistemului imun [4].

## Concluzii

Afectarea hepatică în infecția HBV cronică posedă un caracter imunomediat, deoarece virusul hepatic B nu posedă acțiune citopatică directă. Clearance-ul HBV și afectarea imună a celulelor hepatice se realizează de către limfocitele citotoxice virusspecifice (CTL) ale sistemului imun adaptiv. Persistența HBV reflectă incapacitatea CTL de-a elimina virusul din organism, ceea ce inițiază o reacție cronică necroinflamatoare în ficat, conducând în rezultat la dezvoltarea cirozei sau carcinomului hepatocelular. Afectarea cronică a ficatului în infecția HBV prezintă un proces potențial pretumoral în sine, care decurge cu dereglarea balanței dintre regenerarea hepatocitelor și inflamație. Rolul trombocitelor activate în patogeneza hepatitei virale cronice constă în capacitatea lor de-a atrage în patul microvascular al ficatului celulele CTL. Drept cauze ale perisistenței HBV și HCV se consideră următoarele mecanisme: 1) disfuncția CD4+ și CD8+ celulelor-T în rezultatul replicării virale prelungite – ipoteza "epuizării"; 2) disfuncția celulelor-T regulatoare (T reg) – ipoteza "regulării"; 3) acțiunea de"scăpare" a HBV față de mecanismul de apărare al gazdei prin ocolirea supravegherii imunologice - ipoteza "scăpării" imune (virus escape); 4) modificările în replicarea virală.

Sistarea IFN-α sau a analogilor nucleozidici/nucleotidici la pacienții cu hepatita cronică B poate duce la activarea replicării HBV, însoțită de manifestări clinice și de laborator (acutizarea hepatitei cronice). Răspunsul imunopatologic sub forma acutizării hepatitei cronice cel mai des se dezvoltă peste 8-12 săptămâni, după sistarea tratamentului antiviral.

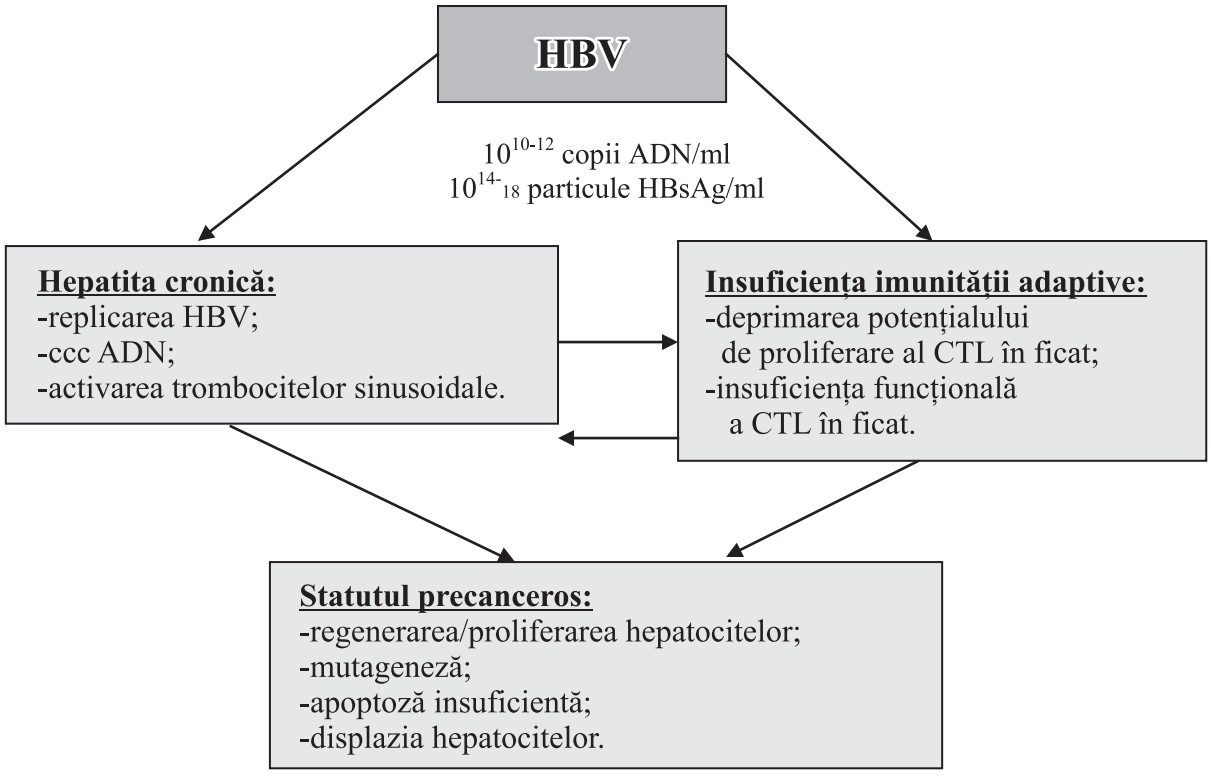


**Fig. 4. Patogeneza hepatitei cronice virale B (V. T. Ivashkin, 2009).**

Acest interval poate fi considerat ca "fereastră terapeutică", în limitele căruia în cazul depistării la pacient a deficitului cantitativ și funcțional al CTL HBV-specifice, este necesar de reînceput tratamentul antiviral.

Întreruperea tratamentului antiviral este însoțită de restabilirea bruscă a activității transcriptazei inverse și replicării ADN HBV. Dar începutul acutizării hepatitei cronice are loc peste 8-12 săptămâni după anularea tratamentului. Acest proces este legat de scăderea cantității celulelor –T citotoxice HBV-specifice și de creșterea în sângele pacienților a hemochinei CXCL-10, indusă de către interferonul-gamma.

Încărcătura virală înaltă servește drept factor-cheie în dezvoltarea insuficienței imunității adaptive, în particular a T-limfocitelor citotoxice (CTL) în ficat. Procesul cronic necrotic-inflamator în ficat persistă datorită replicării treptate a virusului pe matrice episomală (extracromozomială) ciclic covalent circular închisă ADN (cccADN) și insuficienței funcționale a limfocitelor citotoxice din ficat, incapabile de a elimina celulele infectate cu virus. Decurgerea inflamației cronice este susținută de trombocitele sinusoidale, care contribuie la migrarea limfocitelor citotoxice din sânge în ficat. Replicarea noilor hepatocite este însoțită de creșterea mutațiilor în genomul lor și de schimbările displastice, iar eliminarea insuficientă a hepatocitelor cu ADN afectat prin intermediul apoptozei, contribuie la dezvoltarea statutului precanceros.

### Bibliografie

1. Ивашкин ВТ. Иммунная система и повреждения печени при хронических гепатитах В и С. *Рос. Журн. Гастроэнтерологии, Гепатологии, Колопроктологии.* 2009;6:4-10.
2. Boonstra Andre, Woltman Andrea M, Janssen Harry LA. Immunology of hepatitis B and hepatitis C virus infections. *Best Practice& Research Clinical Gastroenterology*. 2008;22(6);1049-1061.
3. Bertoletti Antonio, Gehring Adam J. The immune response during hepatitis B virus infection. *Journal of General Virology.* 2006;87:1439-1449.
4. Rehermann Barbara. Immune Response in Hepatitis B Virus Infection. *Seminars in Liver Disease*. 2003;23(1):21-37.
5. Chien-Fu Huang, Shih-Shen Lin,Yung-Chyuan Ho. The Immune Response Induced by Hepatitis B Virus Principal Antigens. *Cellular&Molecular Immunology.* 2006;3(2):97-106.
6. Ratnam Dilip, Visvanathan Kumar. New concepts in the immunopathogenesis of chronic hepatitis B: the importance of the innate immune response. *Hepatol Int*. 2008;2:S12–S18.
7. Chang J. Judy, Lewin Sharon R. Immunopathogenesis of hepatitis B virus infection. *Immunology and Cell Biology*. 2007;85:16-23.
8. Lang Karl S, Georgiev Panco, Recher Mike. Immunoprivileged status of the liver is controlled by Toll-like receptor 3 signaling. *The Journal of Clinical Investigation*. 2006;116(9):2456-2462.
9. Jung Maria-Christina, Pape Gerd R. Immunology of hepatitis B infection. *The Lancet Infectious Diseases.* 2002;2:43-50.
10. Baumert Thomas F, Thimme Fritz von Weizsäcker Robert. Pathogenesis of hepatitis B virus infection. *World J Gastroenterol.* 2007;13(1):82-90.

# Unele aspecte patogenetice ale hiperplaziei neointimei în cadrul restenozei intrastent

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**Pathogenetic Aspects of the Neointima Hyperplasia in the in-stent Restenosis**

Using the method of hybridization in situ, RT-PCR, confocal scanner microscopy and immunofluorescent microscopy with specific antibodies to matrix metalloproteinase collagenase IV (MMP-2), we analyzed expression of tissue inhibitor (TIMMP-2) and mononuclear cell marker CD68 in restenosed coronary tissue taken from 11 patients who died with in-stent restenosis (ISS). The expression and quantity of micro-ribonucleic acids (micro-RNA/145), MMP-2 and TIMMP-2 were estimated, as well as the number of present macrophages. The micro-RNA/145 expression and quantity are reduced in ISS, and this reduction is proportional to restenosis degree. On the other hand, the MMP-2 expression and quantity in the neointima are increased while that of TIMMP-2 is reduced. With respect to the number of macrophages present in the neointima of ISS tissue, their number is greatly elevated in the areas affected by the restenosis.

**Key words:** restenosis, micro-RNA/145, matrix metalloproteinase.

**Некоторые патогенетические аспекты гиперплазии неоинтимы при внутристентовом рестенозе**

Используя методы гибридизации *in situ* и цепной полимеразной реакции, а также лазерную и иммунофлюоресцентную микроскопию, с использованием специфических антител к матричной металлопротеиназе-2 (ММП-2) или коллагеназа 4, к её специфическому тканевому ингибитору ТИММП-2 и к CD68, маркеру мононуклеарных клеток были определены экспрессия и количество микро-рибонуклеиновой кислоты 145, ММП-2, ТИММП-2, как и число макрофагов в тканевом образце, взятом из стента 11 умерших пациентов с внутристентовым рестенозом (ВСР). Экспрессия и количество микро-РНК/145 снижаются пропорционально степени ВСР, в то время как соответствующие показатели ММП-2 в неоинтиме увеличиваются на фоне уменьшения их величины относительно ТИММП-2. Характерным для ВСР является многократный прирост в неоинтиме числа макрофагов.

**Ключевые слова:** рестеноз, микро-РНК/145, матричная металлопротеиназа.

## Introducere

Restenoza intrastent (RIS), care evoluează de regulă după 4-6 luni de zile de la momentul realizării angioplastiei, rămâne o lansare de alertă a cardiologiei intervenționale, deși trebuie de menționat că incidența acesteia prin aplicarea eluting-stenturilor s-a redus categoric comparativ cu angioplastia cu balon sau stenturi metalice. Destul de frecvent evoluția restenozei intrastent se manifestă prin sindrom coronarian acut, în circa 10% cazuri, fiind constatat infarctul miocardic acut [1]. Potrivit rezultatelor obținute în cadrul trialului PRESTO evoluția clinică a sindromului coronarian acut pe fundalul RIS este asociată cu o incidență sporită a efectelor cardiovasculare adverse [2].

Formarea și hiperplazia neointimei este conceptual vizată drept un aspect oportun al patogeniei RIS, mecanismele inerente fiind tratate vag și ipotetic. Declanșarea proceselor proliferative în peretele arterei coronariene este, în fond, atribuită răspunsului local vascular la impactul traumatic asociat cu alterarea sau chiar denudarea endoteliului coronarian [3]. Activarea plachetelor și a componentelor tisulare ale hemostazei realizează formarea inițială a trombusului parietal și chiar cavitar, fenomen acompaniat de recrutarea celulelor albe sangvine (monocitele, neutrofilele, limfocitele), pasajul cărora printre epavele celulelor endoteliale este facilitat nu numai prin alterarea endoteliului, dar și prin creșterea expresiei chemokinelor (factorii chemoatractanți) și a moleculelor de adeziune, cum ar fi VCAM și ICAM [4]. Infiltrarea și cantonarea acestor celule în spațiul subendotelial amorsează procesul inflamator, susținut de majorarea expresiei citokinelor proinflamatoare și reducerea sintezei constitutive a oxidului nitric (NO), care inhibă activarea factorilor proinflamatori și expresia moleculelor de adeziune. Utilizarea eluting-stenturilor diminuează considerabil activitatea răspunsului inflamator și, respectiv, intensitatea procesului de hiperplazie a neointimei, având repercusiuni benefice asupra riscului RIS [5]. Factorii neuroendocrini (e. g. angiotensina II și endotelina-1) prin acțiunea sa mitogenă, de creștere și de activare a stresului oxidativ potențează formarea neointimei.

Hiperplazia neointimei este concepută nu numai drept rezultatul sintezei *de novo* a diferitor componente ale matricei extracelulare, dar și a migrării celulare. Dovezile acumulate în acest sens aduc la apel implicarea celulei musculare netede vasculare, dată fiind identificată expresia izoformelor embrionare ale proteinelor contractile și prezența în neointimă a celulelor progenitoare pentru miocitele netede [6]. Se consideră că lezarea laminei elastice interne ar fi una din condițiile de coroborare a migrării celulare spre intima coronariană din zona medie adiacentă. Totodată, rezultatele relatate de J. Wilcox și colab. (1996) care demonstrează că circa 50% din celulele neointimei

sunt originare celulelor adventicei (miofibroblaştii), au sugerat semnificaţia lizei diseminate a colagenului matricei extracelulare în procesul de migrare celulară şi formarea şi extinderea neointimei [7]. De remarcat că acestea din urmă sunt asociate cu modificarea calitativă şi cantitativă a cantonului celular al stratului adventicial şi creşterea notabilă a dimensiunii lui [8], fapt care indică şi asupra participării active a adventicei în formarea neointimei. În acest context trebuie de menţionat transformarea fibroblaştilor adventicei sub acţiunea radicalilor liberi de oxigen, citokinelor, leucotrienelor şi a factorilor de creştere (în primul rând factorul de transformare beta) în celule migratoare, miofibroblaşti, care la rândul lor, ajungând în mediul vascular, se transformă în miofibroblaşti contractili ce secretă diferiţi mediatori proinflamatori şi componente ale matricei extracelulare. De altfel, individualizarea miofibroblastului în neointima consecventă angioplastiei experimentale a fost una din primele confirmări asupra capacităţii migratoare a fibroblaştilor adventicei [9].

Formarea neointimei evoluează pe fundalul unui răspuns imun exagerat. Creşterea nivelului circulant al anticorpilor către Heat-Shock-Protein-27, markerul răspunsului imunalergic, se constată deja după 24 de ore din momentul implantării stentului şi se corelează pozitiv cu majorarea conţinutului sanguin la proteinei C reactive, markerul inflamaţiei, disfuncţiei endoteliale şi trombozei intrastent [10, 11]. Celulele prezentatoare de antigen activează limfocitele T, care în consecinţă se transformă în limfocite proinflamatoare Th1, iar persistarea procesului inflamator determină în continuare eliberarea excesivă a radicalilor liberi de oxigen, precum şi a enzimelor proteolitice capabile să influenţeze concludent rata de degradare a colagenului, a laminelor elastice şi, respectiv, a migrării celulare.

