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From its debut in 1958 the journal has striven to support the interests of Moldovan medicine concerning the new concepts of its development. The Editorial Board warmly welcomes both the readers of and the authors for the journal, all those who are enthusiastic in searching the new and more effective ways of solving numerous medicine problems. We hope that those who want to make their contribution into the science of medicine will find our journal helpful and encouraging.

The journal is accredited by the National Council for Accreditation and Attestation. The journal publishes official papers, scientific articles, editorials, clinical studies and cases, lectures, methodological guides, reviews, brief reports and correspondence. The journal welcomes articles in English, Romanian and Russian. The journal editorial policy provides the prompt publication of papers within 12 weeks after receiving them.

Bine ați venit la revista științifică medicală Curierul Medical!

De la prima apariție în 1958, revista susține și dezvoltă noile idei în domeniul medicinii, în Republica Moldova. Colegiul de redacție agrează cu multă considerație atât cititorii cât și autorii articolelor, pe toți acei care cu mult entuziasm caută noi și mult mai efective metode de soluționare a multiplelor probleme ale medicinii. Sperăm, că toți acei care doresc să-și aducă aportul la dezvoltarea științelor medicale, vor găsi revista noastră utilă și atractivă.

Revista este acreditată de către Consiliul Național de Acreditare și Atestare. Revista publică comunicări oficiale și, totodată, sunt editate diverse publicații, inclusiv independente: articole științifice, editoriale, cercetări și prezentări de cazuri clinice, prelegeri, îndrumări metodice, articole de sinteză, relatări scurte, corespondențe și recenzii. Revista publică articole în limba engleză, română și rusă. Politica de editare a revistei prevede examinarea operativă și publicarea articolelor timp de 12 săptămâni după înaintare.

Добро пожаловать в научно-медицинский журнал Curierul Medical!

С первого дня своего выпуска в 1958 году журнал стремится поддерживать и развивать новые идеи в области медицины в Молдове. Редакционная коллегия всегда рада как читателям, так и авторам статей, всем тем, кто с энтузиазмом ищет новые, более эффективные способы решения многочисленных задач медицины. Мы надеемся, что все те, кто хотят внести свой вклад в медицинскую науку, найдут наш журнал полезным и вдохновляющим.

Журнал аккредитован Высшей Аттестационной Комиссией Республики Молдова. В журнале печатаются официальные материалы, научные статьи, наблюдения из клинической практики, обобщающие статьи, краткие сообщения, методические указания, рецензии и корреспонденция. В журнале публикуются статьи на английском, румынском и русском языках. Издательская политика журнала предусматривает оперативное рассмотрение и публикацию статей в среднем в течение 12 недель после поступления.

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RESEARCH STUDIES

Childhood obesity – a major problem of public health

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Abstract

Background: The rate of childhood overweight and obesity is increasing in all countries. Overweight in children represents a very dangerous tendency because for long term it may cause serious health problems such as cardiovascular diseases, type 2 diabetes, hypertension, various forms of cancer and other major chronic diseases, which are generating premature mortality and long-term morbidity.

Material and methods: It has been carried out a secondary study, a narrative review of scientific bibliographical sources dedicated to the problem of obesity in children. The analysis is based on 50 literary sources of foreign authors (USA, Great Britain, Romania, Russia, the Netherlands, Slovenia, Croatia, France, Germany and Italy) and international organizations, published in the period 2000-2014.

Results: Globally, it is estimated that 170 million children under 18 years old are overweight. About 60% of obese children become obese adults. In the Republic of Moldova the statistical records on overweight and obesity among children are incomplete. Many studies show that obesity in children is characteristic for school age, being registered a rate of 10-30%. In order to determine if the excess of adipose tissue can cause health problems it is necessary to calculate the BMI 2-20 years/age/sex according to nomograms CDC 2000 and perform additional investigations: abdominal, tricipital and subscapular skinfold measurement, abdominal circumference, hip circumference, relation between abdomen and hip circumference, determination of adipose tissue percentage by advanced methods, evaluation of nutrition, physical activities and family background. Obesity in children is a risk factor for cardiovascular diseases, hypertension, type 2 diabetes, sleep apnea syndrome, depressions, some forms of cancer, behavioral problems at school and low self-esteem. The performed studies show that the obesity occurred in childhood leads to the decrease of life expectancy due to the complications it determines.

Conclusions: Overweight children face multiple health problems, social and psychological problems. The problem of obesity has a major contribution to the global burden of chronic diseases, with serious social and psychological implications. Thus, research on overweight and obesity in children, with a stress on prevention, has a high priority.

Key words: overweight, obesity, children, health condition, determinants

Introduction

Childhood obesity is a major public health problem worldwide, increasing in all countries [3, 16, 39]. According to the European Commission data for 2014, over 22 million children in the European Union are considered overweight or obese, and this number is increasing each year by 400,000 [16]. The problem of obesity has a major contribution to the global burden of chronic diseases, with serious social and psychological implications, which are practically affecting all ages and socioeconomic groups [39]. Overweight in children represents a very dangerous tendency because for long term it may cause serious health problems such as cardiovascular diseases, type 2 diabetes, hypertension, various forms of cancer and other major chronic diseases [10, 21, 40, 48, 50]. These diseases cause premature mortality and long-term morbidity as well [19]. Additionally, overweight and obesity in children are associated with significant reductions of life quality and a greater risk of social isolation [45].

Material and methods

It has been carried out a secondary study, a narrative review of scientific bibliographical sources dedicated to the problem of obesity in children. The analysis is based on 50 literary sources of foreign authors (USA, Great Britain, Romania, Russia, the Netherlands, Slovenia, Croatia, France, Germany and Italy) and international organizations, published in the period 2000-2014. The analyzed sources were classified into the following sections: background, definition of overweight and obesity in children, evaluation methods of overweight and obesity in children, etiology and determinants, health condition of overweight children, economic impact, and preventive measures against obesity in children. It was applied the qualitative method by comparing the same phenomenon in different countries.

Results and discussion

Epidemiology of obesity in children at international level

Obesity is the most common nutritional disorder of children in many countries, representing a major public health problem in many parts of the world [3, 16, 39]. Currently there is an epidemical and increasing tendency of the frequency of obesity and overweight in children, which have spread worldwide affecting about 20-25% of children and 45-50% of adolescents [16].

Globally, it is estimated that 170 million children under 18 years old are overweight [35]. According to WHO (year 2013) 43 million children under five years old are overweight. Over 60% of obese children become obese adults [16, 17].

The prevalence of obesity and overweight in children is increasing alarmingly in North America, Europe, and recently in Australia, China, South America and North Africa as well. The prevalence varies considerably between different regions and countries, from 5% in Africa and several parts of Asia, to more than 20% in Europe and 30% in America and in several countries in the Middle East [4].

The problem of obesity in children is global and it is spreading increasingly in the developing countries [11, 39]. For example, the prevalence of obesity in children aged 5-12 years old in Thailand has increased from 12.2% to 15.6% in just two years, in Mexico the percentage of overweight and obese children is 41.8%, in Brazil – 22.1%, in India – 22.0% and in Argentina – 19.3%. Sedentary lifestyle and fast change of food practices have resulted in increase of prevalence of obesity in children aged 5-19 years old in developing countries [11].

Obesity in children in Europe has tripled in the last 20 years (European Commission 2007) [7]. In most countries from the Western Europe the obesity has a frequency of 10-25%, in Eastern European countries and Mediterranean countries the frequency is much higher, reaching 40% in female sex. In the Northern European countries, the prevalence of overweight in children is 10-20%, while in the South Europe it reaches 20-35%, the prevalence being in ongoing increase.

According to the European Commission data for year 2014, over 22 million children in the European Union are considered overweight or obese, and this number is increasing each year by 400 000 [16].

Great Britain, Slovenia and the Netherlands are countries in which there have been registered alarming rates of overweight and obesity among children, Great Britain being in the top of the EU countries that are facing this problem [3].

A study performed in Romania on a lot of 7904 children from I-XII grades, coming from 20 schools from Cluj-Napoca, in 2008 showed a prevalence of overweight of 12.8% and a prevalence of obesity of 8.2%. The highest prevalence has been registered in the age group of 6-10 years old for both overweight (15.9%) and obesity (13.3%), and the lowest one in the adolescents (7.6% for overweight and 3.8% for obesity) [46]. Another study performed in Craiova in 2011 established a prevalence of overweight in children of 8.8% (21.2% in preschool children and 7.1% in school children) and a prevalence of obesity of 13.7% (24.2% in preschool children and 12.2% in school children) [4]. According to data provided by the Society of Endocrinology from Romania for 2013, 40% of children are overweight.

In the Republic of Moldova, statistical records on overweight and obesity among children are incomplete. There were considered some particularities that facilitate the occurrence of overweight in the population through Multiple Indicator Cluster Survey (MICS 2012); Study on physical development in children (COSI) from 2013. But it is also known about the alarming situation of overweight and obesity in adults. The data included in the National Strategy of Public Health for the years 2014-2020 show that in the Republic of Moldova about 50% of adults are overweight or obese [12]. In the National Strategy for prevention and control of non-communicable diseases for the years 2012-2020 it is noted that 73.1% of overweight and obese people suffer from hypertension, and 71.6% of them suffer from coronary heart diseases [13]. STEPS 2013 study regarding the evaluation of risk factors for non-communicable diseases in the Republic of Moldova has determined that 56% of the adult population is overweight [36].

Definition of overweight and obesity in children

Obesity is a chronic disorder of nutrition condition characterized by increase of body weight due to the pathogenic adipose tissue, being a consequence of an accumulation of energy, resulted from an imbalance between intake and output of energy [1, 33].

The manifestation of obesity in children has certain tendencies depending on the age and gender of the child [4]. Obesity is common in infancy, especially in the first 6 months, followed by a transient decrease in weight at the age of 4-6 years (adiposity rebound) [21]. This age is considered a critical period with increased risk for obesity in childhood that may persist in the adult (Winickoff et al., 2003). In the period of adiposity rebound changes in the organs and tissues may occur (Fitzgibbon et al., 2002). Children who become overweight during this period are more likely to become obese adults. Obese children up to 3 years old with normal weight parents are exception, having a low risk for obesity in childhood. The probability of the child in becoming obese increases by 4-5 times if this child has obese parents (Summerbell et. al, 2002, Dietz, 2003 Daniels et al., 2005).

It was found out that the increase of prevalence of overweight in boys occurs within prepubertal age and in girls during puberty. Many studies show that obesity in children is common for school age, being registered a rate of 10-30% [21].

Another particularity of the occurrence of obesity in children is a higher prevalence among girls, and this tendency increases with the age. 10.7% boys manifest more frequently a light degree of obesity [67].

Philip R. Nader [33] highlighted some age groups with increased risk of obesity in children aged: 2-4 years, 7-9 years and 12-13 years. If at the age up to 54 months, the BMI value was of 75-85 percentiles, then the child is 6 times more likely to be overweight at the age of 12 years; if at the age of up to 9 years, the BMI value was of 75-85 percentiles, then the child has a chance of 40-50% to be overweight at the age of 12 years.

Krebs F. Nancy et al. [21] investigated the probability of becoming overweight or obese in adult age depending on the age at which overweight and gender occurred. In girls with BMI > 85 percentiles at the age of 3-4 years the probability of becoming obese adult is up to 20%, at the age 5-17 years – 20-

40%. In boys this probability is up to 20% if BMI > 85 percentiles between 3-16 years, 20-40% – at the age of 17 years.

Methods of evaluation of overweight and obesity in children

In children with the age past 2 years the obesity is defined by increased BMI (body mass index) values related to sex and age. The child is obese if BMI ≥ percentile 95 (+ 2DS)/sex/age, is overweight if percentile 85 ≤ BMI < percentile 95/sex/age, has normal weight if percentile 5 ≤ BMI < percentile 85/sex/age, is underweight if BMI < percentile 5/sex/age [4, 21].

For evaluating nutrition condition in children aged past 2 years, CDC and AAP recommend the use of BMI 2-20 years/age/sex charts (CDC 2000) [2, 30].

BMI is not a direct indicator of adipose tissue mass because it does not distinguish the adipose tissue from muscle mass, bones and vital organs. A child may have an increased BMI/age/sex, but to determine if the excess of adipose tissue can cause health problems some additional investigations are necessary: abdominal, tricipital and subscapular skinfold measurement, abdominal circumference, hip circumference, relation between abdomen and hip circumference, determination of adipose tissue percentage by advanced methods (Dual-energy X-ray absorptiometry (DEXA), bioelectric impedance, etc.), evaluation of nutrition, physical activities, family background [23, 43, 50].

Measurement of the thickness of (abdominal, tricipital, subscapular) skinfolds is performed using the caliper and it is an indicator that distinguishes the adipose tissue mass from the muscle mass when a high BMI is established. Expert Committee of 2007 does not recommend the routine use of the measurement of tricipital skinfold for evaluating the obesity in children [21].

Measurement of abdominal and hip circumference is an indirect indicator regarding visceral adiposity. These indicators are commonly used to assess the risk of comorbidities (diabetes type 2, hypertension, hyperlipidemia, hypertriglyceridemia, metabolic syndrome, coronary artery disease) [2].

An important indicator is the ratio between abdominal and hip circumference, which is used in adolescent and adult for evaluating the cardiovascular risk. If this ratio exceeds the value 1 and abdominal circumference ≥ 102 cm in boys, if the ratio between waist and hip circumference ≥ 0.85 and abdominal circumference ≥ 88 cm in girls, then there is a high cardiovascular risk (Cole et al. 2000) [5].

There are advanced methods for the determination of total adiposity, such as abdominal ultrasonography, Dual-energy X-ray absorptiometry (DEXA), bioelectric impedance, densitometry, computed tomography, magnetic resonance imaging are used on a low scale and, especially, for the research purposes, because they have a high cost, poor availability and require a high level of training for users in order to ensure adequate reliability [4].

Determinants of obesity in children

The etiology of obesity is complex. The interaction of several factors, including genetic, metabolic, behavioral and environmental ones, has increased overweight and obesity in children. [1, 8, 28, 50]. The speed of obesity spreading suggests that behavioral and environmental factors, rather than biological factors, have directly contributed to the epidemic [64].

According to the Report of the "First Meeting of the Ad Hoc Working Group on Science and Evidence for Ending Childhood Obesity" (18-20 of June, 2014, Geneva), infant obesity is determined by interactions between biological, behavioral and social factors [38].

Biological factors include maternal malnutrition (unbalanced nutrition, including both underfeeding and overfeeding), obesity, and stress before and during pregnancy and maternal glycemia. Another factor resides in infant feeding, including short duration of breastfeeding (< 6 months); inadequate complementary foods, parents' great care that determines an overconsumption for child [8, 38, 39, 50].

Behavioral risk factors overlap with behavioral and biological factors and include sedentary behavior, sleep duration, fewer opportunities for sport, giving priority to transport, wide interest for broad entertainment provided by TV and computer. Behavioral factors related to food include frequent meals, consumption of foods high in calories, increased consumption of acidulous juices, concentrated sweets, fast foods, consumption of foods with a high degree of processing out of meal times, low consumption of fruits and vegetables, consumption of big portions, some bad eating habits (skipping breakfast, taking dinner in late hours and big portions, diet breach) [10, 28, 37, 38, 49].

Social factors include socio-economic issues, changes in work forms, nutritional education in the family, availability and affordability of healthy food [38, 43].

Excessive food intake and decreased physical activity are the main exogenetic factors that have a role in generating the obesity in children [1, 8, 11, 21, 48].

In a study conducted in Craiova in 2011 it was found out that overweight and obese children compared with normal weight ones, eat sweets daily (55.6%, respectively 42.3%, vs. 11%), acidulous drinks (38.8%, respectively, 38.2% vs. 8.2%), fast foods (31.8%, respectively 34%, vs. 8.3%). The results of the investigation on physical activity showed that 62.9% of overweight children and 64.5% of obese children do not perform physical activities in their free time [4].

Environmental factors that have an important role in the occurrence of overweight in children are family, kindergarten, school and community [21, 42, 43, 47]. Eating habits of their parents influence children's preferences and vice versa. Family behavior is influenced by socio-economical and cultural factors. Children are also influenced by advertising, starting with the school age they become more independent; they buy the desired food by themselves. Another major factor is the kindergarten and school, where the formation of a lifestyle and healthy eating habits takes place. Social environment (community) also may influence the children's behavior by reducing the sedentary life by increasing the accessibility to physical activities [3, 4].

Health condition of overweight and obese children

Overweight children face multiple health problems, social and psychological problems [8, 39, 48]. In particular, child and adolescent obesity is a risk factor for cardiovascular disease, hypertension, type 2 diabetes, sleep apnea syndrome, depression, some forms of cancer, behavioral problems at school and low self-esteem [8, 10, 21, 40, 41, 48, 50]. These diseases cause premature mortality and long-term morbidity as well [59]. The results of studies show that the obesity occurred in childhood leads to the decrease of life expectancy due to complications it determines (OMS 2011) [31]. Additionally, overweight and obesity in children are associated with significant reductions of life quality and a greater risk of social isolation [45].

Topical issue of the problem is determined by the presence of multiple evidences proving an important relation between overweight and cardiovascular diseases. It has been outlined a linear relationship between the increase of BMI and weight of children with BP values past 90 percentiles: 3.45% – in underweight subjects, 14, 22% – in the normal weight subjects, 36.75% – in overweight subjects and 50% - in the obese subjects ($p < 0.001$) [40].

Children with overweight have an increased risk of developing metabolic syndrome, which subsequently develops in type 2 diabetes and predisposes to cardiovascular diseases [32]. At least three indicators of risk are typical for obesity, such as hyperlipidemia, presented by high triglycerides, low density lipoproteins, high LDL, high density lipoprotein, low HDL; increased cholesterol level; glucosemia; insulinemia; hypertension [8].

Zimmet P. et al. [32] found out that in children aged 6-10 years old with BMI ≥ 90 percentiles, metabolic syndrome can be diagnosed, but additional investigation should be conducted if there is a family history of metabolic syndrome, type 2 diabetes, dyslipidemia, cardiovascular diseases, hypertension and/or obesity. At the age of 10-16 years and BMI ≥ 90 percentile, metabolic syndrome is established if triglyceride level ≥ 1.7 mmol/L (≥ 150 mg/dL), HDL < 1.03 mmol/L (< 40 mg/dL), systolic blood pressure ≥ 130/diastolic blood pressure ≥ 85mm Hg, glucose ≥ 5.6 mmol/L (100 mg/dL).

Overweight causes overload of osteoarticular apparatus. Orthopedic disorders suffered by children with obesity include: Genu valgum, flat foot, edema of legs in orthostatism (upright position), joint pain, knee arthritis, aseptic necrosis of the femoral head, hyperlordosis [4, 19, 21].

Obesity in children increases the risk of gastrointestinal diseases, including hepatic steatosis, bile calculus, gastroesophageal reflux [8, 37]. There was detected an increased incidence of fatty liver disease in children in parallel with the increase of obesity incidence in childhood. Thus, the incidence of hepatic disease is assessed at 3% in the general pediatric population and 53% in obese pediatric population.

Obesity has become a major challenge for male and female infertility. Obese girls often face polycystic ovarian syndrome, early menarche, irregular menstrual cycles, unovulation caused by infertility [22, 24].

Obstructive sleep apnea is 4-6 times more common in obese children, being manifested by hypertension, left ventricular remodelling, daytime sleepiness, anxiety and inactivity. Obesity significantly increases the risk of developing asthma [8, 21].

Obesity is associated with some types of cancer, such as colorectal, ovarian, endometrial, cervical, breast and prostate cancer [19].

Economic impact

The results of the studies conducted by the WHO in the European Region showed that the direct costs caused by obesity involve 2-4% from GDP [9]. But there are also indirect costs that are caused by low labor productivity (absence from work for health reasons or premature mortality). Assessments of such losses in England indicate that these costs could be two times higher than the direct costs [14].

According to data from world statistics, presently there are 1 585 768 510 overweight persons, 528589 504 obese persons, and for the treatment of diseases related with obesity in the USA $ 341907 570 are spent [18].

In the USA it has been estimated that the total costs for health services due to obesity will increase to 861-957 billion USD by 2030, totaling 16-18% from GDP [28].

Calculations performed by the United States show that in comparison with persons of normal weight (BMI 20.0-24.9 kg / m2), obese persons (BMI over 30 kg / m2) consume annually by 36% more health services and overweight persons (BMI of 25.0-29.9 kg / m2) – by 10% more. The high costs associated with obesity and unhealthy lifestyle show that the savings could result from health promotion and prevention, at least for short term. Persons who adopt a healthier lifestyle will gain health and will add years to their lives generally [48].

Preventive measures against overweight and obesity in children

National programs for prevention of obesity, particularly in children, treatment of comorbidities, reduction of mortality caused by overweight represent an international priority of public health [3, 44].

Children are victims of an intense advertising marketing for products very rich in fat, sugar and salt. WHO considers this alarming phenomenon is directly related with TV advertising for products that negatively form the food style of the children. [16]

The European Union is actively engaged in the fight against obesity in children. In order to intervene in this field, the European Commission has the Strategy for Europe concerning the issues related to nutrition, overweight and obesity, and two implementation tools, respectively "High level group on nutrition and physical activity" and "EU Platform for diet, physical activity and health". Members of the High Level Group pledged to do more to tackle the alarming tendency related with the increase of obesity among children through an Action Plan on infant obesity (2013) [17].

In order to reduce this risk factor, WHO proposes a series of measures, by inviting the countries to eliminate in-

dustrial fatty acids from foods, promote the display of nutrition information on the labels, reduce the percentage of sugar and salt in foods and sugar in soft drinks or reduce the portion sizes. The action plan also recommends the increase of "financial affordability and consumption of fruits and vegetables" [34].

The tough fight the European authorities must lead is the fight against fast food products, ready-to-eat or frozen food, whose content of sugar, salt and fat is usually very high. Among the solutions currently explored, the scientists analyze a bio-technological process, based on enzymology and fermentation, which would reduce, for example, the presence of sugar in apple juice. Other scientific approaches point to the use of high hydrostatic pressure or a new type of homogenizer in order to improve the distribution of salt and fat in food products; the target is to reduce their presence by up to 30 percent. According to experts, the food can be juicy and tasty without too much salt, sugar or additives, if the processing technologies provide a better distribution of different ingredients [6].

Many EU countries (Great Britain, Slovenia, the Netherlands, Croatia, France, Germany and Italy) have developed and implemented certain strategies and actions in the field of healthy food and physical activity. These countries have concentrated their efforts on creating partnerships between education and health sectors in order to promote health in education institutions [3, 7].

Scottish Ministry of Health and Ministry of Education have joined their forces to create multi-sectoral partnerships aimed at involving schools, families, communities for the facilitation of access to adequate and safe food presented attractively, and also for understanding the role of a healthy nutrition and a healthy lifestyle. The most important actions that were implemented include: provision of free breakfast for children from primary school; stimulation of healthy food marketing within a pilot program conducted at three schools of secondary education; supply of water dispensers, highlighting the benefits of water drinking; stimulation of fruit consumption by placing fruit kiosks in schools; free swimming courses during the holidays; sports programs at the class level by encouraging the participation of the pupils and teachers [3].

In Slovenia a partnership between the Ministry of Health and Ministry of Education and Sport has been created in order to promote health. A special place has the national program on nutrition in schools, which requires all schools to give pupils at least one meal a day [26].

In the Netherlands one of the main national programs is called SchoolGruiten and stands for "fruits and vegetables in school". Within this program the children are acquainted with information on fruits and vegetables through various fun games. Teachers together with the pupils eat fruits and vegetables for two days a week in the classroom. This program has been already proven to be effective. A large study involving the participation of 300 primary schools with a total of about 75 000 children and 7 000 teachers involved in seven different cities, shows that children from Gruitschools really eat more vegetables: from 1.1 portions per day to 1.6 portions per day [27].

Waters E. et al. in review Cochrane "Interventions for preventing obesity in children" have examined the characteristics of programs and strategies in this field [47]. The meta-analysis has included 37 studies (27 946 children) and showed that programs were effective in reducing the overweight and obesity in children. In particular, there were highlighted the most effective actions and strategies: amendments to educational program, which includes the introduction of topics on healthy food, physical activity and body image; increase of the number of hours of physical activity and the development of fundamental movement skills during the week at school; improvement of the nutritional quality of food supplied in schools; introduction of cultural and environmental practices that will encourage children to eat healthy foods and be active every day; training of teachers and medical staff in school in respect with health promotion strategies; involvement of parents in encouraging children to be more active, eat healthy food and spend less time in front of small screens (various brochures, guides, homework).

Generally, namely family and school have a profound influence on the young generation and form the behavior concerning healthy food and physical activity of children and adolescents [31, 43, 50].

Conclusions

1. Childhood obesity is a major public health problem due to the epidemically increased tendency of the frequency of overweight in children that has reached to affect about 20-25% of children and 45-50% of adolescents worldwide.

2. Infant obesity is determined by interactions between biological, behavioral and social factors. Excessive food intake and decreased physical activity are the main exogenetic factors that have a role in generating the obesity in children.

3. Topical issue of the problem is determined by the presence of multiple evidences proving an important relation between overweight and cardiovascular diseases, type 2 diabetes, hypertension, some forms of cancer, hepatic steatosis, infertility and other major chronic diseases that cause premature mortality and long-term morbidity.

4. Epidemiologic and economic impact of obesity is high and the authorities' actions should be directed to its prevention by developing and implementing strategies and actions in the field of healthy food and physical activity for children. In the fight against obesity in children, many countries have focused their efforts on creating multi-sectoral partnerships aimed at involving schools, families, communities for the facilitation of access to adequate and safe food presented attractively, and also for understanding the role of a healthy nutrition and a healthy lifestyle.

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Neuro-vegetative disorders in premenstrual syndrome

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Abstract

Background: The premenstrual syndrome (PMS) is the complex of psychological, emotional and neuro-vegetative symptoms dependent on cyclical fluctuations of reproductive hormones; due to the present study we investigated the peculiarities of neuro-vegetative signs in patients with premenstrual syndrome.
Material and methods: 272 women (136 with moderate/severe premenstrual syndrome and 136 with mild premenstrual symptoms) at the age 18-43 years have been examined. We have used Menstrual Distress Questionnaire (MDQ) to determine clinical profile and intensity of premenstrual symptoms. The pattern of neuro-vegetative signs was assessed by using questionnaire the Motor-Autonomic Profile.
Results: The obtained outcomes indicated that the score of MDQ is markedly increased in patients with moderate/severe premenstrual syndrome. Neuro-vegetative symptoms, assessed by the Motor-Autonomic Profile showed that score in most scales was considerably higher in patients with PMS.
Conclusions: Parameters of the Motor-Autonomic Profile were higher in most scales and subscales in patients with PMS compared with women without PMS, indicating the presence of the broad range of psycho-vegetative disorders.

Key words: premenstrual symptom, premenstrual syndrome, the Motor-Autonomic Profile, neuro-vegetative symptoms, painful symptoms.

Introduction

Premenstrual syndrome is a neuroendocrine syndrome; multifactorial disorder that occurs due to functional insufficiency of hypothalamus-pituitary-ovary system influenced by unfavourable factors or increased sensitivity [2, 13, 15]. There are centres sensitive to steroids located in the hypothalamus, these participate in the mechanism of feeble-back. Due to disturbed neuroendocrine balance, autonomic reactivity increases in the second phase of the menstrual cycle [9, 16]. Vegetative disorders are manifested through emotional symptoms (irritability, depression, anxiety, unstable mood, aggression etc.) and vegeto-vascular signs (headache/ migraine, nausea, vomiting, pain in the heart, etc.) [6].

The autonomic nervous system maintains normal physiological limits of biological parameters and provides adaptation mechanisms of the body. The boundary between adaptation reaction and disease is conventional and depends on intensity and duration of influence of unfavourable factor and the mental and physical condition of the human body. If the woman adjusts to cyclical fluctuations of reproductive hormones her general condition is not affected. Functional disorders of the hypothalamus-pituitary-ovarian axis influence adaptation reaction, therefore, clinical symptoms appear in premenstrual period [6, 8, 13].

One of the objectives of the current study was to determine and assess clinical symptoms occurring in the premenstrual period under vegetative samples (questionnaire the Motor-Autonomic Profile). Comparing autonomic profile in patients with premenstrual syndrome (PMS) and women in the control group allows emphasizing clinical vegetative aspects characteristic for PMS.

Material and methods

Present study was conducted during 2010-2012 at the gynaecological outpatient unit, attended by 272 women: research group included 136 women with moderate/severe PMS, which meet the Diagnosis Criteria and control group – 136 women with mild unique premenstrual symptoms which do not meet the Diagnosis Criteria for PMS. The study included women aged 18-43 years with regular menstrual cycle (23-35 days), no genital or brain injuries, who do not use COCs, are not pregnant or after giving birth (< 6 months).

Medical and social characteristics of participants were obtained from outpatient medical sheets and complex questionnaire that included: age, social status and menstrual function (menstrual cycle and rhythm). Premenstrual clinical profile was assessed by using the Menstrual Distress Questionnaire (MDQ) – questionnaire includes 47 symptoms characteristic for PMS united into 8 groups and valued at 0-3 points (0 points – no symptoms, 3 points – severe symptoms), questionnaire was completed in the premenstrual period at the maximum level of symptoms expression (day 24-26 of menstrual cycle) [5].

Assessment of neuro-vegetative pattern was based on the implementation and completion questionnaire the Motor-Autonomic Profile in the luteal phase of the menstrual cycle (24-26 days). This questionnaire is a clinical tool for quantitative and qualitative assessment of the neuro-vegetative disorders and consists of detailed analysis of bodily sensations (ache, breathless, cold, hot, cool etc) and behaviour (emotional, painful, motor behaviour etc.). The questionnaire consists of 169 statements and questions united into 16 groups, score obtained after calculation is marked by a sign in the Chart [3].

Statistical processing of the survey results was performed using Statistics 7.0 software (StatSoft Inc.). Statistical processing allowed us to calculate rates, averages, indicators of proportion. In order to determine the difference between mean values was used t test and nonparametric tests. Statistically significant differences were considered $p < 0.05$.

Results and discussion

The women involved in the study were comparable by age, social-demographic parameters, physical status and characteristics of menstrual function (tab. 1).

Table 1

General Characteristics of participants in the study

	Study Group n – 136	Control group n – 136
Age, years	30,33 ± 5,68	28,98 ± 5,75
Weight, kg	60,2 ± 4,0	61,24 ± 5,63
Height, cm	165 ± 2,33	162 ± 2,33
Body mass index,kg/m^2	22,31 ± 2,87	22,79 ± 3,11
Menstrual cycle, days	28,66 ± 1,32 (26-35)	28,79 ± 1,44 (25-34)
Living place Urban Rural	 76,47% 23,53%	 84,56% 15,44%
Social status Married Single Divorced	 67,65% 22,79% 9,56%	 64,71% 29,41% 5,88%
Educational level School certificate College University	 44,12% 16,91% 37,5%	 56,62% 23,53% 20,59%**

Diagnosis of PMS is determined in the interview about clinical premenstrual symptoms, Menstrual Distress Questionnaire and meets the Diagnosis Criteria (American College of Obstetricians & Gynaecologists, USA, 2000; Royal College of Obstet&Gynec, The National Association for Premenstrual Syndrome, UK, 2007) [1, 10].

Menstrual Distress Questionnaire (R. Moos) [5] consists of 8 groups of symptoms (47 symptoms), characteristic for PMS. Results showed that parameters in study group were significantly higher compared with parameters in control group (tab. 2).

Table 2

Parametres of Menstrual Distress Questionnaire

Scale	Parameters	Study group n – 136	Control group n – 136	p
1	Pain	8,66 ± 1,43	2,12 ± 1,08	< 0,001
2	Concentration	6,25 ± 1,17	1,03 ± 0,65	< 0,001
3	Behavioural change	5,6 ± 1,32	0,78 ± 0,47	< 0,001
4	Autonomic reactions	4,39 ± 1,47	0,83 ± 0,48	< 0,05
5	Water retention	4,14 ± 1,11	0,96 ± 0,48	< 0,01
6	Negative affect	9,11 ± 1,71	1,59 ± 1,03	< 0,001
7	Arousal	3,87 ± 1,13	2,22 ± 0,98	> 0.05
8	Control	6,08 ± 1,64	1,0 ± 0,64	< 0,01
Total score		47,14 ± 3,67	10,28 ± 1,94	< 0,001

Symptoms included in the Menstrual Distress Questionnaire especially scales 1, 4, 5 and 8 are conducted by autonomic and neuroendocrine mechanisms. Autonomic (vegetative) disorders are often encountered in actually healthy people, disturbing quality of synesthesia (a neurological phenomenon in which stimulation of one sensory or cognitive pathway leads to automatic, involuntary experiences in a second sensory or cognitive pathway). In these cases questionnaire the Motor-Autonomic Profile is the most appropriate structural analysis of the general condition of the subject [3, 4].

Comparing autonomic profile in patients with PMS and women in control group allows emphasizing vegetative clinical aspects characteristic for PMS. It was found that parameters of Motor-Autonomic Profile in patients with PMS were significantly higher than similar parameters in the control group.

Emotional disorders (Scales 1, 2) constitute essential characteristics of the subject and are considered the most common sources producing vegetative symptoms [3]. Clinical symptoms expressed through emotional disorders included: restlessness, anxiety, susceptibility, dissatisfaction, aggression (tab. 3).

General brain disorders (Scale 8) – the most frequent symptoms were mentioned: excessive tiredness, difficulty in concentrating and drowsiness (tab. 3).

Sexual and menstrual disorders (scale 12) were manifested by decrease/lack of sexual desire, discomfort and pain in patients with PMS (tab. 3).

Table 3

Parameters of Motor-Autonomic Profile, Scales 1, 2, 8, 12

Scale	Parameters	Study group n – 136	Control group n – 136	P
1	Anxiety and panic attacks	13,8 ± 1,84	4,0 ± 0,94	< 0,001
2	Depression	10,73 ± 1,44	3,06 ± 1,03	< 0,001
8	Asthenia – hyper-excitability	18,61 ± 1,74	6,26 ± 0,58	< 0,001
	Asthenia	10,59 ± 1,33	4,0 ± 0,79	< 0,001
	Hyper-excitability	7,84 ± 0,82	2,26 ± 0,43	< 0,001
12	Sexual and Menstrual Disorders	7,9 ± 0,71	2,17 ± 0,66	< 0,001
	Sexual	2,75 ± 0,52	0,77 ± 0,31	< 0,001
	Menstrual	5,15 ± 0,69	1,4 ± 0,31	< 0,001

Assessment of vegetative parameters revealed marked clinical symptoms in premenstrual period at scales: respiratory behaviour, hyper-excitability, cerebral vascular disorders, cardiovascular disorders, gastrointestinal disorders, difference between parameters in study group was statistically higher than in control group (tab. 4).

The phenomenon of dyspnoea (respiratory behaviour) occurs in the majority of patients with autonomic disorders (evident when cardiac or respiratory somatic excluded) [3, 4]. The rating scales manifestations of respiratory behaviour (Scales 3, 4) were referred to dyspnoea in emotions, sensations of air failure and intolerance of unventilated spaces.

Table 4

Parameters of Motor-Autonomic Profile, Scales 3, 4, 5, 7, 9, 10

Scale	Parameters	Study group n – 136	Control group n – 136	P
3	Dyspnoea	15,11 ± 1,9	4,04 ± 0,85	< 0,001
4	Respiratory behavior	10,2 ± 1,23	6,02 ± 1,07	< 0,01
5	Tetany – neuromuscular hyper-excitability	18,56 ± 1,82	4,35 ± 0,91	< 0,001
7	Dizziness	11,92 ± 1,34	2,26 ± 0,36	< 0,001
9	Cardiovascular dysfunction	23,67 ± 1,73	7,27 ± 1,37	< 0,001
	Discomfort/palpitations	5,15 ± 0,88	1,1 ± 0,3	< 0,001
	Pulsations	5,63 ± 1,03	1,02 ± 0,27	< 0,001
	Orthostatic phenomena	8,04 ± 1,07	3,08 ± 0,68	< 0,001
10	Gastrointestinal dysfunctions	23,66 ± 1,83	8,4 ± 1,01	< 0,001

Significant differences were found in scale 5 – the phenomenon of neuro-muscular hyper-excitability (tetany) is largely influenced and sometimes caused by the phenomenon of hyperventilation (dyspnoea), therefore this phenomenon (tetany) is analysed in the context of these disorders [3, 8].

Tetany-neuromuscular hyper-excitability – frequently hyper-excitability is manifested by twitching to an unexpected event, twitch of the eyelid and throat feeling in stronger emotions.

Cerebral vascular disorders were manifested by weakness, dizziness, dysfunction of optical analysers («mesh, haze before eyes»), fainting, unreality of the surrounding world, feeling confused, which were included in the composition of scale 7.

Scale 9 – cardiovascular dysfunction is actually a disorder of autonomic cardiovascular function [3]. Significant differences were detected in subscale discomfort/pain at heart and subscale pulsations (in the head, in the whole body).

Gastrointestinal disorders (scale 10) – most commonly were manifested by changes of appetite, gastric and abdominal bloating and discomfort. Scale 10 highlights the most relevant functional gastrointestinal symptoms caused by autonomic disorders.

Disorders in thermoregulatory system – complex scale 11 consists of four subscales: cold, hot, sweating and swelling, the clinical symptoms of this scale refer to dysfunction of the cerebral structures in the hypothalamus and its connections [3] (tab. 5). Thermoregulatory system disorders are characterized by statistical indices significantly higher in patients with PMS compared to women in control group, symptoms were more frequently expressed by feelings of heat and sweating and swelling in emotions.

These results showed significant motor disorders in patients with PMS (sensation of pain and tension in the back muscles, in the lumbar region, excitement in emotions, transient weakness in legs). The set of phenomena included in the scale 14 is meant to highlight the sensorial and motor disorders, which constitute an important part of behaviour and are closely related to the autonomic nervous system [3, 4] (tab. 5).

Table 5

Parameters of Motor-Autonomic Profile, Scales 11, 14

Scale	Parameters	Study group n – 136	Control group n – 136	P
11	Thermoregulation and sweating	25,67 ± 1,75	8,34 ± 1,01	< 0,001
	Cold	6,81 ± 1,21	3,04 ± 0,67	< 0,01
	Hot	5,74 ± 1,15	1,15 ± 0,5	< 0,001
	Sweating	6,16 ± 0,86	2,21 ± 0,57	< 0,001
	Edema	4,08 ± 0,74	0,92 ± 0,36	< 0,001
14	Motor and sensorial disorders	23,6 ± 2,32	5,42 ± 1,3	< 0,001
	Motor disorders	11,18 ± 1,67	2,36 ± 0,39	< 0,001
	Sensorial disorders	4,67 ± 0,94	1,0 ± 0,27	< 0,001
	Disorders of consciousness	1,42 ± 0,53	0,35 ± 0,23	> 0,05
	Confusion disorders	1,2 ± 0,29	0,62 ± 0,23	> 0,05

Painful pattern in PMS includes both muscular-skeletal painful syndrome (muscles and joint pain with various localization) and visceral syndromes (abdominal pain, pain in the region of heart, pain in sexual intercourse, etc.) of different intensity also being worse in patients of study group (tab. 6).