Cu referinţă la migrarea CMNV este de subliniat rolul determinativ al fenotipului celulei. Miocitul matur cu fenotipul contractil nu este activ în vederea migrării şi proliferării, iar miocitul cu fenotip sintetic sau secretor – dimpotrivă. Prin urmare, factorii ce afectează echilibrul fenotipic şi condiţionează preluarea fenotipului secretor al CMNV pot influenţa, în special pe fundalul defrişării barierelor de colagen, migrarea lor spre intima arterei coronariene, cantonarea şi, astfel, formarea neointimei.

Unul dintre factorii care participă la controlul fenotipului CMNV se ataşează la familia de micro-acizi ribonucleici (micro-ARN), oligonucleotide formate din 20-25 de nucleotide [12, 13]. Micro-ARN/143 şi micro-ARN/145 sunt vizaţi drept factori ce realizează menţinerea fenotipului contractil şi contracarează în parte acţiunea factorilor capabili să inducă preluarea fenotipului secretor.

Într-un studiu anterior noi am demonstrat că în RIS expresia micro-ARN/143 este diminuată în raport proporţional cu gradul restenozei, precum şi cu gradul degradării colagenului fibrilar de tip I în media şi adventicea arterei coronariene [14].

***În studiul actual ne-am fixat ca scop următoarele obiective:***

1. Estimarea expresiei şi a cantităţii micro-ARN/145 în paternul tisular al restenozei intrastent.
2. Aprecierea activităţii (sau expresiei) colagenazei (metaloproteinaza matricei-2, MPM-2) şi a inhibitorilor tisulari ai acesteia (ITMPM-2).
3. Evaluarea expresiei unor markeri proinflamatori.

## Material şi metode

Explorările morfologice s-au efectuat pe paternul tisular al restenozei preluat de la 8 pacienţi decedaţi.

**Microscopia fluorescentă**

Criosecţiunile cu grosimea de 5 μm au fost uscate şi spălate în bufer salin fosfat (BSF). După o incubare de 30 min cu albumină de ser bovin, mostrele au fost incubate timp de circa 12 ore cu anticorpi primari către MMP-2 (Biotrend), către ITMPM-2 (Calbiochem), către alpha-actina miocitului neted (Sigma) şi către markerul monocitelor şi a macrofagilor, CD68 (Dako). Apoi, mostrele au fost spălate în BSF şi incubate timp de 60 de min cu anticorpi secundari (IgG) către biotina murină sau de iepure (Dianova), după ce s-au incubat cu streptavidină conjugată (Cy2 sau Cy3). Nucleele au fost colorate în albastru prin DRAQ5 (Alexis) sau DAPI (probe moleculare). Secţiunile tisulare au fost examinate utilizând microscopia confocală laser (Leica TCSSP2). Pentru a individualiza secţiunile optice confocale s-au utilizat linsele Leica Planapo x 40/1,00 sau x 63/1,32. Fiecare imagine scanată avea o rezoluţie de 1024 x 1024 pixels. Pentru a ameliora calitatea imaginilor, fiecare dintre acestea a fost racordată la intensitatea medie a semnalelor şi transferată în dispozitivul grafic de silicon, pentru a obţine imagine tridemensională, utilizând sistemul multi-canal de procesare Bitplane (Elveţia).

**Determinările cantitative imunofluorescente**

Au fost examinate criosecţiunile cel puţin de la 2 blocuri tisulare diferite. Toate mostrele au fost simultan imunologic marcate cu anticorpi primari şi secundari în condiţii identice de fixare şi diluare. Secţiunile prelucrate în BSF şi care n-au fost expuse la anticorpii primari au servit drept martor negativ. La fiecare probă preluată de la pacient au fost analizate randomizat cel puţin 10 câmpuri de viziune prin intermediul microscopului imunofluorescent Leica (Leitz DMRB), aplicând obiectivul Planopo x 40. Criosecţiunile marcate imunologic au fost studiate prin analiza de imagine (Leica) şi Software-ul Image J. Pentru fiecare proteină s-a stabilit un set specific, menţinut constant în toate măsurările. Zona marcajului specific al MPM-2 şi al ITMPM-2 s-a calculat ca valoare procentuală din marcajul pozitiv inerent ariei tisulare.

**Hibridizarea *in situ***

A fost aplicată pentru analiza micro-ARN/145. Prima bandă a ADN-lui complementar s-a sintetizat din ARN miocardial uman. Reacţiile de transcripţie inversă, realizate prin intermediul Supersrcipt II (Invitrogen) şi OligodT (Promega), au fost urmate de reacţia PCR cu polimeraza 5U Taq (0,2 pmol) iminentă micro-ARN/145. Secvenţele amplificate prin PCR au fost purificate, clonate în pCRII-TOPO vector (Invitrogen) şi ordonate. Probele sintetizate pe TOPO-ANP au fost liniarizate prin plasmid Hind III şi EcoRI, utilizând ARN-polimeraza T7 sau Sp6 (Promega) şi marcate prin digoxigenină. Hibridizarea *in situ* s-a efectuat utilizând

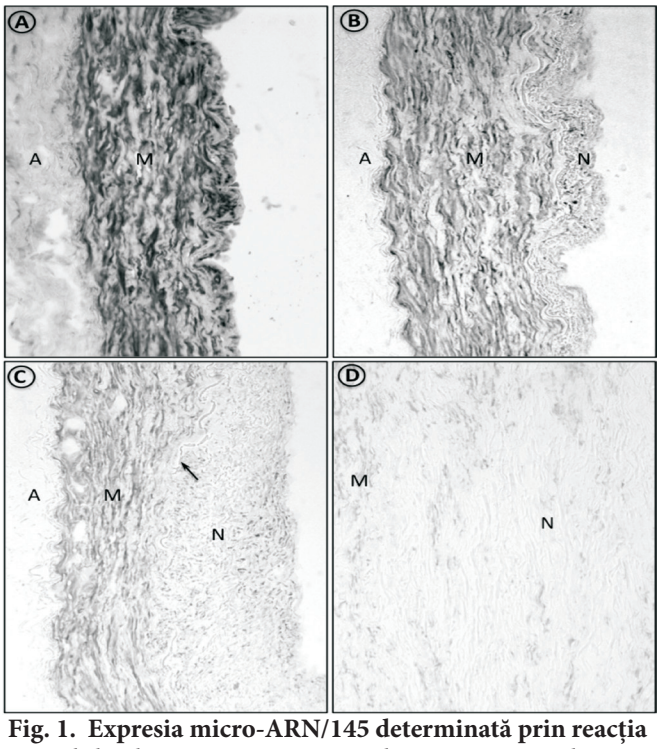


**Fig. 1. Expresia micro-ARN/145 determinată prin reacția hibridizării *in situ*. Panourile: A - vas normal, B - restenoză (grad minim), C – restenoză (grad moderat), D – restenoză (grad sever). Săgeata indică scindarea membranei elastice interne (panoul C). Abreviaturi: A - adventicea; M - media; N - neointima.**

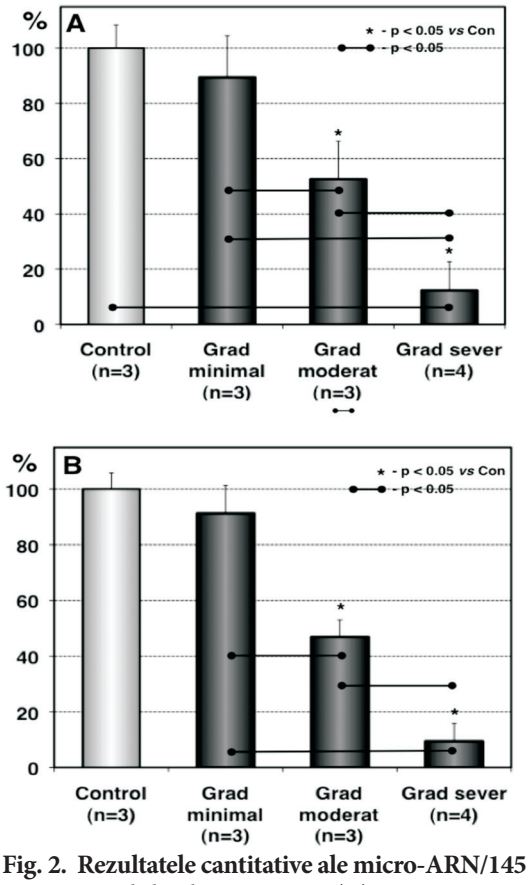


**Fig. 2. Rezultatele cantitative ale micro-ARN/145 determinate prin hibridizarea *in situ* (A) și prin PCR-TR (B). Cantitatea în control a fost standardizată ca fiind de 100%.**

anticorpi către digoxigenina conjugată prin alcalina fosfată [15]. Intensitatea micro-ARN/145 s-a determinat utilizând Imaje J și s-a estimat ca unități de intensitate pe aria tisulară.

**Examenul cantitativ prin PCR în timp real**

ARN-ul total a fost extras din țesutul cardiac utilizând reagentul TRIzol (Invitrogen Life Technologies, USA). După tratarea cu AND-aza (enzima care catalizează clivarea hidrolitică a legăturilor fosfodiesterice ale AND-ului, TURBO DNA-free Ambion, ARN (100 ng) a fost transcris invers (Superscript II, Invitrogen). ADNc a fost amplificat în sistemul PCR (reacția de polimerizare în lanț) în timp real (PCR-TR) iCycler [16]. Mostrele au fost supuse la 3 măsurări cu cel puțin 2 repetări independente ale experimentului. Cantitatea relativă a transcriptului, normalizată cu ARN 18 S, s-a calculat urmând procedurile standarde acceptate [17, 18].

## Rezultate

În cadrul hibridizării *in situ* a fost identificat micro-ARN/145 în vasul fără restenoză și în arterele coronariene cu diferite grade de RIS (fig. 1).

De menționat în acest context expresia marcată a micro-ARN/145 în media vasului fără restenoză, care, totodată, se impune prin absența neointimei. Evoluția restenozei se manifestă prin diminuarea expresiei micro-ARN/145, declinul căruia este în raport direct cu gradul RIS. Astfel, nivelul minim de micro-ARN/145 se constată în restenoza severă. Mai mult decât atât, descreșterea micro-ARN/145 este asociată cu apariția neointimei, expansiunea căreia, de asemenea, este în proporție cu gradul RIS. De remarcat, că reducerea micro-ARN/145 și formarea neointimei sunt însoțite de scindarea membranei elastice interne.

Cuantificarea micro-ARN/145 prin reacția de hibridizare *in situ* și PCR-TR demonstrează valori cantitative similare ale acesteia în arterele coronariene cu RIS (fig. 2).

Important de menționat că cantitatea micro-ARN/145 descrește odată cu avansarea gradului de RIS. Deja în gradul moderat de restenoză reculul devine semnificativ și notează cote de circa 50% ($p < 0,05$). În restenoza severă conținutul de micro-ARN/145 se identifică la o rată de circa 10% din paternul normal. Conținutul de micro-ARN/145 în RIS moderată diferă semnificativ față de indicele obținut în RIS minimă, fiind semnificativă și discrepanța între RIS severă și cea moderată. Rezultatele microscopiei confocale laser privind expresia MPM-2 și a ITMPM-2 sunt prezentate în fig. 3.

Datele obținute indică asupra modificării diferite a expresiei MPM-2 și a ITMPM-2 în cadrul evoluției RIS. Progresarea restenozei de la gradul minim la cel sever se manifestă prin majorarea expresiei MPM-2 și reducerea expresiei ITMPM-2 în media și neointima arterei coronariene. Drept urmare raportul MPM-2/ITMPM-2 este în ascensiune pertinentă odată cu avansarea gradului de restenoză, însoțită de expansiunea zonei neointimei (fig. 4).

Deja în gradul moderat de restenoză acest raport se constată aproape dublu, iar în gradul sever măsoară o creștere de circa 6 ori. Așadar, micșorarea expresiei și a cantității micro-ARN/145 pe de o parte și creșterea raportului MPM-2/

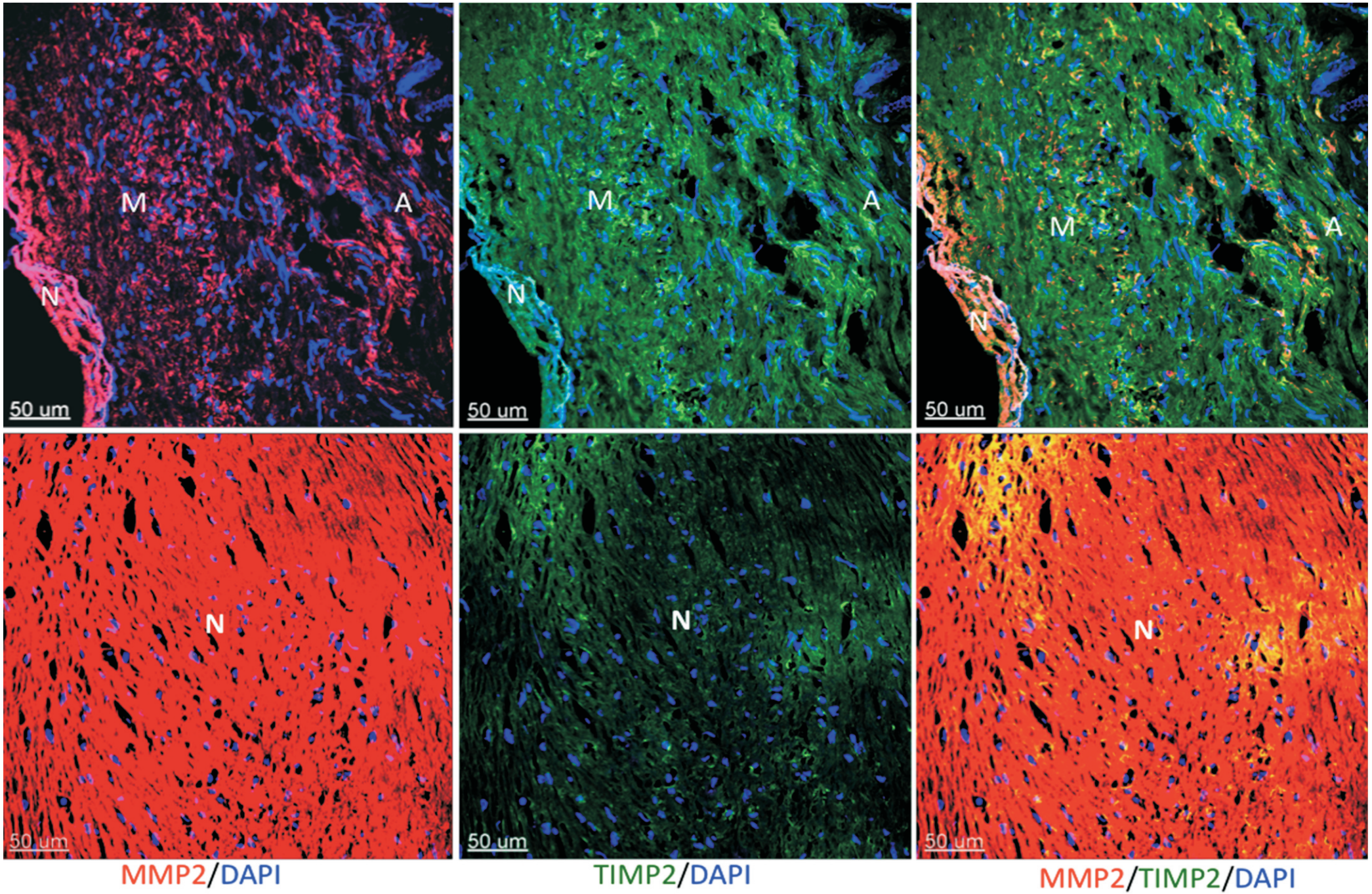


**Fig. 3. Imagini confocale ce demonstrează abundența diferită a expresiei MPM-2 (culoare roșie) și ITMPM-2 (culoare verde) în restenoza de grad minim (panourile de sus) și restenoza severă (panourile de jos). Nucleele sunt colorate in albastru cu DAPI. Toate imaginile din dreapta sunt suprapuneri ale imaginilor care arată expresia MPM-2 și ITMPM-2. A – adventicea; M - media; N - neointima.**