Table 6

Parameters of Motor-Autonomic Profile, Scale 15

Scale	Parameters	Study group n – 136	Control group n – 136	P
15	Painful syndromes	24,59 ± 2,69	7,36 ± 1,19	< 0,001
	Muscular-skeletal syndrome	8,84 ± 1,42	2,23 ± 0,39	< 0,001
	Visceral syndromes	13,21 ± 1,65	4,0 ± 0,87	< 0,001

Pain is concrete body distress, with a broad range of undesirable vegeto-visceral symptoms in patients with premenstrual symptoms and determines the severity of PMS.

It is important to note that PMS is characterized by pronounced premenstrual symptoms that have negative impact for the overall condition and affects the quality of life of women. This is reflected in the scale 16 – Disability. In the study group parameter of this scale was 12.69 ± 1.64, while in the control group – 1.0 ± 0.61 (< 0.001). This data indicates that the scale value in study group is high and affects the quality of life and general condition, so this condition requires treatment.

Low quality of life in patients with PMS is manifested mainly by limiting in daily activities (work, study, house-

work) because of symptoms, difficulties in stealing attention from existing symptoms and focus on something else, and the presence of depression due to feeling disturbing bodily sensations. Not only painful phenomenon causes low quality of life and affective disorders, on the other hand, psycho-vegetative syndrome plays an important role in altering them [2, 9, 12].

All above-mentioned parameters of the Motor-Autonomic Profile in study group demonstrated statistically significant difference, being higher in patients with PMS, this shows a significant autonomic dysfunction in the luteal phase of the menstrual cycle. The study results do not contradict the data reported in the literature.

Disorders in the regulation of neuroendocrine system, established in patients with PMS present a research interest. It is known that autonomic nervous system is involved in the development of moderate premenstrual symptoms [7, 8, 11]. Premenstrual symptoms appear in the presence of parasympathetic autonomic reactivity in the second phase of the menstrual cycle. The basic function of the autonomic nervous system is to maintain normal biological constants and ensuring adaptability of the organism to the environment. In patients with PMS there are vegetative disorders which induce tension in adaptive-compensatory mechanisms [14].

Conclusions

1. The results of this study demonstrated that moderate/severe premenstrual symptoms occur in women with regular menstrual cycle.

2. Parameters of Motor-Autonomic Profile are higher in most scales and subscales in patients with PMS compared with women without PMS, indicating presence of the broad range of psycho-vegetative disorders.

3. Multiple mechanisms taking part in the genesis of autonomic symptoms present in patients with PMS involve painful and affective disorders.

4. Pronounced painful symptoms associated with emotional and neuro-vegetative symptoms in patients with PMS confirm the close connection and presence of common pathogenic links.

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Evaluation of consumption in defined daily doses of drugs used in hospitals

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Abstract

Background: Irrational use of medicines that is wasteful and harmful presents an extremely serious global problem. In developing and transitional countries, in primary care less than 40% of patients in the public sector and 30% of patients in the private sector are treated in accordance with standard treatment guidelines.

Material and methods: For this study we used data of 2009-2013 period, in the Emergency Medicine Institute, which show the dynamics of consumption of medicinal remedies from pharmaco-therapeutic group A – Alimentary tract and metabolism.

Results: The annual consumption of drugs from group A – Alimentary tract and metabolism in the evaluated period varied in value indicators from 999 195 lei in 2009 to 1 042 562 lei in 2013 or by 4.4% and respectively in natural indicators from 123 479 grams in 2009 to 145 092 grams in 2013 or by 117.5%, in total units Defined Daily Doses (DDD)/1000 from 488 in 2009 to 1128 units in 2013 or by 212% and the cost of one DDD/1000 from 5180.7 lei in 2009 to 5397.5 lei in 2013 or by 4.2%. The DDD of 24 medicinal remedies were determined in the Emergency Medicine Institute.

Conclusions: The obtained data reveals that from 3 basic characteristics of drug consumption, DDD is the most objective and can be considered as the cornerstone for making decisions on determining the needs and organization of rational use of medicines.

Key words: drug supplies, defined daily dose, rational drug use, hospitals.

Introduction

In developing and transitional countries, in primary care less than 40% of patients in the public sector and 30% in private sector are treated in accordance with standard treatment guidelines [1]. From 2911 scientific papers devoted to medicines published in China during the years 1990 – 2008, 14.5% were about evaluation of medicinal remedies from pharmaco-therapeutic group A – Alimentary tract and metabolism [2]. There are numerous sources of evaluation data of drugs consumed in hospitals especially of alimentary tract and metabolism group that present a particular permanent scientific and practical interest [3-13]. Nevertheless, in the Republic of Moldova the analysis in Defined Daily Doses (DDD) as an important indicator of optimal rational use of drug remedies generally, and alimentary tract and metabolism ones, in particular, is not studied enough and not highlighted by scientific research literature.

The National Scientific-Practical Centre of Emergency Medicine of the Republic of Moldova reorganized in 2014 in Emergency Medicine Institute (EMI), was founded in 1959. Clinical Services of EMI include: Orthopedic-Traumatology Clinic for 150 beds, Surgery Clinic for 140 beds, Neurosurgery Clinic for 80 beds, Neurology Clinic for 70 beds, Maxillo-Facial clinic for 30 beds, Urology Clinic for 40 beds, Gynecology Clinic for 30 beds, Microsurgery Clinic for 30 beds, Municipal Center with 8 seats hemodialysis and 9 beds, Clinical intensive care unit for 30 beds, in total the above services of the EMI include 600 beds, also include 5 emergency medical help substations and 4 out-patient departments of traumatology and orthopedics [14].

The primary aim of the study was to evaluate institutional representative data on alimentary tract and metabolism drugs utilization for five year (2009-2013) period, in accordance with World Health Organization (WHO) requirements, projected to determine the value of Defined Daily Doses per 1000 Occupied-Bed Days (DDD/1000) [15]. To ensure fuller study it was necessary to determine the DDD of medical remedies for this group of drugs which have been used in EMI, but that are not published by WHO. Based on obtained data, it aimed to make conclusions on the use of alimentary tract and metabolism drugs in the medical institutions and to propose recommendations for ensuring their optimization.

Material and methods

For this study we used data of a five-year (2009-2013) period, in EMI, which show the dynamics of consumption of medicinal remedies from pharmaco-therapeutic group A – Alimentary tract and metabolism, classified as Anatomical Therapeutic Chemical (ATC), classification system of WHO indicating the nature and value. Statistical, analytical, mathematical, comparative, logical and descriptive methods of study were used.

Results and discussion

To determine DDD and compare the consumption of alimentary tract and metabolism drugs for the period of 2009-2013, the statistics data concerning the number of treated patients (patients with health insurance and other free treated by the state categories of citizens were involved), the number of bed/days and total annual quantities of medicines were used. In figure 1 is presented consumption of parenteral form of drugs from Alimentary tract and metabolism group in natural indexes "grams" in EMI for the evaluated period.

From figure 1 we find that the main volume of consumption of medicinal remedies of group A – Alimentary tract and metabolism as parenteral administration in natural indices is subgroup A12 Mineral supplements which increased the consumption volume gradually from 29 560 grams or 47% of all amount in 2009 to 42 495 grams or 63% of all amount

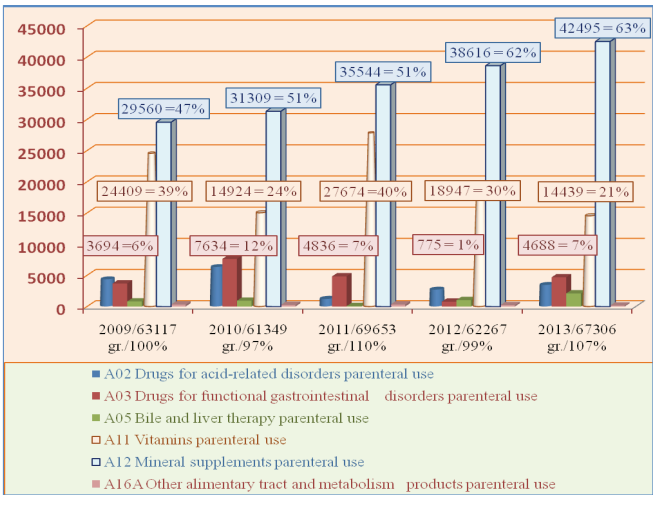

Fig. 1. Parenteral form consumption in natural indexes "grams" of drugs from Alimentary tract and metabolism group in Emergency Medicine Institute for the period from 2009 to 2013.

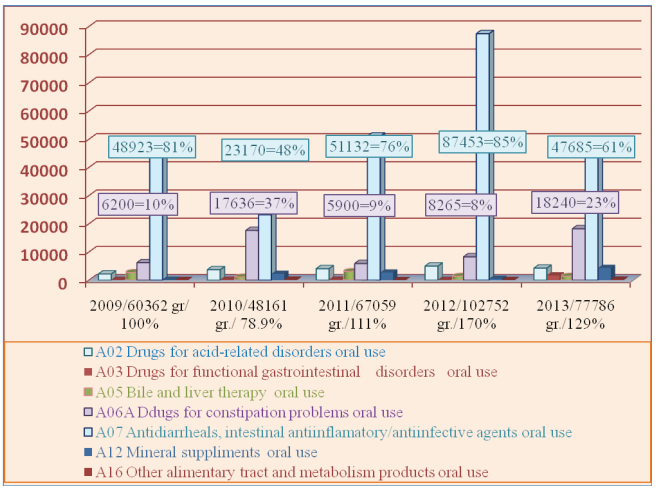

Fig. 2. Oral form consumption in natural indexes "grams" of drugs from Alimentary tract and metabolism group in Emergency Medicine Institute for the period of 2009 – 2013.

in 2013 consisting in a share of 43.8% during the evaluation period. The second subgroup in placement of consumption represents A11 "Vitamins", with a decrease from 24409 grams or 39% of all amounts in 2009, up to 14439 grams or 21% from amount in 2013 consisting in a share of 40.8% during the evaluation period. The rest of subgroups: A02 Drugs for acid-related disorders, A03 Drugs for functional gastrointestinal disorders, A05 Bile and liver therapy, A06 Drugs for constipation problems and A16 Other alimentary tract and metabolism products consumed apart less than 10%, together accounting 14% in 2009 and 16% in 2013.

The parenteral consumption form in value indices "Lei" of drugs Alimentary tract and metabolism in EMI for the period of 2009–2013 is presented in table 1.

The consumption in value indices (lei) for the parenteral use more than 10% per year is represented by four subgroups: A02 Drugs for acid-related disorders, A03 Drugs for functional gastrointestinal disorders, A11 Vitamins and A12 Mineral supplements and is 759 509 lei or 85.7% in 2009 and 856 449 lei or 96.9% in 2013. The rest of subgroups A05 Bile and liver therapy and A16 Other alimentary tract and metabolism products consumption in 2009 amounted to 127 034 lei or 14.3% in 2009 and 27 570 lei or 3.1% in 2013.

In the evaluated period a considerable increase was recorded for subgroups: A03 Drugs for acid-related disorders at 169 150 lei in 2009 to 223 484 lei in 2013 or by 32.2% and A12 Mineral supplements at 147 619 lei in 2009 to 254340 lei in 2013 or by 72.3 %.

The consumption of oral form of administration in natural indexes "grams" drugs of subgroups A Alimentary tract and metabolism in the period of 2009 – 2013 years is presented in figure 2.

From figure 2 it is clear that the main volume of consumption of medical remedies for enteral administration of Alimentary tract and metabolism group in natural indices is drugs of subgroup A07 Antidiarrheals, intestinal antiinflamatory/antiinfective agents, which minimal annual quota changed from 48% or 23 170 grams in 2010 to maximal 85%

Table 1

The parenteral consumption form in value indices "Lei" of drugs Alimentary tract and metabolism used in the period of 2009 –2013

Years	2009	2010	2011	2012	2013
Pharmacotherapeutic groups/value indexes	Lei	Lei	Lei	Lei	Lei
A02 Drugs for acid-related disorders	275306	346071	243018	359853	302621
A03 Drugs for functional gastrointestinal disorders	169150	172073	134006	75917	223484
A05 Bile and liver therapy	84256	89815	22226	8019	15610
A11 Vitamins	167434	75515	103520	84544	76004
A12 Mineral supplements	147619	157817	178154	237089	254340
A16A Other alimentary tract and metabolism products	42778	26420	29972	22189	11960
Total	886543	867711	710895	787610	884019
Percentage	100%	98,00%	80,00%	89,00%	100%

Table 2

The oral form consumption in value indices "Lei" of drugs from Alimentary tract and metabolism group for oral use in the period of 2009–2013

Years	2009	2010	2011	2012	2013
Pharmacotherapeutic groups/values indexes/percentage	Lei	Lei	Lei	Lei	Lei
A02 Drugs for acid-related disorders	9025	4820	54223	17789	87012
A03 Drugs for functional gastrointestinal disorders	12604	3667	10455	2925	3963
A05 Bile and liver therapy	3646	1685	5824	2754	5023
A06A Drugs for constipation problems	5523	14152	5798	6209	16378
A07 Antidiarrheals, intestinal antiinflamatory/antiinfective agents	80650	24735	61257	50839	39864
A12 Mineral suppliments	428	2339	3018	1906	*3905*
A16 Other alimentary tract and metabolism products	773	2582	889	1353	2399
Total	112649	53980	141464	83775	158544
Percentage	100%	48%	126%	74%	141%

or 87 453 grams in 2012. Despite considerable deviations in 2010 and 2012, the consumption of 48 923 grams in 2009 differs slightly from the consumption of 47 685 grams in 2013 that represents a small decrease of 2.5%.

The second subgroup was placed after consumption volume A06 with the minimal annual quota of 8% in 2012 to maximal of 37% in 2010, and with the highest consumption of 18 240 grams in 2013. The overall total consumption in the studied period for enteral form represents an increase from 60 362 grams in 2009 to 77 786 grams in 2013 or by 29%.

Consumption of drugs from subgroups A Alimentary tract and metabolism in oral form in value indices "Lei" for the period of 2009 – 2013 years is presented in table 2.

As shown in table 2, the consumption value for enteral administration form in the evaluated period, with representative annual rate of more than 10% is represented by two subgroups A02 Drugs for acid-related disorders (from 2011 to 2013) and A07 Antidiarrheals, intestinal antiinflamatory/antiinfective agents, which in 2009 amounted to 89 675 lei or 79.6%, and in 2013 constituted an amount of 126 876 lei or 80%. A considerable increase in consumption was for subgroup A02, from 9 025 lei in 2009 to 87 012 lei in 2013 or by 9.7 times. A considerable decrease in consumption was for subgroup A07 from 80 650 lei in 2009 to 39 864 lei in 2013 or by 2.02 times.

Total consumption in value indices "Lei" of drugs from subgroups A Alimentary tract and metabolism for parenteral and oral use in EMI in the period of 2009 – 2013 years is presented in figure 3.

The main assessed indices of volume were determined during the consumption of medicinal remedies of three subgroups A02 Drugs for acid-related disorders, A03 Drugs for functional gastrointestinal disorders and A12 Mineral supplements which showed a consumption respectively in 2009 of 284 331 lei (28.8%), 181 754 lei (17.2%), 148 047 lei (15%), the total 644 132 lei (64.47%) and in 2013 recorded 389 633

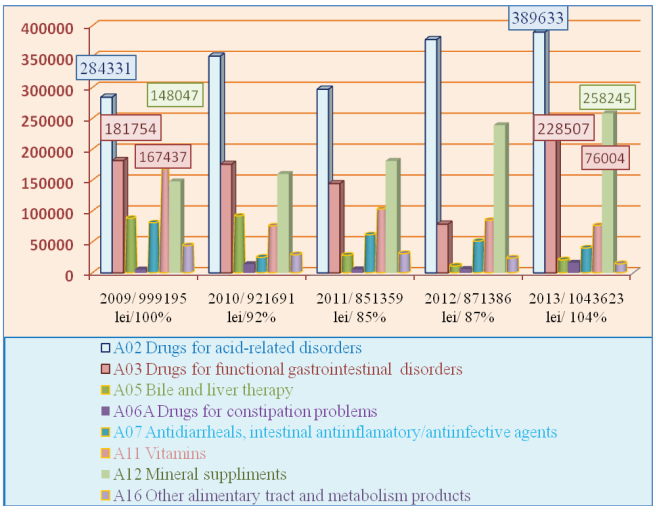

Fig. 3. Parenteral and oral consumption in value indices "Lei" of drugs from Alimentary tract and metabolism group in Emergency Medicine Institute in the period of 2009 – 2013.

lei (37.4%), 228 507 lei (21.9%), 258 245 lei (24.8%), with a total for the mentioned subgroups of 876 385 lei (84.0%).

Each of listed subgroups marked an increase of 37%, 25.8%, 74.5% respectively, or a total increase of 36.1%. A considerable decrease in consumption recorded subgroups A05 Bile and liver therapy, A11 Vitamins and A16 Other alimentary tract and metabolism products, which in 2009 had a total consumption of 298 824 lei or 29.9% for all amount, and in 2013 recorded respectively a total of 110 996 lei or 10.7% for all amount, or a total decrease for the mentioned subgroups of 62.8%. Nevertheless, total consumption of drugs from Alimentary tract and metabolism group during the evaluation period marked an increase of 104.4% in 2013 compared to 2009.

The parenteral and oral form consumption in natural indices "grams" of drugs from Alimentary tract and metabo-

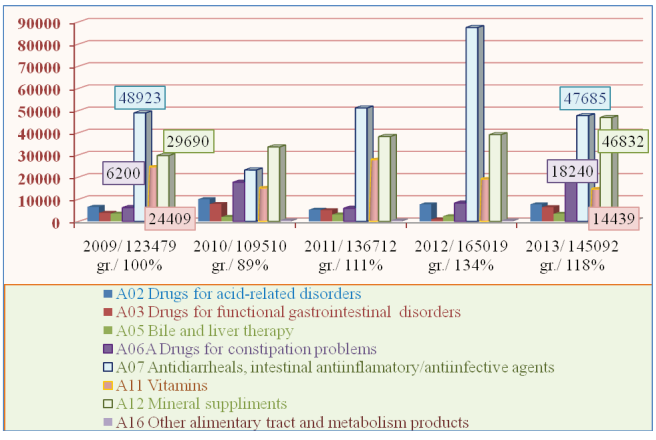

Fig. 4. Parenteral and oral form consumption in natural indices "grams" of drugs from Alimentary tract and metabolism group in Emergency Medicine Institute in the period of 2009 – 2013.

lism group in EMI in the period of 2009 – 2013 years is presented in figure 4.

As it could be seen from figure 4, a steady increase in consumption for the period under review has been seen in subgroup A12 Mineral supplements from 29 690 grams to 46 832 grams or by 57.8%, and less stable for subgroup A03 Drugs for functional gastrointestinal disorders from 3 821 grams up to 6 389 grams or by 67.2% and A06 Drugs for constipation problems from 6 200 grams up to 18 240 grams or by 2.94 times. At the same time, a decrease in consumption during the mentioned period records subgroup A11 Vitamins, from 24 409 grams to 14 439 grams or by 41%. Total consumption of all subgroups in mentioned period has increased by 18%.

To assess the consumption of defined daily doses of drugs recommended by the WHO in case of absence of the drugs in the list of WHO was defined and used the notion of defined daily doses by the Emergency Medicine Institute (EMI) with the abbreviations DDDEMI. These doses were determined after the evaluation from 300 to 500 cases treated in different profile sections and different time periods. A list with nomenclature of drugs with DDD and DDDEMI used for the evaluation of medicines is presented in table 3.

Table 3

Defined Daily Doses determined in Emergency Medicine Institute

International names of drugs or their composition	Route	DDD (EMI)
A ALIMENTARY TRACT AND METABOLISM		
A02A ANTACIDS		
Al. hydroxidum 218 mg + Mg hydroxidum 75 mg + Benzocainum 109 mg/5 ml	O	4824
Aluminii hydroxidum 218 mg + Magnesii hydroxidum 75 mg/5 ml	O	3516
Aluminii hydroxidum 3,5 g + Magnesii hydroxidum + 4 g/100 ml	O	4500
Aluminii hydroxidum 400 mg + Magnesii hydroxidum 400 mg	O	4800
Aluminii hydroxidum/Magnesii carbonas gel 450mg+Magnesii hydroxidum 300 mg	O	5250
Omeprazolum 20 mg + Clarithromycinum 250 mg + Tinidazolum 500 mg	O	1540
A03A DRUGS FOR FUNCTIONAL GASTROINTESTINAL DISORDERS		
Platyphyllini hydrotartras 0,2% 1 ml	P	6
A05B LIVER THERAPY, LIPOTROPICS		
Essential phaspholipids 50 mg, Pyridoxini hydrochloridum 0,5 mg, Cyanocobalaminum 2 mcg, Natrii D-pantothenas 0,3 mg, Nicotinamidum 5 mg, Vehiculum ad 1 ml.	P	750
Phospholipidum hipotalamus 300 mg	O	1800
Argininum 12,5 g + Sorbitolum 25 g	P	2500
Extractum chole 25mg+Extractum Cynarae scolimus 25mg+Curcumae longae pulvis+50 mg	O	3000
Kalii orotas 500 mg	O	1250
Silymarinum 35 mg	O	300
A06A DRUGS FOR CONSTIPATION		
Cassia acutifolia 70 mg	O	140
A07C ELECTROLYTES WITH CARBOHYDRATES		
Hidroreg 18.9g	O	18900
A11D VITAMIN B1, PLAIN AND IN COMBINATION WITH VITAMIN B6 AND B12		
Cyanocobalaminum 50 mg + Pyridoxinum 50 mg + Thiaminum 1 mg	P	101

A11J OTHER VITAMIN PRODUCTS, COMBINATIONS		
Cyanocobalaminum (B12) 0,5 mg, Nicotinamidum (PP) 20 mg, Cocarboxilaza (coenzim of vit. B_1) 50 mg, Adenozintrifosfat disodic trihidrat (ATP) 10 mg	P	81
Magnesii lactas + Magnesii pidolas + Pyridoxinum	O	2850
A12B POTASSIUM		
Kalii chloridum 4% 10 ml	P	1600
Kalii aspartas + Magnesii aspartas 452 mg + 400 mg/10 ml	P	1704
Kalii aspartas + Magnesii aspartas 175 mg + 175 mg	O	1050
Kalii aspartas + Magnesii aspartas 158 mg + 140 mg	O	998
A16A OTHER ALIMENTARY TRACT AND METABOLISM PRODUCTS		
Ademetioninum 400 mg	P	600
Ademetioninum 400 mg	O	800

Note: O – oral, P – parenteral.

To determine the annual number of DDD in the period 2009–2013, the annual consumption in natural indices (grams), separate for each parenteral and enteral form was split to medicinal remedies DDD recommended by WHO, and respectively in their absence to the DDD (EMI) to obtain further amounts for each of the subgroups. The total of these data is shown in table 4.

The DDD consumption for some subgroups in some years varied considerably, but excluding the years when consumption is minimal and least compatible, annual average for A02 Drugs for acid-related disorders is 35423, A03 Drugs for functional gastrointestinal disorders is 64297, A05 Bile and liver therapy is 2483, A06 Drugs for constipation

Table 4

Parenteral and oral form consumption in DDD, of subgroups Alimentary tract and metabolism drugs in Emergency Medicine Institute in the period of 2009–2013

Administered forms	Measure unit	A02	A03	A05	A07	A11	A12	A16
2009								
Parenteral (WHO)	DDD	19947	49477		219	2083	317	60
Parenteral (EMI)	DDD	0	6650		115	110	331	
Oral (WHO)	DDD	7921	2339	600	334			1170
Oral (EMI)	DDD	444	0	57			230	
Total		28312	58466	657		2193	878	1230
2010								
Parenteral (WHO)	DDD	23009	76147	0	0	82769	242	831
Parenteral (EMI)	DDD	0	9143	1160	0	425	18535	
Oral (WHO)	DDD	13275	1337	0	3289			
Oral (EMI)	DDD	395	0	542			4684	
Total	DDD	36679	86627	1702	3289	83614	23461	831
2011								
Parenteral (WHO)	DDD	9630	55623			7241	377	1434
Parenteral (EMI)	DDD	0	10380			12	20346	7
Oral (WHO)	DDD	24806	1387	17	29134			
Oral (EMI)	DDD	704	0	2720	29		5657	120
Total	DDD	35140	67390	2737	29163	7415	26380	1561
2012								
Parenteral (WHO)	DDD	11514	46761	0		7241	20486	236
Parenteral (EMI)	DDD	0	9627	180		12	129	13
Oral (WHO)	DDD	22898	1397	0	2918			
Oral (EMI)	DDD	931	0	183	146		784	10
Total	DDD	35343	57785	363	3064	7415	21399	259

2013								
Parenteral (WHO)	DDD	13346	37928	0		77674	418	37
Parenteral (EMI)	DDD	0	8957	980		550	18739	
Oral (WHO)	DDD	27552	4332	958	17271			20
Oral (EMI)	DDD	742		57	1035		4130	
Total	DDD	41640	51217	3010	18306	78705	23287	57

problems is 3164, A07 Antidiarrheals, intestinal antiinflamatory/antiinfective agents is 22835, A11 Vitamins is 99777, A12 Mineral supplements is 31509, A16 Other alimentary tract and metabolism products is 1396 DDD.

Total annual consumption in DDD of drugs group A Alimentary tract and metabolism for parenteral and oral use in EMI in the period of 2009 – 2013 years is shown in table 5.

From total number of DDD consisting in each subgroup the parenteral form (DDD and DDD(EMI)) represented in sequence by years are 85.7% in 2009, 83.6% in2010; 77.5% in 2011; 76.7% in2012; 72.8% in 2013 and oral form (DDD and DDD(EMI)) respectively 14.3%; 16.4%; 22.5%; 23.3% and 27.2%.

To determine the DDD/1000 during the evaluated period, was taken into account the following data: the number of patients treated in the institution (except those who have no insurance policy and pay for treatment), which in 2009 was 20 946 patients, the median duration of treatment 8.62 days,

Table 5

Total annual parenteral and oral form consumption in DDD of Alimentary tract and metabolism drugs in Emergency Medicine Institute in the period of 2009 – 2013

Administered form	Measure unit	2009	2010	2011	2012	2013
Parenteral (WHO)	DDD	71884	182998	203247	86238	129403
Parenteral (EMI)	DDD	7091	29263	31463	9961	29226
Oral (WHO)	DDD	12269	35067	58662	27333	53087
Oral (EMI)	DDD	876	6621	9645	1913	6165
Total		92120	253949	303017	125445	217881

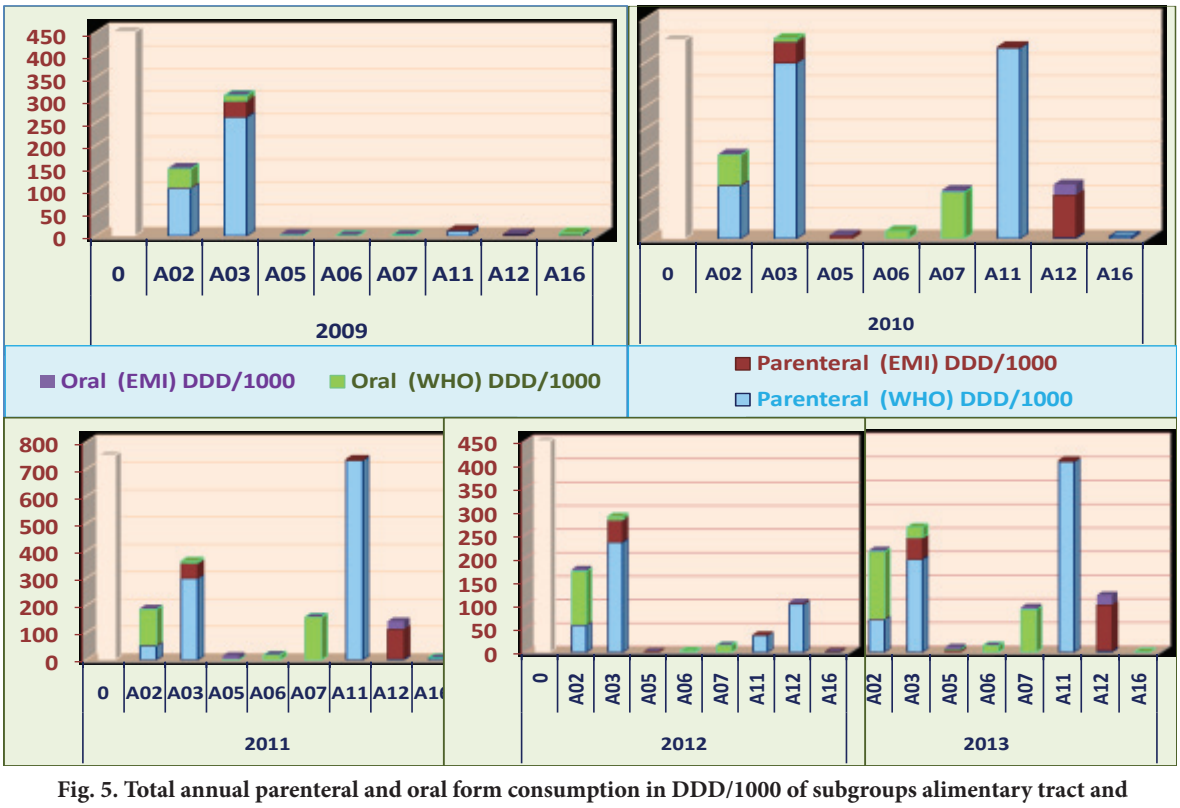

Fig. 5. Total annual parenteral and oral form consumption in DDD/1000 of subgroups alimentary tract and metabolism drugs in Emergency Medicine Institute in the period of 2009 – 2013.

which amounts to 188 762 days/bed; in 2010 their number was 21 341, and the median duration of treatment 8.64 days, which amounts to 191 556 bed/days; in 2011 was 19 913 patients and the median duration of treatment 8.66 days, which amounts to 186 246 bed/days; 2012 was 20 664 and the median duration of treatment 8.82 days, which amounts to 199 816 bed/days, and, in 2013 respectively was 20 830 with a median treatment duration 7.8 days, which amounts to 193 019 bed/days [16].

As one can see from figure 5 in the investigation period the consumption of DDD/1000 more than 100 units were registered for the following subgroups: A02 Drugs for acid-related disorders, A03 Drugs for functional gastrointestinal disorders, A07 Antidiarrheals, intestinal antiinflamatory/ antiinfective agents, A11 Vitamins and A12 Mineral supplements. The consumption of DDD/1000 of oral form for subgroups A02 Drugs for acid-related disorders and A07 Antidiarrheals, intestinal antiinflamatory/antiinfective agents have been constantly increasing over the evaluated period from 44.4 and 1.8 units in 2009 to respectively 146.5 and 94.8 units in 2013.

The total annual parenteral and oral form consumption of DDD/1000 of drugs Alimentary tract and metabolism group in EMI, for the period of 2009 – 2013 is presented in figure 6.

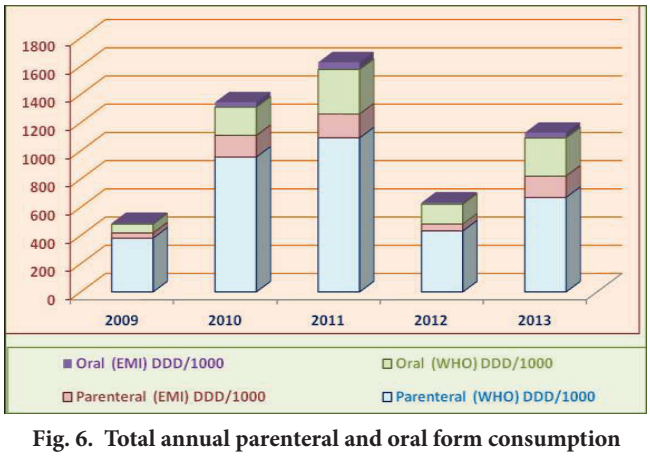

Fig. 6. Total annual parenteral and oral form consumption in DDD/1000 of alimentary tract and metabolism drugs in Emergency Medicine Institute in the period of 2009 – 2013.

A considerable difference between the total DDD consumed per 1 000 occupied bed/days for the period under evaluation can be observed from figure 6. And, if that consumption in 2009 was considered as 1 unit, then this report for 2010 would be 1: 2.75, for 2011 = 1: 3.33, for 2012 = 1: 1.3, for 2013 = 1: 2.31. To assess the cost of one DDD/1000 per year, we divided the value in "Lei" spent by the number of DDD consumed and multiplied by the number of DDD/1000 occupied bed/days determined per year. So in 2009 the cost of DDD/1000 was 5180.7 lei (999 195 lei: 94 120 x 488); in 2010 was 4878.0 lei (921 691 lei: 253 949 x 1344); in 2011was 4571.2 lei (851 359 lei: 303 017 x 1627); in 2012 was 4397.1 lei (871 386 lei: 125 445 x 633) and in 2013 was 5397.5 lei (1 042 562 lei: 217 881 **x** 1128).

Conclusions

1. The obtained data reveals that from 3 basic characteristics of drug consumption [index value, natural indicators (grams), natural indicators DDD], DDD is the most objective and can be considered as the cornerstone for making decisions on determining the needs and organization of rational use of medicines, that in practice means implementation of the "Drug Statistics Methodology" of WHO Collaborating Centre in the Republic of Moldova, first of all in hospital institutions.

2. Taking as a starting point 2009 year the overall assessed consumption of medicinal remedies was presented in:

- index value (lei) of 999 195 lei, the percentage in 2010 amounted to 92.3% (921 691 lei), in 2011 to 85.2% (851 359 lei), in 2012 to 87.2% (871 386 lei) and in 2013 to 104.4% (1042562 lei), or a variation for the evaluated period of 19.2% (from -14,8% to 4.4%);
- natural indicators (grams) of 123479 grams in 2009, which in 2010 amounted to 89% (109510 grams), in 2011 to 110.7% (136712 grams), in 2012 to133.6 % (165019 grams) and in 2013 to 117.5% (145 092 grams) or a variation for the evaluated period of 44.6% (from - 11% to 33.6%).

3. Total number of DDD consisting in each subgroup is 94 120 in 2009, to 253 949 in 2010, to 303 017 in 2011, to 125 445 in 2012 and to 217 881 in 2013, of these totals:

- The parenteral form (DDD and DDDEMI) represented in sequence by years is 85.7% or 78975, 83.6% or 212 261; 77.5% or 234 710; 76.7% or 96 199; 72.8% or 158 629;
- The oral form (DDD and DDDEMI) respectively 14.3% or 13 145; 16.4% or 41688; 22.5% or 68 307; 23.3% 29 246 and 27.2% 59 252.

4. The annual average consumption was recorded for: A02 Drugs for acid-related disorders of 35423 DDD, A03 Drugs for functional gastrointestinal disorders of 64297 DDD, A05 Bile and liver therapy of 2483 DDD, A06 Drugs for constipation problems of 3164 DDD, A07 Antidiarrheals, intestinal antiinflamatory/antiinfective agents of 22835 DDD, A11 Vitamins of 99777 DDD, A12 Mineral supplements of 31509 DDD and A16 Other alimentary tract and metabolism products of 1396 DDD.

5. The number of consumption DDD/1000 occupied bed days in the period under evaluation was:

- 488 in 2009, 1344 in 2010 or an increase of 275%, 1627 in 2011 or an increase of 333%, 633 in 2012 or an increase of 130% and 1128 in 2013 or an increase of 231%. The variation in the evaluated period was from minimal 1.3 times to maximal 3.3 times;
- the cost of one DDD/1000 that was respectively 5180.7 lei in 2009, decreases to 4878.0 lei in 2010, 4571.2 lei in 2011and 4397.1 lei in 2012, respectively and increases to 5397.5 lei in 2013.

6. In order to fully assess drug consumption in DDD for medicinal remedies that WHO has not published, DDD based on the data of Emergency Medicine Institute were determined for 24 drugs.

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Dopplerographic hemodynamic predictive parameters for portal hypertension associated with hepatic cirrhosis

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Abstract

Background: Early diagnosis of diffuse chronic liver pathologies greatly improves the treatment and pathology evolution prior to the installing of the irreversible fibrosis and cirrhosis. Color duplex Doppler ultrasonography appears to offer a number of advantages (accessibility, repeatability, etc.) in identifying asymptomatic patients and a satisfactory accuracy in assessing liver morphology and hepato-lien system hemodynamics. In order to identify hemodynamic indicators with acceptable significance estimating portal hypertension associated with liver cirrhosis in the study a detailed analysis of changes in hepatic vascular flow examined by color duplex Doppler ultrasound is performed.

Material and methods: The research group included 155 patients with varying degrees of fibrosis. Quantification of the fibrosis degree was based on the results of transient Fibroscan elastography, according to the Metavir score (1-4). Evaluation of portal hemodynamics in all patients was done using duplex Doppler ultrasound, hemodynamic indices estimation was performed on arterial and venous side.

Results: Reducing time-weighted average velocity in the portal vein, increasing the flow volume in the lien vein basin, vascular resistance increase at the level of lien artery, offers significant predictive values in identifying portal hypertension associated with liver cirrhosis.

Conclusions: Colored duplex Doppler ultrasound comprehensive approach of splenoportal hemodynamics showed hemodynamic indicators of unimportant significance for the prediction of cirrogene portal hypertension.

Key words: fibrosis, hepatic cirrhosis, hemodynamic indicators, duplex Doppler ultrasound.

Introduction

Diffuse chronic liver diseases play an important role in the morbidity and mortality of the population in many countries economically developed and less developed countries, including the Republic of Moldova. Viral infection, alcohol abuse, metabolic disorders are the primary causes of these liver diseases. The accuracy of laboratory tests and diagnostic imaging methods in identifying asymptomatic patients or with slight expression in a population at high risk is a primary necessity [2,7.8]. Chronic liver disease often has an insidious onset and a slow and progressive evolution. Chronic inflammations are gradually progressing towards hepatic fibrosis potentially reversible at certain stages, eventually resulting in irreversible cirrhosis. Recommended therapeutic algorithms are to reduce the early portal hypertension, which usually has a progressive evolution as they advance and reverse fibrosis treatment stages. Early diagnosis significantly improves the therapeutic behavior benefits prior to irreversible fibrosis and hepatic cirrhosis installation, often associated with fatal complications: depression of liver function, esophageal variceal bleeding, hepatic encephalopathy, hepatocellular carcinoma [3,4,8].

The golden standard for estimating the portal hypertension still remains the catheterization of the hepatic veins with measurement of the pression gradient. Unfortunetly this method is laborious and invasive, with multiple complications and restrictions in use, both technical and often due to serious condition of hepatic patients [1]. Therefore,

this method has not found a desired application in everyday practice, with preponderant use in scientific research studies. The morphological diagnosis also has an important place in the estimating degree of liver tissue damage in chronic diseases, the results of which play a determining role in shaping the conduct of optimal treatment for each patient. However, although the morphological diagnosis is considered competent, liver biopsy remains an invasive method, with well-known complications, filled with difficulties, often common for obtaining the bioptate from the area of interest, such as the need for adequate ultrasound guidance, patients' obesity, and the uncooperative ones. So again, the method can not be widely used [1, 6, 10, 12].

In recent decades, thanks to advances in diagnostic imaging technology, numerous global multicenter studies have shown promising results in the non-invasive assessment of normal liver structure, identification and assessment of morphologically circumscribed and diffuse liver changes, but also in the evaluation of portal hypertension syndrome. Alternative non-invasive diagnostic methods become a vital necessity in monitoring patients with chronic liver diseases, both in accessibility (price/access to equipment) and repeatability.

Non-invasive diagnosis of liver fibrosis and cirrhosis is based mainly on biochemical laboratory tests and transient elastography (Fibroscan) being used successfully in predicting liver elasticity, providing a quantitative assessment of the degree of fibrosis. The degree of liver fibrosis determined by using elastography does not always correlate with the pressure gradient in the hepatic veins, which denotes a special involvement of hemodynamic changes and often it is not only due to advanced fibrosis [5, 9, 11]. Biphasic helical CT with contrasting dynamic magnetic resonance elastography are new methods to assess the stiffness of liver parenchyma. To assess the complications of cirrhosis, portal-systemic collaterals aggravated with bleeding or hepatocellular carcinoma computed tomography angiography with contrast and MRI are used [12, 13, 14]. In the series of diagnostic methods, conventional ultrasound (2D) and duplex Doppler color scheme has a number of advantages as an accessible, non-irradiated, repeatable method, which can be carried out even at the patient's bedside. The method has an acceptable accuracy in liver morphological assessment, favoring more information on hepato-lienal system hemodynamics [2, 3, 4, 10]. However, despite clear progress, noninvasive diagnosis of fibrosis and portal associated hypertension remains a complex issue and requires further studies.

The aim of the study was to make a detailed analysis of changes in hepatic vascular flow assessed by color duplex Doppler ultrasonography in order to identify the hemodynamic indicators with acceptable significance in estimation of portal hypertension associated with liver cirrhosis.

Material and methods

During 2012-2014 years in the Department of Clinical Hepatology at the Republican University Hospital were investigated 155 patients with various degrees of fibrosis. The entire research group was subdivided into group determined with cirrhosis (fibrosis stage 4) – 111 and precirrhotic group, which included patients with various degrees of fibrosis (1-3) – 44. The mean age of selected patients was 48.4 years. Gender distribution was 55 males (35%), women – 100 (65%). Study methods of the group included both traditional analyses made in a patient with liver pathology and modern imaging techniques. Quantification of the fibrosis grade was based on the results of transient elastography Fibroscan according to the Metavir score (1-4). To assess liver structure by conventional echocardiography (2D) were used the following parameters: liver measurements – right lobe, left lobe, caudate lobe, spleen size; contour of the liver was assessed using linear probe. Evaluation of portal hemodynamics was performed in all patients by duplex Doppler ultrasound, estimating hemodynamic indices which were performed on arterial and venous side. Subsequently indicators used in the estimation of portal cirrhogene hypertension were calculated.

Results and discussions

Evaluation of hemodynamic parameters in the portal vein in cirrhotic patients group revealed: dilatation of portal vein in 95 patients (85%), in precirrhotic group in 19 (43%) cases; decrease of time-weighted average velocity (TWAV) in the group of patients with cirrhosis was present in 90 (81%) cases versus to the precirrhotic group – 8 (18%). The increased flow in the portal vein in patients with cirrhosis occurred in 69 (62%) cases, in patients with fibrosis precirrhotic stages in 12 (27%). The values of the statistical indicators used to predict portal hypertension associated with liver cirrhosis are shown in tab. 1.

Table 1

Predictive statistical indicators in portal vein

Indicators	Se	Sp	PPV	NPV	LR+	LR-
Diameter of portal vein (mm)	86%	57%	83%	61%	2	0,24
TWAV (cm/sec)	81%	82%	92%	63%	4,5	0,23
Volume of flow (ml/min)	62%	72%	85%	43%	2,2	0,52

Legend: Se – sensitivity, Sp – specificity, PPV – positive predictive value, NPV – negative predictive value, LR+ – positive likelihood ratio, LR- – negative likelihood ratio.

The evaluation of these parameters in the portal vein showed a very good prediction time-weighted average velocity with a likelihood ratio of 4.5 times compared to only measuring the diameter and volume of vascular flow in the portal vein (fig. 1).

The appreciation of similar dopplerographic parameters at the level of lienal vein settled a dilatation of the lieanal vein in 87 (90%) in group of patients with hepatic cirrhosis compared with 13 (29%) in the group with different stages of fibrosis. Blood flow velocity assessed at this level was characterized by the increase in 83 (86%) in the cirrhotic group and 15 (34%)

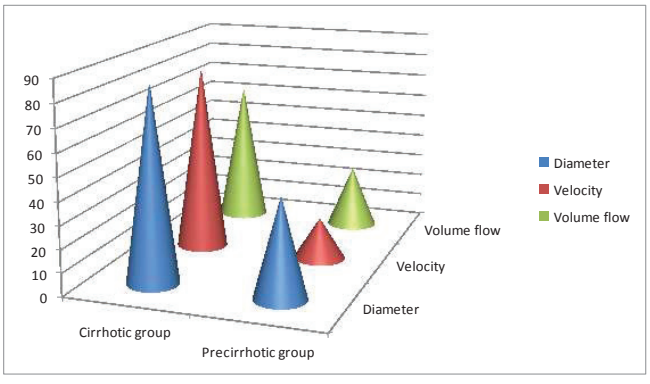

Fig. 1. Comparative analysis of haemodynamic parameters in portal vein.

in the precirrhotic group. Increased volume of the vascular flow in the lienal vein was constant in 92 (96%) in the group with cirrhosis and only 10 (22%) in the other group. Statistically obtained indicators values are shown in tab. 2.

Table 2

Predictive statistical indicators in lienal vein

Indicators	Se	Sp	PPV	NPV	LR+	LR-
Diameter of lienal vein (mm)	90%	70%	87%	77%	3	0,14
TWAV (cm/sec)	86%	65%	85%	69%	2,5	0,21
Volume of flow (ml/min)	95%	77%	90%	89%	4,1	0,064

We can see that practically all the indicators used to assess hemodynamics showed a satisfactory predictive value, especially blood flow volume in lienal vein, values which allow to increase the predicted values by 4,1 times in the diagnosis of disease (fig. 2).

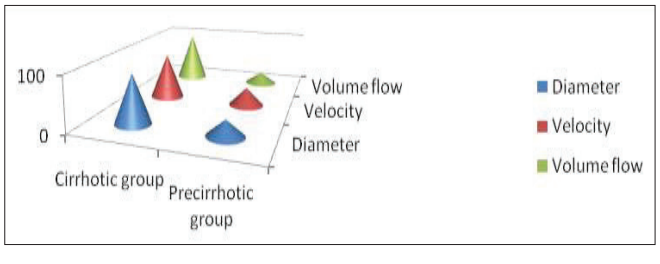

Fig. 2. Comparative analysis of hemodynamic parameters in lienal vein.

Assessing arterial hemodynamics slope impedance the indicators were analyzed: pulsatility index (PI) and resistivity index (RI) at the level of lien and hepatic artery. The obtained statistical indicators values are shown in table 3.

Table 3

Predictive statistical indicators in hepatic and lienal artery

Indicators	Se	Sp	PPV	NPV	LR+	LR-
Hepatic artery (PI)	87%	50%	81%	61%	1,74	0,26
Hepatic artery (RI)	95%	50%	83%	81%	1,9	0,21
Lienal artery (PI)	53%	95%	96%	48%	10,6	0.1
Lienal artery (RI)	83%	95%	97	72	16,6	0,17

So, due to a comparative analysis of isolated hemodynamics of hepatic artery and the lienal artery we can notice a significant increase (16.6 times) of the probability of portal hypertension associated with cirrhosis in the presence of circulatory lien artery disorders (fig. 3).

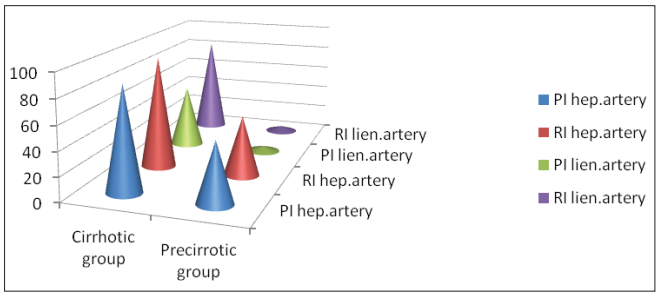

Fig. 3. Comparative analysis of hemodynamic parameters in hepatic and lienal artery.

Also, in this study other utility indicators known in assessing the portal hypertension were evaluated: congestion index (CI), splenoportal index (SPI), portal vascular index (PVI), the index of portal hypertension (IPH), which showed specificity and important positive likelihood ratio. Maximum values were found in CI, with a specificity of 93% and + 11.4 RP. Just PVI – Sp 87%, RP + 5.8.

Splenomegaly, is a mandatory criterion to quantify portal hypertension being analyzed in both groups of patients. It was confirmed that the known experience represents a test of a very high sensitivity, being present in 100 (97%) patients, the cirrhotic group, but a low specificity being present in 19 (43%) patients in group with various degrees of fibrosis (Se 98%, SP 57%). In identifying a more specific criterion we assessed the presence of portosystemic collaterals in both groups of patients, obtaining a positive test in 100 (90%) patients from cirrhosis group and only 2 (4.5%) patients in the precirrhotic group. Thus RP + 18, is an important predictive parameter for portal hypertension associated with cirrhosis (fig. 4.)

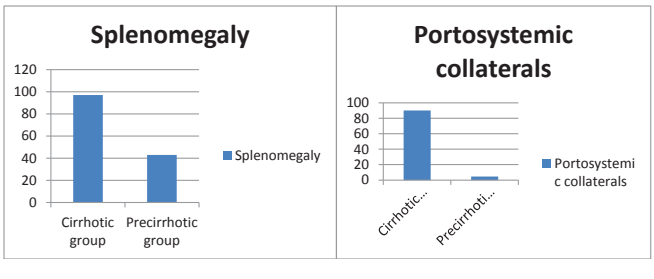

Fig. 4. Comparative analysis of splenomegaly and portosistemic collaterals.

In this research very high predictive indicators for the presence of cirrhosis were revealed, realized by color duplex Doppler ultrasound obtaining ROC curves. Thus, the area under the (AUC) curve for time-weighted average velocity in the portal vein, splenoportal index, the IPH is 1, and the congestion index is 0.976, which shows a great accuracy of using these parameters (fig. 5).

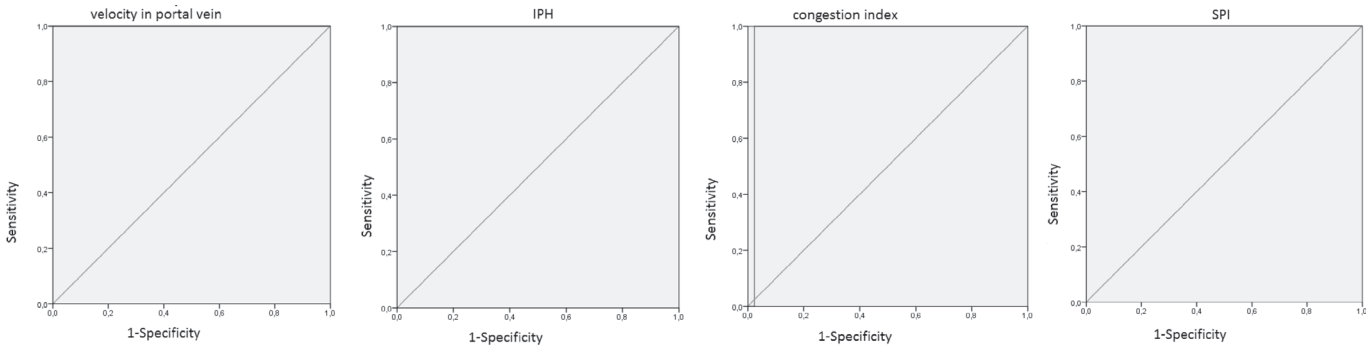

Fig. 5. ROC curves for cirrhotic group.

Conclusions

1. The analysis of hemodynamic indexes by color duplex Doppler ultrasound revealed early emergence of circulatory changes associated with portal hypertension up to the irreversible morphological appearance.

2. Study of circulatory disorders in the portal vein side emphasized the importance of reducing time-weighted average velocity of the cirrhotic patients group.

3. Evaluation of the significance of flow in the lien vein basin determined the superiority of flow volume at this level compared to other indicators.

4. Hemodynamic evaluation of the arterial versant established a major importance of pulsatility and resistance indices at lien artery level predicting portal hypertension associated with liver cirrhosis.

5. Comprehensive approach by color duplex Doppler of splenoportal hemodynamics highlighted several circulatory aspects showing not only academic interest but also practical benefits for patients who can benefit from treatment at a very early stage of fibrosis.

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Public perception as an alternative method for estimating health status

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Abstract

Background: A great number of indicators and quality of life indices in terms of health are reported in the current statistical statements of general morbidity, although having plenty of information; they also have low reliability and poor analytical capabilities. Therefore, the above indices currently lose their relevance, being based only on data of population visits to health care providers for medical advice.
Material and methods: Questioning, as the basic method of sociological research, makes it possible to obtain information quickly and cost-effectively, being recognized as a method with full right to monitor public health.
Results: Subjective information during the accumulation process becomes objective public characteristic and it can be integrated into the actions for planning and evaluation of activities in the field of health care. Insured persons (in comparison with uninsured persons) are more sensitive to their health status also caring about being health insured in any possible demand; insured persons care more about their health, accounting for a higher percentage in the follow-up group of persons with both chronic and acute diseases. This contributed to the fact that the cases of incapacity for work due to illness amongst insured persons were met a third less frequently comparing to uninsured persons.
Conclusions: There were problems identified, which deserve special attention of decision makers in the field of health care. These are: people avoid addressing to the family doctor in case of health concerns, whereas insured persons prefer to appeal mainly to hospital in-patient services, and uninsured persons prefer to appeal for services to private medical centers. In the case of illness almost every third uninsured person (34.8%) does not seek medical attention, and only every fourth uninsured person (25,6%) addresses to the family doctor.

Key words: perception, insured persons, uninsured persons, questionnaire.

Introduction

Accessibility of data on state of health representative for the entire population is the main background for identification and understanding of public health problems and for planning and evaluation of health protection activities [1].

The purpose of human development is the possibility to live a long, fulfilling and healthy life. An essential element of human well-being is considered a good state of health and at societal level it is also a key element of human capital of each country, which contributes to its competitiveness compared to other countries [2]. The statement of some scientists [3] that a good state of health at individual level is an important component of human capital, allowing people to pursue their activities, achieve their goals, have a full life and be members of the society, is indisputable.

One of the most important factors that determine the state of status is health. Health is a vital value ranking the top in the hierarchy of values. Therefore, the importance of identifying and maintaining the state of public health in a country cannot be doubted. Knowing the incidence of certain population groups by age and sex makes it possible to identify those priority groups that need the most attention from the state [4]. Health for most of its representatives is not just absence of diseases, but also presence of vital energy, lack of stress conditions, etc., so it is not a purely physiological well-being, but also a social and psychological one [5]. Health indicators also are a basis for planning health resources required to meet existing needs in different types of medical services.

Throughout several decades of the last century up to the present the main method of determining the state of health of population in our country has been recording morbidity based on referrals of population to medical and sanitary institutions. But estimation of population incidence based only on referrals is now impossible, because a large percentage of population do not seek medical assistance, even if they suffer from serious diseases. People may feel their health deteriorating, but do not seek medical assistance. There is also self-treatment and referral to alternative sources of help. Estimation of the volume and quality of such practices is difficult [6].

More commonly health in researches of scientists on the quality of life is approached from two distinct perspectives [7]. A health dimension consists in considering health as an area of the quality of life being described by indicators by referring to health sector, which are at different levels of analysis: assessment of their own state of health, perceived constraints imposed by the state of health, perception of access to medical services, evaluation or satisfaction with them, life expectancy at birth, morbidity or mortality rates, rate of GDP (gross domestic product) of expenses for medical services, etc. The second dimension in quality of life researches from a health perspective focuses on how patients with different health problems feel the quality of life, people's perceptions that describe different state of health or time of survival of recipients of medical treatment, etc., as a large number of indicators and indices of the quality of life are developed in this approach as well.

Current statistics of total morbidity have information in abundance, but with a low reliability and poor analytical capabilities [8]. Annual general records of data on causes of referrals for medical consultations taken as a source of morbidity study have now lost their relevance. This weakly informative method is maintained only by traditions [9]. This raises a well-founded doubt about the possibility of the estimated incidence calculated based on referrals to be a tool for rapid assessment of the situation related to the health of population in a given administrative area.

A promising direction for estimation of incidence at present consists in using different methods of sociological researches. In economically developed countries data on public health obtained on the basis of sociological surveys became much sought [10].

Perceived state of health reflects the general perception of people of their physical and mental health. Several OECD (Organisation for Economic Cooperation and Development) countries conduct health surveys, which allow respondents to evaluate different aspects of their state of health. Despite the subjective nature of questions of the questionnaire, indicators of perception of the state of health allow making correct forecasts on evolution of morbidity and needed medical services as a whole. Perception or self-evaluation of the state of health is an important indicator of the state of health. It reflects the overall assessment a person makes about his/her health, integrating objective and subjective aspects, especially his/her knowledge and experience about health or disease [11].

The special value of sociological assessments of public health consists in the ability to analyse massive data on pathology, with which people for various reasons do not seek medical assistance. Questioning as a basic method of sociological research makes it possible to obtain information quickly and cost-effectively. Subjective information in the accumulation process becomes an objective population feature [12].

In recent decades in many countries the availability and quality of questioning results on the state of public health have been significantly increased. Currently, questioning is recognized as a method with a full right to monitor health of population, along with recording [1]. Patient's opinion on his/her experiences in the use of health care services becomes an important tool for improving and monitoring access and quality of health services. According to the OECD and WHO (World Health Organization) descriptions the studies of patient's satisfaction with the quality and access to health services is an important part in the overall assessment of the health care system, as well as a foundation for national health policies [13, 14].

According to some authors [15], some countries constantly conduct a systematic monitoring of patient's satisfaction (ex. Denmark, UK, USA, Canada, Norway, the Netherlands). In other countries (ex. Ireland, Czech Republic, Estonia, Spain, Israel, Slovenia, Lithuania) conducting patient satisfaction surveys both at national and institutional level is sporadic. A number of examples illustrate the fact that patients' experience is a tool widely recognized and used in improving the quality of health services.

Material and methods

In this context, we planned to conduct a study on perception of population of the changes of the health system of the Republic of Moldova, with a focus on medical and social factors that influence the state of health, focusing on access and quality of medical services.

We used as a tool a Questionnaire for assessment of medical and social factors influencing health of insured/uninsured persons related to major health problems that the population face, the ways the health system addresses these problems, awareness of new structures and mechanisms involved along with implementation of mandatory health insurance in the Republic of Moldova, insurance coverage and health services, barriers to accessing medical services.

Separate analyses were performed on the main levels of medical assistance, including pre-hospital emergency medical assistance, primary medical assistance, specialized outpatient and hospital medical assistance, evaluating and analysing perception of population of a number of aspects specific to each type of assistance in part, such as: quality of services, waiting time, attitude of medical staff, cooperation between levels of medical assistance, costs of medical assistance.

Data collection was performed by means of survey based on the Questionnaire prepared by the author, of 1067 insured/uninsured persons in 3 geographical areas of the Republic of Moldova both urban and rural ones, such as: Northern Zone (Briceni district), Central Zone (Chisinau municipality, Criuleni and Ialoveni districts) and Southern Zone (Causheni district).

The findings of the study were processed and analysed using Excel, SPSS application and were presented graphically in tables and charts.

The sample of respondents (1067 people) consisted of: 760 insured persons (71.2 ± 1.64%) and 307 uninsured persons (28.8 ± 2.58%) ($t = 13.8459$, $p < 0.001$); by area of residence - 533 persons from urban areas (49.95 ± 2.17%) and 534 persons from rural areas or 50.05 ± 2.16% ($t = 0.0327$, $p > 0,05$). Uninsured persons in proportion of 37.1 ± 2.09% are from rural areas, as compared to 20.5 ± 1.75% of those from urban areas ($t = 6.0909$, $p < 0.001$).

Results and discussion

Perception of the state of health through self-evaluation is considered a relevant indicator of well-being and quality of life and is one of the internationally recommended indicators in the performance of analysis of the state of public health. The fact of influencing the social and cultural environment on perception of the state of health is relevant. Perceived state of health was determined based on 5 variants of answers to the question: "How do you appreciate your current state of health?": very good, good, satisfactory, bad and very bad.

Thus, according to the data of the study (fig. 1), the state of health within the range "satisfactory – very good" is appreciated by 71.7 ± 1.38% of insured persons and by 80.1 ± 1.22% of uninsured persons ($t = 4.5585$, $p < 0.001$), included in the total study group; from urban areas, respectively by 76.4 ± 1.84% of insured persons and 87.2 ± 1.45% of uninsured persons ($t = 4.6165$, $p < 0.001$) and in rural areas – respectively, 66.0 ± 2.05% and 76.3 ± 1.84% ($t = 3.7371$, $p < 0.001$), indicating that insured persons are more sensitive to the state of health, taking care of benefiting from health insurance in case of a possible need. This is also confirmed by assessment of the state of health as "bad" and "very bad" by 10.1 ± 0.92% of uninsured persons of the study group compared to 17.6 ± 1.17% by insured persons ($t = 5.0449$,

p < 0.001); from urban areas - respectively 7.3 ± 1.13% and 14.9 ± 1.54% and in rural areas – 11.6 ± 1.39% and 20.9 ± 1.76%, respectively (t = 2.4076, p < 0.05; t = 2.5643, p < 0.05).

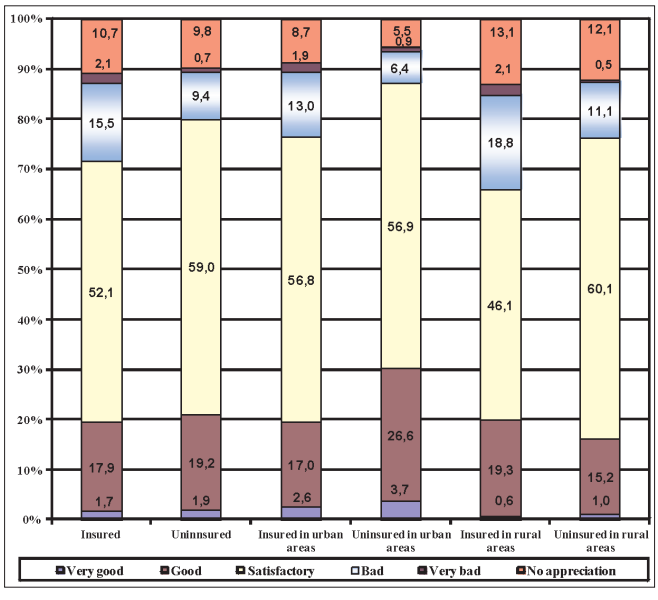

Fig. 1. Perception of current state of health of insured/uninsured persons, %.

Upon conduct of the survey respondents (based on the results of access to the medical system) stated the following: 78.4 ± 1.26% of insured persons suffered from chronic diseases or 56.8% more than uninsured persons (21.6 ± 1.26%) (t = 31.8809, p < 0.001); acute diseases – 65.9 ± 1.45% and 34.1 ± 1.45% (t = 15.4944, p < 0.001) respectively or 31.8% more, confirming a more increased care of insured people for their own health (fig. 2).

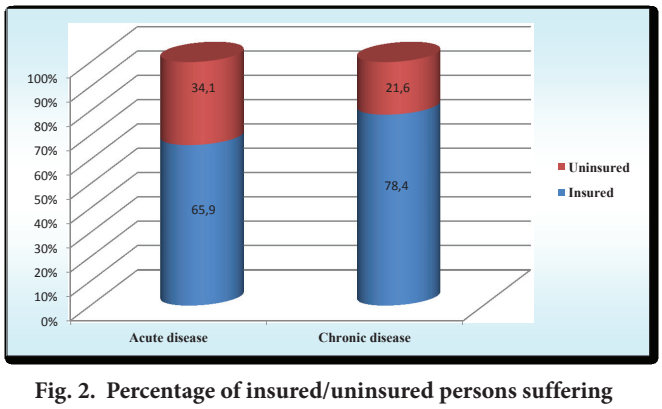

Fig. 2. Percentage of insured/uninsured persons suffering from acute and chronic diseases, %.

Confirmation of the above conclusion is also substantiated by the data on how many times it happened to a person over the last year not to work. Thus the insured 1.5 times less than the uninsured did not work because of diseases.

In case of health problems, depending on availability of health insurance, we find that the insured mainly seek hospital services (90.0 ± 1.09%), consult a family doctor (74.4 ± 1.58%), a medical specialist (74.3 ± 1.57%), seek ambulance services (73.8 ± 1.59%), pharmacy services (62.5 ± 1.76%), and in 65.0 ± 1.73% of cases do not refer to anyone, while the uninsured in these situations refer mainly in 47.1 ± 2.85% to private medical centers, seek the services of nurse – 40.7 ± 2.80% of cases, pharmacy – 37.5 ± 2.76%, ambulance – 26.2 ± 2.51% of cases, consult a medical specialist – 25.7 ± 2.49%, a family doctor – 25.6 ± 2.48% and in 34.8 ± 2.72% of cases do not refer to anyone.

Diseased insured persons from urban areas more frequently seek hospital services (95.1 ± 0.94%), consult a family doctor (83.2 ± 1.62%) and in 77.9 ± 1.79 % of cases seek emergency medical assistance as compared to the uninsured, who in 30.1 ± 1.98% – seek the pharmacy services, in 30.0 ± 1.98% - medical services provided by private centers and 25.0 ± 1.75% of cases – seek the services of nurse or do not refer to anyone (p < 0.001).

Diseased insured persons from rural areas seek mainly hospital services (85.7 ± 1.51%) (t = 5.2802, p < 0,001), ambulance services (69.3 ± 1.99%) (t = 3.2019, p < 0.01), consult a medical specialist (68.4 ± 1.55%) and in 64.7 ± 2.07% of cases - a family doctor (t = 7.0431, p < 0.001), while the uninsured in 71.4 ± 1.96% of cases refer to private medical centers, in 48.3 ± 2.16% of cases – seek nurses' assistance and in 46.7 ± 2.16% of cases – pharmacy services, and 45.5 ± 2.15% of them do not refer to anyone (t = 7.1757, p < 0.001).

The study shows worrying fact that in case of disease almost every third uninsured person (34.8%) does not refer to anyone and only each fourth (25.6%) person consults a family doctor, despite the fact that they benefit from the entire amount of medical services provided for by the Unique Programme of Mandatory Health Insurance (fig. 3).

Conclusions

Perception of state of health, reflects the overall assessment what a person makes about his/her state of health, integrating objective and subjective aspects, especially his/her knowledge and experience about health or disease, becomes an alternative method for estimating state of health and an adequate important indicator of state of health of the population.

Using in research questioning of the population as a basic method becomes particularly valuable by being able to get information quickly and cost-effectively, to analyse large massive data on pathology, while subjective information in the accumulation becomes an objective population characteristic, which generally allows making correct forecasts of evolution of morbidity and needs for medical assistance.

The study's results reveal that the insured are more sensitive to their state of health, taking care to benefit from health insurance in case of a possible need, as confirmed by assessing state of health with the respective qualifications, and namely: state of health within the range "satisfactory - very good" is appreciated by 71.7% of insured persons and by 80.1% of uninsured persons; from urban area by 76.4% of insured persons and 87.2% of uninsured persons respectively, and from rural area by 66.0% and 76.3% respectively. This thesis is confirmed by the assessment of the state of health as "bad" and "very bad" by 10.1% of uninsured persons of the

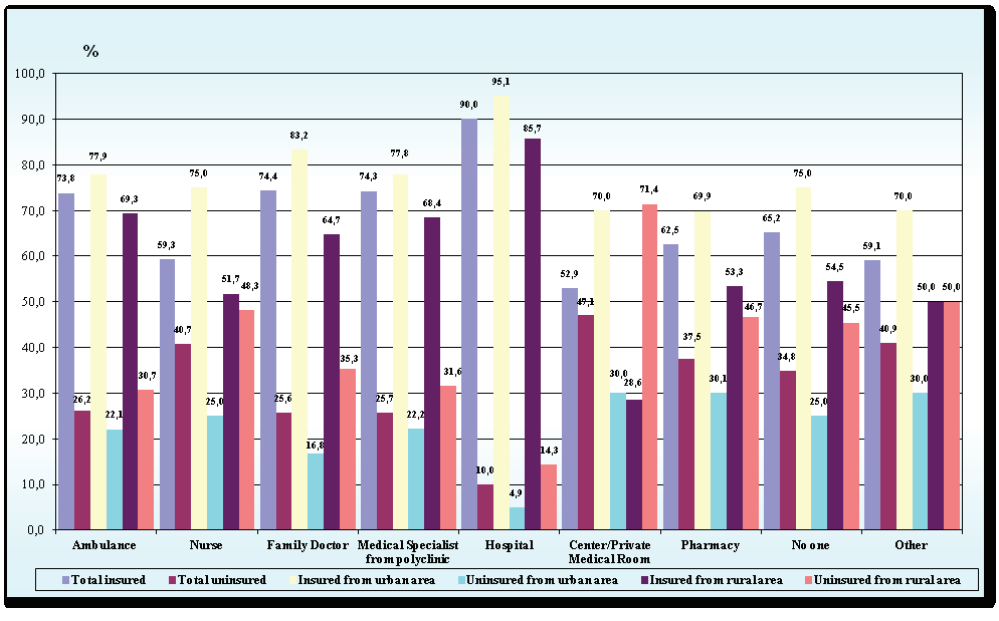

Fig. 3. Medical assistance in case of disease sought by insured/uninsured persons, %.

study group as compared to 17.6% of the insured; from urban area – 7.3% and 14.9% respectively and from rural areas – 11.6% and 20.9% respectively.

It was found that the insured care more for their own health: respondents, based on the results of assessment of the medical system, stated the following: 78.4% of insured persons suffered from chronic diseases or 56.8% more than the uninsured (21.6%); acute diseases – 65.9% and 34.1%, respectively or 31.8% more. Similarly the insured 1.5 times less than the uninsured did not work because of diseases.

In the case of health problems insured persons seek mainly hospital services (90.0%), while uninsured persons in these situations refer mainly to private medical centers (47.1%), avoiding consulting a family doctor.

It is worrying that in case of disease almost every third uninsured person (34.8%) does not refer to anyone and only every fourth person (25.6%) consults a family doctor, despite the fact that he/she benefits from all medical services provided for by the Unique Programme of Mandatory Health Insurance.

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Effect of photodynamic therapy on glutathione S-transferase activity in oral liquid of children with high risk of caries

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Abstract

Background: Pediatric dentistry is looking for new methods to influence the cariogenic microorganisms of dental biofilm without the use of antiseptics and antibiotics, which often have negative side effects. Photodynamic therapy (PDT) is a promising and effective method to influence on the cariogenic microorganisms without using antiseptics and antibiotics.

Material and methods: Forty five children aged 7 to 12 years with high risk of dental caries and pathology of central nervous system were observed during three years of the complex preventive measures, including multivitamins, minerals, antioxidants and PDT of dental biofilm. The activity of glutathione S-transferase (GST), content of reduced glutathione (GSH), thiocyanate (SCN) and total protein in the oral liquid (OL) were determined by spectrophotometry (Diasys Diagnostics, DE). The results were statistically processed using the program Excel Microsoft: Microstat 2007.

Results: In three years the protein content in OL of the children was below the initial content in all groups that may be the confirmation of PDT bacteriostatic effect. In all periods of the study significant changes in the content of GSH, thiocyanate and activity of GST in OL in the children were not observed.

Conclusions: Our results are indicating that complex preventive measures including the non-invasive method of PDT were effective, without any negative side effects and had bacteriostatic action. These complex preventive measures may be recommended for children with high risk of caries and pathology of CNS.

Key words: caries, glutathione, glutathione S-transferase, photodynamic therapy.

Introduction

Currently, pediatric dentistry is looking for new methods to influence the cariogenic microorganisms of dental biofilm to achieve sterility of cavity, root canal, the successful treatment of periodontal disease and oral mucosa without the use of antiseptics and antibiotics, which often have negative side effects. In dental practice photodynamic therapy (PDT) is non-invasive, effective, perspective, and safe method, with a bacteriostatic effect on pathogenic microflora of the mouth [1]. The basis of PDT is a chemical reaction which develops in biological tissues/cells after exogenous application of photosensitizer defined under the action of light energy dose.

The photosensitizer is applied to biological tissue or injected into the tissue where it selectively accumulates in the cells of the pathogen. Then this site is irradiated with a laser using a certain wavelength. As a result, the photosensitizer's photochemical reaction releases oxygen, which acts on the pathologically altered cells or pathogens, destroying them [2]. As a source of laser radiation for PDT the low-powerful semiconductor lasers are used.

In 1990, M. Wilson and G. Pearson demonstrated that PDT can kill the bacteria found in the infected dentin, root canals, and periodontal tissues. Since 1992, the PDT method has been used in clinical medicine, and since 2002 it is a standard of treatment in oncology [1, 5]. Lima J.P. et al. (2009) demonstrated the efficacy of PDT for killing pathogens in the treatment of dentine caries [3].

In the literature, there are a few publications about the usage of PDT in the treatment of dental diseases in children [4, 5, 6, 7], and therefore, further studies on the effect of PDT in children are very important. PDT destroys pathogens, removes the pigment, which are waste products of the microbiota. However, in the process of photochemical reaction the active singlet oxygen releases which is the initiator of radical chain reactions, the products of which are toxic substances, named Reactive Oxygen Species (ROS).

Reduced glutathione (GSH) itself directly and a coenzyme of glutathione transferase (GST), which use it in the conjugation reactions of toxins, are involved in the antioxidant protection of cells against the aggressive oxygen radicals and products of the radical chain reaction. In the literature there is no information on the influence of PDT on the activity of GST in the OL of children with severe disorders of the central nervous system (CNS) and high risk of dental caries.

Objective. Investigation of the PDT influence on the activity of glutathione transferase in the OL of children with severe pathology of CNS and high risk of dental caries.

Material and methods

The study involved 45 children aged 7 to 12 years with severe pathology CNS and high risk of dental caries. All clinical and biochemical studies have been conducted in accordance with ethical and legal standards. As the prevention of dental caries children underwent the complex preventive measures, including the appointment of multivitamins, minerals and antioxidants, as well as conducting PDT on dental biofilm. Children were observed during three years and were divided into four groups depending on the photosensitizer for application: 1st group – 2% methylene blue; 2nd group – 2% anthocyanin; 3d group – 2% toluidine blue, followed by laser irradiation LED 625-635 nm, and the 4th group served as a control group. OL of children were taken after PDT exposition in an hour,

week, month and 3 years. OL was centrifuged at 600 r for 10 minutes and after that the activity of GST [8] content of GSH [9], thiocyanate (SCN) [10] and the total protein (Lowry H., 1951) were determined spectrophotometrically (Diasys Diagnostics, DE). The results are statistically processed using the program Excel Microsoft: Microstat 2007.

Results and discussion

The protein content in OL of patients with caries correlated with the degree and amount of pathogenic microorganisms inhabiting in the oral cavity. One of the tasks was using PDT to destroy cariogenic microflora of dental biofilm in children with high risk of dental caries. Table 1 shows the results of the dynamics of the protein content in OL of children with CNS pathology and high risk of dental caries after PDT influence.

As the table shows, one hour after PDT content of the protein in OL of children slightly increased in the 1st, 3-d and 4th groups, that probably was a result of receipt of proteins and peptides carrying a protective function, in response to the use of PDT. However, during the follow-up after 3 years protein content in OL of children was below the initial content in all groups, that may be the confirmation of PDT bacteriostatic effect.

Active oxygen and its generated radicals due to a photochemical reaction are involved in the oxidative radical chain reactions which result in the formation of toxic products. This leads to the induction of protective antioxidant and antitoxic systems, such as superoxide dismutase, catalase, glutathione peroxidase, glutathione S-transferase and others. GSH has the multifaceted role as an antioxidant. It participates directly in the neutralization reactions of oxygen radicals and their products Reactive Oxygen Species (ROS). Also it is a coenzyme of many glutathione-depending enzymes, including GST.

Figure 1 shows the dynamics of the content of GSH in the OL of children during three years of observation after PDT.

In all periods of the study significant changes in the content of GSH in OL in the children were not observed both in the content per litre of OL (mcmol/l), and per gram of protein (mcmol/g). Results of research of the GST activity of the OL in children varied during the course of PDT (fig. 2).

In an hour after PDT all groups showed a decrease of GST activity both per litre of OL, and per gram of protein. In a week of observation GST activity was also reduced as compared to the baseline in the OL of children before PDT. Apparently, the decrease of GST activity in OL may indicate a partial inactivation of the enzyme by methylene blue [11, 12], anthocyanin [13] and PDT. However, a month later GST activity increased in OL of children, correlating with an increase in the total protein content (tab. 1). After 3 years of observation activity of GST in the first three groups of children was compared with the initial level, which was at the first visit of children, before the influence of PDT. The exception was the 4th group (control) of children who had a low enzyme activity in the OL, but this is not statistically significant ($p_t > 0.05$).

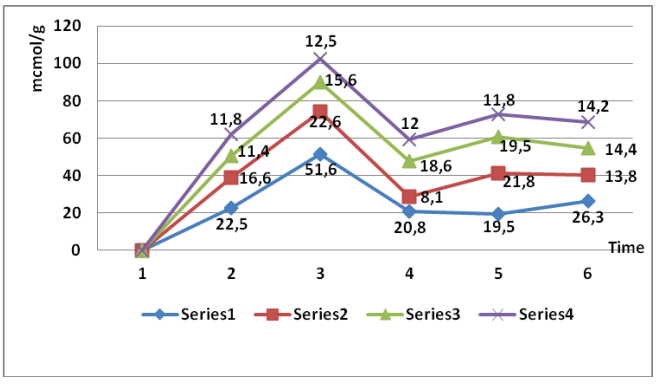

Fig. 1. Dynamics of GSH content in the saliva of children (mcmol/g protein) after PDT. The series correspond to number of groups.

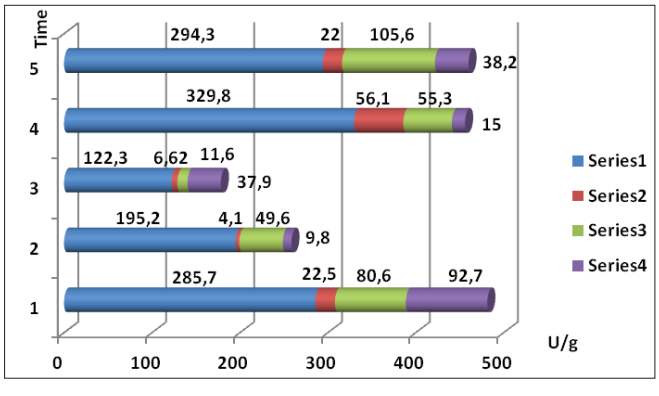

Fig. 2. Dynamics of GST activity in the saliva of children after PDT (U/g protein).