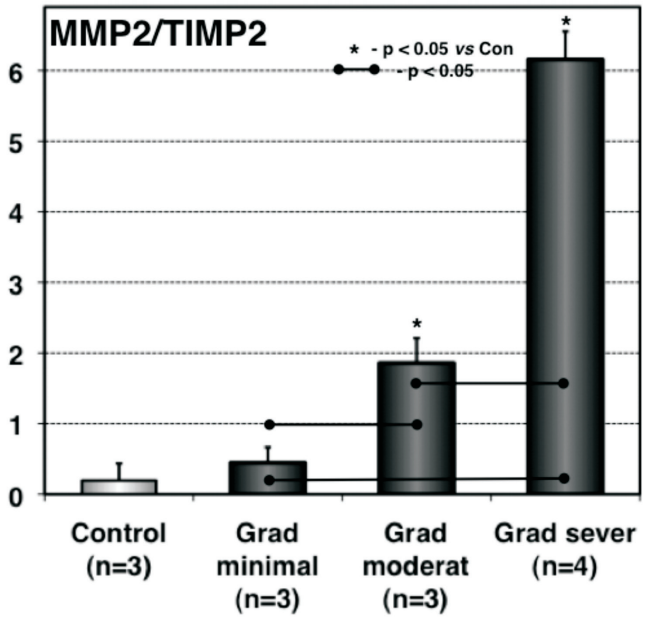


**Fig. 4. Creşterea raportului MPM-2/ITMPM-2 în evoluţia restenozei.**

ITMPM-2, datorită elevării MPM-2 şi descreşterii ITMPM-2, pe de altă parte, sunt evenimente importante, care asociază evoluţia RIS. Utilizarea anticorpilor către CD-68 în cadrul microscopiei confocale laser a permis evidenţierea macrofagilor în paternul tisular al restenozei (fig. 5).

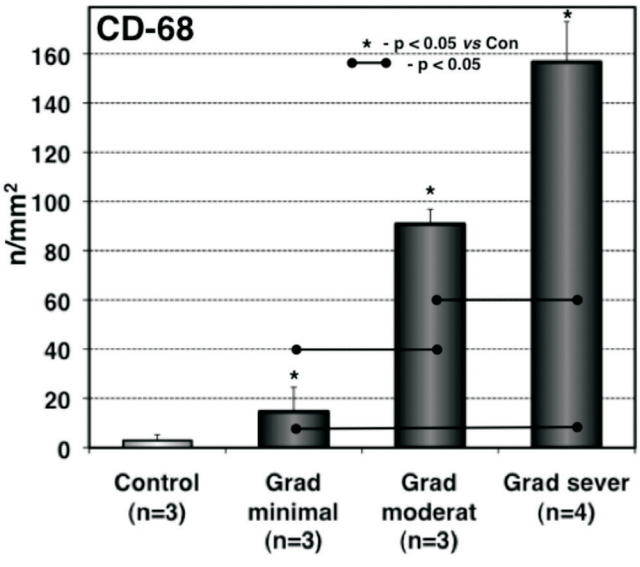


**Fig. 5. Imagini confocale care demonstrează prezenţa macrofagilor (culoarea verde), folosind anticorpul către CD-68: stenturi fără restenoză - panourile de sus, restenoză moderată - panourile din mijloc, restenoză severă - panourile de jos. Culoarea roşie reprezintă imofluorescenţa către SM-actină; în albastru sunt nucleele colorate cu DAPI. Săgeţile din panouirile din mijloc indică aderenţa şi infiltrarea macrofagilor în ţesutul restenozei din partea luminală a stentului. A - adventicea; M - media; N - neointima.**

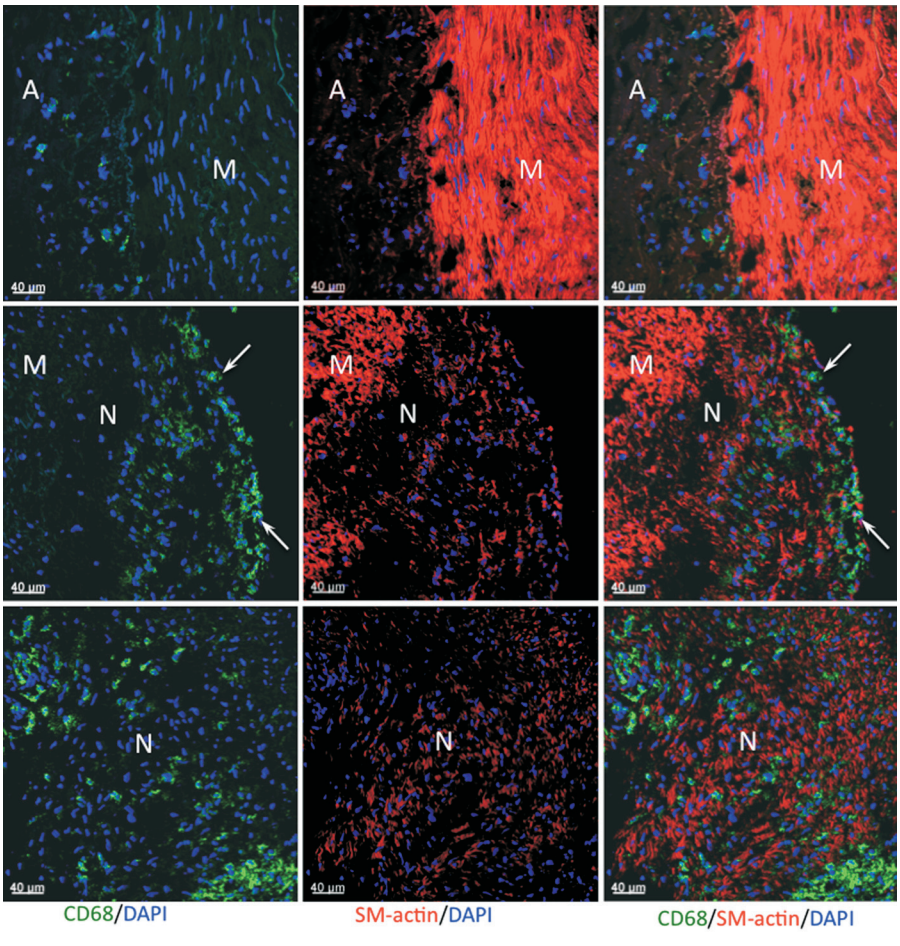


**Fig. 6. Numărul de macrofagi/1 mm² în diferite grade de restenoză.**

În stentul fără restenoză nu sunt individualizați macrofagii în intima vasului, iar prezența lor în adventice este neînsemnată. În restenoză se constată prezența macrofagilor în neointimă, inflitrarea cărora se realizează prin pasajul luminal al monocitelor. Numărul de macrofagi crește proporțional severității RIS (fig. 6). De acum la gradul minim numărul de macrofagi este semnificativ majorat, atingând valoarea medie de 13-15 celule/1 mm$^2$, care de circa 4-5 ori depășește indicele control (stentul fără restenoză). În gradul moderat de restenoză numărul de macrofagi se notează la cote de circa 80 de celule/1 mm$^2$, care practic se dublează în cadrul restenozei severe.

Prezența macrofagilor în neointimă este asociată cu apariția celulelor musculare netede. Potrivit densității culorii albastre (markerul DAPI al nucleului) putem indica asupra creșterii numerice a miocitelor netede în neointimă pe măsura progresării RIS. Prin urmare, extinderea zonei neointimei în restenoză se impune prin evidențe celulare și moleculare distincte: 1) apariția și cantonarea macrofagilor și a celulelor musculare netede, numărul cărora crește proporțional cu gradul de RIS; 2) micșorarea expresiei și a cantității micro-ARN/145 de asemenea în raport direct cu severitatea RIS; 3) elevarea expresiei MPM-2 pe fundalul micșorării expresiei ITMPM-2, raportul MPM-2/ITMPM-2 fiind în creștere odată cu avansarea gradului de restenoză.

### Discuții

Evidențele acumulate privind problema RIS demonstrează că substratul morfologic al restenozei este formarea și hiperplazia neointimei în segmentul arterei coronariene manipulate prin angioplastie. Cu toate acestea, mecanismele celulare și moleculare subtile sunt departe de a fi elucidate. Prezența miocitului neted vascular în zona neointimei se desemnează drept o oportunitate, deși inerențele patogenetice responsabile de acest fenomen rămân incerte. Rezultatele studiului nostru indică pentru prima dată în domeniul acestei materii asupra micșorării expresiei și a cantității micro-ARN/145 în paternul tisular al restenozei, care se produce în raport autentic cu gradul restenozei. Micro-ARN/145 este un oligonucleotid implicat în menținerea fenotipului contractil al miocitului neted vascular matur. Respectiv, diminuarea cantitativă a acestuia este o condiție propice preluării fenotipului secretor (sau sintetic) al celulei musculare netede, predispus la migrare și proliferare. Se sugerează că factorii de bază, care compromit expresia diferitor familii de micro-ARN, sunt angiotensina II, endotelina-1, NO formată pe cale inductibilă, citokinele proinflamatoare, precum și componentele sistemului de semnalizare a stresului mecanic și hemodinamic asupra structurilor peretelui vascular. Fezabilitatea și promovarea migrării miocitului neted coronarian necesită respectiv și anumite circumstanțe de favorizare. La această noimă este, îndeosebi, importantă matricea extracelulară, consolidată prin diferite proteine - scheletice și proteinglicane. Datele noastre, obținute în cadrul microscopiei confocale laser, specifică creșterea expresiei MPM-2 pe fundalul reducerii expresiei ITMPM-2 în intima RIS. Este important de subliniat că majorarea raportului MPM-2/ITMPM-2 se produce proporțional avansării gradului restenozei.

Se știe că MPM-2, definită ca și colagenaza IV, scindează proteinele matricei extracelulare: colagenul fibrilar, (tipurile IV, V, VII, IX, X) și gelatina (colagenul degradat). Analogic enzimei MPM-9 (sau gelatinaza 9 cu care are analogie privind secvența de aminoacizi), colagenaza IV degradează activ laminina-5, proteină importantă a interstițiului diferitor organe. Reglarea activității MPM-2 se datorează mai multor factori, unul dintre cei mai semnificativi fiind ITMPM-2, care atenuează activitatea enzimatică prin intermediul receptorului propriu MMP-14. Creșterea expresiei MPM-2 poate fi și o consecință a acțiunii citokinelor, TNF-alpha și IL-8. De remarcat în acest context, că monocitele pot fi o sursă de sinteză a MPM-2, proces stimulat prin interacțiunea celulelor mononucleare cu celulele endoteliale. Respectiv, macrofagii cantonați în neointimă merită o atenție deosebită în acest sens.

K. Katsaros și colab. (2010) au prezentat dovezi, potrivit cărora creșterea ratei RIS la pacienții care au suportat angioplastie cu *eluting*-stenturi este asociată cu elevarea activității și a nivelelor circulante în ser a MPM-2 și a MPM-9 [19]. Evidențele noastre pot fi semnificative la această conotație, dat fiind faptul că indică asupra fenomenului descreșterii expresiei inhibitorului tisular specific drept un mecanism patogenetic plauzibil al acestui proces. Atenuarea activității MPM-2 și a altor metaloproteinaze poate fi un instrument fiabil de estompare a migrării miocitelor netede vasculare, iar A. Osherov și colab. (2011) consideră în acest context că modularea proceselor de sinteză și degradare a colagenului matricei extracelulare ar fi o țintă terapeutică oportună a RIS.

Reducerea expresiei micro-ARN/145 și activarea procesului de scindare a colagenului sunt asociate cu creșterea numerică a macrofagilor în neointimă. Un mecanism relevant al infiltrării celulare poate fi atribuit pasajului monocitelor sangvine printre epavele endoteliocitelor în urma alterării stratului endotelial. Cantonarea celulelor monoculeare în neointimă este favorizată de degradarea laminei interne prin intermediul MPM-2. Acest fenomen facilitează de asemenea migrarea și cantonarea în neointimă a miocitelor netede vasculare, care au preluat fenotipul secretor datorită afectării expresiei micro-ARN/145. Acumularea celulelor miocitare netede și mononucleare determină în consecință hiperplazia neointimei și riscul la restenoză. Macrofagul, este, totodată, o sursă de eliberare a citokinelor proinflamatoare, care influențează detrimental atât expresia micro-ARN/145, cât și a MPM-2. În plus, citokinele stimulează eliberarea diferitor substanțe biologic active de către miocitul neted vascular cu fenotip secretor și, care periclitează echilibrul de sinteză și degradare a colagenului matricei extracelulare. Posibil, acțiunea antiinflamatoare a *eluting*-stentutilor ar fi una din cauzele principale ale riscului redus al RIS, comparativ cu stenturile metalice. Y. Liu și colab. (2010) acordă monocitelor eterogenitate proinflamatoare, considerând că diferiți markeri ai acestora pot fi predictori independenți ai restenozei arterei coronariene după angioplastie [21].

Aşadar, datele prezentate abordează un aspect inedit al terapiei de prevenire a RIS, care se impune prin explorarea posibilităţilor farmacologice de modulare a expresiei micro-ARN/145. Controlul ei va permite menţinerea fenotipului contractil al miocitului neted coronarian, reducerea ratei de migrare a acestuia şi, respectiv, atenuarea formării şi/sau a hiperplaziei neointimei.

## Concluzii

1. Evoluţia restenozei intrastent se impune prin micşorarea semnificativă a expresiei şi a cantităţii micro-ARN/145 în raport direct cu gradul de restenozare a arterei coronariene expuse angioplastiei cu stent.

2. Expresia MPM-2 în neointima stentului restenozat creşte odată cu avansarea gradului de restenoză, fapt ce ar fi determinat de diminuarea expresiei inhibitorului specific al colagenazei IV. Activarea degradării laminei interne ar fi o cauză ce facilitează migrarea miocitului neted coronarian în neointimă pe fundalul compromiterii controlului fenotipului contractil al acestuia datorită micşorării expresiei micro-ARN/145.