Time: 1- start; 2- in an hour; 3 – in a week; 4 – in a month; 5 – in 3 years. The series correspond to numbers of groups.

One of the defense systems of OL is lactoperoxidase system comprising lactoperoxidase, hydrogen peroxide (H_2O_2) and thiocyanate ions (SCN-), which inhibit cariogenic microflora

Table 1

PDT influence on the content of total protein in the oral fluid of children with pathology of central nervous system and high caries risk

Protein (g/L) inthesaliva	Time of determination/observation				
	1st day	1 hour	1 week	1 month	3 years
1st group	2.65 ± 0.31	2.93 ± 0.14	2.83 ± 0.16	2.82± 0.28	2.36 ± 0.37
2nd group	2.57 ± 0.17	2.39 ± 0.12	2.39 ± 0.46	2.88 ± 0.87	2.57 ± 0.17
3-d group	2.92 ± 0.32	3.27 ± 0.57	2.44 ± 0.25	2.28 ± 0.14	2.52 ± 0.45
4th control group	2.66 ± 0.16	2.93 ± 0.26	2.71 ± 0.19	3.05 ± 0.42	2.27 ± 0.08

Table 2

PDT influence on the content of SCN in the oral liquid of the children with pathology of central nervous system and high caries risk

SCN, mcmol Groups	Time of determination/observation				
	1st day	1 hour	1 week	1 month	3 years
1: mcmol/L	0.280 ± 0.032	0.110 ± 0.015	0.150 ± 0.020	0.185 ± 0.016	0.090 ± 0.028*
mcmol/g	0.102 ± 0.013	0.037 ± 0.005*	0.054 ± 0.010*	0.067 ± 0.009	0.040 ± 0.014*
2: mcmol/L	0.330 ± 0.050	0.219 ± 0.020	0.410 ± 0.056	0.308 ± 0.041	0.264 ± 0.035
mcmol/g	0.128 ± 0.017	0.092 ± 0.012	0.200 ± 0.064	0.124 ± 0.047	0.103 ± 0.012
3: mcmol/L	0.118 ± 0.098	0.217 ± 0.100	0.161 ± 0.047	0.227 ± 0.082	0.145 ± 0.045
mcmol/g	0.053 ± 0.041	0.071 ± 0.039	0.069 ± 0.025	0.102 ± 0.037	0.063 ± 0.024
4: mcmol/L	0.186 ± 0.094	0.106 ± 0.033	0.114 ± 0.063	0.114 ± 0.027	0.140 ± 0.035
mcmol/g	0.068 ± 0.032	0.040 ± 0.008	0.043 ± 0.026	0.039 ± 0.010	0.062 ± 0.015

Symbol * – $P_t < 0.05$

of the oral cavity [14]. Lactoperoxidase, using hydrogen peroxide as oxidant and thiocyanate ions (SCN-), catalyzes the reaction of formation of antimicrobial products, more actively than hydrogen peroxide, such as hypothiocyanate (OSCN-) [15]. The results of determination of the thiocyanate in OL of children after PDT are presented in table 2. In an hour after PDT thiocyanate content slightly decreased, which may indicate a partial inactivation of the lactoperoxidase system. However, subsequently the content of thiocyanate was increased in both methods of calculation and reached levels in the OL of children at their first visit before the PDT (except the first group).

Conclusion

Thus, on the basis of the obtained results, we can conclude that PDT did not have negative effects on the antioxidant systems of glutathione-glutathione S-transferase and antitoxic lactoperoxidase system, the state of which reflects the content of thiocyanate. Bacteriostatic effect of PDT is an established fact that also was confirmed by our results of reducing the protein content in the OL of children. This non-invasive and effective method is particularly important for using in children with disorders of the central nervous system, because it is safe and does not cause negative side effects that can occur with the usage of antibiotics and antiseptics.

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The influence of the tumoral cell proliferation rate in pituitary adenoma on expressing other factors with a prognostic role and therapeutic potential

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Abstract

Background: This article describes the growth rate of pituitary adenomas, their invasion and potential recurrence. Some of them grow rapidly while others remain in a dormant condition for a long time. The recurrence rate is also very hard to forecast. The pituitary adenomas that initially reduced their proliferation rate now acquire a rapid one, producing aggressive recurrences.

Materials and methods: Eighty two cases of pituitary adenomas were studied. The classification of human pituitary adenomas has been profoundly assisted by immunohistochemistry. Ki-67 antibody was used to assess the proliferative index of pituitary adenomas.

Results: Acidophil type adenomas or acidophil cells areas within mixed pituitary adenomas had the highest proliferation rate. Chromophobe pituitary adenomas were Ki67 negative in 90%, as well as the ones that presented the basophilic cells. The pituitary adenomas with a trabecular growth pattern had the lowest rate of proliferation, in about 50% of the cases being zero. The heterogeneity expression of growth factors and corresponding receptors is dependent on hormonal profile of pituitary adenomas.

Conclusion: The pituitary adenomas proliferation rate depends on the type of secreted hormone as well as on the hormonal associations met in some cases of pituitary adenomas.

Key words: pituitary adenoma, cell proliferation, Ki67.

Introduction

Pituitary adenomas are variable in growth rate, potential of invasion and recurrence. Some tumors grow fast; others remain in a dormant stage for a long time. The recurrence rate is also difficult to predict, the pituitary adenomas that initially reduced the proliferation rate acquire a fast proliferation rhythm and produce aggressive relapses. The ability to predict the proliferative potential of pituitary adenomas could have major implications for the clinical management of these tumors. Scientists have focused on identifying the predictive behaviour factors of pituitary adenomas. Ki67 proliferative index has been shown to be a useful tool in the measurement of tissue proliferation and for this reason, has been extensively studied.

Material and methods

Eighty two cases of pituitary adenomas were studied. The classification of human pituitary adenomas has been profoundly assisted by immunohistochemistry. Ki-67 antibody was used to assess the proliferative index of pituitary adenomas. Ki67 proliferation index was performed using Nikon Eclipse E600 microscope at 400X magnification assisted by a specialized cell counting computer program (NSI). The highest immunoreactivity areas were evaluated, an average of 1000 cells per case (about 3 microscopic fields taken as multimedia images) having been studied.

Results

Ki67 proliferation index in the pituitary adenomas was noted as being increased in cases with recurrences. There are no precise data on the pituitary hormones involvement in determining either the proliferation rate of pituitary adenomas or the growth factors. For this reason, the aim of the present study was to identify the potential role of pituitary hormones and growth factors, GFAP and S100 protein in influencing the pituitary adenomas proliferation rate. Proliferating pituitary adenomas represent 73.33% of all cases included in the study. Ki-67 expression was nuclear restricted the reaction intensity being variable, in most cases moderate and intense. The proliferation rate was extremely heterogeneous within this group, ranging between 4/1000 and 49/1000 (Fig. 1 a, b).

The highest proliferation rate was recorded in the acidophil type adenomas or acidophil cells areas within mixed pituitary adenomas. Pituitary adenomas of the chromophobe type were Ki67negative in 90%, like basophilc cell adenomas.

According to the growth pattern, papillary type pituitary adenomas were proliferative, the proliferation rate in most cases being greater than 20/1000.

Pituitary adenomas with trabecular growth pattern showed the lowest proliferation rate, in about 50% of cases it is zero. Pituitary adenomas with compact growth pattern had an average index of proliferation, being located between the papillary and the trabecular types. The proliferation index of compact pituitary adenomas is middle, being located between the papillary and trabecular types.

Afterwards, we analysed the pituitary adenoma proliferation rate in respect of their hormonal status. In 50% of plurihormonal pituitary adenomas there was no proliferation, the index being denoted by 0. The remaining plurihormonal type adenomas had a high proliferation index, 83.33% of which ranges between 21-41/1000.

Pure pituitary adenomas, which immunohistochemically expressed only one hormonal type were proliferating at a rate of 59% of the cases, 41% were not proliferative. Approximately 80% of GH-secreting pituitary adenomas were proliferating at a rate of proliferation medium ranges be-

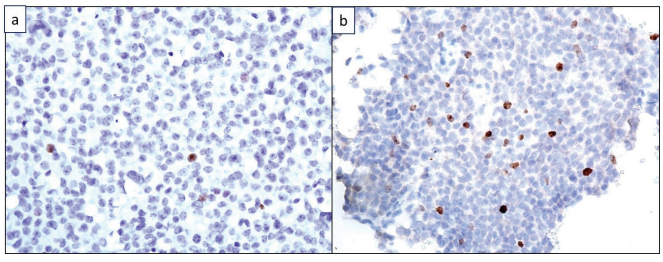

Fig. 1. Variability of proliferation of pituitary adenomas. Pituitary adenomas of low proliferation rate (a) and high proliferative rate (b).

tween 13-24 / 1000. About 80% of GH-secreting pituitary adenomas were proliferating at a medium proliferation rate that ranges between 13-24/1000.

The co-expression of LH – FSH denotes tumor cells proliferation. All LH -FSH positive cases were proliferating, but at a relatively low proliferation rate, in 60% of cases within the limits of 6-12 / 1000.

Non-secreting pituitary adenomas were proliferating in an amount of 77.8%. 57.14% of the proliferating cases had an average rate of proliferation, between 11-20 / 1000. In this study we identified other hormonal combinations, different from those specified before. Their number was reduced so that these particular types required a separate analysis. Thus, the PRL -ACTH hormonal combination was found in one case of papillary adenoma predominantly basophilic with acidophilic areas where the proliferation rate was very high, i.e. 30/1000. Another isolated case of GH-TSH hormone profile also presented a relatively high rate of proliferation of 20/1000. However, in most hormonal profile associations in which one of the hormones had been LH, the tumor cell proliferation rate was 0. In fact, it was the only hormone for which we obtained a statistically significant inverse correlation with Ki67 proliferation index taken from the Table correlation shown in figure 2.

Discussion

In 2004, the World Health Organization included a new subtype – "atypical" adenoma in the classification of pituitary adenomas. This entity has the following characteristics: a tumor proliferation index (Ki67) greater than 3%, p53 over expression and increased mitotic rate [1]. This new variant of adenoma is actually an intermediate step between conventional adenomas and pituitary carcinoma (tumor entity that represents less than 1% of primary pituitary tumors) [2]. In most published studies, the proliferative index Ki-67 is linked to the hormonal profile of adenomas, the size and the degree of invasion thereof as determined by high-resolution imaging. There is no correlation between Ki67 and architectural pattern of adenomas or its tinctorial character at the cytoplasmic level.

In his study Pawlikowski et al. [3] demonstrated that polyhormonal pituitary adenomas show higher Ki67values than the monohormonal ones, in particular those which co-express ACTH. In a previous study, the same researcher evaluated another proliferation nuclear marker – PCNA and the same results were found [4]. The lowest values of proliferation markers were recorded for non-secreting adenomas in both previous studies. Our study has shown similar results; the proliferation marker is highest in polyhormonal adenomas and lower in the case of non-secreting adenomas. On the other hand, there are studies that have shown other types of adenomas to have a higher proliferative index, such

		Ki 67	LH
Ki67	Pearson Correlation	1.000	-.247
	Sig. (1-tailed)	.	.027
	N	61	61
LH	Pearson Correlation	-.247	1.000
	Sig. (1-tailed)	.027	.
	N	61	61

			Ki 67	LH
Kendall's tau_b	Ki 67	Correlation Coefficient	1.000	-.206
		Sig. (1-tailed)	.	.023
		N	61	61
	LH	Correlation Coefficient	-.206	1.000
		Sig. (1-tailed)	.023	.
		N	61	61
Spearman's rho	Ki 67	Correlation Coefficient	1.000	-.265
		Sig. (1-tailed)	.	.020
		N	61	61
	LH	Correlation Coefficient	-.265	1.000
		Sig. (1-tailed)	.020	.
		N	61	61

Fig. 2. The statistical analysis of correlation between Ki67 proliferation index and LH is shown. An inverse correlation between the expression of LH and proliferation rate is noted.

as prolactinoma [5] or ACTH-secreting adenomas [2, 6]. Some studies suggest that prolactinoma with increased proliferation index, besides the risk of recurrence, is pituitary carcinomas precursors [6].

The data given in literature indicate low levels of proliferation marker Ki 67 in both gonadotropic hormone-secreting adenomas, and non-secreting ones [1, 6, 7].

In addition to existing data, the present study has demonstrated that polyhormonal adenomas including co-expression of LH present a low proliferation index.

Conclusions

1. The heterogeneity expression of growth factors and corresponding receptors is dependent on hormonal profile of pituitary adenomas.

2. Secreting GH and PRL pituitary adenomas are the most active in terms of synthesis and release of growth factors.

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The role of microflora in the pathogenesis of burns in different climatic conditions

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Abstract

Background: In all the patients with combustions comprising more than 50% of the body surface, bacteremia takes place, and, during the shock period, its influence is minimal, then, during the toxemia period, when the autolysis process occurs in the affected area, microflora presents a particular danger. Microflora manifests its virulence completely depending on the state of the local and general immunity, which, in its turn, differs in different climatic conditions. Therefore, in order to determine the effective treatment, it is very necessary to study the microflora in patients with combustions in different diametrically opposed climatic conditions – tropical and continental.

Material and methods: The investigations were performed in 287 patients with combustions in tropical conditions (Cuba) and in 133 patients with combustions in continental conditions (Moldova, Moscow) in order to determine the role of microflora in the evolution of combustions in different climatic conditions. The following factors were determined: the microbial type, the sensibility degree to antibiotics, the conditions of favoring microbial invasion and the role of the medical staff in favoring patients with combustions affected by the microbial invasion.

Results: The obtained results showed that the conditions of the tropical climate are very favorable for microflora evolution, and the medical staff plays a very important role in favoring the microbial invasion, in the assessment of septicemia and also in the assessment of the microbial invasions in the pathogenesis of the combustion wounds.

Conclusions: Microflora presents a very big danger in the evolution of combustions. The virulence of microflora in the conditions of the tropical climate is much higher compared to the conditions of the continental climate. The medical staff and the necessary conditions for preserving the asepsis and antisepsis play a very important role in avoiding the microbial invasion in patients with combustions, both locally, and generally.

Key words: microflora, combustion wounds, septicemia, medical staff.

Introduction

Microflora plays a special role in patients with burns. Artz C., Reiss E. (1957) affirm that microflora is one of the major problems in burn patients. A. Yakovlev (1967) considers that bacteremia develops in all patients with burns covering more than 50% of the body surface area, and while during shock its influence is minimal, during toxemia, when the autolytic process occurs in the affected area, microflora poses a particular danger. And not only in terms of bacteremia, that may occur at any time, but rather as a result of the absorption of microbial toxins from the affected area. The severely affected organs may easily decompensate following the local and general microbial invasion.

In all these cases, the microflora displays its virulence entirely depending on the local and general immune status. But

both local and general immunity manifests itself differently in different climatic conditions. In tropical conditions, capillary permeability and plasmorrhea in the affected area are increased and the temperature of the surrounding environment is optimal for microflora vegetation.

Therefore, it was necessary to study microflora in these patients at different stages of burn disease dynamic evolution under absolutely different climatic conditions – tropical and continental.

Material and methods

1147 investigations were performed in 287 patients with burns in tropical conditions and 526 investigations were performed in 133 patients in continental conditions. Microflora type, its sensibility to different local treatment remedies and antibiotics were studied. At the same time, the course of the local process, the general condition of the patient and the efficacy of the surgical interventions and blood culture were also investigated. Periodically, the microflora of the medical staff, instruments and surrounding environment were also studied.

Results

The obtained results showed that all patients had infected wounds upon hospital admission. During the first 2-3 days following the trauma, in most cases, a microbial strain was found on the burn wounds. Shortly after that, its association with other strains increased dramatically. Out of the 287 patients studied in the tropical area, in 137 (48%) of them, staphylococcal microflora was found (tab. 1), in 62 (21.7%) – *Pseudomonas aeruginosa,* in 24 (8.3%) – *Proteus vulgaris,* in 29 (10%) – *Providencia* and 35 (12%) had various associated microflora on hospital admission.

The same situation, without much difference, was found on admission of patients in the continental climate areas (table 1).

The methods of prophylaxis

In order to verify the evolution of pathological processes in burn disease excluding as much as possible the influence of microflora on these processes, in tropical conditions (Cuba), each patient (one or two per ward) was completely isolated and all the necessary conditions were created in the ward: an individual glass table with medicines, a special table for food, sterile conditioned air, individual WC in the ward, individual shower in the ward, sterile linen changed daily, additional ward preparation with quartz lamp, sterile gowns, covers and caps for medical staff daily, a metal medical dressing table sterilized by flame and alcohol and cooled with sterile saline solution after each application of dressing to a patient, and also complete isolation from relatives until patient's discharge. The contact between patients within the department was absolutely excluded. The patients who were able to move within the ward showered 2 or 3 times daily.

In continental conditions, the usual system form was applied: common ward, common dressing ward, common WC, free visitation etc.

Study results and the divergence in appreciating the role of microflora in toxemic stage

During the treatment, the performed investigations showed that: in continental conditions, 4-5-7 days after

Table 1

The microbial type and frequency identified in burn patients in different climatic conditions

Climate area	Number of patients	Microbial strain				
		Staphylococci	Pseudomonas aeruginosa	Proteus vulgaris	Providencia	Various associations
Tropical	287	137 48%	62 21.6%	24 8.3%	29 10%	35 12.1%
Continental	133	63 47.4%	22 16.5%	15 11.3%	12 9%	21 15.8%

Table 2

Microflora type and associations in burn patients in different climatic conditions

Climatic area	Number of patients	On admission	Dynamic evolution, on the 4th-5th-7th day			Among them, associated						
		Form				Staph.+ Pseud. Aerug.	Staph.+ Coli bact.	Staph.+ Proteus vulgaris	Staph.+ Provid.	Pseudom. Aerug.+ Provid.	Pseudom. Aerug.+ Proteus vulg.	Proteus vulg.+ Provid.
		Non-associated	Associated	Non-associated	Associated							
Tropical	287	252 87.8%	35 12.2%	195 67.9%	92 32.1%	18 19.5%	39 42.4%	13 14.9%	5 5.5%	12 13%	3 3.3%	2 2.1%
Continental	133	112 84.2%	21 15.8%	17 12.7%	116 87.3%	26 22.4%	34 29.3%	7 6%	11 9.5%	18 15.6%	13 11.2%	7 6%

the trauma, the microbial association in the affected area increased dramatically (Table 2). While in the tropical area in the above-mentioned conditions, microflora association increased during the treatment from 12.2% to 32.1%, in the continental area, an extremely high virulence was established and its association increased from 15.8 to 87.3%.

In tropical areas, in 67.9% of the patients only one type of microbes remains during the treatment, while, in the continental area, this fact is established only in 12.7% of the patients. The rest of the patients (87.3%) from the continental area show an associated microflora form. This microbial invasion worsened a lot the dynamic evolution of burns in patients in continental conditions as a result of many complications caused by the microflora.

The most frequent microbial invasion in the affected areas, both in tropical and in continental conditions, is the staphylococcal microflora, *Pseudomonas aeruginosa* or *Coli bact*. in different associations. *Proteus vulgaris, Providencia* and others occur less frequently.

The most dangerous manifestation of microbial virulence was established from the 5th to the 9th-12th day after the trauma in both climatic conditions, when local autolytic processes of the necrotized tissues occurred. Once they are removed, the purulent process rapidly regresses and the regeneration in the affected area is clearly improved.

In this short time period, in both climatic conditions, an aggravated general condition of the patients is observed, especially in IIIA-IIIB and IV degree burns involving more than 15-20% of the body surface area. In these patients, 4-5 days after the trauma, the body temperature rises to 39-40-41°C. Patients develop very quickly drowsiness, stupor and delirium and some of them also develop reactive psychosis. At the same time, disturbances of internal organs and systems occur.

It was established that, in tropical conditions, after the removal of necrotized tissues from the wound, the mental lucidity of the patient is recovered in 2-3 days and the function of internal organs – somewhat slower. In continental conditions, this process is much slower, the aggravated general condition may persist up to 20-30 days after the trauma. The disturbance of internal organs function persists for a longer time and it is clear that the background for skin plasty in continental conditions is much less favorable.

That speaks that the main role in the second stage of burns is attributed to toxins accumulated in tissues as a result of increased catabolism and their absorption from the affected area.

There is no doubt that the microbial toxins are also absorbed from the wound, but their role is not so important. This fact was also confirmed during our investigations in tropical conditions, where patients were completely isolated and had their sterile linen changed daily, also having baths and showers daily, and asepsis was observed to the utmost degree. Out of 1286 patients, only 5 had microflora found in their blood, which represents 0.4% of the sepsis conditions. In continental area, under extremely unfavorable conditions as described above, microflora was detected in the blood of 39 out of 363 investigated patients, representing 10.7% of the patients with sepsis condition. The microflora identified in the blood was not always the same as the one in the wound, but it always matched the hospital microflora.

Though the role of microflora in tropical conditions in the studied patients was reduced to a minimum degree, the dynamic evolution of burn disease in tropical conditions is, however, very slowly progressive. Also, both in tropical and in continental conditions, when bacteremia occurred, the reaction of the organism to the infection and to the action of toxins after the reactive "explosion" went shortly after into a state of anergia, followed by patient's death due to the development of respiratory, cardiovascular, hepatic, renal failure etc.

Some authors affirm that sepsis in patients with burns is a persistent complication, but it can be minimized by observing aseptic and antiseptic techniques.

The investigations performed by some authors confirm that the clinical manifestations of the aggravation of the cardiovascular, respiratory, hepatic, renal and other systems' functioning are identical in severe lesions in patients with sepsis and without sepsis.

As a result of the investigations performed at the medical staff in continental conditions, pathogenic microflora was detected in oral and nasal cavities in 68.2% of the cases, on hands in 52.3% cases and on personal objects in 78.7% cases, as well as in the surrounding environment. This means that hospital microflora represents a major danger. In 83.4% cases, hospital microflora was resistant to most cephalosporin antibiotics.

Microflora, both in tropical and in continental conditions, remains to be an extremely dangerous factor in the aggravation of the course of burns. It is severe not only during the preoperative period, but during the postoperative period too, when, as a result of the virulent microflora invasion, a marginal lysis of transplant occurs or even a complete lysis.

Pneumonias, abscesses, phlegmons, thrombophlebitis, pyelonephritis etc. frequently occur as a result of generalization of infection (sepsis). Sometimes, on the general background of microflora invasion, multiple microabscesses in the intact skin areas with a marked marginal inflammation occurred.

These disorders disappear once the sepsis is under control. The result of the investigations we performed confirmed that microflora invasion can be reduced to minimum, once the asepsis and antisepsis are strictly observed, which was proved by our studies in tropical conditions.

Conclusions

1. Both local and general microbial invasion is extremely dangerous in the dynamic evolution of burn disease.

2. The virulence of microflora in tropical climate conditions is much higher compared to the continental climate conditions.

3. The conditions required for maintaining the asepsis and antisepsis, described in this paper, allow avoiding the microbial invasion in patients with burns, both locally and generally.

4. At the second stage of burns, the main cause of patient's general condition aggravation is intoxication with autolytic products of the necrotized tissues from the affected area, but not microbial invasion, as it is stated by some authors.

5. The role of the living and treatment conditions of the burn patient is extremely important in avoiding both local and general microbial invasion.

6. The medical staff plays a very important role in intra-hospital microbial invasion in burn patients.

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Retroorbital pain and autonomic dysfunctions in patients with migraine

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Abstract

Background. A part of migraine patients complain of unilateral or bilateral ocular pain during migraine access, which may be associated with some vegetative disorders: ptosis, mydriasis, conjunctival congestion, photo and phonophobia, lacrimation, unvoluntary periorbital muscle contractions, nasal hypersecretion. It is important to analyze the frequency of ocular pain in patients with migraine, laterality, character and their association with other autonomic manifestations.

Material and methods: 91 patients with migraine (9.9% men, 90.1% women), out of them 51.6% with chronic migraine, 34.1% with episodic migraine and 14.3% with rare episodic migraine. Patients' age was 18-63 years. The study included only patients with migraine without other associated neurological or ocular pathology. Ophthalmologic examination included assessment of visual acuity, perimetry, intraocular pressure measurement in migraine crisis and lucid period (air-push N 10-21 mm Hg), ophthalmoscopy, biomicroscopy, refractometry if necessary.

Results: According to the statistical analysis of data, 48.4% patients had bilateral ocular pain during migraine attack; 26.3% unilateral headache and eye pain, 25.3% did not experience pain during the migraine attack. By the type of eye pain 34.1% had non-pulsating retro-orbital pain; 18.7% –pulsating retro-orbital pain; 22% - had superficial eye pain. During the migraine attack 18.7% of migraine patients had unilateral conjunctival congestion, ipsilateral of headache; 33% bilateral congestion and congestion absent in 48.4% patients. Unilateral lacrimation – 11.4%, bilateral lacrimation in – 25.6%. Photophobia between attacks of migraine accounted for 38.6% of patients. Unilateral ptosis – 8.4% during the attack, bilateral ptosis – 10.8%. Periorbital muscle tics during the migraine attacks were observed in 42.2% patients. We found a statistically significant correlation ($P < 0.001$) between the type of eye pain and intraocular pressure values measured during the migraine attacks and between them.

Conclusions: Migraine attacks are often associated with different character of ocular pain, autonomic disorders and with increasing of intraocular pressure.

Key words: ocular pain, migraine.

Caracterul durerilor retroorbitale şi tulburărilor vegetative la pacienţii cu migrenă

Introducere

O parte dintre pacienţii cu migrenă acuză dureri oculare uni- sau bilaterale în timpul crizei migrenoase, care se pot asocia cu unele tulburări vegetative: ptoză, midriază, congestie conjunctivală, foto- şi fonofobie, lăcrimare, contracţii involuntare ale musculaturii periorbitale, hipersecreţie nazală [1, 2, 5]. Durerile oculare pot fi uni- sau bilaterale, independent de localizarea cefaleei şi se deosebesc după caracterul durerii şi intensitatea acesteia. Varietatea senzaţiilor oculare,

descrise de către pacienți ca „dureri", se pot diviza în 2 grupuri: dureri retrobulbare, de caracter pulsatil sau nepulsatil, caracterizate de către pacienți ca „dureri în fundul ochilor"; dureri la suprafața ochiului, caracterizate ca senzații de "frigere" a regiunii pleoapelor și suprafeței anterioare a globului ocular [3, 4].

Obiective: analiza frecvenței durerii oculare la pacienții cu migrenă, lateralitatea durerii oculare, caracterul ei și asocierea cu alte manifestări vegetative. Compararea valorilor tensiunii intraoculare în dependență de tipul durerii oculare și intensitatea cefaleei.

Material și metode

În cadrul Institutului de Neurologie și Neurochirurgie, au fost examinați oftalmologic 91 de pacienți cu migrenă (9,9% bărbați; 90,1% femei) dintre care 51,6% cu migrenă cronică, 34,1% cu migrenă episodică frecventă și 14,3% cu migrenă episodică rară. Vârsta pacienților 18 – 63 de ani. În studiu au fost incluși doar pacienții cu migrenă, fără altă patologie oculară sau neurologică asociată. Pacienții au fost interogați conform chestionarului despre durata cefaleei, intensitatea, lateralitatea, caracterul acesteia, prezența unor tulburări vegetative asociate cefaleei [1]. Examenul oftalmologic a inclus aprecierea acuității vizuale, perimetria, măsurarea tensiunii intraoculare (TIO) în criză migrenoasă și perioada lucidă (air-push N = 10-21 mm Hg), oftalmoscopia, biomicroscopia, refractometria la necesitate [6].

Rezultate și discuții

Conform analizei statistice a datelor 48,4% pacienți au prezentat dureri oculare bilaterale în timpul crizei migrenoase; 26,3% dureri oculare unilaterale ipsilaterale cefaleei și 25,3% nu acuzau dureri oculare în timpul crizei migrenoase. După tipul de dureri oculare, 34,1% au prezentat dureri retroorbitale nepulsatile; 18,7% – dureri retroorbitale pulsatile; 22% – dureri superficiale. În timpul crizei migrenoase, 18,7% pacienți au prezentat congestie conjunctivală unilaterală, ipsilaterală cefaleei; congestie bilaterală – la 33% și congestie absentă – la 48,4% pacienți. Lacrimație unilaterală – 11,4%, bilaterală – 25,6%. Fotofobie în timpul crizei migrenoase – la 82%, fotofobie între accesele migrenoase au acuzat 38,6% pacienți. Ptoză unilaterală în timpul crizei migrenoase -8,4%, ptoză bilaterală – 10,8%. Ticuri ale musculaturii periorbitale în timpul crizei migrenoase – 42,2% pacienți.

Prezența fenomenelor vegetative periferice unilaterale este, probabil, condiționată de activarea reflexului trigemino-vegetativ sub influența unei stimulări excesive a căilor trigeminale aferente [2, 5, 6]. Acest reflex constă din conexiuni funcționale între aferențele trigeminale și eferențele parasimpatice, care părăsesc trunchiul cerebral prin *n.cranian* VII, traversează ganglionul geniculat și efectuează sinapse în ganglionii sfenopalatin, otic și carotid, asigurând astfel inervarea secretomotorie a unor structuri precum glandele lacrimale și mucoasa nazală [2, 3, 8].

Ochiul și orbita au o inervație vegetativă abundentă, fiind în interferență cu ramurile nervului trigemen. Neuronii ganglionului trigeminal sunt atât senzitivi cât și efectori. Când sunt activați de stimuli nociceptivi, ei eliberează mediatori inclusiv peptida asociată genei calcitonina și oxidul nitric. Acest *feedback* pozitiv stă la baza reflexului *trigeminal-vascular*, prin care survine o dilatare a vaselor craniene după un stimul nociceptiv, cauzând durerea retroorbitală [2, 7].

Reflexul *trigeminal-autonom* este multisinaptic, implicând activarea nucleelor salivator superior și *Edinger-Westphal* de către colateralele nucleului trigeminal caudal. Fibrele nucleului salivator superior activează efectorii parasimpatici în ganglionul pterigopalatin, ceea ce produce vasodilatare și în ganglionul ciliar și provoacă lacrimație [5, 7].

Fibrele nucleului *Edinger-Westphal* mediază constricția pupilară. Reflexele trigemino-vascular și trigeminal-autonom stau la baza congestiei conjunctivale, lacrimației și durerii periorbitale în migrenă și cefalee *cluster* care, de regulă, sunt associate cu fotofobia [8].

Mai mult de 80% dintre pacienții cu migrenă suferă de fotofobie în timpul atacului. Un studiu recent relevă o pondere a fotofobiei de 98% [10].

Drummond în studiul său arată, că pacienții cu migrenă sunt mai sensibili la lumină, atât în perioada atacului migrenos, cât și între atacuri în comparație cu persoanele ce nu suferă de migrenă [11].

Vanagaite et al. au examinat paienții cu migrenă ce suferă de fotofobie și au ajuns la concluzia că fotofobia „pare să fie o proprietate intrinsecă a pacienților cu migrenă" și este asociată cu disfuncția căilor vizuale de la nivelul retinei până la lobul occipital [12]. Diverși stimuli vizuali pot provoca atacul migrenos: lumina solară, blicurile și reflecțiile emise de televizor și computer, lumina fluorescentă etc, 30–60% din atacurile migrenoase au fost provocate de lumină puternică sau sclipiri [11, 12].

Migrena nu este unicul tip de cefalee asociat cu fotofobia. Subiecții cu cefalee de tensiune, de asemenea, sunt mai sensibili la lumină decât grupul de referință cu subiecți sănătoși [12]. Fotofobia unilaterală este caracteristică penru cefaleea *cluster*, hemicrania continuă, și alte tipuri de cefalee trigeminal-autonomă [13, 14].

Orbita are și o inervație simpatică abundentă. Nervii ciliari scurți includ fibre simpatice pentru vasele orbitei și nervii ciliari lungi – pentru inervația pupilei. În timpul atacului migrenos are loc o disfuncție simpatică asociată de mioză și ptoză. Vasodilatația intensă la nivelul tunicii vasculare a globului ocular produce creșterea intermitentă a tensiunii intraoculare în timpul atacului migrenos [5, 7]. La pacienții cu durere oculară în timpul crizei migrenoase, TIO medie OU = 18,5 mm Hg, pe când la pacienții, care nu acuzau durere oculară, TIO medie OU = 14,0 mm Hg ($p < 0,0001$) (tab. 1). Pacienții care prezentau durere retrobulbară pulsatilă sau nepulsatilă în timpul atacului migrenos, au prezentat valori mai mari ale TIO decât pacienții fără dureri sau dureri superficiale cu caracter de frigere (tab. 1).

Reglarea tensiunii oculare are loc prin mecanisme locale, însă este dependentă de sistemul nervos simpatic și parasim-

Tabelul 1

Valorile TIO în dependență de tipul durerii oculare

	Tipul durerii	TIO în criză	TIO între crize
1	Durere oculară superficială	14,2 mmHg	13,7 mmHg
2	Durere retrobulbară pulsatilă	20,5 mmHg	19,7 mmHg
3	Durere retrobulbară nepulsatilă	20,3 mmHg	19,2 mmHg
4	Absența durerii oculare	14,03 mmHg	13,8 mmHg
5	Durere oculară unilaterală	20,1 mmHg	19,0 mmHg
6	Durere oculară bilaterală	17,7 mmHg	17,0 mmHg

p1/p2 < 0,0001 p1/p3 < 0,0001 p2/p4 < 0,0001
p3/p4 < 0,0001 p5/p6 < 0,005

patic prin intermediul nervilor ciliari lungi și scurți. Excitația simpaticului cervical provoacă o scădere a tensiunii intraoculare, iar excitația parasimpaticului provoacă o hipotonie cu scăderea de scurgere a umorii apoase. Datorită contracției mușchiului ciliar, care trece prin pintenul scleral și lărgește porii trabeculari, excitația trigemenului produce o creștere rapidă a tensiunii oculare asociată cu mioză.

Concluzii

1. Durerile și discomfortul ocular sunt destul de frecvente la pacienții cu migrenă și pot fi uni- sau bilaterale.

2. Manifestările vegetative cum ar fi lacrimația, congestia conjunctivală, ptoza, fotofobia în timpul și între crizele migrenoase pot fi uni- sau bilaterale și se asociază frecvent durerilor oculare.

3. Accesul de migrenă provoacă creșterea tensiunii intraoculare în migrena asociată cu dureri retrobulbare (pulsatile și nepulsatile).

4. Tensiunea intraoculară, în migrena asociată cu dureri retrobulbare, este mai înaltă decât în cea asociată cu dureri oculare superficiale și fără dureri oculare.

5. TIO în migrena asociată cu dureri oculare unilaterale este mai înaltă decât în cea cu dureri oculare bilaterale.

6. Toate modificările TIO în criză și între crize sunt în limitele normei (N = 10-21mmHg).

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Clinical and immunological predictive factors for antituberculosis pulmonary treatment failure

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Abstract

Background: The Rrepublic of Moldova reports the biggest incidence of tuberculosis and the lowest success treatment rate among European region countries. In the most of patients the antituberculosis treatment failure is correlated with social risk factors (social, economical low status, social-epidemiological category of the population) and biological (young age, male sex, some physiological conditions, associated diseases). Clinical factors (extensive forms, chronic evolution, immune disturbances), therapeutical factors (treatment errors and interruptions, individualised regimens) and administrative factors (interrupted supplying, suboptimal drug quality) prevail in regions with defficiencies in heath care delivery. Risk factors association is more evident than the severity of one risk factor. Clinical and immunological assessement is important before initiation of the treatment for establishing risk reduction measures and increasing success rate.

Material and methods: The study was conducted on 201 cases with treatment failure and 105 with successfuly ended treatment. Cases were investigated according to national standards and local specific immune procedures.

Results: High clinical risk factors were revealed: late detection, extensive and bilaterally localised tuberculosis, lung tissue destructions and dissemination, complications, comorbidities; as well as high immune risk factors were established: low cellular resistance, high degree of intoxication, low preimmune resistance.

Conclusions: The study at least of high clinical and immune risk factors must be performed before the treatment initiation for increasing the treatment success rate.

Key words: tuberculosis, immune reactivity, treatment, failure, risk factors.

Factorii clinici şi imunologici predictivi ai eşecului tratamentului tuberculozei pulmonare

Introducere

Tuberculoza reprezintă o provocare pentru sistemul sănătăţii publice a oricărui stat. Conform ultimului raport al OMS pentru anul 2011, au fost estimate 12 milioane de cazuri de TB, corespunzător unei prevalenţe de 178/100 000 populaţie şi au fost înregistrate 1,1 milioane de decese, 455 000 fiind HIV pozitive [4]. Majorarea continuă şi rapidă a incidenţei TB a început în anii 1990, odată cu debutul crizei socio-economice şi reducerii drastice a examenelor radiologice profilactice, realizate întregii populaţii. În 2001, a fost aprobat Programul Naţional de Control al Tuberculozei (PNCT), care a început printr-un proiect pilot în mun. Chişinău şi a fost extins pe întreaga ţară în 2005 [2]. La baza PNCT stau 2 principii: depistarea a cel puţin 70% din cazurile noi prin microscopia sputei şi atingerea ratei de 85% a succesului terapeutic. În pofida tuturor intervenţiilor realizate de Guvern, Ministerul Sănătăţii, nici unul din obiective nu a fost atins [4]. Astfel, o treime dintre cazurile depistate revin celor mai contagioase forme şi anume: cota parte de 37,4% revine cazurilor cu microscopia sputei pozitivă, iar cota de 38,0% – cazurilor cu forme distructive ale parenchimului pulmonar (înregistrate 2011), iar ratele succesului terapeutic se menţin constant la cele mai mici valori înregistrate în Regiunea Europeană (rata maximă în 2006-62%, de atunci având tendinţă descendentă de 57,8%-2008, 57,3%-2009, 52,3%-2010, 53,4%-2011). Indicatorii direct corelaţi cu rata redusă a succesului terapeutic sunt ratele înalte ale eşecului terapeutic: 2001 – 18,5%, 2002 – 10,4%, 2003 – 12,1%, 2004 – 12,4%, 2005 – 10,9%, 2006 – 10,9%, 2007 – 9,2%, 2008 – 7,4%, 2009 – 6,2%, 2010 – 19,6%, 2011 – 3,4% şi abandonului 2011 – 7,7% [1]. Cauzele ratei crescute ale eşecului terapeutic includ mai mulţi factori, clasificaţi în: factori biologici şi clinici, factori imunogenetici, factori terapeutici şi farmacologici, factori administrativi, factori social-epidemiologici [2].

Factorii biologici şi clinici sunt: vârsta cu risc ftiziogen maxim, sexul masculin, anumite stări fiziologice, bolile asociate procesului specific [5]. S-a raportat că vârsta tânără conferă risc mare pentru dezvoltarea eşecului datorită particularităţilor socio-economice agravante la 90% dintre bolnavii tineri: neîncadrarea în câmpul muncii, migraţia masivă, populaţia tânără numeroasă din penitenciare. Anumite stări fiziologice, asociate vârstei reproductive la femei, precum sarcina şi lactaţia, sunt factori de risc pentru eşec la gravida bolnavă de TB. Studiile de complianţă terapeutică relatează despre frecventele întreruperi ale tratamentului de către gravidă, din considerente că este toxic pentru făt, nounăscut şi din cauza stigmatizării femeii bolnave de TB [2]. Anumite co-morbidităţi cum ar fi: sindromul imunodeficienţei dobândite prin infecţia HIV şi patologiile sistemului limfoganglionar (limfomul Hodgkin şi non-Hodgkin), sau secundar tratatamentului cronic cu corticosteroizi şi imunomodulatoare (blocantele TNF-αα), bolile respiratorii nespecifice cronice, patologiile aparatului digestiv, patologiile infecţioase cronice hepatice, diabetul zaharat (tip I/II), bolile renale cronice, patologiile sistemului nervos central şi patologiile psihiatrice sunt factori contributivi ai eşecului. Aceste patologii scad reactivitatea imună şi rezistenţa nespecifică,

scad concentraţia şi biovalabilitatea medicamentelor, cresc rata efectelor adverse, determină întreruperi de tratament şi individualizarea tratamentului, predispune aderenţei reduse şi în consecinţă conferă risc crescut eşecului terapeutic [8].

Factorii clinici aparţin particularităţilor clinico-radiologice ale bolii: formele clinice extinse, cu afectarea a mai mult de 3 segmente pulmonare, diseminarea extinsă sau generalizată a infecţiei, asocierea complicaţiilor cu caracter de urgenţă (hemoptizii, pleurezie, pneumotorax, hidropneumotorax).

Imunitatea mediată celular, definită ca hipersensibilizare întârziată, este baza răspunsului imun în infecţia tuberculoasă şi determină particularităţile patogenezei, tabloului clinic şi evoluţiei bolii [6]. S-a determinat că evoluţia acut progresivă a TB, cu distrucţii parenchimatoase extinse şi multiple focare de diseminare, au rezultate scăzute ale tratamentului şi în majoritatea cazurilor sunt determinate de tulburările sistemului imun: deficitul răspunsului imun celular prin limfopenia limfocitelor T, deficitul subpopulatiilor CD4, CD8, CD72, inversarea raportului limfocitelor T helper/T supresor, creşterea numărului monocitelor, hiperactivitatea limfocitelor B, eliberarea exagerată a enzimelor proteolitice, kininelor, prostaglandinelor, peptidelor vasoactive, care duc la progresia procesului tuberculos, apariţia distrucţiilor masive parenchimatoase şi eşecul terapeutic [9]. Asocierea factorilor de risc, conferă un risc mai mare, decât severitatea unui singur factor de risc. Studiul factorilor predictivi ai eşecului este important înaintea iniţierii tratamentului fiecărui pacient cu TB, pentru iniţierea măsurilor de reducere a intensităţii riscurilor şi creşterea eficacităţii terapeutice.

Scopul cercetării: evaluarea factorilor clinici şi imunologici predictivi eşecului în tratamentul tuberculozei pulmonare.

Obiectivele: 1) Studierea caracteristicilor generale, particularităţilor de depistare, a manisfestărilor clinice şi aspectelor radiologice ale bolnavilor de TB pulmonară cu eşec terapeutic. 2) Evaluarea statutului imun al bolnavilor de TB pulmonară cu eşec terapeutic în dependenţă de administrarea tratamentului antituberculos şi imunocorector. 3) Stabilirea factorilor predictivi ai dezvoltării eşecului terapeutic.

Material şi metode

Lucrarea a fost compartimentată într-un studiu clinic şi imunologic. Studiul clinic a fost selectiv, descriptiv şi retrospectiv, de tip caz-control, efectuat în baza a 201 cazuri noi de TB pulmonară cu eşec terapeutic şi 105 cazuri noi de TB pulmonară, care au finalizat cu succes tratamentul antituberculos, înregistrate şi tratate în cadrul IMSP Spitalul Municipal de Ftiziopneumologie. Cazurile au fost investigate conform Protocolului Clinic Naţional-123 „Tuberculoza la adult".

Studiul imunologic a inclus 88 de cazuri noi de TB pulmonară, distribuiţi în eşantionul de studiu, format din 54 de bolnavi, care în cursul tratamentului antituberculos au dezvoltat eşec terapeutic şi eşantionul de control, format din 34 de bolnavi, care au finalizat cu succes tratamentul. Pentru comparaţie, a fost utilizat un eşantion martor, format din 50 de persoane sănătoase. Investigaţiile imunologice au fost efectuate înaintea iniţierii tratamentului anti-tuberculos şi la finele fazei intensive de tratament, conform procedurilor adoptate în cadrul Laboratorului de Imunologie şi Alergologie a IFP „Chiril Draganiuc". Tehnicile şi indicatorii imuni investigaţi: reacţia de transformare blastică a limfocitelor la fitohemaglutinină (PHA), la antigenele micobacteriene (tuberculina), stafilococului, streptococului şi pneumococului, s-a utilizat pentru caracterizarea activităţii funcţionale a limfocitelor T şi a sensibilizării celulare specifice. Reacţia de formare a rozetelor s-a aplicat pentru estimarea cantitativă a conţinutul limfocitelor T şi B. Evaluarea titrului anticorpilor şi imunoglobulinelor s-a efectuat prin analiza imunofermentativă pe suport solid. Activitatea fagocitară a neutrofilelor s-a evaluat cu ajutorul testului reducerii nitro-blue-tetrazolium [7].

Indicele leucocitar de alergizare (ILA) a fost calculat conform formulei 1:

$$\mathbf{ILA = \frac{MIE + CP + NT + NN + NS}{(L + M)\ x\ (E + B + 1)}, (1)}$$

unde: MIE-mielocite, CP-celule plasmatice, NT-neutrofile tinere, NN-neutrofile nesegmentate, NS-neutrofile segmentate, L-limfocite, M-monocite, E-eozinofile, B-bazofile. La cei sănătoşi ILA = 0,96 UC, cu (IÎ: 0,55-1,39 (±± 1 ES), ILA < 0,05 semnifică alergizare.

Indicele Leucocitar de Intoxicaţie *Я.Я. Кальф-Калиф* (ILI_K) s-a calculat conform formulei 2:

$$\mathbf{ILI_K = \frac{(4MIE + 3T + 2N + S) + (CP + 1)}{(L + M)\ x\ (E + 1)}, (2)}$$

unde, MIE-mielocite, T-neutrofile tinere, N-neutrofile nesegmentate, S-neutrofile segmentate, CP-celule plasmatice, L-limfocite, M-monocite, E-eozinofile, B-bazofile. Valorile normale sunt de 0,62 ± 0,083 UC.

Rezultate şi discuţii

Particularităţi generale, clinice şi radiologice ale bolnavilor cu eşec tarepeutic

Distribuţia pacienţilor conform tipului de personal medical implicat, cu rol major în depistarea cazului nou de TB am apreciat, că majoritatea covârşitoare, 193 (96,02%) bolnavi ai eşantionului de studiu au fost depistaţi de medicul de familie, iar 8 (3,98%) bolnavi – de către specialişti. În eşantionul de control 94 (89,52%) pacienţi au fost depistaţi de medicul de familie, iar 11 (10,48%) – de către specialişti. Comparând datele obţinute la analiza celor două eşantioane, s-a stabilit, că depistarea cazului nou de TB de către medicul de familie a predominat semnificativ în eşantionul de studiu 193 (96,02%) *vs* 94 (89,52%), ($p < 0,05$).

Durata trenantă a simptomatologiei mai mult de 3 luni până la adresarea la medic, a caracterizat concludent bolnavii eşantionului, care au evoluat spre eşec, 113 (56,21%) *vs* 34 (32,38%), ($p < 0,01$) şi demonstrează adresabilitatea întârziată la serviciile specializate. Apreciind datele anamnezice şi ale examenului clinic am constatat, că toate componentele

clinice ale sindromului de intoxicație au predominat concludent la bolnavii evoluați cu eșec: astenia – la 199 (99%) *vs* 97 (92,38%) pacienți (p < 0,05), scăderea în greutate – la 193 (96,02%) *vs* 81 (77,14%) pacienți, (p < 0,001), inapetența – la 194 (96,51%) *vs* 82 (78,09%) pacienți, (p < 0,001), transpirațiile nocturne – la 189 (94,03%) *vs* 66 (62,86%) pacienți, (p < 0,001), febra vesperală – la 109 (54,23%) *vs* 34 (32,38%), (p < 0,001). Câteva semne clinice ale sindromului bronhopulmonar, au predominat în eșantionul evoluat spre eșec: tuse – la 199 (99,0%) *vs* 95 (90,47%) pacienți (p < 0,01), expectorațiile – la 196 (97,51%) *vs* 87 (82,86%) pacienți (p < 0,001), durerea toracică – la 43 (21,39%) *vs* 10 (9,52%) pacienți (p < 0,01). Deci, bolnavii în stare generală alterată, cu numeroase acuze vor evolua mai frecvent cu eșec, ceea ce demonstrează ținta măsurilor pentru optimizarea rezultatului terapeutic.

Bolile concomitente tuberculozei s-au diagnosticat mai frecvent la bolnavii eșantionului de studiu, respectiv 143 (71,14%) *vs* 51 (48,57%), (p < 0,001). Totuși, evaluând diagnozele, nu am constatat vreo veridicitate statistică la repartizarea bolilor asociate între eșantioane. Astfel, în eșantionul evoluat spre eșec, am constatat că 51 (35,66%) pacienți au prezentat boli asociate: patologii ale tractului gastrointestinal, dintre care 22 (15,38%) pacienți au avut ulcer gastroduodenal/gastrită cronică/rezecție gastrică, iar 27 (18,89%) – boli cronice hepatice. Boli cronice respiratorii nespecifice s-au diagnosticat la 23 (16,08%) pacienți, fiind asociate tabagismului activ. S-au determinat 4 pacienți cu boli renale cronice. Diabetul zaharat s-a depistat la 14 (9,79%) pacienți. Boli ale sistemului nervos central, inclusiv polineuropatii și stări post-traumatism cerebral s-au diagnosticat la 13 (9,09%) pacienți. Bolnavi cu alcoolism cronic au fost 12 (8,40%). Infecția HIV s-a confirmat la 8 (5,59%) bolnavi, toți în stadiul clinic C3. Un bolnav (0,5%) avea concomitent limfomul Hodgkin. Două bolnave (0,95%) în stări fiziologice au fost: o pacientă cu sarcină la notificare și o lăuză. În categoria „alte boli" s-au apreciat 15 bolnavi: 5 (2,48%) – cu patologie cronică a urechii medii, 3 (1,49%) – boli ale aparatului locomotor, 3 (1,49%) paciente – boli genitale, 3 (1,49%) – boli ale aparatului cardiovascular, 1 (0,5%) pacientă cu cataractă.

În eșantionul control, din cei 51 (48,57%) pacienți, cu comorbidități, 13 (25,49%) pacienți au avut boli asociate patologiilor tractului gastro-intestinal (7 pacienți cu ulcer gastro-duodenal/gastrită cronică/rezecție gastrică și 6 cu boli cronice hepatice), 13 (25,49%) bolnavi – patologii cronice respiratorii nespecifice. Diabetul zaharat s-a diagnosticat la 5 (9,80%) și boli ale sistemului nervos central la 7 (13,72%) pacienți. Infecția HIV s-a depistat la 2 (3,92%) bolnavi, toți în stadiul clinic C3. S-au determinat 3 (5,88%) bolnavi cu pielonefrită cronică și 3 (5,88%) bolnavi cu alcoolism cronic. Comparând statistic eșantioanele, s-a stabilit că bolile aparatului gastrointestinal și alcoolismul cronic au predominat nesemnificativ în eșantionul evoluat spre eșec, iar bolile cronice nespecifice ale aparatului respirator au predominat nesemnificativ în eșantionul, care a finalizat tratamentul cu succes. Celelalte boli au fost aproximativ identic repartizate la bolnavii eșantioanelor cercetate.

Explorările radiologice obligatorii au permis stabilirea următoarelor diagnostice clinico-radiologice: majoritatea bolnavilor din ambele eșantioane au fost diagnosticați cu tuberculoză infiltrativă, 179 (88,05%) *vs* 85 (80,95%), particularitate specifică epidemiologiei actuale a tuberculozei, totuși tuberculoza fibrocavitară s-a diagnosticat doar la bolnavii, care au evoluat cu eșec 6 (2,99%) cazuri, iar tuberculoza nodulară a predominat semnificativ în eșantionul de control 8 (7,61%) *vs* 1 (0,95%), (p < 0,01). Localizarea tuberculozei în ambii plămâni și procesul tuberculos extins a predominat semnificativ în eșantionul de studiu 163 (81,10%) *vs* 36 (34,29%), (p < 0,01), respectiv 187 (93,03%) *vs* 58 (55,24%), (p = 0). Toate fazele evolutive, care conferă gravitate – distrucțiile și diseminația pulmonară, au predominat semnificativ la bolnavii eșantionului de studiu 144 (71,64%) *vs* 49 (47,14%), (p < 0,001) și, respectiv, 135 (67,16%) *vs* 34 (32,31%), (p < 0,001). Concomitent, distrucții masive și pneumonia cazeoasă s-au identificat doar în acest eșantion: 7 pacienți (3,48%) cu pneumonie și, respectiv, 20 (9,95%) pacienți.

Sinteza studiului clinic a confirmat, că bolnavul care a evoluat cu eșec terapeutic, a fost depistat mai frecvent de către medicul de familie, pe cale pasivă, tardiv, prin examenul suspectului simptomatic, cu simptomatologia sindromului de intoxicație tuberculoasă și sindromului bronho-pulmonar bine exprimat, evoluând mai mult de 1 lună, agravat de boli concomitente. Studiul radiologic a conturat ponderea mai mare a bolnavilor cu proces tuberculos sever, bilateral și extins, agravat de distrucții și diseminație bronho-pulmonară în eșantionul de studiu.

Studiul răspunsului imun în dependență de administrarea tratamentului antituberculos

Imunitatea celulară a fost apreciată prin prisma activității funcționale a limfocitelor T, a ponderii procentuale a limfocitelor T și subpopulațiilor acestora (limfocitele T helper și limfocitele T supresor) și a ponderii procentuale a limfocitelor B, ceea ce caracterizează asociat și imunitatea umorală. Activitatea funcțională a limfocitelor T, evaluate prin reacția de transformare blastică a limfocitelor cu fitohemaglutinină (RBTL cu PHA) până la tratament, la bolnavii eșantionului de control (EC) a fost semnificativ statistic redusă (t = 8,57; p < 0,001) față de același indicator evaluat după tratament. Acest indicator a avut un nivel mai redus în comparație cu cei sănătoși (t = 13,4; p < 0,001). Indicatorul activității funcționale a limfocitelor T în eșantionul de studiu (ES), a fost semnificativ mai redus și comparativ cu bolnavii eșantionului de control (t = 4,86; p < 0,001). După tratament, RBTL în ambele eșantioane a crescut, însă mai mult - la bolnavii eșantionului de control (t = 3,03; p < 0,01 pentru EC și t = 2,25; p < 0,05 pentru ES). După tratament activitatea funcțională a limfocitelor la bolnavii eșantionului de studiu, a rămas semnificativ mai redusă decât la bolnavii eșantionului de control (t = 4,24; p < 0,001). Valorile sunt oglindite în tabelul 1.

Tabelul 1

Caracteristica imunității celulare (M ± m)

Indicatori	Sănătoși	Eșantion control		Eșantion studiu	
		Înainte	După	Înainte	După
RTBL cu PHA	79,9 ± 1,16	65,1 ± 1,23●	68,9 ± 0,18♦	56,3 ± 1,33●	61,7 ± 1,21♦
Limfocite T %	60,2 ± 0,75	63,3 ± 1,24●	68,5 ± 1,52♦	52,2 ± 0,94●	56,6 ± 0,78♦
Limfocite Th %	43,7 ± 0,85	42,3 ± 1,20	44,1 ± 1,39	34,1 ± 0,81●	36,7 ± 0,73♦
Limfocite Ts %	16,6 ± 0,72	20,9 ± 0,83●	24,4 ± 1,23♦	18,1 ± 0,64	19,8 ± 0,55♦
Limfocite B %	24,9 ± 0,70	26,8 ± 0,34●	23,5 ± 0,51♦	28,8 ± 0,71●	26,1 ± 0,58♦

Notă: ● – diferență statistic semnificativă în comparație cu eșantionul persoanelor sănătoase.

♦ – diferență statistic semnificativă între subeșantioane, înainte și după tratament.

Cantitatea limfocitelor T până la tratament, la bolnavii eșantionului de control, a fost semnificativ mai mare, decât la cei sănătoși (t = 2,1; p < 0,05), iar la bolnavii eșantionului de studiu a fost semnificativ mai mic decât la cei sănătoși (t = 6,65; p < 0,001). După tratament, cantitatea limfocitelor T în ambele eșantioane a crescut, însă semnificativ – la bolnavii eșantionului de control (t = 3,58; p < 0,001 pentru EC și t = 2,68; p < 0,01 pentru ES). Dar și după tratament, cantitatea limfocitelor T în eșantionul de studiu a fost mai redusă decât în eșantionul de control (t = 6,97; p < 0,001).

Cantitatea limfocitelor T helper până la tratament, la bolnavii eșantionului de control, a fost aproximativ similară cu cea a eșantionului sănătoșilor, iar la bolnavii eșantionului de studiu a fost semnificativ mai mică, comparativ cu cei sănătoși (t = 8,18; p < 0,001). După tratament, cantitatea limfocitelor T helper nu s-a modificat semnificativ, însă la bolnavii eșantionului de studiu a crescut semnificativ (t = 2,39; p < 0,01). Totuși, chiar și după tratament, cantitatea limfocitelor T helper în eșantionul de studiu a rămas mai mică, decât la bolnavii eșantionului de control (t = 4,71; p < 0,001).

Până la tratament, cantitatea limfocitelor T supresor la bolnavii eșantionului de control, a fost semnficativ mai mare decât la cei sănătoși (t = 3,9; p < 0,001) și semnificativ mai mare decât la bolnavii eșantionului de studiu (t = 2,72; p < 0,01). Cantitatea limfocitelor T supresor la cei sănătoși, comparativ cu bolnavii eșantionului de studiu, nu s-a diferențiat. După tratament, cantitatea limfoctelor T supresor în ambele eșantioane a crescut semnficativ, însă mai important la bolnavii eșantionului de control (t = 2,33; p < 0,05 pentru EC și t = 2,07; p < 0,01 pentru ES). Dar și după tratament, cantitatea limfocitelor T supresor în eșantionul de studiu a rămas semnficativ mai redusă decât la bolnavii eșantionului de control (t = 3,37; p < 0,01). Aceste rezultate au demonstrat un deficit mai sever și rigid al reactivității limfocitelor T în eșantionul de studiu și o reducere mai puțin importantă a reactivității și cantității limfocitelor T la bolnavii eșantionului de control.

Cantitatea limfocitelor B până la tratament în ambele eșantioane a fost semnficativ mai mare, comparativ cu cei sănătoși (t = 2,4; p < 0,05 pentru EC și t = 3,9; p < 0,01 pentru ES). După tratament, cantitatea limfocitelor B s-a redus, însă mai important în eșantionul control (t = 5,4; p < 0,001 pentru EC și t = 2,96; p < 0,01 pentru ES). Concomitent, cantitatea limfocitlor B la bolnavii eșantionului de control a fost semnficativ mai redusă, decât la bolnavii eșantionului de studiu, atât până la tratament, cât și după (t = 2,51; p < 0,05 până la tratament și t = 3,36; p < 0,01 după tratament). Aceste rezultate, demonstrează activarea mai importantă a imunității limfocitelor B la bolnavii eșantionului de studiu.

Nivelul seric al IgG în ambele eșantioane, până la tratament, a fost semnificativ mai mare decât la cei sănătoși, de asemenea s-a constatat mai mare la bolnavii eșantionului de studiu, comparativ cu indicatorul eșantionului de control (t = 11,0; p < 0,001 pentru EC și t = 14,0 p < 0,001 pentru ES). După tratament, s-a determinat reducerea semnficativă în ambele eșantioane a cantității IgG (t = 2,48; p < 0,01 pentru EC și t = 2,41 p < 0,01 pentru ES). Totuși, chiar și după tratament, nivelul seric al IgG în eșantionul de studiu s-a constatat a fi mai mare, decât la bolnavii eșantionului de control (t = 2,48; p < 0,05).

Nivelul seric al IgA în ambele eșantoane, până la tratament a fost semnificativ mai mare decât la cei sănătoși, de asemenea, s-a constatat a fi mai mare la bolnavii eșantionului de studiu, comparativ cu bolnavii eșantionului de control (t = 4,0; p < 0,001 pentru EC și t = 5,8 p < 0,001 pentru ES). După tratament, în ambele eșantioane, s-a determnat reducerea semnficativă IgA (t = 3,58; p < 0,001 pentru EC și t = 2,41 p < 0,01 pentru ES). De asemenea, chiar și după tratament, nivelul seric al IgA în eșantionul de studiu, a rămas semnficativ mai mare, decât în eșantionul de control (t = 3,55; p < 0,001).

Nivelul seric al IgM în eșantionul de control, până la tratament, nu s-a deosebit de același indicator al celor sănătoși, iar la bolnavii eșantionului de studiu, nivelul seric al IgM a fost semnficativ mai mare, comparativ cu cel al sănătoșilor (t = 6,4; p < 0,001 pentru ES). După tratament, în ambele eșantioane s-a determinat reducerea semnificativă a nivelului seric al IgM (t = 3,19; p < 0,01 pentru EC și t = 2,11 p < 0,05 pentru ES). De asemenea, nivelul seric al IgM la bolnavii eșantionului de control a fost semnificativ mai redusă, decât la bolnavii eșantionului de studiu, atât până la tratament, cât și după tratament (t = 3,96; p < 0,001 până la tratament și t = 5,11; p < 0,001 după tratament). Aceasta demonstrează o activare mai importantă a imunității limfo-

Tabelul 2

Caracteristica imunității umorale a bolnavilor eșantioanelor evaluate (M ± m)

Indicatori	Sănătoși	Eșantion control		Eșantion studiu	
		Înainte	După	Înainte	După
IgG g/l	12,3 ± 0,27	17,2 ± 0,33●	15,7 ± 0,40♦	18,2 ± 0,31●	17,1 ± 0,31♦
IgA g/l	2,6 ± 0,10	3,2 ± 0,11●	2,6 ± 0,13♦	3,6 ± 0,14 ●	3,2 ± 0,11♦
IgM g/l	1,4 ± 0,06	1,6 ± 0,09	1,2 ± 0,07♦	2,2 ± 0,11●	1,9 ± 0,10♦
Ac naturali	2,5 ± 0,08	1,7 ± 0,10●	2,1 ± 0,11♦	1,3 ± 0,09●	1,7 ± 0,09♦

Notă: ● – diferență statistic semnificativă în comparație cu eșantionul persoanelor sănătoase.
♦ – diferență statistic semnificativă între subeșantioane, înainte și după tratament.

citelor B în eșantionul de studiu. Indicatorii sunt expuși în tabelul 2.

Cantitatea anticorpilor naturali la bolnavii ambelor eșantioane, până la tratament a fost mai redusă decât la cei sănătoși ($t = 6,64$; $p < 0,001$ pentru EC și $t = 10,4$ $p < 0,001$ pentru ES). La bolnavii ambelor eșantioane, după tratament, s-a indentificat creșterea cantității anticorpilor naturali ($t = 2,6$; $p < 0,01$ pentru EC și $t = 2,91$ $p < 0,01$ pentru ES). De asemenea, cantitatea anticorpilor naturali, la bolnavii eșantionului de control, a fost semnficativ mai înaltă decât la bolnavii eșantionului de studiu, atât până la tratament, cât și după ($t = 3,28$; $p < 0,01$ până la tratament și $t = 3,09$; $p < 0,01$ după tratament). Acest fenomen demonstrează utilizarea mai rapidă a anticorpilor naturali circulanți la bolnavii eșantionului de studiu, probabil, datorită numărului mai mare al antigenelor circulante în sânge.

Analiza sensibilizării celulare și umorale, la diferite antigene utilizate, realizată prin reacția de transformare blastică a limfocitelor până la tratament, a determinat că sensibilizarea la antigenele micobacteriene a fost semnificativ mai înaltă la bolnavii ambelor eșantioane, decât la cei sănătoși ($t = 3,0$; $p < 0,01$ pentru EC și $t = 3,7$ $p < 0,001$ pentru ES). După tratament, în ambele eșantioane a fost identificată creșterea sensibilizării la antigenele micobacteriene ($t = 3,27$; $p < 0,01$ pentru EC și $t = 2,89$ și $p < 0,01$ pentru ES), date oglindite în tabelul 3.

Nivelul sensibilizării la antigenele stafilococice, până la tratament a fost semnificativ mai înalt doar la bolnavii eșantionului de control, comparativ cu cei sănătoși ($t = 2,3$; $p < 0,05$). După tratament, nivelul sensibilizării la antigenele stafilococice în eșantionul de control, a crescut semnificativ comparativ cu eșantionul de studiu ($t = 2,21$; $p < 0,05$). Sensibilizarea la antigenele streptococice până la tratament, în ambele eșantioane, nu a stabilit divergență față de indicatorul analog al eșantionului de sănătoși. După tratament, în ambele eșantioane, s-a determinat o creștere a sensibilizării la antigene streptococice ($t = 2,72$; $p < 0,01$ pentru EC și $t = 3,16$ și $p < 0,01$ pentru ES). Nivelul sensibilizării la antigenele pneumococice până la tratament, în ambele eșantioane nu s-a diferențiat de indicatorul analog al eșantionului sănătoșilor. După tratament, în ambele eșantioane s-a determinat o creștere a sensibilizării la antigene pneumococice ($t = 2,79$; $p < 0,01$ pentru EC și $t = 2,82$ și $p < 0,01$ pentru ES). Rezultatele expuse au demonstrat prezența unui nivel mai înalt al sensibilizării celulare la bolnavii eșantionului de control, în comparație cu eșantionul de studiu.

Cantitatea anticorpilor antimicobacterieni până la tratament, în ambele eșantioane, a fost semnificativ mai mare decât la cei sănătoși ($t = 8,3$; $p < 0,001$ pentru EC și $t = 9,1$; $p < 0,001$ pentru ES). De asemenea, la bolnavii eșantionului de studiu, cantitatea anticoprilor antimicobacterieni a fost semnficativ mai mare ($t = 2,2$; $p < 0,05$) și comparativ cu

Tabelul 3

Caracteristica unor indicatori ai hipersensibilizării celulare, umorale și intoxicației (M ± m)

Indicatori	Sănătoși	Eșantion control		Eșantion studiu	
		Înainte	După	Înainte	După
RBTL Ag micobacteriene %	2,0 ± 0,21	3,4 ± 0,42●	5,6 ± 0,54♦	3,3 ± 0,28●	4,4 ± 0,28♦
RBTL Ag stafilococic %	1,7 ± 0,21	2,6 ± 0,34●	4,3 ± 0,47♦	2,1 ± 0,19●	3,1 ± 0,24♦
RBTL Ag streptococic %	1,3 ± 0,18	1,6 ± 0,28	2,7 ± 0,26♦	1,3 ± 0,14	2,1 ± 0,20♦
RBTL Ag pneumococic %	0,7 ± 0,12	0,5 ± 0,11	1,0 ± 0,15♦	0,5 ± 0,06	0,8 ± 0,08♦
Ac antimicobacterieni UDO	2,3 ± 0,09	4,5 ± 0,25●	3,6 ± 0,27♦	5,4 ± 0,33●	5,0 ± 0,31
IgE total IU/ml	17,4 ± 1,28	98 ± 11,0●	51 ± 6,9♦	118 ± 13,6●	74 ± 9,6♦
ILA UC	0,19 ± 0,061	0,81 ± 0,161●	0,22 ± 0,051♦	0,83 ± 0,115●	0,72 ± 0,104
CIC %	49,3 ± 2,38	52,2 ± 4,53	33,8 ± 3,10♦	97,3 ± 5,5●	76,8 ± 4,23♦
Indicele *Kalf-Kalif* UC	0,9 ± 0,04	0,9 ± 0,18	0,3 ± 0,06♦	0,9 ± 0,13	0,8 ± 0,11

Notă: ● – diferență statistic semnificativă în comparație cu eșantionul persoanelor sănătoase.
♦ – diferență statistic semnificativă între subeșantioane, înainte și după tratament.

indicatorul înregistrat la bolnavii eșantionului de control. După tratament, s-a identificat reducerea anticorpilor antimicobacterieni la bolnavii ambelor eșantioane, însă doar la bolnavii eșantionului de control, această reducere a fost semnificativă statistic (t = 2,94; p < 0,01). Cantitatea anticorpilor antimicobacterieni în eșantionul de studiu după tratament, a rămas semnificativ mai mare decât în eșantionul de control (t = 3,44; p < 0,01). Aceasta a demonstrat activarea mai importantă a imunității umorale la bolnavii eșantionului de studiu, ca rezultat al reducerii imunității celulare.

Cantitatea IgE-total în ambele eșantioane, până la tratament, a fost semnificativ mai mare, comparativ cu a celor sănătoși (t = 3,3; p < 0,001 pentru EC și t = 7,4 și p < 0,001 pentru EC). După tratament, s-a determinat reducerea IgE-total la bolnavii ambelor eșantioane (t = 3,67; p < 0,001 pentru EC și t = 2,68 și p < 0,01 pentru ES), dar mai importantă – la bolnavii eșantionului de control.

Indicele leucocitar de alergizare (ILA), până la tratament, a fost mai mare în ambele eșantioane (t = 2,21; p < 0,05 pentru EC și t = 2,99 și p < 0,01 pentru ES), comparartiv cu a celor sănătoși. După tratament, s-a determinat reducerea ILA, însă doar la bolnavii eșantionului de control, această diminuare a atins pragul semnificației statistice (t = 3,08; p < 0,01), ceea ce a demonstrat o păstrare mai bună a reactivității imune a bolnavilor eșantionului de control.

Analiza unor indicatori, care denotă intoxicația organismului, a demonstrat că cantitatea complexelor imune circulante (CIC), până la tratament, în eșantionul de control, nu s-a diferențiat de indicatorul analog al eșantionului celor sănătoși, iar la bolnavii eșantionului de studiu a fost semnificativ mai mare (t = 7,9; p < 0,001). După tratament, s-a determinat reducerea cantității complexelor imune circulante în ambele eșantioane (t = 3,35; p < 0,01 pentru EC și t = 2,94; p < 0,01 pentru ES). Totuși nivelul complexelor imune circulante după tratament, la bolnavii eșantionului de studiu, a rămas mai mare, decât la cei sănătoși (t = 8,2; p < 0,001), ceea ce demonstrează prezența unei intoxicații mai severe a bolnavilor eșantionului de studiu, atât înainte, cât și după tratament. Indicele leucocitar de intoxicație Kalf-Kalif nu s-a diferențiat între eșantioanele de bolnavi și sănătoși, însă a crescut după tratament doar la bolnavii eșantionului de control (t = 3,05; p < 0,01).

Reactivitatea preimună a fost apreciată prin testul de reducere a sării de blue-nitro-tetrasolium (NBT) și s-a calculat numărul și indicele fagocitar.

Activitatea funcțională a neutrofilelor, evaluată prin testul de reducere a sării de blue-nitro-tetrasolium până la tratament, la bolnavii eșantionului de control, a fost identică cu același indicator obținut la cei sănătoși. La bolnavii eșantionului de studiu, activitatea funcțională a neutrofilelor a fost semnificativ mai redusă (t = 2,51; p < 0,05) decât la cei sănătoși. După tratament, la bolnavii ambelor eșantioane, s-a determinat creșterea activității funcționale a neutrofilelor (t = 2,66; p < 0,01 pentru EC și t = 2,15; p < 0,05 pentru ES). Indicatorii sunt apreciați în tabelul 4.

Activitatea fagocitară, apreciată prin indicele fagocitar până la tratament, la bolnavii ambelor eșantioane, nu s-a diferențiat semnificativ de același indicator al celor sănătoși. Dar s-a evidențiat tendința de reducere al acestui indicator la bolnavii eșantionului de studiu și de creștere – la bolnavii eșantionului de control. După tratament, la bolnavii ambelor eșantioane a crescut indicele fagocitar (t = 3,5; p < 0,001 pentru EC și t = 2,86; p < 0,01 pentru ES). Însă la bolnavii eșantionului de studiu, atât până la tratament, cât și după, indicele fagocitar a fost seminificativ mai redus (t = 2,41; p < 0,05 până la tratament și t =4,93; p < 0,001 după tratament). Datele obținute au demonstrat, că la bolnavii eșantionului de control, activitatea fagocitară a fost practic nemodificată înaintea începerii tratamentului, iar după tratament a crescut semnificativ, în comparație cu bolnavii eșantionului de studiu.

Cantitatea neutrofilelor, capabile să fagociteze (numărul fagocitar), a fost semnificativ mai redusă la bolnavii ambelor eșantioane, comparativ cu același indicator al eșantionului celor sănătoși. După tratament, la bolnavii ambelor eșantioane, s-a demonstrat creșterea numărului fagocitar (t = 4,24; p < 0,001 pentru EC și t = 4,48; p < 0,05 pentru ES). Însă la bolnavii eșantionului de studiu, atât până, cât și după tratament, numărul fagocitar a rămas semnificativ mai redus (t = 2,4; p < 0,05 până la tratament și t = 2,01; p < 0,05 – după tratament). Astfel, am demonstrat că la bolnavii eșantionului de control, numărul fagocitar de la debut a fost practic nemodificat, iar după tratatament a crescut semnificativ, comparativ cu bolnavii eșantionului de studiu.

Analiza de ansamblu a indicatorilor rezistenței preimune a demonstrat, că reactivitatea preimună la bolnavii eșantionului de control a fost înainte de tratament nemodificată, dar după tratament, s-a activat mai eficient, iar la bolnavii eșantionului de studiu, activarea rezistenței preimune a decurs mai lent.

Tabelul 4

Caracteristica unor indicatori ai rezistenței preimune (M ± m)

Indicatori	Sănătoși	Eșantion control		Eșantion studiu	
		Înainte	După	Înainte	După
NBT-test UC	0,14 ± 0,006	0,14 ± 0,008	0,17 ± 0,008♦	0,12 ± 0,005●	0,17 ± 0,023♦
Numărul fagocitar %	76,9 ± 0,86	77,9 ± 1,08	85,2 ± 1,31♦	73,9 ± 1,25●	81,6 ± 1,17♦
Indicele fagocitar UC	4,61 ± 0,17	5,1 ± 0,23	6,2 ± 0,21♦	4,4 ± 0,16	5,0 ± 0,13♦

Notă: ● – diferență statistic semnificativă în comparație cu eșantionul persoanelor sănătoase.

♦ – diferență statistic semnificativă între subeșantioane, înainte și după tratament.

Stabilirea factorilor predictivi ai dezvoltării eşecului terapeutic

Rezultatul analizei logistice regresive a demonstrat, că calea pasivă de depistare a fost evaluată ca factor predictiv cu risc redus pentru eşec, apreciată cu RP = 1,302 (IÎ 95%: 0,774-2,189). Depistarea tardivă, strâns corelată cu latenţa simptomatologiei până la adresarea la medic, a fost evaluată în calitate de factor predictiv cu risc mare pentru eşec, RP = 2,681 (IÎ 95%: 1,635-4,398). Evaluând particularităţile radiologice ale procesului tuberculos la depistare, am determinat că localizarea bilaterală a tuberculozei a fost determinată ca factor predictiv major pentru eşecul terapeutic, RP = 8,221 (IÎ 95%: 4,811-14,048). Tuberculoza extinsă pe mai mult de 3 segmente pulmonare s-a stabilit ca factor predictiv major, apreciat cu RP = 10,823 (IÎ 95%: 5,564-21,058). Complicaţiile asociate evoluţiei tuberculozei au fost apreciate ca factori de risc mediu pentru eşecul terapeutic, RP = 2,090 (IÎ 95%: 1,362-6,212).

În consecinţă, stabilim că depistarea tardivă a formelor severe de tuberculoză pulmonară, cu localizare în ambii plămâni şi extinse, agravate de complicaţii şi boli asociate sunt factorii cei mai importanţi în dezvoltarea eşecului.

Pentru stabilirea căror indicatori imunologici le revine calitatea de factor predictiv al eşecului tratamentului tuberculozei, am calculat coeficientul raportului probabilităţilor doar a indicatorilor, care au etalat o diferenţă statistic semnificativă între eşantioanele de bolnavi investigaţi. Analizând indicatorii imunităţii celulare, am constatat că conţinutul redus al limfocitelor T a fost considerat cu risc major pentru eşecul terapeutic, RP = 62,5 (IÎ 95%: 14,231-274,49). Asemănător, conţinutul redus al limfocitelor T helper a fost apreciat cu risc major, RP = 12,5 (IÎ 95%: 3,42-45,04). Conţinutul crescut al limfocitelor T supresor a fost analizat cu RP = 2,10 (IÎ 95%: 0,62-7,14), valoare cu risc mediu pentru eşecul terapeutic.

Evaluând imunitatea umorală, am constatat că cantitatea crescută a IgE total a fost estimată prin RP = 1,131 (IÎ 95%: 0,098-13,114) cu valoare neutră pentru eşec. Conţinutul crescut al limfocitelor B a fost apreciată ca factor neutru, RP = 1,158 (IÎ 95%: 0,348-3,847). Cantitatea crescută a IgA a fost evaluată ca factor de risc mic RP = 1,545 (IÎ 95%: 0,241-9,905). Informativitatea redusă a indicatorilor imunităţii umorale este explicată prin implicarea primară a imunităţii celulare în cursul infecţiei tuberculoase.

Nivelul intoxicaţiei specifice, apreciat prin cantitatea crescută a complexelor imune circulante, a fost dovedită ca factor de risc major RP = 9,801 (IÎ 95%: 2,895-33,175) pentru eşecul terapeutic.

Deficitul rezistenţei preimune, apreciată prin activitatea funcţională redusă a neutrofilelor, a fost considerat ca factor neutru, RP = 1,158 (IÎ 95%: 0,341-3,847). Cantitatea redusă a neutrofileor capabile să fagociteze, estimată prin indicele fagocitar, a fost apreciată ca factor de risc mare RP = 2,875 (IÎ 95%: 0,926-8,928).

Severitatea sindromului inflamator, apreciată prin viteza crescută a sedimentării hematiilor, a fost estimată prin RP = 5,213 (IÎ 95%: 1,626-30,131), avînd valoare cu risc mare pentru eşecul terapeutic. Ierarhia factorilor de risc este expusă în tabelul 5.