3. Creşterea cantonului macrofagilor în neointimă se constată proporţional gradului de restenoză şi poate fi consemnat drept un mecanism al activării procesului inflamator în peretele arterei coronariene restenozate.

## Bibliografie

1. Nayak A, Kawamura A, Nesto R, et al. Myocardial infarction as a presentation of clinical in-stent restenosis. *Circ J.* 2006;70(8):1026-1029.
2. Assali A. Acute coronary syndrome may accur with in-stent restenosis an disassociated with adverse outcomes (the PRESTO trial). *Am J Cardiol*. 2006;98(6):729-733.
3. Costa MA, Simon DI. Molecular basis of restenosis and drug-eluting stents. *Circulation.* 2005;111:257-2273.
4. Li JJ, Ren Y, Chen KJ, et al. Impact of C-reactive protein on in-stent restenosis: a meta-analysis. *Tex Heart Inst J.* 2010;37(1):49–57.
5. Wessely R, Hausleiter J, Michaelis C, et al. Inhibition of neointima formation by a novel drug- eluting stent system that allows for dose-adjustable, multiple and on- site stent coating. *Arterioscler Thromb Vasc Biol.* 2005;25:748-753.
6. Van Oostrom O, Fledderus J, Kleijn D, et al. Smooth muscle progenitor cells: friend or foe in vascular disease? *Current Stem Cell Research and Therapy*. 2009;4:131-140.
7. Wilcox JN, Waksman R, King SB, et al. The role of the adventitia in the arterial response to angioplasty: the effect of intravascular radiation. *Int J Radiat Oncol Biol Phys Radiat med Nonmalignant Dis.* 1996;36:789-796.
8. Maiellaro K, Taylor WR. The role of the adventitia in vascular inflammation. *Cardiovasc Res.* 2007;75:640-648.
9. Shi Y, Brain JE, Fard A, et al. Adventitial myofibroblasts contribute to neointimal formation in injured porcine coronary arteries. *Circulation*. 1996;94:1655–1664.
10. Moohebati M, Falsoleiman H, Deghani M, et al. Serum Inflammatory and Immune Marker Response After Bare-Metal or Drug-Eluting Stent Implantation Following Percutaneous Coronary Intervention. *Angiology*. 2011;62:184-190.
11. Niccoli G, Montone RA, Ferrante G, et al. The evolving role of inflammatory biomarkers in risk assessment after stent implantation. *J Am Coll Cardiol.* 2010;56(22):1783-1793.
12. Zhao Y, Srivastava D. A development view of microRNA function. *Trends Biochem Sci.* 2007;32:189-197.
13. Chua JH, Armugam A, Jeyaseelan K. MicroRNAs: Biogenesis, function and applications. *Current Opinion in Molecular Therapeutics*. 2009;11:189-199.
14. Popovici I. The role of the micro-RNA/143/145 in intra-stent restenosis evolution. *Kardyologia*. 2011, in press.
15. Schneider M, Kostin S, Strom C, et al. S100A4 is upregulated in injured myocardium and promotes growth and survival of cardiac myocytes. *Cardiovasc Res.* 2007;75:40-50.
16. Troidl C, Troidl K, Schierling W, et al. Trpv4 induces collateral vessel growth during regeneration of the arterial circulation. *J Cell Mol Med.* 2008;DOI:10.1111/j.1582-4934.2009.00707.x.
17. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res.* 2001;29:e45.
18. Polyakova V, Loeffler I, Hein S, et al. Fibrosis in endstage human heart failure: Severe changes in collagen metabolism and MMP/TIMP profiles. *Int J Cardiol.* 2010;DOI:10.1016/j.ijcard.2010.04.053.
19. Katsaros K, Kastl S, Bsci G, et al. Increased restenosis rate after implantation of drug-eluting stents in patients with elevated serum activity of matrix metalloproteinase-2 and -9. *Clinical Res*. 2010;DOI: 10.1016/j.jclin.023.
20. Osherov A, Gotha L, Cheema A, et al. Proteins mediating collagen biosynthesis and accumulation in arterial repair: novel targets for anti-restenosis therapy. *Cardiovasc Res.* 2011;DOI: 10.1093/cvr/cvr012.
21. Liu Y, Imanushi T, Ikejima H, et al. Association between circulating monocyte subsets and in-stent restenosis after coronary stent implantation in patients with ST-elevation myocardial infarction. *Circ J.* 2010;74(12):2585-91.

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# National System for Preparedness and Response to Public Health Emergencies

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**Abstract**

In this article the risks and hazards, and their possible impact on Moldova's territory and population are briefly described. The need to merge the preparedness and response measures to potential public health emergencies in an integrated system involving both the health system components, as well as other relevant bodies is argued. The concept of a National System for Disaster Preparedness and Response to Public Health Emergencies is defined. Also the role, structure and main tasks of the system as a whole, as well as its components are described. An overview of all medical response forces to emergencies is being made. Some visions and proposals for the coordination of health care institutions' activities at the local level for triggering the hazard or public health emergencies are expressed.

**Key words**: system, preparedness, response, public health emergencies

**Национальная система готовности и реагирования на неотложные ситуации в общественном здоровье**

В данной статье в сжатой форме представлены потенциальные риски и опасности для населения и территории Республики Молдова, а также их возможное пагубное воздействие. Дана аргументация необходимости объединения мер по подготовке и реагированию на неотложные ситуации в общественном здоровье в единую интегрированную систему, включающую как компоненты системы здравоохранения, так и другие соответствующие структуры. Дано определение Единой Национальной Системы подготовки и реагирования на неотложные ситуации в общественном здоровье, изложены роль, структура и основные задачи как Системы в целом, так и её отдельных компонентов. Представлен обзор сил и средств медицинского реагирования на чрезвычайные ситуации. Изложены некоторые видения и предложения по координации действий учреждений здравоохранения на территориальном уровне в случае опасности или возникновения неотложных ситуаций в общественном здоровье.

**Ключевые слова**: система, подготовка, реагирование, неотложные ситуации в общественном здоровье.

## I. General overview

The Republic of Moldova's territory is at risk of impact of a series of hazards, be those natural, manmade or biosocial, which may lead to emergencies or even disasters. The geographical proximity of Moldova to the seismic region of the Carpathian Mountains poses a threat of earthquakes of up to a magnitude of 7-9 on the Richter scale. More than 200 locations and extensive lands are vulnerable to flooding caused by water overflows, hydrotechnical node accidents or by damages to the protective dams located on the Dniester and Prut rivers. About 40% of the country's communities are at risk of landslides. Moldova is situated at the crossroads of several paths for the transportation of up to 400-450 thousand tons of highly flammable and/or harmful substances per year. A current threat is posed by potential epidemics and imported highly pathogenic conditions caused by high population migration rates. Nuclear power plants and chemical processing companies in the neighboring countries may pose a threat of radioactive or chemical pollution of the country in case of possible breakdowns occurring at such structures. A major threat to the country's population and economy is posed by heavy snow falls, frost, hailstorms, hurricanes, fires, droughts and other natural adverse phenomena.

Along with the high density of the population and limited economic resources of the country, these factors maintain a high level of risk to human life and health and represent the main cause which could lead to eventual public health emergencies, which, under article 2 of Law No.10 from 03.02.2010 regarding the state surveillance of public health, represent "the occurrence or imminent risk of spreading a disease or health event that causes the high probability of a large number of deaths and/or a large number of disabilities among the affected population or determine the broad exposure to the action of a biological, chemical or physical agent which can cause significant risks in the future for a substantial number of persons affected among the population".

As a result of these risks, one of the essential tasks of the country's Health System is to ensure a high degree of readiness to respond promptly and appropriately to eventual emergency situations with consequences resulting in public health emergencies.

Obviously, in case of hazards or public health emergencies the Ministry of Health's institutions organize and carry out a complex of response measures, but they could be successful only in case they are coherent, well coordinated and directed, integrated into a unique system involving both the health system forces and means, as well as other relevant bodies (Civil Protection and Emergency Situations Service, local and central government, law enforcement bodies, army forces, etc.).

At the same time, the Health System in the Republic of Moldova does not currently have a framework document that would formally establish an integrated national system of preparedness and medical response to extraordinary situations, disasters and public health emergencies, and would establish clear and unambiguous role, tasks, structure, activities, responsibilities and interaction between its components. Partially this gap is offset by some legislative and regulatory acts governing the activity of various structures involved in the health care response and liquidation of consequences of eventual extraordinary situations, disasters and public health emergencies, as well as by the Plan for the delivery of health care to the population of the Republic of Moldova in case of Emergency Situations. However, despite of this, the problem of coordination and integration of response activities of the health system components remain important for the time being.

In the analysis below the current institutional framework predesigned to accomplish the preparedness and response activities to emergency situations are reflected and some views and proposals regarding the integration of the existing structures in a National System for Preparedness and Response to Public Health Emergencies are exposed. Also the role, tasks, structure and activities are stipulated.

## II. The definition, role and basic tasks of the National System for Preparedness and Response to Public Health Emergencies

**The National System for Preparedness and Response to Public Health Emergencies** (further **System**) is a complex of structures, forces, mechanisms and relationships, integrated into a single system and destined for organizing and carrying out measures to ensure preparedness, prevention, mitigation, response and recovery from the consequences of extraordinary situations and public health emergencies.

**The basic tasks of the System are as follows:**

- Multisectoral mobilization in order to ensure a proper preparedness degree of leadership bodies, medical facilities, services and formations, medical transport, material resources, warning and communication systems, etc. for a prompt response to extraordinary situations and public health emergencies, disasters and public health emergencies (further - public health emergencies);
- Ensuring an all-hazards approach and assessment of their risks for public health;
- Development of national preparedness and response plans for public health emergencies;
- Assessment of health system components and health facilities preparedness level for response to public health emergencies;
- Accumulation, generalization and analysis the information regarding population protection from factors that may generate public health emergencies, predict their possible consequences for the human health;
- Development and implementation of measures aimed to reduce vulnerabilities and mitigate hazards which could provoke public health emergencies;
- Planning, organization, coordination and implementation of preparedness and response measures, and liquidation of public health emergencies consequences;
- Estimating the damage caused to population health and medical facilities by the impact of public health emergencies;
- Needs assessment, planning, creation, maintenance and continuous renewal of the stocks of medicines, supplies, disinfectants, medical equipment, medical and sanitary means and sanitary-household means needed for the response to public health emergencies;
- Developing and implementing modern methods and procedures of medical assistance to the population in emergencies;
- Training of leadership bodies, medical facilities, medical personnel and population on preventive and response actions to public health emergencies;
- Drafting legislation and regulations on the preparation, prevention and response to public health emergencies;
- Collaboration in the field of emergency preparedness and response with public health structures of central and local authorities, and other relevant bodies in the country and abroad.

## III. System's Structure

The System's Structure (fig. 1) consists of:

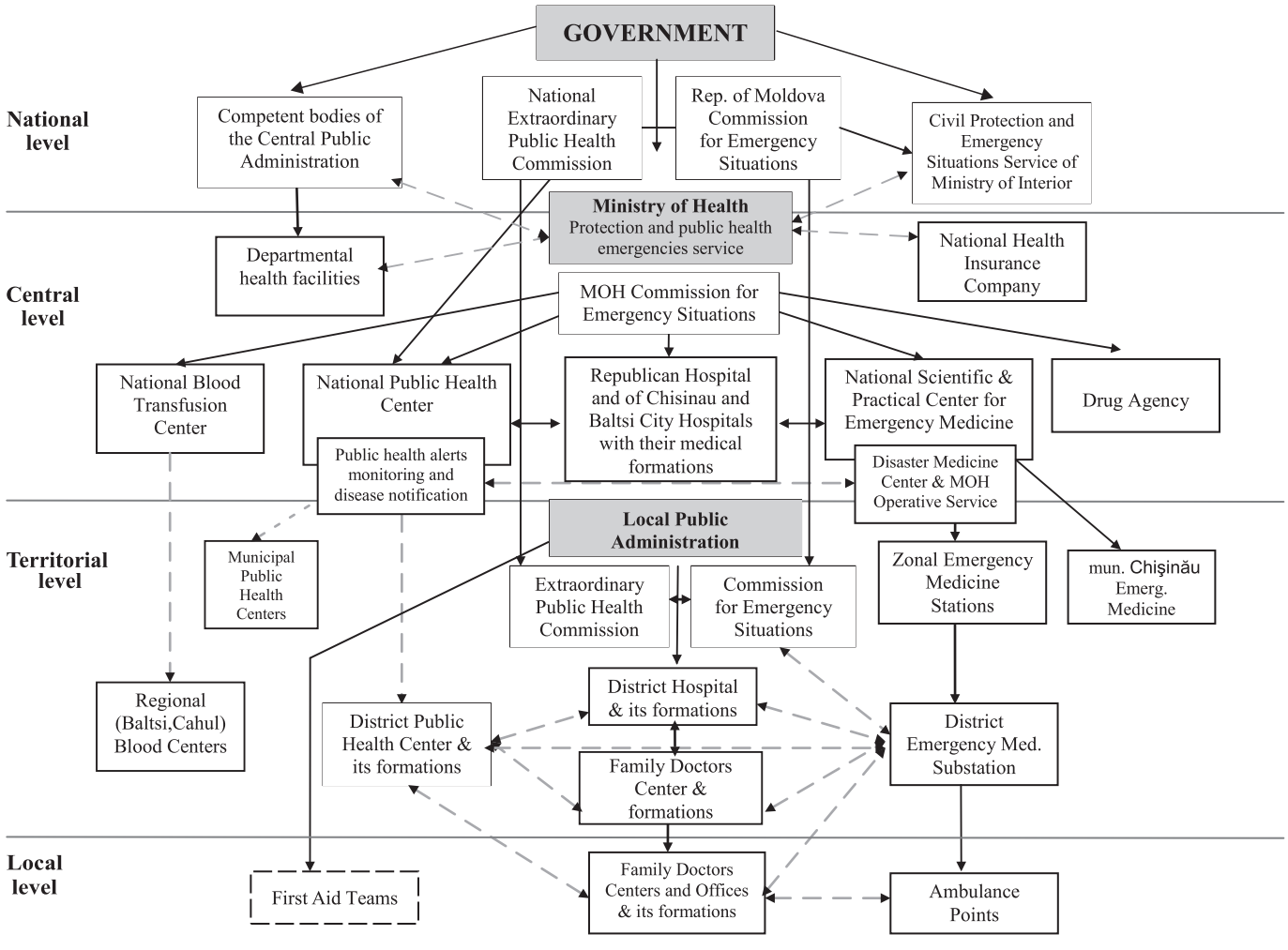
- directing and coordinating bodies;
- medical response forces (health services, institutions and formations);
- the warning and communication system;
- the training system.

Conventionally the system is structured in four levels - national, central, territorial and local.

**Directing and coordinating bodies**

At national level the overall coordination of activities related to emergency preparedness and response to public health emergencies is done by the Government of the Republic of Moldova and its two specialized commissions: the Commission for Emergency Situations of the Republic of Moldova and the National Extraordinary Public Health Commission. The direct coordination is carried out by the Ministry of Health.

The Commission for Emergency Situations of the Republic of Moldova has been established under Article 17 of Law No. 271 from 09.11.1994 regarding Civil Protection for the purpose of performing directing and executive functions for the prevention and acting in emergency situations generated by natural disasters, large-scale damages, fires, epidemics, epizootics, epiphytotic diseases and other dangerous phenomena. The Commission's activity is regulated by the Government's Decision of 04.12.2001 nr. 1340 on the Commission for Emergency Situations of the Republic of Moldova. The Commission is chaired by the Prime Minister. The Deputy-Prime Ministers, the Minister of Internal Affairs and the Head of Civil Protection and Emergency Situations Service of the MIA are Vice-Chairmen. The Commission's composition includes the heads of relevant Central Public Authorities. The




working body of the Commission is the Civil Protection and Emergency Situations Service of the MIA.

The National Extraordinary Public Health Commission has been established under Article 55 of Law no. 10 from 03.02.2009 on the State Surveillance of Public Health for the purpose of ensuring an adequate degree of preparedness for public health emergencies and their management. The Commission's activity is regulated by The Government's Decision nr.820 from 14.12.2009 on the National Extraordinary Public Health Commission. One of the Deputy-Prime Ministers is appointed as Commission's Chairman and Minister of Health is appointed as Vice-Chairman. The Commission's composition includes heads (or deputies) of relevant Central Public Authorities, as well the chiefs of central level health structures. In accordance with Articles 58 and 59 of the Law on the State Surveillance of Public Health, the National Extraordinary Public Health Commission has the right to declare/cancel, through its decision, the State of emergency in Public Health. The working body of the National Extraordinary Public Health Commission is the National Center of Public Health.

The Ministry of Health is the central body of public administration in the field of health. One of its tasks is to develop policies and coordinate the preparation and response activities in case of public health emergencies. For this purpose in the central office of the Ministry of Health a special service is established – the Service for Protection and public health emergencies. The Ministry of Health, like other central public authorities, has its own Commission for Emergency Situations. For non-stop communication a subdivision of the Republican Disaster Medicine Centre – the Operative Service of the Ministry of Health is located in the Ministry of Health. The Service is operational 24 hours a day and plays the role of "focal point" for the exchange of information in case of emergency situations between the Ministry of Health and other central and local public authorities, as well as all health institutions from the country.

The Ministry of Health's Commission for Emergency Situations is a coordinating body created to ensure an adequate degree of the Health System preparedness for any extraordinary situations and public health emergencies, as well as to fulfill the general management of actions on prevention, mitigation, response and recovery in case of their occurrence.

The Commission is chaired by the Minister of Health. The Commission consists of vice-chairmen, secretary and members (the heads of key departments of the Ministry of Health and the relevant central level medical institutions). One of

the vice-chairmen is designated as prime vice-chairman. At the Commission's meetings, other persons may be also invited through the decision of its chairman, given the specific situation or problem discussed. The Commission's activity is regulated by law, decisions of the Commission for Emergency Situations of the Republic of Moldova and the National Extraordinary Public Health Commission, the Regulation of the Commission, the orders, disposals and indications of the Minister of Health.

**The main tasks of the Commission for Emergency Situations of the Ministry of Health are as follows:**

- Mobilization and coordination of measures undertaken within the country health system activities in order to ensure an adequate degree of preparedness for eventual exceptional situations and public health emergencies;
- Performance of general management and joint efforts of all components of the health system aimed at prevention, reduction, prompt and effective response, recovery and subsequent liquidation of consequences of public health emergencies;
- Providing public information about the causes and dimensions of public health emergencies, and measures undertaken to prevent and liquidate their consequences, familiarization of people with the rules of behavior in exceptional situations and public health emergencies.

**To fulfill its tasks the Commission is entitled with the right:**

- To adopt decisions within its competences and to issue them as minutes or directives that is mandatory for the chiefs of all health institutions and formations from the country system;
- To take decisions on the use of financial and material means for overcoming the consequences of public health emergencies and providing the necessary medical care to the affected population;
- To control the activity of health institutions' commissions for emergency situations and to examine their chairmen reports;
- To carry out checks and surveys, involving institutions and specialists in the field, in order to prevent and/or decrease the impact of accidents, catastrophes, disasters, outbreaks of infectious disease, mass poisoning of the population, to detect their causes and consequences, increase the level of protection of population and environment, as well as to ensure the operational stability of health facilities;
- To involve the necessary health system's forces and means in order to liquidate the medical consequences of emergencies;

**Organization of the Commission's activity:**

The Commission's working meetings are convened whenever necessary, but at least once per semester. In special cases, at the discretion of its Chairman, the Commission's meetings may take place out of the capital city, in territories. Issues discussed at meetings and its decisions have to be recorded in minutes signed by the chairmen and the secretary.

In the event of a threat or outbreak of major emergency situations, at the Commission's decision, an Operative Command Center is deployed in the Ministry of Health. The Center's main tasks are: to organize the implementation of the plans for medical care to the population in emergency situations; to ensure continuous and operative management of health services, formations and institutions involved in medical response measures and liquidation of consequences of extraordinary situations and public health emergencies; to control implementation of hierarchically superior bodies' decisions and of carried out measures; to accumulate information from outbreaks, analyze it and assess the effectiveness of activities undertaken; to put forward proposals aimed at rapid improvement of the situation; to permanently keep informed the Ministry of Health's leadership on the progress of work.

An important role in the coordination and management of the process of preparedness and response measures to public health emergencies plays the National Health Insurance Company.

At the central level the role of coordinating and directing bodies are performed by some health institutions of different destination, directly subordinated to the Ministry of Health, which simultaneously carry out the executive functions, thus being part of System's Forces and Means. The number of such institutions includes the following: the National Scientific and Practical Centre of Emergency Medicine, the Emergency Medicine Zonal Stations, the National Centre of Public Health, the National Blood Transfusion Center and the Drug Agency.

National Scientific and Practical Centre of Emergency Medicine (NSPCEM) is a tertiary level public medical institution, providing emergency and planned medical care to the population of the country, as well as methodological support in organizing and performing emergency medical care, including mass casualty incidents. NSPCEM is a coordinating body of the Medical Emergency Service and Disaster Medicine Service. A specialized subdivision activates within NSPCEM – the Republican Centre for Disaster Medicine (RCDM) responsible for directing organizational-methodical and coordinating work of all components of the Republican Disaster Medicine Service. During the daily work RCDM accumulates, generalizes and analyzes information on possible risks triggering emergency situations, plans the organization of medical care to the population in cases of mass casualty incidents, takes part in the training of medical personnel of the health institutions in the preparation and response to exceptional situations and public health emergencies, assess the level of preparedness of medical institutions to work in crisis situations, etc. According to the decision of the Commission for Emergency Situations of the Ministry of Health, in case of threat or outbreak of large-scale emergency situations RCDM is deployed to the MoH and provides operational guidance to the Commission's Operative Command Center.

Emergency Medicine Zonal Stations are functional and structural components of the Medical Emergency Service at the pre-hospital stage. They are accountable to the Ministry of Health and provide emergency medical care to the population

in the served territory (zone), in exceptional circumstances and beyond. In the composition of Emergency Medicine Zonal Stations are included district Emergency Medicine Substations and Emergency Medicine Points, located in the served area. Territories of service boundaries are determined by the Ministry of Health. Currently there are 4 Emergency Medicine Zonal Stations in the country: North, Central, South and Autonomous Territory Gagauzia.

National Centre of Public Health (NCPH) is a scientific, practical, methodological and coordinating institution of the Service of State Surveillance over Public Health. NCPH provides substantiation for public health policies and strategies, develops drafts for sanitary regulations, methodologies and other acts on public health, ensures research and development of highly specialized expertise, provides methodological and practical support in the field of public health and perform other activities on State Surveillance over Public Health. Among the NCPH's directions of activity is included also the ensuring of the emergency preparedness and public health interventions in public health emergencies, in collaboration with the relevant services of other ministries and central administrative authorities. NCPH is designated as National Focal Point for the implementation of International Health Regulations (IHR) and is responsible for notifying the World Health Organization on events that may constitute a public health emergency of international importance. For this purpose within NCPH a special subdivision is established - the section of public health alerts monitoring and disease notification, which monitors on 24 - hours basis the situation in the country and is permanently ready to receive and provide information to both the Ministry of Health's leadership, and to WHO.

The National Blood Transfusion Centre is the coordinating institution responsible for planning, monitoring, evaluation and coordination of the Blood Service's activity in Moldova; for labile and stable blood preparations and diagnostics production for the purpose of satisfying the real needs of medical institutions; for blood transfusion assistance in emergency situations; for monitoring of opportunity, feasibility and correctness of blood therapy in medical institutions; for the promotion of voluntary and non-remunerated blood donation; and for the training of personnel in transfusion medicine.

Drug Agency is a public institution subordinated the Ministry of Health and responsible for carrying out state policy on drug and pharmaceutical activities; authorization (expertise, certification and registration) of medicinal products and their quality monitoring; supervision and control of pharmaceutical activities; monitoring and coordinating the supply medicines and pharmaceutical assistance at national level; regulation in the field of drug and pharmaceutical activities; methodological, organizational and consulting activity in the pharmaceutical companies and health care providers.

At the territorial and local level (district, locality) the general management of response to public health emergencies is carried out by organs of local government (district councils, municipal councils, municipalities, mayor's office) through local commissions for emergency situations, which are established in each administrative-territorial unit and territorial extraordinary public health commissions, which are established in each municipality and each level II administrative-territorial unit.

The direct coordination of the medical components of the response to health emergencies system in municipalities Chisinau and Balti, and Autonomous Territory Gagauzia is carried out by local bodies governing the health sector, namely: the Department of Health of Chisinau Municipal Councils, the Medical Section of City Hall Balti and Department of Health and Social Protection of the Autonomous Territory Gagauzia.

In regard to the coordination at the district level, this is complicated by the fact that basic institutions providing health services in the district (District Hospital, Medical Center of Family Doctors, District Public Health Center and District Emergency medicine Substation) have different administrative subordination, legal form and type of ownership and their coordination is not institutionalized - in districts currently there are not health sector coordination bodies. In order to solve the problem and taking into account that the principle of unified leadership is one of the key principles underlying the implementation of measures in response to crisis situations, the Ministry of Health, through its order No 454 from 10.12.2007 "On planning the medical care to the population in emergency situations", designated the district hospital director as responsible for directing and coordinating the response to the emergency situations of all district health system's components. However, checks and tactical exercises took place in several districts, and the experience of liquidating the consequences of emergency situations, such as for example the recent floods, have demonstrated that in exceptional circumstances, especially if they are causing a large number of victims, the volume of work which goes to the hospital director in part concerning hospital care is very large, which inevitably complicates his/her work as medical response actions' coordinator on the entire district. In this context and taking into account the new role, tasks and responsibilities that are put by legislation on the Service of State Surveillance over Public Health, we consider it advisably to study the possibility of designating the District Public Health Center as a medical coordinator of all components of the health system at district level. Obviously, to successfully accomplish the tasks attributed to the Centre, it should be vested with respective authority and strengthened with medical personnel trained in crisis management and material-technical base (transport, transmission equipment, computers, etc.).

Health Response Forces are represented by health care institutions (public, departmental and private) and formations created and maintained by them for the purpose of providing medical assistance to population in emergency situations. More or less these activities are shared by all health care institutions within the country, each being awarded certain tasks depending on the activity and specificity. However, a

particularly important role plays the institutions of the Medical Emergency Service, Service of State Surveillance over Public Health and the Hospital Sector.

Emergency Medical Assistance Service is part of the Health System that provides emergency medical assistance to population in the pre-hospital stage (including in cases of mass casualty events) and assisted medical transportation of patients from the accident or illness aria till respective health facilities. Structurally the Service consists of NSPCEM, which is both a specialized hospital and organizational and methodical coordination body of the Service, 4 Emergency Medicine Zonal Stations with 41 Emergency Medicine Substations and 88 Emergency Medicine Points. Daily in the country there are about 250 ambulance team on duty, which will be involved first for emergency medical assistance of population in case of mass casualty events.

Among the State Surveillance over Public Health Service's institutions are included the National Center for Public Health, Public Health Centers of Chisinau and Balti municipalities and 34 public health centers at district level. In case of emergency situations outbreaks all levels of public health, centers organize and carry out anti-epidemic and sanitary-hygienic measures such as: epidemiological intelligence in the disaster aria, increased sanitary monitoring over objectives with major importance, measures aimed to detect, localize and liquidate the outbreaks of infectious diseases, permanent control of the environment in the outbreak area, informing people about the dangers of infection/contamination, preventive and precautionary measures to be done, etc. Public health centers are part of the National Network of laboratory observation and prevent environment contamination (pollution) with radioactive substances, poisonous, highly toxic and biological agents.

In addition to their basic functions, most medical institutions are required to create and maintain formations of different healthcare profiles in state of readiness for action in emergency situations. According to the Plan of the Republic of Moldova's population health care in the event of Emergency Situations health care institutions in the country are responsible to maintain the following number of formations:

- Paramedical teams – 620.
- Medical teams – 488.
- Specialized medical teams – 90.
- Preventive medicine teams – 42.
- Medical detachments – 3.

Paramedical and medical teams are created by family doctors' centers and offices, health centers, district and municipal hospitals for the purpose to provide in emergency situations, conjunctively with ambulance teams, pre-hospital medical and emergency care (as a rule at the borders of the outbreak and in the places where casualties evacuated from disaster area are concentrated).

Specialized medical teams are usually created by republican and municipal hospitals in order to strengthen the capacity of medical institutions, directly involved in providing medical assistance in emergency situations, by organizing and providing specialized medical care. Specialized medical teams include the following profiles: trauma, combustiologic, neurosurgical, surgical, radio-therapeutic, pediatric, infectious diseases, psychotherapeutic, toxicology and obstetrics and gynecologic.

Medical detachments are created by general profile hospitals (republican and municipal) in order to provide emergency medical care with elements of qualified and specialized medical care in case of emergency situations with a significant number of injured. One of the medical detachments tasks is to substitute a district hospital when it becomes out of function.

Among the Health Response Forces are also included the Civil Protection's first aid teams, which represent formations created on voluntary principle by the economic units, enterprises, educational institutions, local authorities, etc. for the purpose of providing first aid to injured in places of emergency situations, their evacuations outside the outbreak zone and their concentration in maximum protected of danger and accessible for the transport places (points for casualty concentration).

The responsibility for the health formations preparedness for actions according their destinations lies on the directors of facilities which form them. In the event of threat or emergency outbreak the formations are passed under the directing body responsible for organizing medical assistance to the population in emergency situation in accordance with the decision of the Minister of Health or the respective emergency situation's commission. Formations are working in the disaster area until the completion of the rescue, treatment and rehabilitation of injured following the task to health care institutions in which they were evacuated.

**Warning and communication system**

The warning and communication system is based on the Emergency Medical Assistance Service dispatch services and transmission networks, through which information regarding the danger or the occurred emergencies is sent and medical care activities for the population are conducted and coordinated. To achieve this task in all Emergency Medical Assistance substations, special points for the reception and distribution of urgent information have been created by the order of the minister of health nr.382 from 11.08.2009 "On the mode of reception of emergency information by medical and medical education institutions, located in the level II administrative-territorial units".