Tabelul 5

Factorii de risc ai eşecului în tratamentul tuberculozei pulmonare

Grad	Factor de risc	Raportul probabilităţilor (IÎ 95%)
Risc mare	Tuberculoză extinsă	10,823 (IÎ 95%: 5,564-21,058)
	Localizare bilaterală	8,221 (IÎ 95%: 4,811-14,048)
	Depistare tardivă	2,681 (II 85%: 1,635-4,398)
	Co-morbidităţi	2,611 (IÎ 95%: 1,611-4,259)
	Conţinut redus al limfocitelor T	62,5 (IÎ 95%: 14,231-274,49)
	Conţinut redus al limfocitelor T helper	12,5 (IÎ 95%: 3,42-45,04)
	Cantitate crescută a CIC	9,801 (IÎ 95%:2,895-33,175)
	Viteză crescută a VSH	5,213 (IÎ 95%: 1,626-30,131)
	Reducerea indicelui fagocitar	2,875 (IÎ 95%: 0,926-8,928)
Risc mediu	Complicaţii	2,090 (ÎI 95% 1,362-6,212)
	Co-morbidităţi	2,611 (IÎ 95%: 1,611-4,259)
	Conţinut crescut al limfocitelor T supresor	2,10 (IÎ 95%:0,62-7,14)

Notă: IÎ – intervalul de încredere.

Deci, indicatorii clinici şi imunologici cu valoare prognostică majoră pentru eşecul terapeutic, sunt: depistarea tardivă a cazului nou, tuberculoza extinsă, localizarea bilaterală, deficitul imunităţii celulare (conţinutul mic al limfocitelor T şi a subpopulaţiei T helper), indicatorii majoraţi ai intoxicaţiei specifice, nivelul înalt al complexelor imune circulante, indicatorii reduşi ai rezistenţei preimune şi viteza de sedimentare a hematiilor crescută.

Concluzii

1. Calea pasivă de depistare, realizată tardiv de către medicul de familie, a formelor severe, extinse de tuberculoză cu localizare bilaterală, cu componente distructive şi diseminaţie, agravate de complicaţii şi co-morbidităţi, au constituit cauzele eşecului terapeutic. Factorii predictivi cu risc mare au fost: tuberculoza pulmonară extinsă RP = 10,823, localizarea bilaterală a procesului specific RP = 8,221, depistarea tardivă a tuberculozei RP = 2,681, bolile asociate tuberculozei RP = 2,611.

2. Modificările imune, evidenţiate la bolnavii care au dezvoltat eşec terapeutic, au fost: perturbări importante şi rigide ale tuturor parametrilor reactivităţii imune (imunităţii celulare, umorale şi specifice), rezistenţei preimune (indicele şi numărul fagocitar) şi indicatorii crescuţi ai intoxicaţiei endogene.

3. Bolnavii, care au finalizat cu succes tratamentul antituberculos, au demonstrat indicatori ai rezistenţei preimune normali sau slab modificaţi, au fost mai labili şi o parte din ei, 8 din 43 (18,6%), au revenit la normalitate.

4. Indicatorii imunologici cu valoare prognostică de factori cu risc mare au fost: deficitul rezistenței imune celulare (diminuarea limfocitelor T (RP = 62,5) și T helper (RP = 12,5), nivelul intoxicației endogene (cantitatea crescută a complexelor imune circulante (RP = 9,801), deficitul rezistenței preimune (indicele fagocitar redus (RP = 2,875)), sindromul inflamator (viteza de sedimentare a hematiilor crescută (RP = 5,213).

Recomandări practice

1. Măsurile de reducere a probabilității dezvoltării eșecului terapeutic trebuie inițiate tuturor bolnavilor de tuberculoză pulmonară, depistați pasiv, tardiv, cu forme severe și extinse de tuberculoză, cu localizare bilaterală, cu evidente componente de distrucție pulmonară și diseminație bronhopulmonară, agravate de complicații și co-morbidități.

2. Pentru optimizarea rezultatului terapeutic, se recomandă efectuarea investigațiilor de identificare a perturbărilor rezistenței imune celulare (conținutul limfocitelor T și subpopulației T helper), indicatorul intoxicației (conținutului complexelor imune circulante), indicatorii reactivității preimune (indicele fagocitar și activitatea funcțională a neutrofilelor) și evaluarea sindromului inflamator (viteza de sedimentare a hematiilor).

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Differential diagnosis of the pulmonary infiltrate in actual epidemiological state of tuberculosis

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Abstract

Background: Tuberculosis (TB) is a big challenge for public health in the Republic of Moldova, with an incidence recored on the third place among high TB-burden countries. Pneumonia is a distinct nosologic entity included in the frame of respiratory pathologies with an impact on public health, showing a high risk for a poor outcome.
Material and methods: The conducted study was retrospecitive, selective and descriptive realised on a sample of 194 patients (study group – 125 patients with pulmonary tuberculosis and reference group – 65 patients with community acquired pneumonia).
Results: It was established that younger age, urban overcrowding, harmful habits, disadvantageous economic status, sigle matrmonial status, epidemiological high risk conditions characterized patients with tuberculous pulmonary infiltrate. Old age, comorbidities, reduced availability of highly qualified medical care, characterized patients with non-specific pulmonary infiltrate.
Conclusions: Complex approach to patients with pulmonary infiltrative opacities, indivudalised according to social, demographic, epidemiological and biological features must be performed appropriately, considering the severity of the epidemiogical situation of tuberculosis in the Republic of Moldova.
Key words: tuberculosis, pneumonia, risk factors.

Diagnostricul diferențial al infiltratului pulmonar în situația epidemiologică actuală a tuberculozei

Introducere

Tuberculoza reprezintă o provocare pentru Sistemul de Sănătate al oricărui stat și a fost declarată de către OMS urgență modială în 1993 [10]. Conform raportului OMS actual, o treime din populația mondială este infectată cu *M. tuberculosis*, anual înregistrîndu-se peste 9 milioane de cazuri noi de tuberculoză și 2 milioane de decese prin progresia tuberculozei. Republica Moldova se situează pe locul 3 din cele 18 țări cu povară înaltă a tuberculozei din regiunea europeană [10]. Criza social-politică apărută în anul 1990 a determinat continua agravare a indicatorilor epidemiologici ai tuberculozei, cauzând valoarea maximă a incidenței în 2006 de 134 la 100 000 populație și o mortalitate de 19 la 100 000 populație. Acești indici manifestă o descreștere lentă, ca în 2013 să se înregistreze o incidență a tuberculozei de 110 la 100 000 populație și o mortalitate de 11 la 100 000 populație. Cauza agravării situației epidemiologice a tuberculozei constă în reducerea considerabilă a examenelor radiologice profilactice, în special al radiofotografiei medicale datorită „restructurării" serviciului administrativ, începând cu anii 1990 [5]. Mai mult ca atât, începând cu ianurie 2014, aceasta nu se mai efectuează populației de pe teritoriul Republicii Moldova. În consecință a crescut numărul bolnavilor simptomatici de tuberculoză, cu modificări radiologice specifice, care se adresează în instituțiile medicale teritoriale cu profil general (asistență medicală primară teritorială) [4]. Datorită schimbării particularităților de management al bolnavilor de tuberculoză, medicii generaliști la momentul inițierii reformelor nu posedau cunostințe sufieciente despre metodele de diagnostic diferențial al tuberculozei, stabilind diagnosticul tardiv al formelor grave, extinse, frecvent cu evoluție cronică și deces rapid [6]. În 2001, Republica Moldova a adoptat strategia recomandată de OMS, numită DOTS (*Direclty Observed Treatment Short Course Chemotherapy*). Programul Național de Control al Tuberculozei, la baza căruia stă strategia DOTS, este adoptat la fiecare 5 ani și are la bază 2 obiective: să atingă depistarea a 70% din cazurile noi de tuberculoză pulmonară prin microscopia frotiului sputei și să vindece cel putin 85% din cazurile noi de tuberculoză pulmonară. În pofida tuturor investițiilor financiare, măsurilor de optimizare a depistării bolnavilor de tuberculoză și instruirea personalului asistenței medicale primare, nici depistarea prin microscopia frotiului sputei și nici rata succesului terapeutic nu a atins obiectivele stabilite [6]. Conform datelor raportate, ponderea formelor de tuberculoză pulmonară bacilară în Republica Moldova rămâne fără modificări semnificative: 2007 – 44,1%, 2008 – 44,1%, 2009 – 39,5%, 2010 – 38,1%, 2011 – 37,4%, 2012 – 39,2%, deși se atestă a rată îngrijorător de mare a formelor de tuberculoză pulmonară cu distrucții parecnhimatoase: 2007 – 43,7%, 2008 – 46,3%, 2009 - 40,1%, 2010 - 40,3%, 2011 – 38,0%, 2012 – 37,3%. Conform Protocolului Clinic Național-123, diagnosticul tuberculozei se efectuează conform unui algoritm bine stabilit, ce include examenul sputei prin colorația Ziehl – Neelson, radiografia toracică, cultura bacteriologică și testarea sensibilității pentru preparatele antituberculoase de linia 1-a [8]. Actualmente, a fost inclus în acest algoritm testarea sensibilității la rifampicină prin intermediul testului Xpert-MTB/RIF. În pofida unui algoritm de diagnsotic bine prestabilit, în circa 30% din cazurile de tuberculoză, diagnosticul prezumptiv eronat stabilit de „pneumonie comunitară" cauzează tergiversarea depistării tuberculozei, astfel condiționând dezvoltarea formelor severe, extinse, cronice cu

evoluție rapid fatală [3]. Însă, gravitatea majoră a bolnavilor cu diagnostic eronat constă în pericolul epidemiologic, pe care aceștia îl expun asupra populației sănătoase în calitate de sursă de infecție tuberculoasă.

Pneumonia reprezintă o entitate nosologică distinctă printre patologiile respiratorii cu impact asupra sănătății publice, având un risc major pentru o evoluție nefavorabilă [2]. Astfel, în țările regiunii europene, pneumonia se situează pe locul IV în structura generală a mortalității, fiind precedată de boala ischemică, bolile cerebrovasculare și cancerul pulmonar [1]. De asemenea, pneumonia este complicația terminală, determinantă a decesului la pacienții cu afecțiuni oncologice, cardiovasculare, infecțioase și chirurgicale preexistente [2]. Multiplele studii epidemiologice au constatat o incidență înaltă a bolii la adulți (5-16 cazuri la 1000 populație), cu valori variind în dependență de grupurile de vârstă, comorbidități și regiuni geografice [1]. Astfel, la vârstnici sunt înregistrate 25 040 de cazuri la 1000 populație. În Republica Moldova, în ultimii 10 ani, incidența pneumoniilor variază mult cu tendință spre majorarea de la 19 177 până la 23 022, cu un indice al morbidității de la 4 la 5,9 la 1000 populație. În SUA, anual se înregistrează 3-4 mii cazuri de pneumonii (indicele morbidității fiind 10-16 la 1000 locuitori), dintre care 900 000 se spitalizează.

Anual, în lume se înregistrează peste 3 mln cazuri de îmbolnăviri prin pneumonie. Estimativ, se consideră că acest număr este determinat de formele cu evoluție moderată și severă, deoarece formele ușoare sunt tratate în condiții de ambulator, unde diagnosticul de „pneumonie" este subapreciat. Conform PCN, investigațiile obligatorii realizate pacientului cu opacități infiltrative pulmonare sunt: hemoleucograma, glicemia, examenul radiologic al cutiei toracice în 2 incidențe, analiza sputei la BAAR, sumarul urinei, spirografia și puls-oximetria. Investigațiile recomandate sunt: analiza biochimică a sângelui, bacterioscopia sputei precedată de evaluarea citologică, examenul bacteriologic al sputei (doar în pneumonii cu evoluție severă și de gravitate medie). În cazurile anterior menționate se pot recomanda selectiv, în dependență de anamneză și particularități clinice, aprecierea anticorpilor IgM (ELISA) către agenți atipici, antigenele specifce urinare (ELISA, testul imunocromatografic al legionelei și pneumococului), hemoculturi, toracocenteză, gazimetria sângelui arterial, examenul ecografic al organelor interne, ECG și doar în cazuri complicate cu dificutăți de diagnostic, se recomandă tomografia computerizată [7]. În pofida tuturor investițiilor manageriale și financiare, în activitatea clinică, specificitatea infiltratului pulmonar nu este apreciată în o treime din cazuri datorită tabloului clinic necaracteristic, dar mai mult ca atât, informativitatea redusă a metodelor microbiologice utilizate [3].

Scopul cercetării: studierea particularităților clinice și paraclinice ale tuberculozei pulmonare infiltrative în contextul strategiei DOTS.

Obiective: studierea particularităților generale, sociale, economice și epidemiologice ale bolnavilor de tuberculoză pulmonară infiltrativă și pneumonie comunitară; evaluarea manisfestărilor clinice, indicatorilor de laborator ai bolnavilor de tuberculoză pulmonară infiltrativă și pneumonie comunitară; elucidarea dificultăților de diagnostic diferențial al tuberculozei pulmonare infiltrative cu pneumonia comunitară;

Material și metode

A fost îndeplinit un studiu retrospectiv, selectiv și descriptiv al unui volum total de 194 bolnavi cu infiltrate pulmonare parenchimatoase, în vârsta de 18-70 de ani, internați în IMSP Institutul de Ftiziopneumologie „Chiril Draganiuc", în perioada 01.01.2011-01.01.2013, distribuți în 2 eșantioane: eșantionul de studiu – 192 de bolnavi de tuberculoză pulmonară infiltrativă și eșantionul de comparație - 65 de bolnavi de pneumonie comunitară. Criteriile de includere în eșantionul de studiu: vârsta de > 18 ani; tip pacient „caz nou". Pentru colectarea materialului primar a fost utilizată metoda extragerii informației din formularele medicale și cele statistice. A fost efectuată o analiză minuțioasă a documentației medicale. Toate datele cercetărilor clinice, de laborator, instrumentale și ale documentației medicale au fost incluse în fișa individuală de studiu, formată din următoarele compartimente: date generale; statut educațional, statut economic, statut civil, condiții de viață, metoda depistării, diagnostic clinic (conform protocoalelor clinice naționale); diagnosticul bolilor asociate, tabloul clinic; rezultatele examinărilor de laborator și instrumentale. Metode de analiză: de comparație; de sinteză; determinarea veridicității; analiza discriminantă; prelucrarea matematico – statistică a materialului a fost efectuată prin verificarea cantitativă și calitativă a materialului acumulat, mai apoi s-a procedat la repartizarea materialului în grupări simple și grupări complexe. Materialul prelucrat a fost tabelat, folosind tabele simple, de grup și combinate. Prelucrarea statistică a rezultatelor studiului s-a efectuat computerizat, utilizând aplicațiile programelor Microsoft Excel XP și Statistica 10,0.

Rezultate și discuții

Particularități sociodemografice, economice, epidemiologice și manageriale

Conform repartiției după gen, am apreciat o predominare semnificativă a bărbaților, comparativ cu femeile în ambele eșantioane, astfel că în eșantionul de studiu au fost 97 (75,19 ± 3,80%) bărbați și 41 (63,07 ± 5,98) femei, iar în cel de comparație – 41 (63,07 ± 5,98%) bărbați și 24 (36,92 ± 5,98%) femei, cu același grad de concludență $p < 0,01$. Comparând raportul de genuri (bărbați/femei) am constatat că acesta a fost similar în ambele eșantioane (2,31 în eșantionul de studiu și 1,71 în eșantionul de comparație). Vârsta medie a pacienților eșantionului de studiu a constituit 35,6 ani și a eșantionului de comparație 54,5 ani, cu diferență semnificativă atestată ($p < 0,01$). Conform locului de trai, am apreciat o predominare a pacienților veniți din mediul rural în eșantionul de comparație 54 (83,07 ± 4,65%) bolnavi, comparativ cu 77 (59,69 ± 4,31%) bolnavi din eșantionul de studiu, $p < 0,001$). Această constatare demonstrează accesibilitatea redusă a serviciilor medicale a populației rurale și aglomerarea surselor de infecție tuberculoasă în grupurile populaționale urbane.

Analizând ponderea bolnavilor defavorizaţi social prin categorisirea lor în dependenţă de antrenarea în câmpul muncii, am constatat că persoanele neangajate, respectiv fără poliţă de asigurare medicală obligatorie, a predominat în eşantionul de studiu 109 (84,49 ± 3,18%) cazuri, comparativ cu 38 (58,46 ± 6,11%) cazuri în eşantionul comparaţie), iar persoanele angajate au predominat în eşantionul de comparaţie 27 (41,53 ± 6,11%) cazuri faţă de 20 (15,50 ± 3,18%) cazuri în eşantionul de studiu cu acelaşi gradient de semnificaţie statistică, p < 0,001). Această constatare demonstrează inaccesibilitatea serviciilor de asistenţă medicală primară a bolnavilor de tuberculoză, datorită absenţei poliţei de asigurare obligatorie la majoritatea acestora.

Am apreciat ocupaţia de bază a pacienţilor investigaţi, care a constatat că muncitorii necalificaţi au predominat în eşantionul de studiu 54 (41,86 ± 4,34%) cazuri, comparativ cu 14 (21,53 ± 5,09%) cazuri în eşantionul comparaţie), iar muncitorii calificaţi au predominat în eşantionul de comparaţie 30 (46,15 + 6,19%) cazuri faţă de 34 (26,35 ± 3,87%) cazuri în eşantionul de studiu. La fel şi funcţionarii au predominat în eşantionul de comparaţie 11 (16,92 ± 4,65%) cazuri, comparativ cu 4 (3,10 ± 1,52%) cazuri în eşantionul de studiu, p < 0,001). Persoanele pensionate au preodminat în eşantionul de comparaţie cu acelaşi grad de concludenţă 10 (15,38 ± 4,47%) cazuri, comparativ cu 6 (4,65 ± 1,85%) cazuri în eşantionul de studiu, p < 0,001, datorită vârstei mai înaintate a bolnavilor. Următoarele categorii profesionale au fost similar distribuite între eşantioane: agricultori 23 (17,82 ± 3,37%) şi 10 (15,38 ± 4,47%), invalizi 7 (5,42 ± 1,99%) şi 5 (7,69 ± 3,30%), studenţi 1 (0,77 ± 0,77%) şi 2 (3,07 ± 2,14%), respectiv. Aşadar, persoanele cu un nivel socio-economic jos sunt predispuse dezvoltării tuberculozei pulmonare, iar cei cu un nivel relativ optim fac mai frecvent pneumonii comunitare. Vârsta înaintată, expusă prin grupul numeros al pensionarilor demonstrează necesitatea vigilenţei clinice în acest grup populaţional.

Evaluarea statutului educaţional a constatat o distribuţie a grupurilor de şcolarizare fără atingerea pragului statistic. Totuşi, s-a apreciat tendinţa de predominare a studiilor incomplete în eşantionul de studiu: 40 (31,00 ± 4,07%) cazuri, comparativ cu 14 (21,53 ± 5,09) cazuri în eşantionul de comparaţie şi a studiilor primare 31 (24,03 ± 3,76%) cazuri, comparativ cu 10 (15,38 ± 4,47%) cazuri în eşantionul de comparaţie, fapt care demonstrează gradientul jos al culturii igieno-sanitare a bolnavilor de tuberculoză. În eşantionul de comparaţie, s-a demonstrat predominarea concludentă a studiilor superioare în 11 (16,92 ± 4,65%) cazuri, comparativ cu 4 (3,10 ± 1,52%) cazuri în eşantionul de studiu.

Nivelul de trai, apreciat ca satisfăcător, a predominat în eşantionul de comparaţie – 64 (98,46 ± 1,52%) cazuri comparativ cu doar 50 (38,76 ± 4,29%) cazuri în eşantionul de studiu, şi la acelaşi nivel de concludenţă, nivelul de trai nesatisfăcător a predominat în eşantionul de studiu 79 (61,24 ± 4,29%) cazuri şi doar 2 (3,07 ± 2,14%) cazuri în eşantionul de comparaţie, p < 0,001. În precaritate extremă, prin absenţa locului de trai s-au identificat 12 (9,30 ± 2,55%) bolnavi ai eşantionului de studiu.

Referitor la apartenenţa la anumite grupuri de risc sporit de îmbolnăvire am apreciat că acestea au predominat la acelaşi grad de concludentă statistică (p < 0,001) în eşantionul bolnavilor de tuberculoză: migranţi au constituit 44 (34,10 ± 4,17%) cazuri în eşantionul de studiu, comparativ cu 11 (16,92 ± 4,65%) cazuri în eşantionul de comparaţie, fumătorii 87 (67,44 ± 4,12%) cazuri în eşantionul de studiu, comparativ cu 16 (24,61 ± 5,34%) cazuri în eşantionul de comparaţie, consumatorii cronici de alcool în 60 (46,512 ± 4,39%) cazuri, comparativ cu 6 (9,23 ± 3,59%) cazuri în eşantionul de comparaţie. Foşti deţinuţi ai instituţiilor penitenciare au fost identificaţi doar în eşantionul de studiu 7 (5,42 ± 1,99%) cazuri, iar utilizatori de droguri intravenoase (UDI) s-au depistat într-un număr redus în ambele eşantioane 3 (2,32 ± 1,32%) şi 1 (1,53 ± 1,52%) caz. Datele sunt reprezentate în tabelul 1.

Cercetarea statutului matrimonial, diferenţiat în categorii conform celor expuse anterior, a demonstrat că statutul vulnerabil de persoană celibatară a predominat în eşantionul de studiu 38 (29,45 ± 4,01%) cazuri, comparativ cu 7 (10,76 ± 3,84%) cazuri în eşantionul de comparaţie, asemănător şi cel de persoană divorţată 18 (13,95 ± 3,05%) cazuri, comparativ cu 1 (1,53 ± 1,52%) caz în eşantionul de comparaţie, atingând acelaşi prag statistic (p < 0,001). Iar persoanele căsătorite au

Tabelul 1

Factorii de risc de îmbolnăvire

Semne clinice	Eşantion de studiu n = 129		Eşantion de comparaţie n = 65		P
	n	M ± m (%)	n	M ± m (%)	
Neangajat	109	84,49 ± 3,18	38	58,46 ± 6,11	< 0,001
Studii incomplete	71	55,04 ± 4,39	24	36,92 ± 2,44	< 0,01
Munci necalificate	54	41,86 ± 4,34	14	21,53 ± 5,09	< 0,01
Civil solitar	54	41,86 ± 4,34	8	12,30 ± 4,08	< 0,001
Contact tuberculos	43	33,33 ± 4,15	0	0	< 0,001
Migraţie	44	34,10 ± 4,17	11	16,92 ± 4,65	< 0,001
Detenţie	7	5,42 ± 1,99	0	0	< 0,001
Consum de alcool	60	46,512 ± 4,39	6	9,23 ± 3,59	< 0,001
Tabagism	87	67,44 ± 4,12	16	24,61 ± 5,34	< 0,001

predominat în eșantionul de comparație – 51 (78,46 ± 5,09%) cazuri, comparativ cu 68 (52,71 ± 4,39%) cazuri în eșantionul de studiu, la un prag statistic înalt (p < 0,001). Deci, statutul civil de persoană vulnerabilă a predominat la bolnavii de tuberculoză.

Factorul de risc epidemiologic major de îmbolnăvire de tuberculoză reprezintă contactul tuberculos, fiind stabilit la o treime din bolnavii de tuberculoză 43 (33,33 ± 4,15%) pacienți și 33 (25,58 ± 3,84%) pacienți au provenit din focare epidemice de TB. Iar în eșantionul de comparație nu s-a constatat niciun contact cu sursă de infecție tuberculoasă și niciun pacient n-a provenit dintr-un focar epidemic de tuberculoză.

Sinteza rezumativă a particularităților generale, a caracteristicilor sociale, economice și civile ale bolnavilor cu pneumonie și tuberculoză pulmonară a demonstrat, că genul masculin și vârsta tânără a caracterizat eșantionul bolnavilor de tuberculoză, iar genul masculin și vârsta înaintată a particularizat eșantionul bolnavilor de pneumonie comunitară. Statutul economic de persoană neangajată a predominat la bolnavii de tuberculoză. Ocupațiile de bază ale persoanelor investigate au fost mai frecvent muncile necalificate în eșantionul cu tuberculoză pulmonară și munci calificate – la pacienții cu pneumonie severă. Nivelul de școlarizare incomplet s-a apreciat mai frecvent la bolnavii de tuberculoză, iar nivelul optim – la majoritatea bolnavilor de pneumonie. Statutul civil de persoană solitară a predominat la bolnavii de tuberculoză, iar de persoană căsătorită s-a evidențiat într-o proporție majoritară la bolnavii eșantionului cu pneumonii. Grupul migranților a predominat în eșantionul bolnavilor de tuberculoză. Foști deținuți au reprezentat doar eșantionul bolnavilor de tuberculoză. Deprinderile nocive cum ar fi consumul cronic de alcool și fumatul activ au predominat în eșantionul cu tuberculoză. Indicatorii cum ar fi contactul tuberculos și apartenența la focarul epidemic de tuberculoză nu au fost reprezentativi pentru pacienții cu pneumonii comunitare cercetați.

Particularitățile de management al cazului, aspectele clinice și de diagnostic ale bolnavilor de tuberculoză și pneumonie

Studiind managementul cazului, am constatat că nu este vreo diferență semnificativă între tipul personalului medical calificat, care a identificat și diagnosticat bolnavii cu pneumonie sau tuberculoză. Majoritatea acestora s-au adresat serviciilor medicale primare pentru acordarea îngrijirilor în sănătate 95 (73,64 ± 3,87%) bolnavi ai eșantionului de studiu și 51 (78,46 ± 5,09%) bolnavi ai eșantionului de comparație. Specialistul pneumoftiziolog a confirmat mai frecvent diagnosticul de pneumonie comunitară în cercetarea expusă 14 (21,53 ± 5,09%) cazuri) decât a depistat cazurile de tuberculoză 13 (10,07 ± 2,65%) cazuri, însă gradul de concludență statistică nu a fost atins. O particularitate distinctă este durata de evoluție a simptomatologiei evocatoare. Astfel, o simptomatologie evocatoare cu o durată de până la 7 zile a predominat în eșantionul pneumoniilor comunitare 61 (93,84 ± 2,98%) cazuri, iar o simptomatologie cu o durată de mai mult de 1 lună a predominat în eșantionul bolnavilor cu tuberculoză în 110 (85,27 ± 3,12%) cazuri.

Deoarece severitatea stării clinice este un criteriu al calității vieții, am constatat că starea clinică mediu alterată a bolnavilor a predominat nesemnificativ în eșantionul de studiu: 31 (24,03 ± 3,76%) cazuri și, respectiv, 11 (16,92 ± 4,65%) cazuri în eșantionul de comparație. Iar în eșantionul de comparație au predominat bolnavii în stare gravă 33 (50,76 ± 6,20%) cazuri și extrem de gravă în 21 (32,30 ± 5,80%) cazuri, comparativ cu 53 (41,082 ± 4,33%) și, respectiv, 45 (34,88 ± 5,80%). Deci, gravitatea stării generale este o particularitate definitorie a pneumoniei comunitare tratate în condiții de staționar.

Diferențierea aspectelor clinice a determinat o prezență similară a sindromului de intoxicație și bronhopulmonar la întreg contingentul ambelor eșantioane 129 (100%) cazuri în eșantionul de studiu și 65 (100%) cazuri în eșantionul de comparație. Anumite componente ale sindromului de intoxicație au fost similar repartizate între eșantioane, cum ar fi: astenia – 129 (100%) cazuri în eșantionul de studiu și 65 (100%) cazuri în eșantionul de comparație, inapetența în 114 (88,37 ± 2,82%) cazuri în eșantionul de studiu și 53 (81,53 ± 4,81%) cazuri în eșantionul de comparație.

Scăderea în greutate, ca indicator al persistenței sindromului de intoxicație, s-a constatat la majoritatea pacienților cu tuberculoză pulmonară 109 (84,49 ± 3,18%) cazuri și doar la o mică parte din cei cu pneumonie 8 (12,30 ± 4,07%) cazuri. Transpirațiile profuze ca componentă a sindromului de intoxicație tuberculoasă s-au identificat la fiecare al doilea pacient al eșantionului de studiu 76 (58,91 ± 4,33%) bolnavi) și doar la o mică parte din eșantionul de comparație în 16 (24,61 ± 5,34%) cazuri, atingând un prag înalt al concludenței statistice (p < 0,001). Febra, ca indicator al unui proces infecțios acut și indicator al severității lui a predominat în eșantionul de comparație 54 (83,07±4,85%) cazuri, comparativ cu 26 (20,15 ± 3,53%) cazuri în eșantionul de studiu), pe când subfebrilitatea s-a apreciat într-o proporție similară în ambele eșantioane (11 (8,52 ± 2,45%) cazuri în eșantionul de studiu și 11 (16,92 ± 4,65%) cazuri în eșantionul de comparație). Explicăm prezența subfebrilității în eșantionul de comparație ca o febră decapitată de antibioterapie și antipiretice, iar în eșantionul de studiu – ca un indicator al intoxicației tuberculoase. Spectrul clinic a fost expus în tabelul 2.

În privința componentelor sindromului bronhopulmonar am constatat prezența acestora la întregul contingent al ambelor eșantioane. Repartiția componentelor acestuia nu a demonstrat diferențe semnificative între eșantioane, fapt ce demonstrează că aparatul respirator răspunde quasiidentic în orice afecțiune bronhopulmonară. Deci, tusea s-a determinat la întreg contingentul de bolnavi. Expectorațiile seromucoase s-au identificat într-o proporție similară în ambele eșantioane, în eșantionul de studiu în 40 (31,00 ± 4,07%) cazuri și 19 (29,23 ± 5,64%) cazuri în cel de comparație, expectorațiile mucopurulente au predominat în eșantionul de comparație în 46 (70,76 ± 5,64%) și în 75 (60,00 ± 4,38%) cazuri în eșantionul de studiu, durerea toracică s-a apreciat similar în 8 (6,20 ± 2,12%) cazuri în eșantionul de studiu și 3 (4,61 ± 2,60%) cazuri în cel de comparație, hemoptiziile s-au apreciat similar între eșantioane 14 (10,85 ± 2,73%) cazuri în eșantionul de studiu

Tabelul 2

Spectrul simptomatologiei clinice

Semne clinice	Eșantion de studiu n = 129		Eșantion de comparație n = 65		P
	n.	M ± m (%)	n.	M ± m (%)	
Astenie	129	100	65	100	> 0,05
Scădere în greutate	109	84,49 ± 3,18	8	12,30 ± 4,07	< 0,001
Inapetență	114	88,37 ± 2,82	53	81,53 ± 4,81	> 0,05
Transpirații	76	58,91 ± 4,33	16	24,61 ± 5,34	< 0,001
Febră	26	20,15 ± 3,53	54	83,07 ± 4,85	< 0,001
Subfebrilitate	11	8,52 ± 2,45	11	16,92 ± 4,65	> 0,05
Tuse	129	100	65	100	> 0,05
Expectorații seromucoase	40	31,00 ± 4,07	19	29,23 ± 5,64	> 0,05
Expectorații mucopurulente	75	58,14 ± 4,34	46	70,76 ± 5,64	> 0,05
Durere toracică	8	6,20 ± 2,12	3	4,61 ± 2,60	> 0,05
Hemoptizii	14	10,85 ± 2,73	5	7,69 ± 3,30	> 0,05
Dispnee	95	73,64 ± 3,87	59	90,76 ± 3,59	< 0,01
Dispnee MRC2	45 din 95	47,36 ± 5,12	6 din 59	10,16 ± 3,93	< 0,001
Dispnee MRC3	40 din 95	42,10 ± 5,06	39 din 59	66,10 ± 6,16	< 0,001
Dispnee MRC4	10 din 95	10,52 ± 3,14	20 din 59	33,89 ± 6,16	< 0,001

și 5 (7,69 ± 3,30%) cazuri în eșantionul de comparație 2. Însă, este necesar de menționat că hemoptiziile în tuberculoză au avut aspectul unor filamente de sânge proaspăt integrate în spută, iar în pneumonie au avut aspectul unei spute ruginii.

Este importantă ponderea bolnavilor cu dispnee în eșantionul de comparație 59 (90,76 ± 3,59%) cazuri, comparativ cu 95 (73,64 ± 3,87%) cazuri în eșantionul de studiu, p < 0,01. Această constatare devine o particularitate evidentă a eșantionului cu pneumonii tratate în condiții de staționar. Diferențiind gradul dispneei conform criteriilor MRC, am apreciat că dispneea la efort fizic de urcare pantă (gradul II) a predominat semnificativ în eșantionul de studiu 45 (47,36 ± 5,12%) cazuri, comparativ cu 6 (10,16 ± 3,93%) cazuri în eșantionul de comparație), dispneea la mers de 100 m (gradul III) a predominat în eșantionul de comparație 39 (66,10 ± 6,16%) cazuri, față de 40 (42,10 ± 5,06%) cazuri în eșantionul de studiu), iar dispneea de repaus (gradul IV) a predominat în eșantionul de comparație 20 (33,89 ± 6,16%) cazuri, comparativ cu 10 (10,52 ± 3,14%) cazuri în eșantionul de studiu. Toți indicatorii s-au diferențiat la același grad de concludență statistică (p < 0,001), datele fiind prezentate în tabelul 2.

Am constatat, că bolnavi cu comorbidități au predominat semnificativ în eșantionul de comparație 65 (100%) cazuri comparativ cu 93 (74,40±3,90%) cazuri în eșantionul de studiu, atingând pragul de p < 0,01). Spectrul patologiilor în eșantionul de comparație este mai mare fiind condiționat de vârsta mai înaintată a bolnavilor. Deși, se atestă diferențe în repartiția pe grupuri nosologice, pragul semnificației statistice îl ating doar câteva dintre acestea. Deci, bolile cronice respiratorii nespecifice (BCRN, cu includerea bronșitei cronice, BPOC) au predominat în eșantionul de studiu în 19 (20,43 ± 4,18%) cazuri, comparativ cu 11 (16,92 ± 4,65%) cazuri în eșantionul de comparație. Bolile aparatului gastrointestinal (BAGI), cu includerea ulcerului gastroduodenal, gastritei cronice, s-au determinat într-o proporție similară în 17 (18,28 ± 4,00%) cazuri în eșantionul de studiu și 9 (13,84 ± 4,28%) cazuri în cel de comparație. Bolile hepatice cronice, precum hepatita cronică și ciroza hepatică au predominat nesemnificativ în eșantionul de comparație 2 în 12 (18,46 ± 4,81%) cazuri și 9 (9,67 ± 3,06%) cazuri în eșantionul de studiu. Bolile renale cronice, precum pielonefrita cronică a fost diagnosticată într-o proporție similară 4 (4,30 ± 2,10%) cazuri în eșantionul de studiu și 4 (6,15 ± 2,98%) cazuri în eșantionul de comparație). Infecția HIV (B20) a predominat însă nesemnificativ în eșantionul cu tuberculoză 5 (3,876 ± 1,69%) cazuri și 1 (1,538 ± 1,53%) caz în cel de comparație). Apreciem că extinderea numerică a populației HIV infectate se răsfrânge asupra creșterii prevalenței tuberculozei, care reprezintă prima cauză de deces la persoanele HIV infectate. O singură gravidă s-a diagnosticat cu pneumonie severă și 2 bolnavi psihici 2 (2,15 ± 1,50%) cu retard mintal în eșantionul cu tuberculoză.

O diferență concludentă s-a determinat prin ponderea mărită a bolnavilor cu diabet zaharat în eșantionul de comparație 11 (16,92 ± 4,65%) cazuri și 4 (4,30 ± 2,10%) cazuri în eșantionul de studiu, p < 0,05). Deci, perturbările endocrine condiționează imunosupresia secundară, ceea ce predispune dezvoltarea pneumoniilor. Bolile cardiovasculare (în baza hipertensiunii arteriale și cardiopatiei ischemice) au predominat în același eșantion 12 (18,46 ± 4,81%) cazuri, comparativ cu 9 (9,67 ± 3,06%) cazuri în eșantionul de studiu, p < 0,05. Iar alcoolismul cronic, prin diagnostic confirmat, a predominat în eșantionul de studiu 28 (21,70 ± 3,63%) cazuri și 6 (9,23 ± 3,59%) cazuri în eșantionul de comparație, p < 0,01. În ordine descrescândă, rata comorbidităților a fost oglindită în tabelul 3.

Tabelul 3

Structura ponderală a bolilor asociate şi stărilor medico-biologice

Indicatori	Eşantion studiu n = 93		Eşantion control n = 65		P
	n	M ± m (%)	n	M ± m (%)	
BCRN	19	20,43 ± 4,18	11	16,92 ± 4,65	< 0,05
BAGI	17	18,28 ± 4,00	9	13,84 ± 4,28	< 0,05
Alcoolism cronic	28	21,70 ± 3,63	3	4,61 ± 2,60	< 0,01
Boli hepatice	9	9,67 ± 3,06	12	18,46 ± 4,81	> 0,05
Boli renale cronice	4	4,30 ± 2,10	4	6,15 ± ,98	> 0,05
Diabet zaharat	4	4,30 ± 2,10	11	16,92 ± 4,65	< 0,05
B 20	5	5,37 ± 2,33	1	1,53 ± 1,52	> 0,05
Boli cardiovasculare	9	9,67 ± 3,06	12	18,46 ± 4,81	< 0,05
Boli psihice	2	2,15 ± 1,50	0	0	> 0,05
Graviditate	0	0	1	1,53 ± 1,52	> 0,05

Aprecierea rezumativă a particularităţilor biologice, caracteristicilor sociale, economice şi civile ale bolnavilor cu pneumonie severă şi tuberculoză pulmonară a demonstrat, că vârsta comparativ mai tânără, neangajarea în câmpul muncii, ocupaţiile de muncitor necalificat, consumul cronic de alcool, migraţia au caracterizat bolnavii de tuberculoză pulmonară. Atât medicul de familie cât şi specialistul pneumoftiziolog au fost implicaţi, de asemenea, în îngrijirea medicală specializată. Totuşi, bolnavii de tuberculoză manifestau o simptomatologie de o durată mult mai mare decât cei cu pneumonie comunitară. Gravitatea stării generale a fost apreciată drept criteriu de includere în eşantioane, totuşi, febra înaltă şi prevalenţa dispneei a predominat semnificativ în eşantionul bolnavilor cu pneumonii comunitare. Afectarea polisegmentară şi bilaterală a plămânilor, cu componente distructive şi de diseminaţie au caracterizat tuberculoza pulmonară. Evoluţia rapid pozitivă spre o resorbţie considerabilă cu o ulterioară vindecare a predominat în eşantionul bolnavilor cu pneumonii comunitare, iar rata înaltă a deceselor şi a bolnavilor pierduţi din supravegherea medicală a caracterizat eşantionul de tuberculoză.

Studiul comparativ al indicatorilor formulei leucocitare

Studiul indicatorilor formulei leucocitare a determinat, că numărul leucocitelor în ambele eşantioane de bolnavi înaintea iniţierii tratamentului, a fost statistic semnificativ mai mare decât la bolnavii eşantionului martor (t = 5,965; p < 0,001 pentru eşantionul de studiu şi t = 4,43; p < 0,001 pentru eşantionul de comparaţie). La finele tratamentului, numărul leucocitelor în ambele eşantioane s-a redus, însă nesemnificativ în eşantionul de studiu şi semnificativ în eşantionul de comparaţie (t = 2,767; p < 0,05). Indicatorul a rămas la nivel mult mai înalt faţă de indicatorul celor sănătoşi (t = 4,977; p < 0,001 pentru eşantionul de studiu şi t = 3,513; p < 0,01 pentru eşantionul de comparaţie). Comparând eşantioanele de bolnavi, am constatat că numărul leucocitelor a fost nesemnificativ mai mare în eşantionul de comparaţie înaintea iniţierii tratamentului, iar la finele său a devenit mai mare, însă nesemnificativ în eşantionul de studiu.

Numărul neutrofilelor segmentate la bolnavii eşantionului de studiu până la tratament, a fost mai redus, comparativ cu al eşantionului de persoane sănătoase (t = 2,885; p < 0,01 pentru eşantionul de studiu) şi mai mare nesemnificativ în eşantionul de comparaţie. La finele tratamentului, numărul acestora s-a redus, totuşi, rămânând la un nivel mai mare, comparativ cu al eşantionului de control (t = 4,179; p < 0,001 pentru eşantionul de studiu şi t = 2,272; p < 0,05 în eşantionul de comparaţie). Comparând indicatorii fiecărui eşantion, până şi după tratament, am constatat că reducerea numărului segmentatelor a fost semnificativă în ambele grupuri (t = 2,649; p < 0,05 pentru eşantionul de studiu şi t = 2,687; p < 0,05 în eşantionul de comparaţie). Comparând eşantioanele de bolnavi, am constatat că înaintea iniţierii tratamentului şi la finele său, numărul segmentatelor a fost mai mare nesemnificativ în eşantionul de comparaţie.

Cantitatea neutrofilelor nesegmentate la bolnavii ambelor eşantioane a fost semnificativ mai înaltă decât la eşantionul de control (t = 4,28; p < 0,01 pentru eşantionul de studiu şi t = 4,0257; p < 0,001 pentru eşantionul de comparaţie). După tratament, cantitatea neutrofilelor nesegmentate s-a redus semnificativ în ambele grupuri (t = 2,348; p < 0,05 pentru eşantionul de studiu şi t = 3,845; p < 0,01 pentru eşantionul de comparaţie). Dar în pofida tratamentului administrat, ponderea nesegmentatelor a rămas mai mare decât la cei sănătoşi în eşantionul de studiu (t = 2,001; p < 0,05) şi s-a normalizat în eşantionul de comparaţie. Între eşantioane s-a apreciat o diferenţă semnificativă, documentată printr-un număr mai mare al indicatorului în eşantionul de studiu doar la finele tratamentului (t = 2,051; p < 0,05).

Cantitatea neutrofilelor tinere a fost mai mare semnificativ faţă de indicatorul persoanelor sănătoase doar în eşantionul de studiu. (t = 3,590; p < 0,01). Comparând eşantioanele, am constatat că neutrofilele tinere au fost semnificativ mai multe în eşantionul de studiu, doar înaintea iniţierii tratamentului (t = 3,321; p < 0,01). Datele sunt reflectate în tabelul 4.

Rezultatele obţinute demonstrează labilitatea vădită cu tendinţă spre ameliorare a conţinutului tuturor tipurilor de

Tabelul 4

Caracteristrica formulei leucocitare (M ± m)

Indicatori	Eşantion martor	Eşantion studiu		Eşantion de comparaţie 2	
		1	2	1	2
Leucocite 10^9/l	6,2 ± 0,19	9,41 ± 0,50●■	8,79 ± 0,48	11,21 ± 1,09●	7,78 ± 0,418●♦■
Neutr. segm. %	63,0 ± 1,00	61,03 ± 1,99●♦	54,79 ± 1,75●♦	65,69±2,123♦■	56,375 ± 2,739●
Neutr. nesegm.%	1,8 ± 0,18	5,74 ± 0,546●	5,74 ± 2,292●♦■	5,91±1,014●♦	1,82 ± 0,301●♦■
Neutr. tinere %	0	0,06 ± 0,04●■	0,035 ± 0,0218	0,03 ± 0,02■	0
Bazofile %	0,17 ± 0,043	0,382 ± 0,121●	0,323 ± 0,082●	0,06± 0,044●	0,218 ± 0,192●
Eozinofile %	1,8 ± 0,20	3,38 ± 0,88●♦	6,264 ± 0,46♦■	1,875±0,594●	2,718 ± 0,551●
Mielocite %	0	0,058 ± 0,238	0,054 ± 0,229	0,062±0,0442	0,062 ± 0,04420
Plasmocite %	0	0,029 ± 0,028	0	0,093±0,09	0,093 ± 0,09
Limfocite 10^9/l	2,15 ± 0,045	1,94 ± 0,162	2,066 ± 0,112	1,76±0,157	2,49 ± 0,2265♦
Limfocite %	27,3 ± 0,98	21,8 ± 1,598●	24,588 ± 1,557●■	18,22±1,469●♦	31,25 ± 2,513♦■
Monocite %	6,0 ± 0,35	9,294 ± 0,970●	11,06 ± 0,985●■	8,07±0,814●	7,468 ± 0,728■
VSH mm/oră	7,2 ± 0,47	41,12 ± 3,207●	31,50 ± 3,460●♦	42,125±3,281●	31,25 ± 3,266♦●

Notă: ● – diferenţă statistic semnificativă în comparaţie cu eşantionul persoanelor sănătoase;
♦ – diferenţă statistic semnificativă în eşantioane înainte (1) şi la finele tratamentului (2);
■ – diferenţă statistic semnificativă între eşantionul de studiu şi de control.

neutrofile în patologiile parenchimatoase nespecifice, ceea ce predispun evoluţia bolii spre vindecare. Devierea la stânga a formulei leucocitare şi dinamica nesemnificativă în eşantionul de studiu demonstrează cauza menţinerii stării grave a pacienţilor cu tuberculoză.

Apreciind cantitatea eozinofilelor am determinat o creştere nesemnificativă în eşantionul de studiu şi un număr egal cu cel al sănătoşilor în eşantionul de comparaţie. La finele fazei intensive, s-a constatat o creştere concludentă a cantităţii eozinofilelor în cadrul eşantionului de studiu ($t = 2,231$; $p < 0,05$). Respectiv, la finele antibioterapiei, cantitatea acestora a crescut nesemnificativ în eşantionul de comparaţie. Comparând indicatorul cu cel al sănătoşilor la finele tratamentului, am apreciat o cantitate mărită concludent în eşantionul de studiu ($t = 4,617$; $p < 0,001$) şi neconcludentă în eşantionul de comparaţie. Aceasta demonstrează acţiunea hipersensibilizantă a triggerilor antigenici la bolnavii de tuberculoză. Între eşantioane s-a constatat un număr mai mare de eozinofile doar la finele tratamentului în eşantionul de studiu ($t = 3,237$; $p < 0,01$).

Numărul bazofilelor s-a apreciat înalt concludent, comparativ cu cel al sănătoşilor în ambele eşantioane ($t = 6,119$; $p < 0,001$ pentru eşantionul de studiu şi $t = 17,97$; $p < 0,001$ pentru eşantionul de comparaţie). Tratamentul a diminuat numărul lor, totuşi rămânând la un nivel înalt concludent, comparativ cu al eşantionului persoanelor sănătoase ($t = 9,084$; $p < 0,001$ pentru eşantionul de studiu şi $t = 4,832$; $p < 0,01$ pentru eşantionul de comparaţie). Până la iniţierea tratamentului şi la finele fazei intensive, nu s-a demonstrat nicio dinamică concludentă în ambele eşantioane. Comparând indicatorii între eşantioanele de bolnavi, am constatat că cantitatea acestora a fost mai mare semnificativ în eşantionul de studiu până la tratament ($t = 2,473$; $p < 0,05$) şi nesemnificativ după tratament.

Mielocitele s-au identificat într-un număr foarte redus în ambele eşantioane, fără a atinge pragul concludenţei statistice nici înaintea iniţierii, nici la finele fazei intensive în cadrul fiecărui eşantion şi nici comparativ cu persoanele sănătoase.

Plasmocitele s-au identificat într-un număr foarte mic la bolnavii investigaţi. Nu s-au determinat diferenţe statistice nici comparativ cu persoanele sănătoase, nici comparativ cu rezultatele obţinute înaintea şi la finele fazei intensive a tratamentului.

Numărul absolut al limfocitelor a fost diminuat înaintea iniţierii tratamentului antituberculos în ambele eşantioane ($t = 2,05$; $p < 0,05$ pentru eşantionul de studiu şi $t = 2,369$; $p < 0,05$ pentru eşantionul de comparaţie). Tratamentul a contribuit la majorarea numărului acestora, însă fără a atinge pragul statistic în eşantionul de studiu şi a atins pragul în eşantionul de comparaţie ($t = 2,347$; $p < 0,05$). Diferenţe semnificative între eşantioane nici până la iniţierea, nici la finele tratamentului nu s-au identificat.

Ponderea procentuală a limfocitelor a demonstrat cantitatea lor mult redusă înaintea iniţierii tratamentului antituberculos în ambele eşantioane ($t = 3,361$; $p < 0,001$ pentru eşantionul de studiu şi $t = 5,141$; $p < 0,001$ pentru eşantionul de comparaţie). Tratamentul a contribuit la majorarea numărului acestora, însă fără a atinge pragul statistic în eşantionul de studiu şi a atins pragul în eşantionul de comparaţie ($t = 4,474$; $p < 0,001$). Între eşantioane s-a apreciat un număr mai mare al limfocitelor în eşantionul de comparaţie la finele tratamentului ($t = 3,689$; $p < 0,01$).

Cantitatea monocitelor a fost mărită înaintea iniţierii tratamentului antituberculos semnificativ statistic ($t = 3,193$; $p < 0,01$ pentru eşantionul de studiu şi $t = 2,326$; $p < 0,05$ pentru eşantionul de comparaţie). La finele tratamentului s-a demonstrat că numărul acestora a crescut nesemnificativ în eşantionul de studiu şi s-a redus nesemnificativ în eşantionul

de comparaţie. Comparativ cu sănătoşii, după tratament acest indicator a atins un grad înalt al concludenţei statistice în eşantionul de studiu (t = 4,836; p < 0,001) şi a fost mai mic nesemnificativ în eşantionul de comparaţie. Eşantioanele fiind comparate între ele, s-a constatat că numărul monocitelor a fost mai mare nesemnificativ în eşantionul de studiu înaintea tratamentului. Iar după tratament, numărul acestora a fost mai mare în eşantionul de comparaţie (t = 2,929; p < 0,05).

Viteza de sedimentare a hematiilor înaintea iniţierii tratamentului a fost mult crescută, comparativ cu acelaşi indicator al sănătoşilor (t = 10,464, p < 0,001 pentru eşantionul de studiu şi t = 10.536; p < 0,001 pentru eşantionul de comparaţie). După tratament s-a redus, însă a rămas la un grad înalt al concludenţiei statistice (t = 6,973; p < 0,001 pentru eşantionul de studiu şi t = 7,2882 p < 0,001 pentru eşantionul de comparaţie). Comparând valorile obţinute înaintea şi la finele tratamentului s-a obţinut o descreştere a indicatorului semnificativ statistic (t = 2,038; p < 0,05 pentru eşantionul de studiu şi t = 2,348; p < 0,05 pentru eşantionul de comparaţie). Comparând eşantioanele de bolnavi, nu s-au constatat diferenţe nici până, nici după tratament.

Concluzii

Cercetarea aspectelor comparative ale bolnavilor cu procese infiltrative pulmonare de diferită geneză a stabilit, că vârsta comparativ mai tânără, statutul economic defavorizat, statutul civil vulnerabil, deprinderile nocive şi anumite particularităţi epidemiologice agravante au caracterizat bolnavii cu tuberculoză pulmonară. Iar vârsta avansată, statutul civil şi profesional optim, comorbidităţile au caracterizat bolnavii cu pneumonie comunitară

Rapiditatea evoluţiei clinice până la stabilirea diagnosticului de pneumonie comunitară şi latenţa depistării simptomatice a cazului de tuberculoză este particularitatea definitorie a fiecărui eşantion. Într-o stare alterată de mediu s-au aflat mai frevent bolnavii de tuberculoză, iar starea generală gravă a caracterizat bolnavii eşantionului cu pneumonii. Unele componente ale sindromului de intoxicaţie au caracterizat eşantionul bolnavilor de tuberculoză (scăderea marcată în greutate, transpiraţiile profuze), iar febra a predominat concludent în eşantionul bolnavilor de pneumonie. Bolnavii dispneici şi mai ales cu un grad înalt al dispneei au fost mai mulţi în eşantionul cu pneumonii.

Corelaţia clinico-paraclinică a apreciat un răspuns inflamator sistemic la debutul bolii cu mult mai intens în eşantionul bolnavilor cu pneumonie severă. Iar devierea la stânga a formulei leucocitare a fost identificată la un grad similar în ambele eşantioane. Deşi a fost demonstrată o uşoară ameliorare a indicatorilor neutrofilici sub acţiunea tratamentului antituberculos, în eşantionul bolnavilor cu tuberculoză severă, aceştia s-au normalizat în eşantionul pneumoniilor comunitare severe. De asemenea, atenţionăm asupra dinamicii rapide de optimizare a răspunsului inflamator în cursul tratamentului nespecific (durata medie de tratament 12 zile), comparativ cu dinamica lentă sub acţiunea tratamentului specific (durata medie a tratamentului fazei intensive în condiţii de staţionar a constituit 67 de zile). Fenomenul hipersensibilizării, apreciat prin creşterea eozinofilelor şi bazofilelor, a fost evident atât înainte cât şi la finele fazei intensive a tratamentului bolnavilor cu tuberculoză severă. În eşantionul cu pneumonii severe astfel de modificări nu s-au înregistrat. Supresia imunităţii celulare, apreciată prin cantitatea absolută şi relativă redusă a limfocitelor, a fost evidentă în eşantionul bolnavilor cu tuberculoză severă. În eşantionul pneumoniilor comunitare, deşi s-a apreciat la debutul tratamentului o limfopenie evidentă, măsurile terapeutice complexe au ameliorat perturbările imune, astfel că s-a demonstrat o limfocitoză la finele tratamentului.

Deci, devierea la stânga a formulei leucocitare şi imunosupresiei celulare au fost similar modificate în eşantioanele cu patologii specifice şi nespecifice, însă dinamica pozitivă a fost mai evidentă în eşantionul pneumoniilor comunitare, iar dinamica lentă a caracterizat eşantionul bolnavilor de tuberculoză.

Abrodul complex al pacienţilor cu opacităţi infiltrative pulmonare, necesită a fi individualizat conform particularităţilor sociale, economice, demografice, epidemiologice şi biologice, luând în cosideraţie gravitatea situaţiei epidemiologice a tuberculozei în Republica Moldova.

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REVIEW ARTICLES

Some aspects of biomedical preclinical research involving laboratory animals

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Abstract

Background: Animal research has enormous utility as understanding the complex interactions of molecular mechanisms, biochemical and physiological study ultimately depends on intact organisms *in vivo*. The use of animals for biomedical research was the subject of much debate and continues to be today. The main question of this debate is whether or not correct, morally speaking, to conduct animal research. Scientists who use animals for these purposes mean that experiments should be done as humanely as possible. They agree that animals should not be used as long as there are testing methods producing similar results. Internationally these principles are reflected in the rules developed by the Organization for Economic Cooperation and Development for Good Laboratory Practice. However, the implementation of this legislation in our country aims at purchasing proper equipment of scientific laboratories for preclinical research, monitoring biomedical ethics and ensures quality results. The experimental ethics is a prerequisite for ensuring good scientific practice.

Conclusion: The existence of a regulatory body to provide advice and evaluation of experimental design becomes mandatory enforcement of the concept of ethics in laboratory animal experiments.

Key words: animals, ethics, preclinical research.

Introduction

Scientific value and integrity of biomedical research is closely related to responsible and humanitarian treatment of animals. Animal research is enormously useful because understanding the complex interactions of molecular, biochemical and physiological mechanisms ultimately depends on the study of the intact organisms in vivo. To be carried out, such researches involve genetic and environmental factors that are difficult, if not impossible to control by studies on humans – though such experiences are valuable only if these controls are maintained with care. In addition, an experimental design that produces major pain or suffering declines subjects, if not eliminates the scientific value of the experiment. Finally, irresponsible or inhuman treatment of animals diminishes the reputation of scientific institutions, jeopardizing funding and threatens the public image of science.

Animal experiments had a remarkable contribution to the discovery of treatments for various diseases. It is enough to remember Paulescu's researches, and especially the success of the Canadian student Charles Herbert and his tutors, Dr. Banting and Prof. MacLeod (last two Nobel laureates) who, experimenting on dogs, discovered insulin hormone drug that saved in the last hundred yeas millions of lives. Animal experiments are necessary, as the experiments held on sick or healthy volunteers are necessary, even though there is no immediate benefit.

In the Helsinki Declaration, adopted in 1964 by the World Medical Association (WMA), which is a guide with recommendations for physicians involved in biomedical research on human subjects is mentioned that research on human subjects must be based on accepted scientific principles and evidence obtained through laboratory experiments on animals [1].

Animal protection legislation exists over 80 years (in the UK from 1911) and is functional in many European countries. Subsequently, many countries have adopted codes of practice to protect animals used in research. Each Ethics Committee monitors compliance with codes of practice and codes of ethics regarding animal experiments [2].

Since the twentieth century, the use of animals for experimental purposes extends beyond the fields of pharmacology and physiology, being used in fields such as psychology, cosmetics testing, testing different medicinal products and other consumer products.

It is impossible to do research using laboratory animals and not to create a minimum of discomfort to them. The need to reduce animal suffering probably arose with the need to use them, but today this is of major importance. As we move into an accelerated pace of scientific investigations, new problems for the care and use of laboratory animals continue to occur. The main question that gives rise to these debates is that whether it is correct or not correct, morally speaking, to conduct animal research. Scientists who use animals for such purposes know the problem and understand that experiments should be done as humanely as possible and that we should not use animals as long as the testing methods produce similar results.

There are two positions in animal experiments: in favor of animal experiments, only under the following conditions:

if their suffering is very small and if the human benefits derived from experiments could not be detected by other methods; against animal testing: if the experiments cause suffering, if human benefits are not proven and if you can make these experiments by other methods [3].

The use of animals in research is essential for the development and production of new drugs. Animal experiments are provided by the laws of all countries, because it is not allowed that any potential drug substance to be tested directly on humans. In order to reduce the number of animals, the "rule of three R" (replacement, reduction, refinement) is applied [4]. Russell and Burch (1959) proposed three specific strategies to minimize animal pain and stress research:

- Replacement: the researcher has to ensure that the objectives of the study cannot be achieved by alternative methods. Where possible, conscious animals should be replaced by unconscious animals or by insensitive material and higher-ranking animals should be replaced by lower-ranking ones.
- Reduction: if the number of animals involved in the experiment is higher, there is more suffering and more costs. Experiments that have a good design can be performed with a smaller number of animals without reducing scientific expectations. If the significance or accuracy of a study is not compromised, fewer animals should be used.
- Refinement: change of accommodation and experimental procedures so as to minimize the pain and stress and to promote the welfare of animals used in research, from birth until their death.

Strategies for reducing, replacing and refining not only have ethical bases, but also practical advantages. If the experiments can be made, for example, using the mice instead of the dogs, with less or no animals, the cost of these trials will be lower.

Successful implementation of the 3Rs principle strictly depends on the education and training of personnel involved in animal testing [5].

Continuous training is necessary for minimizing animal pain and the distress caused to animals, accumulation of theoretical and practical knowledge for the safety of the personnel, all leading to satisfactory scientific expectations. Increasing scientific quality is achieved only when the unit activities are preceded, the staff is trained, equipped with responsibility and strict records exist [6].

Another critical step in performing the experiment is given by the extraction, processing and dissemination. Results must be reliable, reproducible and provide repetition. Dissemination is mandatory regardless of the conclusions. Regardless of the form of dissemination, disseminating material must contain the following data about animals (species, line, strain, source, type, number, age, sex, weight and clinical status), the design of the experimental protocol (procedures used, time periods, equipment used) and analytical methods (including statistical methods). Even though a study had an appropriate design, it is useless if the results were not processed fairly and were not disseminated [7].

Organization for Economic Cooperation and Development (OECD) – a unique forum where the governments of 33 democracies work together to respond to the economic, social, those related to globalization and exploitation of globalized opportunities has developed and approved in 1981, the principles of good laboratory Practice (GLP) [OECD. Principles of Good Laboratory Practice (GLP)] use organizational and scientific methods, and experiences of various national and international sources, the specific objectives are mentioned related to the growth and maintenance of laboratory animals. The increased interest in recent years to medical experiments on animals is caused by a new scientific understanding; sustainable, humanistic and integrative view characterizing the millennium. The use of animals for research is under control in some EU countries under the provisions of the "European Convention for the protection of vertebrate animals used for experimental and other scientific purposes" [8].

In the Republic of Moldova, Law on the protection of animals nr.265 used for experimental and other scientific purposes was adopted by Parliament in July 2006 [9].

At the same time implementation of the recommendations of ISO/IEC 17025, 15190 in biomedical research centers of the country has as a goal the proper management of clinical and laboratory facilities needed to ensure their objects of study. In the researches conducted on animals, it would be ideal that the animal preserve stability in physiological status such as the response to variability of interest not to be interfered with unwanted influence. If animal welfare is compromised, consequences may include: high variability in results, the need to increase the number of animals, incomplete data, data that cannot be analyzed with low credibility, results that cannot be applied in other situations and the impossibility of publishing. Maintaining the animal welfare, identifying, controlling and eliminating factors that cause physiological and behavioral disturbances show good scientific practice. Under this context the Scientific Center of Drug Research team has set as a goal and is working on the implementation of standards GLP (Good Laboratory Practice) in preclinical study in practical science activities of Nicolae Testemitsanu State University of Medicine and Pharmacy. An important role in this direction is the work of the Ethics Committee of the university research.

In EU countries there is a tendency of standardizing the functionality of Ethics Committees, the European Federation of Associations of Laboratory Animal Science (FELASA) has published "Principles of ethical review practice for animal experiments in Europe" [10]. Regardless of the name, a control organization to assess animal research is required to ensure implementation of the concept of ethics in animal experiments and should be structured in several categories of people (doctors, researchers, neutral staff, staff from the animal protection associations, lawyers, philosophers, priests, etc.) to be neutral to the institutions initiating animal studies.

Conclusions

1. Human attitude towards animals is a relevant marker of ethical posture and level of civilization.

2. Experimental Ethics is prerequisite for ensuring good scientific practice. The existence of a regulatory body to provide advice and evaluation of experimental design becomes mandatory enforcement of the concept of ethics in animal experiments.

3. In order not to eliminate the scientific value of animal experiments in our country, it is necessary to implement Good Laboratory Practice standards.

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Tuberculosis and hard-to-reach group – migrant population

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Abstract

Background: The Republic of Moldova reports the biggest incidence of tuberculosis and the biggest rate of migrants among European Region countries. For the most of migrants the risk for TB development is correlated with social risk factors (low life conditions, overcrowding, disruption from the health care services), epidemiological risk factors (infectious contact) and biological features (young age, male sex, some physiological conditions, associated diseases). Risk factors association is more evident than the severity of one risk factor. The review study was conducted using relevant scientific resourses. **Conclusions:** TB is a big challenge worldwide. Despite high trends of migration noted in the 20th century the phemonen of migration as a risk factor for TB development is studied insufficienly. Immigrants are the majority of TB patients in high-income countries. The irregular emigrants are the most of TB patients from high-burden countries. Radiological and immunological screening in pre-departure phase is the most important procedure for decreasing of TB rates by providing latent TB infection treatment. Raising awareness among migrants about TB, emphasizing that diagnosis and treatment are free of charge and independent regarding migration status are important TB control actions performed in this hard-to-reach population.

Key words: tuberculosis, migration, risk factors.

Introduction

Labor market – vector of the mobility and migration. There is an estimated one billion migrants in the world today, which includes 232 million international migrants and 740 million internal migrants. *Mycobacterium tuberculosis* (TB) caused 9 million people ill with TB worldwide in 2013, with 1.5 million deaths [32]. TB particularly affects poor and vulnerable populations, migrants being assessed as key affected population [34]. In the framework of the 61st World Health Assembly of the WHO, was approved a resolution (61.17 from 2008) regarding the health of migrants, asking Member States to promote health care policies and practices aiming at migrant population [20]. Consecutively in June 2012, the working group headed by UN General Secretary Ban Ki-moon, approved the Millennium Development Goals (MDGs) where migration was recognized officially as an important factor of development, with a total impact of about one trillion dollars. On May 19, 2014, in the framework of the 67th World Health Assembly were adopted the new post-2015 global TB targets and strategy [32]. The strategy aimed to end the global TB epidemic with specific bench-marks and targets till 2035. It is built on a

"know-your epidemic" approach and focuses particularly on serving those not reached/hard-to-reach as well as most vulnerable and marginalized populations [33]. That strategy highlighted the needs of migrants and the necessity of the cross-border collaboration regarding the health of migrants. It was established that migrants' vulnerability to TB is caused by discriminatory policies in non-health sectors such as immigration service, labor and social protection departments. Absence of collaboration with these institutions and the absence of targeted TB prevention and control strategies regarding migrants create significant barriers in reaching TB elimination targets [1, 28].

For on average 1 billion migrants, migration is an effective and immediate tool to reduce poverty, conflict and escape for improving the life condition for their families [32]. Migrants are the backbone of health systems in all OECD countries and the safety valve in the global economy [30]. An important vector for migration from low to high-income contruies is the price of a labor hour. Explaining the economic role of labor migration we note the differences between wages: for India and China it is 0.25 USD/hour, Moldova 0.51 USD/hour, Russia 0.6 USD/hour, and Poland 2.09 USD/hour, USA 17.2 USD/hour, Japan 23.66 USD/hour, France 17.66 USD/hour, and Germany 33.88 USD/hour [19]. A big difference between monthly salaries is registreted in the CIS region. The medium salary in the economy in Russia constitutes 119 USD, in the Ukraine 66 USD, in Moldova 69 USD, in Kirgistan 31 USD, in Tadjikistan 13 USD. Adjusted to the financial role the differences in work time between EU countries and other states are noted. In EU countries the work time per year is less than 650 hours comparing with Japan and less than 340 comparing with the US [17]. In Europe it is established a specific work segregation according to the national criteria. So, migrant workers are employed in hard work conditions with low wages [25]. Gender inequality is also well expressed and appreciated by the employment of majority of men in construction, transportation, agriculture, and women in home care, service and entertainment [25].

One of the objectives of EU policy is to remove barriers for job mobility. Work mobility is essential for a proper function of the internal economy and for origin country as well as for destination country. Currently, only 2% of EU citizens are exercising their right to work in another country. Despite this, over one million European people cross the border every day for work searching and every year 250 000 people get their pension in other Member States of the European Union but not in their own country [25].

The migration is a necessity for a Moldovan migrant worker and other individuals from low-income countries. The negative repercussion of the migration is the severe damage of the origin country economy by "drain and export of the human skills" [8]. This type of migration lasts long periods of time or is a permanent emigration (such as the emigration for the US or Canada) [27]. This emigration has not a compensation recovery for balancing the national budget of the origin country [12]. According to the concept of knowledge and experience exchange, people migrate for searching new place of work, taking with them their profession and qualifications. Based on the concept of brain wasting, intellectual emigration is seen as a "cleaning" phenomen of the exporting countries. "Brain-drain" manifests economically negative tendency leading to the diminishing of the life standard of the citizens from origin country [8]. It was established that the emigration of 3-5% of skilled migrants is equal to the loss of 10% of the gross domestic product of the exporter country [7]. Factors that determine the "drain and export of human skills" are: a) personal – the need of high wages, restructuring and loss of jobs in the origin country, information from immigrated relatives and friends, curiosity and spirit of adventure, fear of war persecution; b) general – global demographic changes, that diminish the working population in high-income countries, economic globalization, visa liberalization, progress in transportation and communication, deep economic disparities between countries, possibility to create jobs through investment and development of multinational companies [26]. Analyzing the labor market situation in Moldova during 2000-2010, it was noted the decreasing number of active population from 1 654 662 to 1 422 300 people due to the intensive process of emigration [38].

For the developed countries the mobility/migration of the employees is integrated in the "mondialisation of the competences" [7]. As the workers have a similar economic high level, they do not migrate to gain more money but to change the professional experience. Their absence is recompensated rapidly by arrived foreign migrants. There is no evident economic repercussion on the export country, but is established a professional gain for the accepting country. Studying the real causes of migration in the European Union, the European Commission made the following general recommendations: a) to develop a national strategy for migration control in EU Member States; b) to promote information services in third countries to boost the cooperation between diplomatic organizations and local authorities; c) to develop policies for prevention of illegal migration (sanction of the illegall transportation of persons); d) to promote appropriate policies of labor attraction; e) to develop and to apply simple and transparent policies for obtaining of work permition, by this way combating undeclared work; f) to improve the status of migrant women g) to pay particular attention to unaccompanied minors who migrate from third countries into the European Union; h) apply the rules for marriage convenience of a person from a Member State with a person from a third country [25].

Finding work in a foreign country for a labor migrant is complicated by a multitude of problems: firstly – the residence visa issues, secondly – problems with the police, thirdly – problems with the crime organizations, fourthly – low salary from the employer, fifthly – the problem with healthcare services. A serious problem for a migrant in this regard is the lack of health insurance (56%), its high price (32%) and the absence of a residence address (4%) [20]. Research

data showed that Moldovan migrants have long absences at the medical addressing. Migrants visit health care services 2 times less frequently than the population not involved in migration. The number of visits to the general practitioner of migrants is on average 1.5 visits/year *versus* 3.2 visits/year of the individuals not involved in migration. That is reflected on the life expectancy indicator. For Moldova it is about 10.8 years lower in comparison with the average for EU, but is about 1.5 years higher than on average for CIS countries [35]. Other problems for migrant population are: low quality feeding, low psychosocial climate, stigma, etc. [19].

Migration is a big challenge, especially for the epidemiological security. Massive immigration automatically involves reducing of the public health actions for providing the epidemiological security goals. If the epidemiological security of the population immigrated from the country of origin is lower than the epidemiological security of the host country, migrants will bring with them infections and will be defined as public threat for the hosting population. Regardless of this, if new arrivals during the time will follow standards of personal hygiene their epidemiological security will increase and the epidemiological load on the hosting population will decrease. This phenomenon is well established for EU countries, where massive migration of groups originally from high burden for TB, especially MDR-TB countries, such as CIS countries, decreased the epidemiological security [1, 11].

If the epidemiological security in the hosting country is low, migrants after crossing the borders are not in secure epidemiological conditions and put themselves in a doubtful safety being the first to contact the infectious diseases. This phenomenon is described in Russia, regarding HIV infection. East-Asian migrants just arrived in Russia frequently contact HIV infection due to their free sex behaviour. Even within the state there are epidemiological security oscillations between different regions according to their economical, climatic, epidemiological particularities that are reflected on the epidemiological indicators.

Migration policies describe several types of migrants: individuals with specific legal and social status – labor migrants and undocumented migrants, trafficked and detained migrants. In the Republic of Moldova the highest level achieves illegal labor emigration. Labor migration involves not only skilled people but more evident healthy people. It is estimated that till 2050 every second citizen of the Republic of Moldova will have the status of migrant obtained due to internal and external migration [38]. A specific type of migration for our country is so called "commuting" or internal migration, defined as a commutation from the village to the city. Villages in the Republic of Moldova were always providing labour population to cities. On average for the entire country, every fifth person has performed an internal migration and every second citizen changed domicile [38].

A detrimental aspect of internal economy is that Moldova is considered a country with the lowest degree of urbanization in Europe (41.4%). The fact has major implications for employment policies, which must provide the creation of new jobs places in rural areas and jobs in the frame of alternative sectors of agriculture [29]. Due to a low economical growth, the Moldovan rural population rests without jobs being pushed to leave the family and to perform internal or external migration with the aim for finding a well paid place of work. Actually Moldova experiences a mass migration phenomenon due to its weak economy [29]. Migrants are the major source of income for the national economy, with total remittances from those living abroad estimated at 1 billion dollars per year. During 2000-2010, the number of legal Moldovan migrants working abroad increased from 138 300 to 311 000 thousand persons, which represents about 27% of the working age Moldovan population [30]. This process is increased due to expanding of European Union border in 2006 and due to changing of visa status for some category of population, for example for Moldovans with Romanian passports (fig. 1).

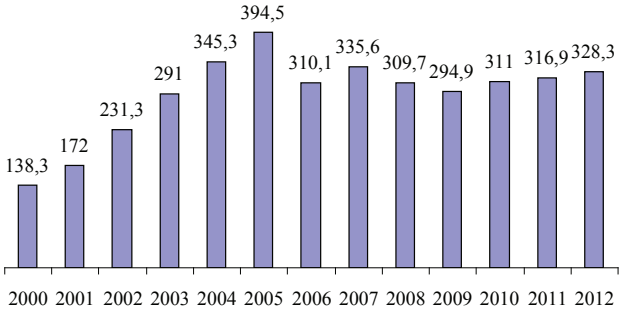

Fig. 1. The dynamics of the number of Moldovan citizens working abroad, (thousands of persons, absolute number). National Bureau of Statistics [38].

A high risk consequence for the next generation is the increasing number of minors whose parents are gone to work abroad, so-called phenomen of "home alone children". In Moldova this phenomenon achieved the highest level in Europe and is caused by low social services, vague knowledge, erroneous attitudes and practices regarding child rights [35].

At this moment can be identified three types of international migration specific for Moldovan citizens [34]: 1) short international migration – Moldovan labour migrants go to work in the Community of Independent States (CIS); 2) long international migration – Moldovan labour migrants go to work in the states of the European Union 3) long legal international migration – Moldovan migrants go to live in the US and Canada. More recently were observed the following trends and changes in the general pattern of international migration: changing the preference from CIS region to the countries of the EU; changing the short migration to long-term migration; increased migration to the US and Canada [8]. Moldova has considerable experience with emigration to the US and Canada where depart 3000 Moldovan citizens annually.

According to the research conducted by the National Bureau of Statistics, 60% of Moldovan migrants leave for CIS countries, mainly to the Russian Federation and 40% of

them to the EU countries, mainly to Italy. Characteristics of migrants working in CIS are the predominance of male and people from rural area, while to the EU countries go to work more frequently women and skilled young people [39]. As following of labor migration it was observed the reduction of unemployment rate in the Republic of Moldova due to reducing of economically active age population. Also, due to labor migration in the Moldovan population is actively manifesting the phenomenon of «demographic aging». The process of demographic aging is reflected by growing of the rate of population aged over 60 years, due to the migration of young people (70% of migrants are aged form 16 to 29 years, and 60-70% of them are women) [38].

Only less than 3% of immigrants will rest in the destiny country for all their life. Illegal status pushes migrants to return home. It was noted, that skilled migrants more frequently returned home than unskilled, being equipped with additional education, training and work experience that they have gained abroad [8]. Upon returning to the origin country, migrants who lived in poor housing, received low wages and had limited access to health services are returning home less healthy than when they left. When migrants return to their place of origin with detected, untreated TB, erroneously treated TB, or complications, they become an important load on their health care system and manifest profound health implications for their families and communities. In this way migrants determine high financial burden on their households if they do not have adequate health and social protection upon returning to their places of origin [1]. Another negative economical effect of migration is increasing of consumption, non-productiveness and the remittance depending by the non-migrant population.

Why are migrants vulnerable to tuberculosis?

There are several risk factors for TB development in migrants. First of all, migrants face a higher exposure to TB infection due to overcrowded living and working conditions. Due to poverty they have increased vulnerability to HIV infection contact, malnutrition and substance use. Delays in TB diagnosis among migrants are commonly associated with difficulty in healthcare access, lack of education, poor health-seeking behaviors, cultural beliefs, stigma and marginalization. Due to social risk factors, migrants often do not have access to correct TB-related information as the consequence of language barriers and cultural beliefs. Social-related factors: stigma, lack of awareness of health services, low health-related spending capacity, as well as migrant-unfriendly health services, all lead to delaying in seeking for health care [20].

Nkulu F. in the study regarding social features of migrants living in Sweden demonstrates their low degree of knowledge, low degree of healthcare seeking behavior, several misconceptions and negative attitudes regarding TB [26]. The author established that 23% of Swedish TB cases are foreign-born residents established in Sweden for more than 10 years. It was established that implicated risk factors for TB are the language barrier and unfamiliarity with the Swedish Health Care System. The study demonstrated that including TB education measures for accepting the screening will help to improve TB control in the country through early detection.

In high-burden countries TB-related morbidity and mortality among migrants have negative economic effects at household level for them and for their families. At societal level sick migrants will cause the loss of productivity and loss of revenue in the industries and at national government level. The negative economic effect is manifested in both - source and destination countries (loss of productiveness for destination country and loss of remittances for origin country).

Among migrant workers with a legal status, their access to TB diagnosis tools and health care is determined by the health insurance coverage, provided by the State or by the employer [2]. Irregular migrants face the fear of deportation that reduces their access to diagnostic and treatment services. If TB is diagnosed, the migrant is pushed to leave the destination country. Deportation during the anti-TB treatment causes the incompliance or interruption of anti-TB treatment which leads to development of drug resistant TB and increases danger of such patients for the health systems of origin, transit and destination countries. In Western Europe, more frequently multidrug-resistant TB (MDR-TB) is diagnosed in immigrants from Eastern European countries. MDR-TB is very difficult and expensive to treat. Resistant TB determines a substantial economic impact on the hosting country (ex. EU countries are low TB-incidence countries). As the transmission of TB infection from foreign-born to native European populations is well documented, in this way is established the epidemiologic danger of immigrants.

There are data showing that in low TB-burden countries, from 20% to 70% of notified TB cases are foreign-born individuals. Posey D. L. in the study of the implementation of new TB screening requirements for the US immigrants and refugees established that during 2013 in the US 64.6% of TB cases were diagnosed among foreign-born individuals [27]. Farah M. G. studying tuberculosis in migrants from Norway determined that the incidence of TB in originals from Africa is 190/100 000 and from Asia 80/100 000 population [13].

The risk for development is the highest among foreign-born groups and is up to 50 times higher than in native populations. The increased risk among foreign-born individuals may continue for 20 years after migration due to reactivation of latent TB infection contacted in their country of origin. Kruijshar M. E. in a study regarding TB in migrants from the UK established that 28% of migrants had positive results at interferon gamma release assays, that means that one third of migrant population of the UK have latent TB infection and a high risk for TB development [21].

Even during the transit to destiny country the TB risk of migrants is high especially when the travel occurs under precarious conditions. Irregular migrants may face violence and be held in detention centers with poor nutrition and ventilation, often in close proximity with others with preexisting TB. At destination migrants' integration into the host

country's health system, social services, accessing of housing, jobs is difficult. The continuous risk to be expulsed from the arrival country diminishes the health care seeking due to mistrust. As well as migrants' own health-seeking behavior and cultural practices may affect their use of TB services. Discriminatory practices such as deportation after positive TB diagnosis are an important barrier for migrants for seeking TB services in the country of destination [23].