**Training System**

Training system is represented by the Chairs of the Nicolae Testemitanu State Medical and Pharmaceutical University (Medical Emergencies, Military and Extreme Medicine, Traumatology, Orthopedics and Military Surgery), medical colleges and emergency medicine training centers (republican and regional), where students and medical personnel potentially involved in population medical care in emergency situations are trained.

## References

1. Republic of Moldova Law No. 411 from 03.28.1995 on Health Care.
2. Republic of Moldova Law No. 271 from 11.09.1994 on Civil Protection.

3. Republic of Moldova Law No. 10 from 02.03.2009 on the State Surveillance over Public Health.
4. Government Decision No. 1340 of 12.04.2001 on the Commission for Emergency Situations of the Republic of Moldova.
5. Government Decision No. 820 from 14.12.2009 on the National Extraordinary Public Health Commission.
6. Government Decision No. 777 from 27.11.2009 on approving the Regulation on organization and functioning of the Ministry of Health, its structure and central office staff.
7. Government Decision No. 384 12.05.2010 on the State Surveillance over Public Health Service.
8. Government Decision No.891 from 17.07.2003 on the creation of the Emergency Medical Assistance Service of Moldova.
9. Government Decision No. 475 from 26.03.2008 on the approval of the Action Plan for implementing the International Health Regulations in the Republic of Moldova.
10. Government Decision No. 1252 from 12.01.2005 on approving the Regulation, structure and staff limit of Drag Agency.
11. Minister of Health Order No.85 from 30.03.2009 on organization and functioning of the Emergency Medical Assistance Service of the Republic of Moldova.
12. Minister of Health Order No. 369 from 06.03.2010 on the State Service for Public Health Surveillance.
13. Minister of Health Order No. 317 from 02.08.2007 on the reorganization of the Ministry of Health Emergency Medical Assistance Service for Emergency Situations in the Republican Disaster Medicine Service.
14. Minister of Health Order No.443 from 03.12.2007 on the establishment of the Operative Service of the Ministry of Health.
15. Minister of Health Order No. 454 from 10.12.2007 on planning of population medical care in emergency situations.
16. Minister of Health Order No.382 from 08.11.2007 on the mode of reception of emergency information by medical and medical education institutions, located in the level II administrative-territorial units.

# Aspectele epidemiologice şi manageriale actuale ale leucemiei mieloide cronice

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## Updated Epidemiologic and Management Aspects of Chronic Myeloid Leukemia

Chronic myeloid leukemia is a clonal myeloproliferative disorder resulting from the stem cell neoplastic transformation caused by translocation between the long arms of chromosomes 22 and 9. Chronic myeloid leukemia accounts 15–20% of leukemias in adults. This myeloproliferative malignancy occurs mostly in workable population with the age of 25–50 years old. Male: female ratio may reach 1.4:1. A higher incidence of chronic myeloid leukemia is registered among persons heavily exposed to radiation, including survivors of the atomic bomb blasts in Japan and patients undergoing radiotherapy. The contemporary management of CML diversifies the diagnostic and treatment options in regard with the level of medical assistance. Glivec® International Patient Assistance Program (GIPAP) is one of the most generous and far-reaching patient assistance programs ever developed for cancer therapy, axed on the insurance of treatment with Imatinib mesylate of different malignant neoplasms.

**Key words:** chronic myeloid leukemia, epidemiology, management, level of medical assistance.

## Актуальные аспекты эпидемиологии и мэнэджмента хронического миелолейкоза

Хронический миелолейкоз представляет собой клональный опухолевый процесс системы гематопоэза, развивающийся в результате злокачественной трансформации стволовых клеток как следствие транслокации между длинными плечами хромосом 22 и 9. Хронический миелолейкоз составляет 15–20% всех лейкозов у взрослых. Эта злокачественная миелопролиферативная опухоль возникает преимущественно у трудоспособного населения в возрасте 25–50 лет. Соотношение мужчин и женщин достигает 1,4:1. Более высокая частота хронического миелолейкоза регистрируется среди лиц подвергавшихся ионизирующему облучению, включая выживших после ядерных взрывов в Японии и пациентов, которым проводилась радиотерапия. Современный мэнэджмент хронического миелолейкоза диверсифицирует диагностические и лечебные мероприятия в зависимости от уровня медицинской помощи. Glivec® International Patient Assistance Program (GIPAP) является одной из наиболее благородных и далеко идущих программ помощи больным, разработанной для противоопухолевой терапии и направленной на обеспечение Иматиниб мезилатом при лечении различных злокачественных опухолей.

**Ключевые слова:** хронический миелолейкоз, эпидемиология, мэнэджмент, уровень медицинской помощи.

### Introducere

Leucemia mieloidă cronică (LMC) constituie un proces neoplazic clonal al sistemului hematopoietic, care rezultă din transformarea malignă a celulei stem, cu menținerea capacității de diferențiere pentru toate liniile celulare. Patologia se caracterizează în special prin multiplicarea necontrolată a celulelor seriei mieloide, cu creșterea masei granulocitare totale și a celei circulante. Drept marcher citogenetic al acestei leucemii cronice servește cromozomul Philadelphia t (9; 22), care se formează în urma translocației reciproce a unei părți de material genetic dintre cromozomii 9 și 22 [2, 3, 5, 6, 18, 22, 23]. Identificarea genei de fuziune BCR/ABL și a proteinei himerice p210 cu activitatea tirozinkinazică conturează LMC la nivel molecular [3, 5, 21, 23]. LMC constituie 0,6–1,6 cazuri la 100000 de populație [3, 5, 18, 22, 23]. LMC constituie o patologie oncologică frecvent întâlnită în structura morbidității prin hemoblastoze, constituind 15–20% din toate leucemiile la adulți și fiind una dintre cele mai grave și invalidizante maladii umane [3, 5, 6, 22, 23]. Cauza LMC nu este bine cunoscută. Iradierea ionizantă pare să reprezinte un factor favorizant. Sunt identificate 3 stadii clinico-evolutive ale LMC: cronică, de accelerare și acută [7, 8, 10, 14, 16, 21]. În faza cronică oncopatologia respectivă poate evolua asimptomatic. Tratamentul contemporan al LMC cuprinde: chimioterapie, imunoterapie, citokine și transplant medular alogenic [2, 3, 4, 5, 6, 8, 9, 10, 11, 13, 14, 15, 20, 22]. Transplantul medular, precum și inhibitorii tirozinkinazei sunt opțiunile curative de vindecare a pacienților cu LMC în faza cronică [3, 4, 5, 6, 8, 15, 16]. Imatinib Mesilat sau Glivec® este un inhibitor al tirozinkinazei, produse de gena himerică BCR-ABL, situată pe cromozomul 22 și se consideră ca și o „terapie în țintă" [8]. Glivec® a fost implementat în practica clinică în anul 2001 și în prezent constituie în multe instanțe ca tratament de primă linie al LMC [3, 4, 5, 6, 8, 9, 10, 11, 16]. Medicamentul Glivec® se aplică cu succes în toate fazele leucemiei mieloide cronice, cel mai înalt răspuns clinico-hematologic și citogenetic fiind obținut în faza cronică a maladiei [2, 5, 10]. În rezultatul studiilor clinice ulterioare s-a constatat eficacitatea medicamentului în formele recidivante și refractare ale leucemiei acute limfoblastice pozitive la Ph cromozom, în tumorile gastro-intestinale stromale maligne pozitive la Kit (CD 117) și dermatofibrosarcoma protuberans (DFSP) inoperabilă, recidivantă și/sau metastatică.

Printre problemele majore ale serviciului oncologic, inclusiv în domeniul leucemiilor, pot fi considerate depistarea preponderent tardivă, creșterea indicilor morbidității în rândul populației apte de muncă, gradul sporit de invaliditate, mortalitatea relativ înaltă, lipsa serviciilor și a infrastructurii medico-sociale de suport al bolnavilor oncologici. În pofida progreselor esențiale în crearea noilor agenți chimioterapeutici, nu este elaborat algoritmul eficient de conduită a bolnavilor în diferite faze ale LMC. Întârzierea în administrarea chimioterapiei „de țintă" explică creșterea cheltuielilor de conduită a pacienților, ceea ce determină managementul deficitar al LMC. Publicațiile care reflectă cercetări demonstrative privitor la eficacitatea nemijlocită și la distanță, precum și inofensivitatea medicației cu inhibitorii tirozinkinazei nu sunt numeroase, majoritatea dintre acestea fiind bazate pe loturi nesemnificative de cazuri. Nu este relatată dinamica clinico-hematologică pe fond de terapie cu Imatinib Mesilat în raport cu chimioterapia convențională. Nu s-au elaborat definitiv principiile de dozare a medicamentului în funcție de eficacitatea și durata chimioterapiei anterioare și de datele examinărilor citogenetice și moleculare ale măduvei osoase.

### Material şi metode

Este programat un studiu secundar – reviul literaturii de specialitate. Acumularea informației pentru cercetare s-a efectuat prin analiza datelor literaturii de specialitate internaționale și ale statisticii oficiale pe entitatea nosologică respectivă [19]. Au fost studiate peste 20 de surse bibliografice de referință. Tipul procesului mieloproliferativ cronic a fost identificat în conformitate cu Clasificarea Internațională a Neoplasmelor Mieloide, propusă de O.M.S. în anul 2001 [7, 20].

### Rezultate şi discuţii

Studiul literaturii de specialitate la tema dată a demonstrat că incidența LMC în Europa și America de Nord poate varia între 0,6–2 cazuri la 100000 de adulți pe an [3, 5, 6, 16, 17, 18, 22, 23]. În Republica Moldova morbiditatea de LMC se cifrează la 0,6 cazuri la 100000 de populație [5, 13, 14]. În România LMC are o incidență de 200 de noi cazuri pe an. Aglomerări de cazuri sau particularități geografice semnificative de răspândire a acestei leucemii nu sunt înregistrate. Morbiditatea prin LMC crește odată cu vârsta, cu incidența maximă cuprinsă între 25–50 de ani, ceea ce denotă afectarea preponderentă a persoanelor apte de muncă. La majoritatea bolnavilor înrolați în studiile chimioterapeutice vârsta variază între 50–60 de ani, cu media de ≈ 53 de ani [4, 6]. LMC este întâlnită rar la vârsta sub 18 ani și apare ca o excepție sub 5 ani (când se descrie forma „juvenilă", atipică). S-a constatat o prevalență ușoară a pacienților de sex masculin (bărbați:femei = 1,4:1). Nu s-au raportat transmiteri de la un individ la altul și nu s-au descris cazuri familiale [2, 3, 5, 6, 18, 22]. Aproximativ 4600 de cazuri noi de LMC au fost diagnosticate în anul 2004 în SUA, și în 1570 de cazuri a survenit decesul din cauza progresării procesului leucemic. În România sunt afectați aproximativ 1500 de pacienți, iar dintre aceștia aproximativ jumătate sunt incluși în tratamentul cu Imatinib Mesilat [1, 12].

Cauza LMC nu este bine cunoscută, ceea ce induce dificultăți de screening în această oncopatologie. Iradierea (în special în doze mari) se consideră un factor etiologic favorizant. Argumentele sunt de ordin statistic: incidența crescută la personalul medical din radioterapie/radiologie care au activat fără protecție adecvată, la pacienții tratați cu radioterapie și la populația din Hiroshima și Nagasaki după explozia bombei atomice [3, 5, 6, 16, 18, 22, 23]. Nu sunt evidențiate dovezi demonstrative și argumentate precum că agenții chimici sau virusuri ar reprezenta factorii favorizanți ai LMC.

În faza cronică oncopatologia respectivă este asimptomatică în 15 – 40% cazuri, fiind depistată ocazional prin analiza

generală a sângelui (leucocitoză, devierea leucogramei în stânga) şi ultrasonografia abdominală (splenomegalie), ceea ce explică diagnosticarea ei preponderent tardivă.

Analiza datelor din sursele bibliografice referitoare la managementul actual al pacienţilor cu patologii maligne cronice relevă că Imatinib Mesilat se consideră opţiunea chimioterapeutică de primă linie în tratamentul LMC. Doza medicamentului se stabileşte în funcţie de stadiul clinico-hematologic evolutiv al bolii, constituind 400 mg în faza cronică, 600 mg - în faza de accelerare şi 800 mg - în criza blastică [2, 5, 6, 8, 9, 10, 21, 22, 23]. În scopul monitorizării răspunsului citogenetic după 6–8 luni de tratament se efectuează examinarea repetată a celulelor medulare la Ph-cromozom şi gena BCR-ABL p210 [6, 10, 14, 16, 18, 21]. Ca o opţiune curativă în LMC ar mai putea fi şi monochimioterapia cu Busulfan, Hidroxicarbamidă şi/sau α-Interferon (α-IFN) în faza cronică, monochimioterapia cu Citarabină în faza de accelerare şi polichimioterapia conform diverselor scheme în cea acută, reieşind din tipul crizei blastice.

Prezintă interes experienţa mondială reflectată în publicaţiile de specialitate despre tratamentul LMC, care relevă rezultatele aplicării diverselor opţiuni chimio- şi imunoterapeutice. În faza cronică a LMC pe fond de medicaţie cu α-IFN răspunsul clinico-hematologic complet poate fi obţinut în 81% cazuri, răspunsul citogenetic complet – în 26% cazuri [17]. Supravieţuirea de peste 5 ani a bolnavilor trataţi cu α-IFN constituie 57%, fiind mai relevantă faţă de cazurile tratate cu chimioterapie convenţională (42%) [2, 6, 18]. În cadrul tratamentului chimioterapeutic convenţional longevitatea medie a pacienţilor cu LMC variază între 4–5 ani, la 30% dintre ei, depăşind termenul de 10 ani [5]. Totodată sunt descrise cazuri cu o durată a vieţii de 15–20 de ani după administrarea tratamentului. Durata crizei blastice constituie în medie 4,5 luni, cu extreme de 0,5 – 15 luni. Imatinib Mesilat se aplică cu succes în toate fazele LMC, cel mai elocvent răspuns clinico-hematologic şi citogenetic fiind atins în faza cronică a patologiei. Remisiunea clinico-hematologică completă pe fond de medicaţie cu Imatinib Mesilat poate fi obţinută în timp de 1 – 2 luni. Spre deosebire de chimioterapia convenţională şi α-IFN, Imatinib Mesilat contribuie la atingerea remisiunii citogenetice majore în 65–85% cazuri şi celei complete în 45–80% cazuri [6]. Supravieţuirea fără recidive constituie 89%, peste 18 luni de tratament cu Imatinib Mesilat. Până la faza de accelerare, calitatea vieţii bolnavilor este satisfăcătoare, cu păstrarea capacităţii de muncă.

Managementul LMC în ţările în curs de dezvoltare derulează prin implementarea Glivec® International Patient Assistance Program (GIPAP), care a fost lansat în anul 2001 de Novartis Pharma AG ca program de donaţie şi înrolează bolnavii cu diferite faze ale leucemiei mieloide cronice, ale leucemiei acute limfoblastice şi tumorilor gastro-intestinale stromale (GIST) maligne [9, 10, 16, 17]. GIPAP prezintă unul dintre cele mai generoase şi de lungă durată program în domeniul terapiei anti-cancer, axat pe asigurarea tratamentului cu Imatinib Mesilat a pacienţilor cu procese neoplazice maligne [9, 13, 14, 17]. Identificarea şi recomandarea instituţiilor medicale, evaluarea şi calificarea pacienţilor pentru GIPAP, suportul informaţional şi logistic sunt efectuate de TMF şi Axios International. Novartis Pharma AG este responsabilă de aprobarea instituţiilor medicale pentru GIPAP şi de expedierea loturilor de medicamente în calitate de donaţie [13, 17]. Peste 280 de centre medicale de referinţă specializate în hematologie/oncologie sunt implicate în acest program internaţional. Din momentul lansării, GIPAP a aprovizionat cu Imatinib Mesilat mai mult de 10000 de pacienţi, din peste 80 de ţări care nu aveau acces la acest remediu eficient şi bine tolerat.

GIPAP a fost lansat oficial în Republica Moldova în martie 2006 prin semnarea Memorandumului de Înţelegere (MOU) între managerul GIPAP şi administraţia Institutului Oncologic din Moldova [13]. Institutul Oncologic a fost calificat ca instituţie medicală de referinţă pentru GIPAP, iar dl Vasile Musteaţă, doctor în medicină, Catedra Hematologie, Oncologie şi Terapie de Campanie a USMF „Nicolae Testemiţanu" este numit în funcţie de administrator GIPAP în Moldova. Implementarea eficientă a acestui program internaţional în Moldova se datorează eforturilor Ministerului Sănătăţii, administraţiei Institutului Oncologic şi ale şefului Catedrei Hematologie şi Oncologie, membru corespondent al AŞRM, prof. univ. Ion Corcimaru.

GIPAP prezintă un program internaţional non-profit, flexibil şi bine monitorizat. Regulamentul acestui program prevede dări de seamă la fiecare 3 luni, care să reflecte numărul total al pacienţilor trataţi cu Glivec®, cantităţile de medicament recent expediate, utilizate şi rămase în stoc, necesităţile de medicamente în perioada imediată de 3 luni, reieşind din numărul de cazuri incluse în GIPAP, detaliile referitoare la autorizarea importului medicamentului. În cadrul programului funcţionează serviciul, care acordă suport informaţional şi managerial medicilor responsabili de GIPAP şi bolnavilor. GIPAP este înalt apreciat atât de pacienţii incluşi, cât şi de medicii curanţi şi de autorităţile din sfera ocrotirii sănătăţii. Bolnavii dispun de acces gratuit şi sigur la medicamentul de primă linie pentru tratamentul unor neoplazii maligne, care asigură un răspuns clinic şi molecular net superior în raport cu chimio- şi imunoterapia convenţională, precum şi calitatea bună a vieţii. Prevalenţa crescută a leucemiei mieloide cronice şi a leucemiei acute limfoblastice, resursele financiare limitate ale ţărilor în curs de dezvoltare în aprovizionarea cu tratamente costisitoare determină actualitatea şi necesitatea imperioasă a acestui program. GIPAP a perfecţionat semnificativ standardele de diagnostic şi tratament al maladiilor oncologice în Republica Moldova. Programul exercită impact pozitiv asupra activităţii ştiinţifice în cadrul Institutului Oncologic şi USMF „Nicolae Testemiţanu" deoarece stimulează aplicarea tehnologiilor noi în diagnosticarea şi tratamentul tumorilor de geneză diversă. GIPAP constituie o arenă internaţională optimă pentru cooperare în domeniile actuale ale oncologiei şi hematologiei, luând în consideraţie şi necesităţile psihologice şi sociale ale pacienţilor.

Referitor la managementul LMC se comunică că în România sume importante se irosesc deoarece se întârzie trece-

rea de la tratamentul de primă linie la cel de linia a doua în cazul acestei patologii mieloproliferative [12]. De asemenea, cresc cheltuielile legate de LMC din cauza că, în continuare investigaţiile necesare pentru un diagnostic corect sunt considerate un lux. L. Caban în cadrul Conferinţei Internaţionale „Managementul bolilor cronice - reconfigurarea sistemelor de sănătate" subliniază importanţa principiului cost – eficienţă în conduita pacienţilor cu LMC. În acest sens, medicul şi pacientul sunt întotdeauna pe aceeaşi parte a baricadei, ambii fiind interesaţi de creşterea supravieţuirii şi a calităţii vieţii. De cealaltă parte a baricadei se vor afla întotdeauna guvernanţii şi decidenţii din sistemul sanitar, care urmăresc scopul de a avea rezultate bune cu cheltuieli reduse. Personajele poziţionate de o parte şi de cealaltă a baricadei au scopuri diferite, care totuşi se pot armoniza prin dialog pentru a ajunge la un act medical de calitate cu costuri rezonabile. Pentru aceasta trebuie de acţionat în direcţia profilaxiei bolii prin campanii de descurajare a unor vicii, prin limitarea expunerii la investigaţii imagistice, la noxe la locul de muncă. Dacă în LMC tratamentele de primă linie nu mai dau rezultate după un an-un an şi jumătate de administrare, este necesară trecerea la linia a II-a de tratament. Dar, din cauza circuitului complicat al dosarelor şi al aprobărilor de la casele de asigurări de sănătate, aceste tratamente sunt mult întârziate, cu consecinţe grave pe de o parte, pe de altă parte este compromis şi tratamentul de primă linie, iar pacientul dezvoltă complicaţii. Aceste întârzieri în administrarea tratamentelor de linia a II-a explică creşterea cheltuielilor şi, implicit, un management deficitar al LMC.

O altă cauză a creşterii cheltuielilor în cazul LMC o reprezintă concepţia total greşită, potrivit căreia investigaţiile medicale ce se impun pentru stabilirea unui diagnostic precoce, corect şi complet, sunt considerate încă un lux [12]. Un diagnostic precoce presupune o încărcătură tumorală ce poate fi mai uşor eradicată. Un diagnostic corect şi complet oferă posibilitatea utilizării unei terapii individualizate care ar putea asigura controlul maladiei, evitarea tratamentelor inadecvate, reducerea numărului zilelor de spitalizare, în consecinţă producându-se reducerea cheltuielilor. Se menţionează că inhibitorii de tirozin-kinază constituie, la ora actuală, un remediu foarte eficient care a dat rezultate remarcabile în tratamentul LMC. Astfel, există studii care demonstrează că unii pacienţi au supravieţuit între 7 şi 10 ani prin administrarea acestor tratamente, fără să mai prezinte simptomatologia bolii. De asemenea, la unii pacienţi a fost înregistrată trecerea de la faza acută la cea cronică a bolii, cu o calitate a vieţii cvasinormală. Se consideră că un management eficient al LMC impune crearea unei reţele de centre de diagnostic complex, care să beneficieze de aparatură performantă şi personal specializat [12].

O importanţă practică şi ştiinţifică majoră în managementul LMC o are implementarea Programului European pentru Tratamentul Leucemiei mieloide cronice (EUTOS) [1]. Tratamentul şi studiile în cadrul EUTOS pentru LMC au fost iniţiate în octombrie 2007. Obiectivul proiectului este o mai bună înţelegere a bolii, evaluare, standardizare şi monitorizare pentru optimizarea diagnosticului şi tratamentului în întreaga Europă. De asemenea, se urmăreşte şi elaborarea protocoalelor pentru tratament. Proiectul are ca scop gestionarea acestei boli în Europa prin patru programe-cheie, respectiv un registru european al pacienţilor, monitorizarea evoluţiei bolii, un program privind rezultatele clinice şi teste pentru evaluarea efectelor farmaceutice. Pentru continuarea acestui proiect, Novartis alocă şase milioane de euro. Prof. Michele Baccarani (Universitatea Bologna, Italia) relevă că sunt selectaţi 2330 de pacienţi în studii academice, iar 1307 - în studii clinice medicale, dintre aceştia 13 fiind din România. Guido Guidi, coordonatorul diviziei de oncologie Novartis pentru Europa, a declarat într-o conferinţă de presă că un alt obiectiv al studiilor este atingerea unui standard molecular similar în toate ţările europene. Guido Guidi relatează că România se numără printre ţările incluse în studiul EUTOS pentru leucemia mieloidă cronică cu trei centre şi 13 pacienţi în studiul clinic şi subliniază că, în pofida greutăţilor economice, autorităţile române au manifestat deschidere pentru acest program european [1, 12]. Programul respectiv urmăreşte obiectivul ca laboratoarele să fie standardizate pentru managementul calitativ al bolii. Şi chiar dacă în România standardizarea nu este realizată, tratamentul aplicat pacienţilor pentru leucemia mieloidă cronică este acelaşi ca în orice altă ţară europeană, la fel ca şi calitatea diagnosticului şi a tehnologiei.

Prof. Rudiger Hehlmann (Universitatea Heidelberg, Germania) susţine că prin continuarea acestui program se poate studia mai bine boala, se poate extinde registrul european şi se poate urmări riscul de progresie a bolii, dar şi influenţa medicamentului asupra altor tratamente pentru boli diferite, pe care pacientul le mai poate avea [1]. El a mai subliniat că accentul se pune pe monitorizarea moleculară, în diferite faze, a cromozomului Philadelphia, responsabil de apariţia afecţiunii.

Prof. Michele Baccarani susţine că studiile clinice ajută la implementarea ghidurilor şi uniformizarea tratamentului pe regiuni, adăugând că în perioada ianuarie - septembrie 2010 au fost înregistraţi 500 de noi pacienţi, iar în cei doi ani care urmează se aşteaptă ca numărul acestora să ajungă la 3000 [1]. Specialistul italian a evidenţiat importanţa studiului pentru lumea ştiinţifică medicală, accentuând importanţa monitorizării tratamentelor, având în vedere că este vorba despre pacienţi care nu reacţionează toţi la fel la acelaşi tratament. Tratamentul actual a dus la o supravieţuire a pacienţilor la 5 ani în 93% cazuri, faţă de o rată de supravieţuire de 38% în perioada 1983 – 1994, când existau alte tratamente [2, 18, 22].

În rezultatul studierii literaturii periodice internaţionale, evaluării dinamice a datelor clinico-hematologice şi imagistice, precum şi a rezultatelor tratamentului pacienţilor cu LMC din subloturile investigate, s-au conturat premisele pentru elaborarea algoritmului de conduită în oncopatologia respectivă, care include algoritmul diagnostic şi cel de tratament.

În calitate de indicatori pentru monitorizarea implementării algoritmului de tratament pot fi utilizate: proporţia pacienţilor cu LMC, la care s-a efectuat chimioterapia „de ţintă", medicaţia citoreductivă şi/sau imunoterapia cu normalizarea analizei generale a sângelui şi a mielogramei în decurs de 3

luni, pe parcursul unui an; proporția pacienților cu LMC, la care s-a efectuat chimioterapia „de țintă" sau/și imunoterapia cu dispariția Ph-cromozomului și genei de fuziune BCR-ABL, respectiv în 12 și 18 luni, pe parcursul unui an; proporția pacienților cu LMC, la care s-a menținut răspunsul clinico-hematologic, citogenetic și molecular complet pe parcursul unui an; proporția pacienților cu LMC, la care s-a abrogat sau s-a redus gradul de invalidizare, pe parcursul unui an.

## Concluzii

1. LMC reprezintă o patologie oncohematologică relativ frecvent înregistrată în structura morbidității prin tumorile sistemului hematopoietic, constituind 15 – 20% din toate leucemiile la adulți și afectând preponderent persoanele apte de muncă.

2. Managementul contemporan al LMC diversifică opțiunile diagnostice și curative în funcție de nivelul asistenței medicale.

3. Un management eficient al LMC impune organizarea screening-ului, axat pe depistarea precoce a splenomegaliei și a modificărilor în sângele periferic, precum și la utilizarea indicatorilor obiectivi pentru monitorizarea implementării algoritmului de tratament.

4. GIPAP constituie sursa de perfecționare a standardelor de diagnostic și tratament al LMC în Republica Moldova.

5. Diagnosticul corect, complet și precoce oferă posibilitatea aplicării terapiei individualizate care poate asigura controlul maladiei, evitarea tratamentelor inadecvate, reducerea numărului zilelor de spitalizare, ceea ce va duce la optimizarea cheltuielilor.

6. Tratamentul LMC în faza cronică și de accelerare, fără complicații poate fi efectuat în condiții de ambulator sau de staționar de zi. Tratamentul LMC în faza de accelerare, cu complicații (hemoragice, trombotice, infecțioase) și acută se efectuează în secțiile specializate de hematologie.

7. Medicamentul Imatinib Mesilat constituie o opțiune terapeutică de primă linie în faza cronică și de accelerare a LMC, fiind net superioară în raport cu chimioterapia convențională și α-IFN, prin posibilitatea atingerii răspunsului clinico-hematologic complet și rapid, a răspunsului citogenetic complet și creșterea semnificativă a calității vieții și longevității pacienților.

## Bibliografie


1. Agerpres. *Știri externe.* 2010, 22 octombrie.
2. Baccarani M, Saglio G, Goldman J, et al. Evolving concepts in the management of chronic myeloid leukemia: recommendations from an expert panel on behalf of the European LeukemiaNet. *Blood*. 2006;108(6):1809–1820.
3. Butoianu E, Niculescu-Mizil E. Leucemia mieloidă cronică. In: Coliță D. Medicină Internă. Hematologie. Partea a II-a. București: Editura medicală, 1999;48–68.
4. Castagnetti F, Palandri F, Amabile M, et al. Results of high-dose imatinib mesylate in intermediate Sokal risk chronic myeloid leukemia patients in early chronic phase: a phase 2 trial of the GIMEMA CML Working Party. *Blood*. 2009;113(15):3428–3434.
5. Corcimaru I. Leucemia granulocitară cronică. In: Corcimaru I. Hematologie. Chișinău: CEP Medicina, 2007;178–189.
6. Cortes JE, List A, Kantarjian H. Chronic myelogenous leukemia. In: Pazdur R, Coia LR, Hoskins WJ, et al. Cancer Management: A Multidisciplinary Approach. 8th Edition. New York: CMP Healthcare Media, 2004;773–786.
7. Cortes JE, Talpaz M, O'Brien S, et al. Staging of chronic myeloid leukemia in the imatinib era: an evaluation of the World Health Organization proposal. *Cancer*. 2006;106(6):1306–1315.
8. Dressman MA, Malinowski R, McLean LA, et al. Correlation of major cytogenetic response with a pharmacogenetic marker in chronic myeloid leukemia patients treated with imatinib (STI 571). *Clin. Cancer Res*. 2004;10:2265–2271.
9. Durosinmi MA, Faluyi JO, Okany CC, et al. Preliminary experience with imatinib mesylate therapy of Ph+ chronic myelocytic leukaemia in Ile-Ife Nigeria. *Journal of Clinical Oncology*. 2005;23(16S):3216.
10. ESMO Gudelines Working Group, Chronic myelogenous leukemia: ESMO Clinical Recommendations for diagnosis, treatment and follow-up. *Annals of Oncology*. 2007;18(2):ii51–ii52.
11. Kosmidis PA, Schrijvers D, André F, et al. ESMO Handbook of Oncological Emergencies. Taylor & Francis Group, 2005;158.
12. Marincovici M. In: *Viața sănătoasă. Jurnal național.* 2010, 24 septembrie.
13. Musteață V, Corcimaru I, Sofroni M, ș. a. GIPAP în Republica Moldova: realizări și perspective. *Buletinul Academiei de Științe a Moldovei. Științe Medicale*. 2008;2(16):226–228.
14. Musteata V, Corcimaru I. Targeted therapy of chronic myelogenous leukemia: experience of the Institute of Oncology of Moldova. *Archives of the Balkan Medical Union*. 2008;43(3):154–155.
15. Niederwieser D. HSCT for chronic myeloid leukemia in adults. In: Apperley J, Carreras E, Gluckman E, et al. Haematopoietic Stem Cell Transplantation. European School of Haematology. The EBMT Handbook. 5th Edition. Paris: Herissey, 2008;388–396.
16. O'Brien S, Berman E, Devetten MP, et al. Chronic myelogenous leukemia. NCCN Clinical Practice Guidelines in Oncology. V 2.2009. National Comprehensive Cancer Network, Inc., 2008;1–47.
17. Ramos JD. Gleevec Patient Assistance Program USA. Patient Guide. Seattle: Cancer Resources & Advocacy, 2004;1–5.
18. Richard RE, Linenberger M. Chronic myeloid leukemia. In: American Society of Hematology Self-Assessment Program. Blackwell Publishing, 2005;178–189.
19. Spinei L, Lozan O, Badan V. *Biostatistica.* Chișinău: Tipografia Centrală, 2009;186.
20. Vardiman JW, Harris NL, Brunning RD. The World Health Organization (WHO) classification of the myeloid neoplasms. *Blood*. 2002;100:2292–2302.
21. Xenocostas A. Chronic myelogenous leukemia (CML). Hematology Practice Guideline. London Health Sciences Centre. London Regional Cancer Program, 2007;1–10.
22. Масляк ЗВ. Хронічний мієлоїдний лейкоз. In: Гайдукова СМ. Гематологія та трансфузіологія. Київ: ВПЦ «Три крапки», 2001;239–251.
23. Туркина АГ. Хронический миелолейкоз. In: Воробьёв АИ. Руководство по гематологии. Москва: Ньюдиамед, 2003;251–264.

## ANNIVERSARIES


La acest început de cireşar, în 5 iunie a anului curent, academicianul Victor Lacusta, Om Emerit al Republicii Moldova, figură reprezentativă a cercetării naţionale în domeniul medicinii, rotungeşte onorabila vârstă de 60 de ani. Fiind alături de el, aducându-i sincere urări de sănătate şi realizării frumoase, la acest popas sexagenar, noi, colegii din domeniul ştiinţelor fizice şi inginereşti, venim cu câteva creionări de alternativă a portretului Dumnealui, ţinând cont de faptul că şi domeniul lui profesional de activitate ţine în mare măsură de medicina alternativă, bazată pe metodele fizice.

Făcând primii paşi în activitatea ştiinţifică sub tutela academicienilor Natalia Gheorghiu şi Eva Gudumac, după absolvirea cu brio în 1974 a Institutului de Stat de Medicină, Victor Lacusta este captivat „incurabil" de aplicarea metodelor nonfarmacologice în tratamentul a diverse maladii. Încă în anul 1976 realizează cu succes câteva anestezii sub influenţa hipnozei. Ulterior activează în calitate de asistent la Catedra de Psihiatrie a Institutului de Stat de Medicină şi colaborator ştiinţific la Institutul de Fiziologie al Academiei de Ştiinţe, unde a început investigaţiile clinico-experimentale sistematice în domeniul medicinii alternative (acupunctura). Sub conducerea Domniei sale au fost susţinute 11 teze de doctor şi 2 teze de doctor habilitat la specialităţile Medicină netradiţională, Fiziologia omului şi animalelor.

Firește, că aflându-te pe tărâmul medicinii tradiţionale, nu poţi să avansezi şi să devii profesionist recunoscut la nivel internaţional fără a fi în contact şi fără a te inspira din originile acestui domeniu miraculos, care pornesc din China. Academicianul Victor Lacusta i-a avut ca dascăli pe talentaţii specialişti chinezi, profesorii Lin Peigen şi Xang Ping de la Universitatea de Medicină Tradiţională din Nanging. Venind în mediul autohton cu o pregătire vastă şi cu aprecieri elogioase din partea profesorilor chinezi, s-a încadrat plenar în zbuciumul creator, compilat în cele peste 400 de publicaţii ştiinţifice, 20 de monografii, elaborări metodice, programe ale catedrei pe care o conduce. Victor Lacusta este autorul unor studii de sinteză a surselor antice de medicină tradiţională chineză, aflate în arhivele celor mai prestigioase biblioteci din China şi a monografiilor, bazate pe cercetări experimentale proprii. "Tratatul de acupunctură clinică", elaborat de Victor Lacusta este prefaţat, înalt apreciat şi recomandat de Organizaţia Mondială a Sănătăţii pentru medicii din Europa. Academicianul Victor Lacusta este un generator de idei, un neobosit cercetător, deţine 16 brevete de invenţie, este laureat al Premiului Academiei de Ştiinţe a Moldovei pentru cea mai valoroasă lucrare ştiinţifică (2004).

Investigaţiile clinico-experimentale de mai mulţi ani au dat posibilitatea elaborării unui concept, în corespundere cu care acupunctele şi organele interne ale organismului formează un sistem integru funcţional, relativ autonom de reglare a unor funcţii fiziologice. Graţie acestui concept, medicina tradiţională milenară capătă noi perspective de aplicare practică în domeniul diagnosticului, tratamentului şi profilaxiei unui spectru întreg de maladii.

Extinderea experienţei aplicării acupuncturii în diverse centre medicale din străinătate şi dezvoltarea colaborării între specialiştii din mai multe ţări europene au pregătit terenul pentru fondarea Asociaţiei Europene de acupunctură - *European Association of Acupuncture (EAA)*. Şi ca o recunoaştere internaţională a profesionalismului academicianului Victor Lacusta a fost alegerea dlui în 1994 în calitate de vicepreşedinte al *EAA*, iar în 1997 - ca preşedinte al *EAA*. Concomitent este membru al *Liga Medicorum Homoeopathica Internationalis*.

Virtuţile de expert şi profesionalist academicianul Victor Lacusta le manifestă pregnant şi la nivel naţional. Deja de mai mulţi ani, el este preşedinte al Comisiei de Experţi al CNAA, fiind responsabil, obiectiv şi amabil. Prin efortul Dumnealui, în premieră în Europa a fost organizat Consiliul Ştiinţific Specializat de susţinere a tezelor de doctorat în domeniul Medicinii Alternative. Este Preşedinte al Seminarului Ştiinţific de profil din cadrul Institutului de Fiziologie şi Sanocreatologie al AŞM, membru al Consiliului ştiinţific universitar şi al Senatului Universităţii de Medicină şi Farmacie „Nicolae Testemiţanu".

Este fondator şi redactor-şef al revistei ştiinţifico-practice Medicina Alternativă (editată din 1997); redactor-şef al revistei internaţionale „*The Bulletin of the European Postgraduate Center of Acupuncture and Homeopathy*" (1996-2001); membru al colegiului de redacţie al Buletinului Academiei de Ştiinţe a Moldovei. Ştiinţele vieţii; al revistei internaţionale *Traditional Medicine East and West*, al Revistei Române de Acupunctură.

Mai mulţi cercetători din domeniul istoriei ştiinţelor şi artelor înclină spre ideea că medicina, fizica şi muzica sunt dintre cele mai vechi pe Tera. Fizica a fost integrată în activitatea sa de academician prin medicina alternativă şi metodele fizice de tratament. Este pasionat de şah şi muzică, în duet cu soţia Djulieta Lacusta (flaut, pian) interpretează piese muzicale etno-jazz pe scene profesioniste. Astfel, primii paşi spre integrarea ştiinţei şi artei în activitatea lui sunt făcuţi. Rămâne să vedem completată această integrare la următoarele popasuri jubiliare.

Ajungând la ultima creionare a portretului academicianului Victor Lacusta, cu ocazia împlinirii celor 60 de ani, îi urăm multă sănătate, succese în activitatea profesională, prosperitate şi realizări frumoase spre binele ţării şi al cetăţenilor ei.

**Valeriu Canţer**,
*Doctor habilitat în ştiinţe fizico-matematice, profesor, academician*
*Preşedinte al Consiliului Naţional de Atestare*
*şi Acreditare al Republicii Moldova*
*Membru al Consiliului Suprem pentru*
*Ştiinţă şi Dezvoltare Tehnologică AŞM*


Eugen Guțu este un nume de referință în mediul chirurgical autohton. La o vârstă nu atât de înaintată a reușit deja să facă ceva foarte important, salvând multe vieți și făcând din această activitate un sens al existenței lui. La mijlocul lunii lui cuptor va împlini frumoasa vârstă de 50 de ani domnul Eugen Guțu, doctor habilitat în medicină, profesor universitar, șeful catedrei Chirurgie generală și semiologie a USMF "Nicolae Testemițanu".

S-a născut la 15 iulie 1961, în familia unor studenți ai Institutului de Medicină din Celeabinsk (Rusia). Tatăl dumnealui, Vasile Guțu care avea să devină mai târziu fondatorul serviciului de endoscopie în Republica Moldova, după absolvirea institutului a lucrat ca medic-chirurg în Spitalul Regional Central al orașului Kurgan (Rusia). Mama, Nina Guțu a activat o perioadă îndelungată ca medic terapeut. În anul 1974 familia Guțu revine pe meleagurile natale, stabilindu-se în Chișinău. Eugen Guțu este înmatriculat la facultatea Medicină generală a Institutului de Stat de Medicină din Chișinău, pe care a absolvit-o cu succes în 1984. Primii pași întru însușirea acestei profesii dificile, tânărul chirurg i-a făcut sub supravegherea unor medici experimentați precum L. Enzin, V. Iaz, S. Chiriac, M. Lumei.

În perioada anilor 1987-1990, chirurgul Eugen Guțu își perfecționează cunoștințele în cadrul aspiranturii, efectuate în condițiile secției chirurgie septică („Раны и раневая инфекция") a Institutului de Chirurgie „A. V. Vișnevskii" din Moscova. Munca zilnică asiduă, alături de așa specialiști cu renume precum M. I. Kuzin, B. M. Kostiucionok, V. A. Karlov, S. M. Beloțkii, a contribuit la formarea domnului Eugen Guțu ca chirurg și om de știință. Cercetările fundamentale în domeniul imunologiei la bolnavii cu infecție chirurgicală și evaluarea detaliată a evoluției procesului de plagă i-au permis domnului Eugen Guțu să susțină cu succes teza de doctor în medicină "Influența tratamentului chirurgical activ asupra statusului imun general și local în caz de infecție purulentă chirurgicală", eveniment ce a avut loc în 1990, sub conducerea profesorilor V. A. Karlov și S. M. Beloțkii. Tânărul și energicul cercetător reușea să combine lucrul asupra tezei cu munca grea în secție, tratând un contingent extrem de dificil de pacienți. În această perioadă, în carnetul său de muncă va apărea inscripția de mulțumire "pentru acordarea ajutorului medical sinistraților ce au suferit în urma seismului din Armenia".

În anul 1990 Eugen Guțu revine la Chișinău și își începe activitatea în calitate de asistent la catedra Chirurgie facultativă. Sub conducerea unor profesori excelenți și chirurgi virtuoși, precum Gh. Ghidirim și E. Cicală, asistentul universitar Guțu acumulează rapid experiență în chirurgia abdominală. În scurt timp i se încredințează efectuarea de sinestătător a intervențiilor chirurgicale la pacienții extrem de gravi cu hemoragii, consultarea bolnavilor din raioanele Republicii în cadrul serviciului "Aviasan".

Din anul 1993 baza clinică a catedrei devine spitalul de urgență, iar doctorul Guțu însușește un alt compartiment vast al chirurgiei – tratamentul pacienților cu traumatism toracic și abdominal. Zi și noapte, în condițiile sălii de operație chirurgul își perfecționează măiestria, acumulează experiență și sporește încrederea în propriile forțe. În 1994 obține un grant de participare la conferința tinerilor savanți în SUA. Eugen Guțu împărtășește cu drag cunoștințele acumulate medicilor tineri și studenților, încadrându-se perfect în activitatea pedagogică și în anul 1997 i se conferă titlul de conferențiar universitar.

Doctorul Guțu urmărește cu viu interes dezvoltarea chirurgiei mondiale și participă cu rapoarte la congrese internaționale în SUA, Norvegia, Spania, Italia, România, Turcia, Rusia și alte țări. Sub conducerea continuă a academicianului Gh. Ghidirim, domnul Guțu însușește cele mai laborioase intervenții chirurgicale pe ficat și pancreas, chirurgia laparoscopică, elaborează și implementează metodele contemporane de hemostază endoscopică. Rezultatele activității practice și ale lucrului științific din acea perioadă sunt oglindite în multiple publicații, inclusiv în reviste internaționale de prestigiu.

Totalizarea unei vaste experiențe a clinicii de chirurgie "N. Anestiadi" în tratamentul hemoragiilor gastro-duodenale, i-a permis domnului Eugen Guțu să susțină în 2005 teza de doctor habilitat în medicină cu tema "Prognozarea și prevenirea recidivei hemoragiei ulceroase gastroduodenale", sub conducerea academicianului Gheorghe Ghidirim. În același an, doctorul Eugen Guțu devine șef al catedrei Chirurgie generală și semiologie, iar în 2009 obține titlul științifico-didactic de profesor universitar.

Grație talentului de conducător și muncii asiduie zi de zi, domnul profesor Eugen Guțu a reușit "să ridice" catedra, condusă de domnia sa, la un nivel calitativ nou. În clinică se efectuează un spectru larg de intervenții chirurgicale pe organele cavității abdominale și vasele sangvine, sunt implementate metode noi de tratament – operații videoendoscopice în caz de reflux gastro-esofagean și insuficiență venoasă, este organizată activitatea serviciului endoscopic, se efectuează operații complexe în caz de cancer al pancreasului, ficatului, intestinului și al căilor biliare.

Domnul profesor Eugen Guțu este președintele comisiei de experți în chirurgie în cadrul Consiliului Național pentru Acreditare și Atestare, președintele comisiei de calificare a cadrelor didactice ale USMF "Nicolae Testemițanu", membru al Asambleei Academiei de Științe din Republica Moldova, membru al Societății chirurgilor "N. Anestiadi", al Societății Române de Chirurgie, Asociației Internaționale de chirurgie hepato-pancreato-biliară, Societății Internaționale de Chirurgie. Profesorul Eugen Guțu este autorul a peste 250 de lucrări științifice, 9 elaborări metodice și 4 invenții. Sub conducerea domniei sale au fost susținute 3 teze de doctor în medicină și altele 3 se apropie de finalizare.

Jubileul l-a surprins pe profesorul Guțu în plină putere, cu maximă energie și planuri creative noi de activitate. Deci îi dorim multă sănătate, prosperitate, realizări frumoase în activitatea sa nobilă, noi succese și fericire alături de cei dragi.

Vivat! Crescat! Floriat!

**Ion Ababii**, *dr. h., profesor, academician*
*Rector al USMF „Nicolae Testemițanu"*

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