Priimac A. A. established that among multiple causes that influence the epidemiological situation of tuberculosis in Russia should be noted the main influence of migration, constituted by the influx of persons released from prison from ex-USSR countries, migrant workers, refugees from the former Soviet Republics and from the regions of the ethnic conflicts of Russia [36]. Since 90-es the law in the Russian Federation does not require the annual chest radiography of persons with the risk for TB including migrants. Consecutively migration contributes to the deterioration of the epidemiological situation of tuberculosis in Russia. It was noted that the TB morbidity rate in migrant population is 6.7 times higher than in the resident population. More severe is the situation that annually about 30% of Russian patients with active TB migrate within the country. Moving to a new place of residence, such patients often remain unaware of new TB facilities and, therefore, do not receive an appropriate treatment, also in their environment are not carried out the necessary preventive measures. To resolve this situation the author recommends to reorganize the migration service in Russia, to adopt a proper legislation for a strict control of the state on the migration, obliging migrants to be screened timely for tuberculosis, and detected sick migrants to be treated.

It was noted that migrants dramatically worsened the epidemiological situation in Petersburg. As an example was shown St. Petersburg where in 2012 came 228 000 migrant workers. During this period of time TB was detected in 352 migrants and only 150 of them were deported or left the country on their own wish to perform the treatment. Also in 2012 the diagnosis of HIV infection in native St. Petersburg population was recorded at 60.0/100 000 and among migrants 83.2/100.000; TB in native St. Petersburg population was recorded at 32.4/100 000 and among migrants – 154.2/100 000 [35]. According to the epidemiological situation the most dangerous for the epidemiological situation are migrant workers from Central Asia. They have origin in high TB-burden region and being infected with *M. tuberculosis*, put themselves to risk for developing of active disease. Sick migrants expose a high epidemiological danger on the Russian native population.

Rospotrebnadzor noted that in Russia the epidemiological situation is aggravated by the fact that about 80% of migrants work on social significant objects such as: construction, selling, markets and are in constant contact with a lot of people [36]. Coordinator of the regional program in the field of labor migration J. Zelensky noted that Tajikistan is one of the largest suppliers of cheap labor in Russia. In this country the incidence of TB is much higher than in Russia, representing 204/100 000, but in Russia – about 85/100 000 people. According to this fact the individuals arrived from Tajikistan represent danger for epidemiological security of Russia being more frequently ill.

More severe is the epidemiological situation in Moscow. Among newly diagnosed patients with TB in Moscow during 2008, 16.7% of them were migrant-foreigners, 9% – were internal migrants (citizens of other regions of Russia), and about 10% – homeless people. Among foreign-born TB patients identified in Moscow, the proportion of citizens of Tajikistan was 21.3%, of Uzbekistan –20.3%, Kyrgyzstan – 14.4%, Ukraine – 14.1%. From the total number of TB patients detected in Russia, only 11.7% were deported to their origin country and 20.8% of them were admitted to the Russian hospitals for the treatment of TB [36]. The rest of migrants with TB remain on the territory of Russia continuing to spread the infection.

Local studies noted a high prevalence of Moldovan migrants with tuberculosis that returned from Russia (64%), Ukraine (12.1%), and other countries such as Italy, Turkey. It was established the prevalence of severe forms: caseous pneumonia and fibrocavitary tuberculosis in 19.6% of migrants. Low success rate, high default rate (10.6%) and deaths (5%) were more evident than in local resident population [10].

Regarding HIV infection, J. Zelensky noted that migrants from Central Asia have a greater risk to contact HIV in Russia, because sex is much freer than in the East and 30% of migrants have a high risk behavior [36]. Condom use among the population from the East is very low, as well as general knowledge about the HIV infection is precarious. The author established that adjusted to life threatening infections such as TB and HIV in migrant population demonstrate the epidemiological load on the Russian health security.

No studies were found about the TB among migrants from detention. Migrants in detention centers or trafficked persons often live in unsanitary and unhealthy conditions for extended periods of time, that increase their vulnerability to TB infection and active disease.

It was established that forced displacement of persons after an army conflict or a natural disaster is often associated with an increased TB risk due to malnutrition, overcrowding in camps and disruption from the health care services.

International experience of screening for tuberculosis in migrant population

Migration is a social determinant of the increased TB-related morbidity and mortality. There are specific differences in migrant screening procedures in the pre-departure phase and post-arrival phase for each country. Those differences influence TB-related morbidity and potential public health impact on the health system [28].

Rizo M. established that screening in pre-immigration settings may reduce the risk of TB development compared with post-immigration screening. Also the author noted that the new entrants more frequently complete the screening procedures than the old-established migrants [28].

The majority of European countries continue to use chest radiography for screening and detection of TB among applicants for permanent residence [24]. However, chest radiography has a low sensitivity and specificity for detection of TB. Chest radiography is even less sensitive and less specific for TB in HIV-positive individuals. Irregular migrants cannot benefit of such screening care in origin and destination countries, due to the lack of health insurance. The fear to perform the screening for TB is amplified by discriminatory practices such as deportation or interdiction to work if an active disease is established. Moldova experiences the pre-arrival screening in 3000 citizens annually of emigrants with intention to leave the country for the US and Canada. They perform the digital chest radiography, serological testing for hepatitis B and HIV infection.

Posey D. L. in the study of the implementation of new TB screening requirements for the US noted that beginning from 1991 the algorithm for TB diagnosis among adult migrants includes chest radiography and microscopic sputum examination of those with findings suggestive for TB [27]. In 2007 the national algorithm was enhanced by including additionally sputum cultures as a diagnostic tool for TB screening of those with suggestive findings for TB. In pediatric population TB screening was performed through tuberculin skin testing. From 2009 tuberculin skin test was replaced by interferon gamma release assays (IGRAs). This serological technique of TB screening demonstrated a high sensibility for identifying persons with latent TB infection. The author demonstrated the importance of serological screening of foreign-born migrants through IGRAs for identifying the latent TB infection [27].

Kruijshar M. E. in a study regarding serological screening of new entrants in the UK established that 20-28% of migrants had positive results at IGRAs [21], in this way demonstrating that one third of migrants have latent TB infection. The author noted high trends of migration to England from countries of Sub-Saharian Africa and continuously increased trends of migration from the Indian Subcontinent Countries, regions with high TB-burden epidemiological situation.

Farah M. G. studying the long-term risk of tuberculosis in immigrants in Norway determined that the incidence of TB in Norwegian migrants from Africa is 190/100 000 and among migrants from Asia 80/100 000 population [13]. These rates were 90 times higher than the crude TB incidence in Norway, and the rate of TB development was 2 times higher in the first two years after arrival of migrant in Norway [13]. The author recommended the vigilance regarding migrants due to the high risk for TB disease that lasts many years after arriving in the hosting country.

It is well established the negative influence of migration on the anti-TB treatment compliance. Chen Jing evaluated TB among migrants in Shanghai China [41]. In China there are registered one million TB cases, the second largest number in the world with a global incidence of 75/100 000 population. In China, the most frequent type of migration is commuting from rural to urban areas. In total there were registered 145 million internal migrants in China during the period of 2009. From the total number of TB patients 52% were internal migrants. Among outcomes of antituberculosis treatment predominated default (52% of treated cases) and death (30%). There were established risk factors for default of anti-TB treatment in Chinese migrants: self-administrated treatment, retreatment, and age of the patient over 60 years. The national study assesses that almost one third of patients with TB who interrupted or defaulted the antiTB treatment were migrants.

Conclusions

TB is a big challenge worldwide. Immigrants are the majority of TB patients in high-income countries. The irregular emigrants are the most of patients of high-burden countries. Radiological and immunological screening in pre-departure is the most important procedure for decreasing the rates of TB. Raising awareness among migrants about TB, emphasizing that diagnosis and treatment are free of charge and independent regarding migration status are important TB control actions. Considering high relevance of migration on the risk of TB development a national survey in the actual geopolitical context must be performed in the Republic of Moldova.

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Hemorrhagic transformation of ischemic stroke – prediction and evaluation with different computed tomography modalities

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Abstract

Background: Hemorrhagic transformation (HT) of ischemic stroke is a complex and heterogeneous phenomenon, which involves numerous parameters whose knowledge remains partial. Large HT is often associated with poorer outcome and higher mortality, especially parenchymal hematoma type 2, that's why the search of strong HT predictors is very important and can improve management of ischemic stroke patients. Our aim was to review the literature regarding computed tomography (CT) imaging predictors of HT and possible input of different computed tomography modalities in diagnosis and evaluation of HT following acute ischemic stroke. The contribution of non-contrast computed tomography, computed tomography angiography (CTA) and dynamic Perfusion CT (PCT) investigation in the prediction of the hemorrhagic transformation risk were studied. Multiple multicentre studies revealed useful information on different CT patterns predictors of symptomatic intracerebral hemorrhage after stroke, which is the most important type of hemorrhagic transformation from a clinician's point of view.

Conclusions: Data from the multiple studies and trials revealed that different CT modalities show high potency in HT prediction and evaluation. Non-contrast CT standard investigation showed high accuracy in HT prediction by assessment of early ischemic signs, quantification of the Alberta Stroke Program Early CT Score (ASPECTS), grading of leukoaraiosis severity. CTA is useful in HT prediction by the assessment of collateral vessels; intra-arterial occlusion and ASPECTS score calculated from the CTA source images. PCT showed the best predictive values by the measurement of blood-brain barrier permeability.

Key words: acute ischemic stroke, hemorrhagic transformation, non-contrast computed tomography, computed tomography angiography, perfusion computed tomography.

Introduction

Stroke is the third leading cause of death in industrialized countries and the most frequent cause of permanent disability in adults worldwide [1, 2]. One of the most undesirable complications of ischemic stroke is hemorrhagic transformation (HT), which may further complicate already devastating clinical condition. HT after acute ischemic stroke is known to associate with poor outcome and delays the initiation of proper anticoagulation treatment for stroke with cardioembolism [3]. Historically, hemorrhagic transformation, initially designated as "red softening," has long been recognized by neuropathologists to occur as a natural consequence of ischemic brain injury. To search for new treatments as well as intervention measures for HT, it is important to understand its underlying mechanism and identify its predictors [4].

Epidemiology. The true incidence of hemorrhagic transformation remains uncertain, and reported frequencies vary depending on the methodology used and the underlying pathogenesis of the ischemic insult [5]. Autopsy series have reported secondary bleeding from 51% to 71% of recent embolic infarctions as compared to a 2% to 21% incidence in non-embolic strokes [6, 7]. Computed tomography (CT) studies have reported hemorrhagic infarction from 26% to 43% of non-anticoagulated patients with predominantly embolic infarcts [8, 9]. With the recent large use of new different treatment modalities of acute ischemic stroke (AIS), HT rates were in detail evaluated in multiple clinical trials of therapeutic (intravenous and intra-arterial fibrinolysis) and surgical (mechanical thrombectomy and arterial stenting) AIS interventions.

Basic rates reported in those trials for asymptomatic hemorrhagic transformation (AHT) and symptomatic hemorrhagic transformation (SHT) constitute: National Institute of Neurological Disorders and Stroke (NINDS) – 4.5% (14 patients/ from 312 cohort size) AHT and 6.4% (20/312) SHT [10]; European Co-operative Acute Stroke Study-II (ECASS-II) – 39.6% (161/407) AHT and 8.8% (36/407) SHT [11]; European Co-operative Acute Stroke Study-III (ECASS-III) – 27% (113/418) AHT and 2.4% (10/418) SHT [12]; Alteplase thrombolysis for acute noninterventional therapy in ischemic stroke (ATLANTIS) – 11.4% (31/272) AHT and 7.0% (19/272) SHT [13]; Safe Implementation of Thrombolysis in Stroke-Monitoring Study (SITS-MOST) – 9.6% (617/6438) AHT and 7.3% (468/6483) SHT [14]; Prolyse in Acute Cerebral Thromboembolism II (PROACT-II) – 68% (73/108) AHT and 10% (11/108) SHT [15]; the Interventional Management of Stroke I (IMS I) – 43% (34/80) AHT and 6.3% (5/80) SHT [16]; the Interventional Management of Stroke III (IMS III) – 27.4% (119/434) AHT and 6.2% (27/434) SHT [17]; Safety and efficacy of mechanical embolectomy in acute ischemic stroke (MERCI Trial) – 27.7% (39/141) AHT and 7.8% (11/141) SHT [18]; The Penumbra Pivotal Stroke Trial (Penumbra System for Clot Removal in Intracranial Large Vessel Occlusive Disease)-16.8% (21/125) AHT and 11.2% (14/125) SHT [19]; Solitaire flow restoration device versus the Merci Retriever in patients with acute ischemic stroke (SWIFT Trial) -15.5% (9/58) AHT and 2.0% (1/58) SHT [20]; Trevo versus Merci retrievers for thrombectomy revascularization of large vessel occlusions in acute ischemic stroke (TREVO-2) – 40.9% (36/88) AHT and 4.5% (4/88) SHT [21].

CT classification. There is a broad spectrum of severity of hemorrhagic transformation, ranging from subtle petechial hemorrhage within infarcted tissue to large-volume hematoma extending beyond the borders of the infarction [22]. All types of HT are classified regarding their Computed Tomography (CT) patterns in 2 large groups: hemorrhagic infarction (HI) and parenchymal hematoma (PH) and include two subtypes of HI (HI1 and HI2) and two subtypes of PH (PH1 and PH2) (Table 1) [23]. The term *hemorrhagic infarction* describes heterogeneous hyperdensity occupying a portion of an ischemic infarct zone on CT-imaging, whereas *parenchymatous hematoma* refers to a more homogeneous, dense hematoma with mass effect [24].

Table 1

Radiographic classification of the spectrum of hemorrhagic transformation, based on criteria proposed by Fiorelli et al. (1999)[23]

Hemorrhage classification	Radiographic appearance
Hemorrhage infarction type 1 (HI1)	Small hyperdense petechiae
Hemorrhage infarction type 2 (HI2)	More confluent hyperdensity throughout the infarct zone; without mass effect
Parenchymal hematoma type 1 (PH1)	Homogeneous hyperdensity occupying <30% of the infarct zone; some mass effect
Parenchymal hematoma type 2 (PH2)	Homogeneous hyperdensity occupying >30% of the infarct zone; significant mass effect. Or, any homogenous hyperdensity located beyond the borders of the infarct zone

Figure 1 represents examples of four patients hospitalized at the Institute of Neurology and Neurosurgery (Chisinau, Moldova), between 01.2015-03.2015, diagnosed with acute ischemic stroke in the right middle cerebral artery vascular territory. During hospital treatment was registered hemorrhagic transformation, proved by the presence of characteristic Computed Tomography pattern – hyperdense inclusions of different size and intensity, supporting various types of HT from HI1 to the extensive PH2.

Risk factors and predictors of HT. Several factors are associated with or predict HT. Although the usefulness of some of these markers in clinical practice might be limited, the development of imaging techniques and the identification of predictive biomarkers might help in the selection of patients at increased risk of HT [25]. Reliable clinical and radiologic predictors are needed to identify patients at highest risk for hemorrhagic transformation in order to guide the safe use of anticoagulants or thrombolytic therapy [26]. Initial stroke

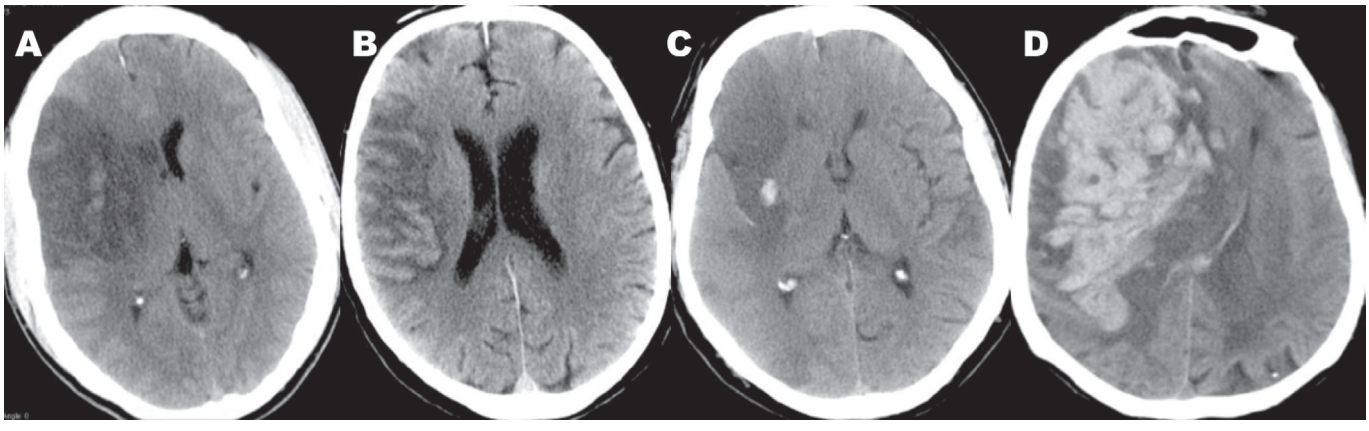

Fig. 1. Non-contrast Multislice Computed Tomography.

A – Male 51 years, 3 days after symptoms (acute left hemiplegia) onset, HI type 1 transformation (small hyperdense petechiae in the center of large right parietal hypodensity with edema and contralateral shift of median brain structures). B – Male 71 years, 10 days after symptoms onset, HI type 2 (confluent hyperdensities throughout the ischemic right parietal zone, without mass effect). C – Male 60 years, 24 hours of onset (acute deep left hemiplegia, sopor), PH type 1 transformation (homogeneous hyperdensity 5 mm in diameter in the projection of the external capsule on the medial contour of large right hemispheric infarction). D – Woman 85 years, 2 hours after symptoms onset (The Glasgow Coma Scale 3), Right hemorrhagic transformation – hemispheric parenchymal hematoma type 2, death in a few hours of onset.

severity, older age, heart disease, high blood pressure, male gender, obesity, baseline hyperglycemia or history of diabetes, uncontrolled hypertension at presentation, antiplatelet therapy prior to hospitalization were reported to have sensitivity in HT prediction [27-31]. But the strongest predictors are characteristic imaging patterns, which are useful in diagnosis, evaluation and prognosis assessment in all cases of ischemic stroke and especially in such complication as HT. Computed tomography (CT) is recommended by the American Heart Association as the initial modality of choice for stroke investigation.

CT imaging in HT diagnosis and prediction. It is usually assumed that CT is the gold standard for the detection of ICH. The diagnostic performance of CT stroke protocols is improved with modification of window and level settings [32] interpretation of CT angiographic source images, and CT perfusion. Imaging of ischemic stroke and its hemorrhagic transformation has 4 main priorities: 1) Imaging the Cerebral Parenchyma (mostly is provided by non-contrast CT (NCCT), but additional information is supplied by Angiography CT source images (CTA-SI) and Perfusion CT (PCT) maps), 2) Intracranial Vascular Evaluation (CTA), 3) Assessment of cerebral perfusion parameters (basic PCT maps), 4) Blood-Brain Barrier Permeability evaluation (permeability surface (PS) PCT product).

Non-contrast CT (NCCT). Non-enhanced, or non-contrast, CT represents the first step of all more complicated CT modalities (angiography and neuroperfusion), or can be used alone as an urgent tool in stroke management protocol. NCCT is the preferred modality because of its accessibility, speed, and patient's tolerance, thereby permitting the rapid triage of patients suspected of having experienced a stroke [33]. Imaging the Cerebral Parenchyma by NCCT combines three basic roles in assessing the status of brain tissue in the acute stroke patient: the exclusion of hemorrhagic stroke, the detection of the ischemic tissue, and the exclusion of conditions that mimic acute cerebral ischemia. The perfection of multidetector technology has enabled a CT scan of the head to be obtained with submillimeter slice thickness in a few seconds and with superior tissue differentiation (contrast resolution). Besides pure exclusion of brain hemorrhage, however, early data about site and severity of brain ischemia and their respective pathogenesis are clinically requested.

Early computed tomographic (CT) signs of cerebral infarction seen within 6 hours after onset of symptoms of stroke may be predictive of poor functional outcome and clinically significant hemorrhagic transformation of the infarct [34, 35, 36]. Early CT signs include: dense MCA, loss

Table 2

Definition of Early CT Signs of Ischemic Stroke [37]

CT Sign	Definition	Type of Measure
Hypoattenuation in thirds of middle cerebral artery territory	Decreased attenuation of less or more than one-third of the presumed middle cerebral artery territory	Semiquantitative
Obscuration of lentiform nucleus	Decreased attenuation involving the lentiform nucleus and inducing the loss of the precise delineation of this area	Qualitative
Cortical sulcal effacement	Decreased contrast, loss of precise delineation of the gray-white interface in the margins of the cortical sulci, corresponding to localized mass effect	Qualitative
Focal hypoattenuation	Increased radiolucency of brain structures relative to other parts of the same structure or to contralateral counterparts	Qualitative
Loss of insular ribbon, obscuration of sylvian fissure	Decreased precision in delineation of gray-white interface at lateral margin of the insula	Qualitative
ASPECTS value*	One point is subtracted for each area of hypoattenuation in a defined region (normal = 10).	Semiquantitative
Hyperattenuation of vessel	Attenuation higher than that in any other visualized artery or vein	Qualitative
Loss of gray and white matter differentiation in the basal ganglia	Decreased contrast, loss of precise delineation of the gray-white interface of the basal ganglia	Qualitative
Hypoattenuation of basal ganglia	Decreased attenuation (measured in Hounsfield units) in basal ganglia	Quantitative

* ASPECTS = Alberta Stroke Programme Early CT Score

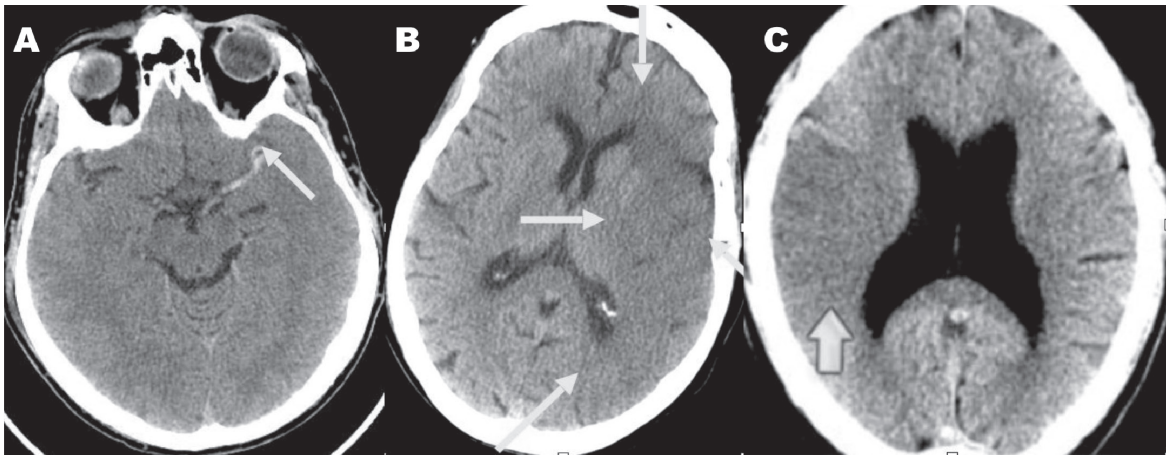

Fig. 2. Non-contrast Computed Tomography. Early CT signs of acute ischemic stroke.
A – dense middle cerebral artery sign (arrow). B – early ischemic signs in left hemisphere, slice on the level of basal ganglia: loss of gray and white matter differentiation, large hypodensity, effacement of sulci, loss of lentiform nucleus outline (arrows). C – early ischemic signs in right hemisphere, slice on the level of the body of the lateral ventricles: loss of gray and white matter differentiation, hypodensity, effacement of sulci (arrow).

of basal ganglia outline, loss of insular ribbon, hypodensity, effacement of sulci or ventricles, dense cortical sulci, loss of gray and white matter differentiation [38, 39, 40] (definition of early CT signs is shown in tab. 2).

One of the most important early CT sign is the increased density within the occluded vessel, which represents the thrombus. When this is the MCA, it is called the hyperdense MCA sign [42, 43], and it is seen in one third to one half of all cases of angiographically proven thrombosis (Fig. 2 A). Hence, it is an appropriate indicator of thrombus when present, but its absence does not exclude thrombus.

Another significant early CT sign of cerebral ischemia within the first few hours after symptom onset is loss of gray-white differentiation (fig. 2 B, C), because there is an increase in the relative water concentration within the ischemic tissues. This sign includes loss of distinction among the nuclei of the basal ganglia and a blending of the densities of the cortex and underlying white matter in the insula and over the convexities. The subsequent swelling of the gyri produces sulcal effacement, which may lead to ventricular compression. The sooner these signs become evident, the more profound is the degree of ischemia. However, the ability of observers to detect these signs on NCCT is quite variable, depending on the size of the infarct, the time between symptom onset and imaging, and the methodology of the trial itself; the detection rate appears to be 67% in cases imaged within 3 hours. The rate of detection increases to 82% at 6 hours [41]. To offer the reliability and utility in assessing early ischemic changes on NCCT examination was developed special score system – The Alberta Stroke Program Early CT Score (ASPECTS) with a reproducible grading system of acute ischemic stroke in the middle cerebral artery (MCA) circulation territory.

The Alberta Stroke Program Early CT Score (ASPECTS). The ASPECTS is a 10-point quantitative topographic CT scan score used in patients with MCA stroke. Segmental assessment of MCA territory is made and 1 point is removed from the initial score of 10 if there is evidence of infarction in that region [44, 45]. A normal CT scan receives ASPECTS of 10 points. A score of 0 indicates diffuse involvement throughout the MCA territory. An ASPECTS score less than or equal to 7 predicts worse functional outcome in 3 months as well as symptomatic hemorrhagic transformation.

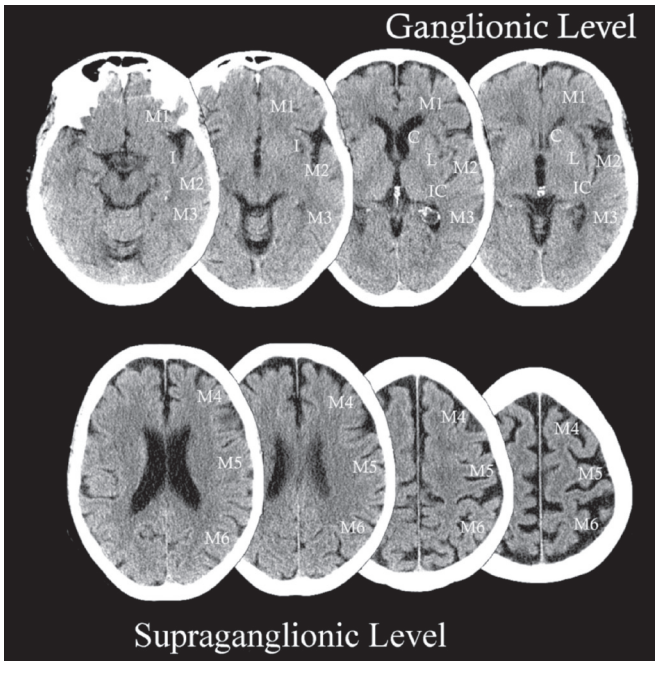

Fig. 3. Axial NCCT images showing the MCA territory regions as defined by ASPECTS. C – Caudate, I – Insular ribbon, IC – Internal Capsule, L – Lentiform nucleus, M1 – Anterior MCA cortex, M2 – MCA cortex lateral to the insular ribbon, M3 – Posterior MCA cortex, M4, M5, M6 are the anterior, lateral and posterior MCA territories immediately superior to M1, M2 and M3, rostral to basal ganglia. Subcortical structures are allotted 3 points (C, L, and IC). MCA cortex is allotted 7 points (insular cortex, M1, M2, M3, M4, M5and M6).

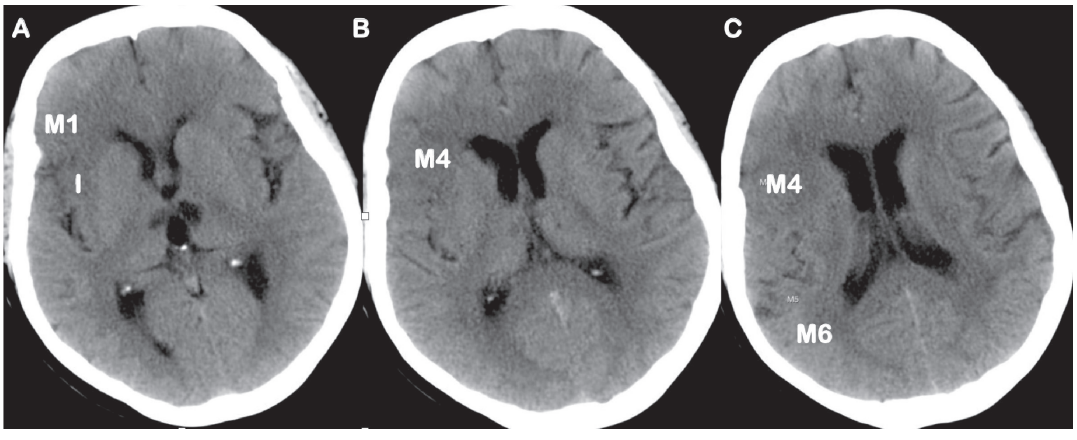

Fig. 4. Female, 55 years. NCCT in hyperacute phase of stroke (2 hours of symptoms onset – left hemiparesis, right pyramidal insufficiency, visual disorders, aphasia).
A, B – ganglionic level. C – supraganglionic level. Early signs of stroke in right MCA, ASPECTS = 6 (M1, M4, M6, I). Chronic bilateral lacunar infarct in thalamus.

ASPECTS is determined from evaluation of two standardized regions of the MCA territory: the basal ganglia level, where the thalamus, basal ganglia, and caudate are visible, and the supraganglionic level, which includes the corona radiata and centrum semiovale (fig. 3). All cuts with basal ganglionic or supraganglionic structures visible are required to determine if an area is involved. The abnormality should be visible on at least two consecutive cuts to ensure that it is truly abnormal rather than a volume averaging effect (fig. 4).

The ECASS I and ECASS II studies have shown that the presence of early CT signs occupying more than one-third of the MCA territory is accompanied by an increase in the hemorrhagic transformation risk (especially of PH- 2 type in the ECASS II study) and poor clinical outcome [11]. The multicenter analysis of 1205 patients routinely treated by intravenous tPA within 3 h shows that the symptomatic HT rate is multiplied by more than 4 in patients with early signs in >1/3 of the MCA territory [47]. In the MAST-E study, the presence of early signs was also a strong predictor of hemorrhagic transformation and symptomatic ICH [29]. In contrast, other thrombolysis studies have shown that the presence of early signs was related to stroke severity but was not independently associated with the occurrence of side effects [14, 15]. The ASK (Australian Streptokinase Trial) study, evaluating treatment with intravenous streptokinase within the first 4 h of ischemic stroke, did not show a significant association between the presence of early signs and the occurrence of a major PH.

Hyperdense middle cerebral artery sign. It has been shown that the presence of an arterial hyperdensity on the pre-therapeutic brain CT scan is an independent predictive factor of any tPA related to HT [46]. It was previously demonstrated that this sign was frequently observed in patients developing an asymptomatic HT after thrombolysis by intravenous tPA within the first 3 h. In other study, the presence of an arterial hyperdensity on brain CT scan is associated with MCA or internal carotid artery occlusion in most cases and with a specific MRI pattern consisting of a large pretreatment diffusion and perfusion abnormality volume [48]. The presence of a proximal arterial hyperdensity, as the severity of perfusion reduction suggests, is probably associated with a limited collateral blood supply. The maximal haemodynamic consequence lies in the territory of the perforating lenticulostriate arteries and the severity of the ischemic injury in this territory probably induces the hemorrhagic transformation [14].

Leukoaraiosis (LA). Originally noted as diffuse, nonspecific subcortical white matter lesions on CT, leukoaraiosis is a common finding among patients with ischemic stroke and has been associated with poor post-stroke outcomes as well as increased risk for HT [51-52]. LA represents cerebral white matter changes that are frequently observed on CT and MRI scans of elderly individuals, are seen as bilateral, patchy, or diffuse areas of hypodensity on CT involving the periventricular and centrum semiovale white matter. These lesions have irregular margins and do not follow specific vascular territories [53].

The largest visual scales used for assessment of LA on CT are: 1) Van Swieten et al., (score 0-4). The severity of LA is graded separately for the regions anterior and posterior to the central sulcus added together: 0 – no lesion, 1 – partial involvement of the white matter, and 2 – extending up to the subcortical region [54]. 2) Blennow et al., (score 0-3). The final score is the mean value between the extension and severity scores. Extent of LA: 0 – no decrease in the attenuation of white matter; 1-decreased attenuation of white matter at the margins at the frontal and occipital horns of the lateral ventricles; 2-decreased attenuation of white matter around the frontal and occipital horns of the lateral ventricles with some extension toward the centrum semiovale; and 3-decreased attenuation of white matter extending around the whole lateral ventricles and coalescing in the centrum semiovale. Severity of LA: 0-none, 1-mild, 2-moderate, and 3-marked decrease in the attenuation of white matter [55].

A recent retrospective multicenter study showed, that leukoaraiosis is a risk factor for symptomatic HT in 449 patients

treated with thrombolysis for anterior circulation stroke less than 6 h after symptom onset [49]. For the analysis, leukoaraiosis in the deep white matter was dichotomized into absent or mild versus moderate or severe. The rate of symptomatic HT was significantly higher in patients with moderate to severe leukoaraiosis of the deep white matter (n = 12 of 114; 10.5%) than in patients without relevant leukoaraiosis (n=13 of 335; 3.8%). In a logistic regression analysis (including age, NIHSS score on admission and type of thrombolytic treatment), leukoaraiosis remained an independent risk factor (p = 0.03). Other large study of 1,153 consecutive patients with imaging-confirmed ischemic stroke concluded, that presence of coexisting severe leukoaraiosis predicts poor functional 90-day outcome (modified Rankin Scale) in patients with acute intracranial large artery occlusion in anterior circulation independent of other known important outcome predictors (70% of patients in this subgroup were either severely disabled or died at 90 days) [50].

Computed Tomography Angiography (CTA). CTA provides excellent imaging of intra- and extra-cranial vasculature, which is essential in diagnosis of every type of cerebrovascular disease, especially acute ischemic stroke. Recent studies show that CTA is a safe and accurate technique for imaging most extracranial and intracranial vessels for stenosis/occlusions with the sensitivity and specificity equal to or superior to that of MRA (Magnetic Resonance Angiography) in most circumstances, and in some cases, its overall accuracy approaches or exceeds that of DSA [56, 57]. For the acute stroke patient, vascular imaging plays several roles: 1) differentiation of real occlusion from transient ischemic attack, or other cerebrovascular disease and establishing the mechanism of ischemia to prevent subsequent episodes, 2) visualization of the localization of the site of vascular occlusion, extent and morphology of thrombus, 3) assessment of collaterals status and 4) analysis of cerebral parenchyma on CTA source images (CTA-SI).

A recent retrospective multi-center study of 263 patients showed, that the quantification of intracranial thrombus extent predicts functional outcome, final infarct size and risk of HT [58]. This study group developed the clot burden score (CBS) -a semiquantitative CTA grading system in acute anterior circulation ischemic stroke to quantify the extent of ipsilateral intracranial thrombus, allotting major arteries 10 points for the presence of contrast opacification on CTA. 2 points each were subtracted for absence of contrast opacification in the complete cross-section of any part of the proximal M1 segment, distal M1 segment or supraclinoid ICA and 1 point each for M2 branches, A1 segment and infraclinoid ICA (Fig 5, A). A score of 10 indicates absence of a visible occlusion on CTA, a score of 0 indicates occlusion of all major intracranial anterior circulation arteries. Study group found, that patients with lower CBS were more likely to have hemorrhagic infarct transformation (P = 0,003) and parenchymal hematoma (P = 0,008) on follow-up scans [58].

Collateral flow. Next extremely important CTA parameter is the development grade of **collateralls** in the infarcted hemisphere (fig. 5, B, C, D)**.** The presence of robust collateral flow is associated with rapid recanalization in acute ischemic stroke and reduction of infarct size [60]. The PROACT II trial investigators semiquantitatively analyzed pial collateral formation on angiography and categorized them as full, partial, or none and found that presence of good collaterals influences NIHSS score at initial presentation, infarct volume and HT rate on 24-hour CT scan in patients with MCA occlusion. The presence of collaterals has also been associated with better outcomes, reduced infarct size, and faster recanalization [15]. In the study Lin K. et al., 2012, the CTA collateral score was graded using a 4-point scale from 0 to 3 ("0"–absent collateral supply; "1"– collateral supply filling >0 but ≤ 50%; "2"– collateral supply filling >50 but <100%; and "3"– collateral supply filling 100% of the occluded MCA territory compared to the contralateral side) and compared with HT rate. Data analysis revealed significant associations between symptomatic HT and proximal occlusion (p = 0.049) and collateral score (p = 0.017) [59].

Computed tomography angiogram-source images (CTA-SI). The source images of the brain during CTA acquisition make a focus of hypoperfusion much more detectable than does the NCCT (Fig 6). Lev et al., [32] demonstrated the very high correlation between size of the infarct on CTA-SI and that which was demonstrated on follow-up CT studies. Hypoattenuation on brain CTA source images is attributable to early ischemic edema and a diminished con-

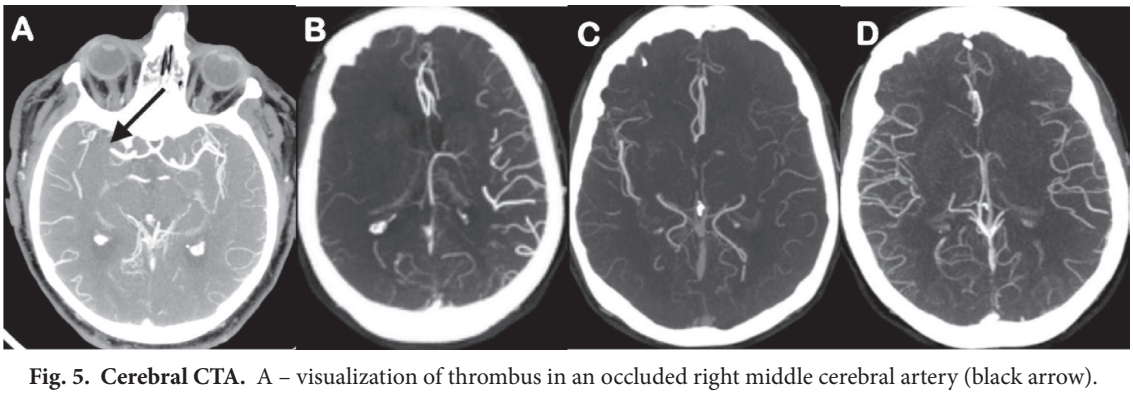

Fig. 5. Cerebral CTA. A – visualization of thrombus in an occluded right middle cerebral artery (black arrow). 3 grades of collaterals development: B – poor collaterals in right ischemic hemisphere, C – intermediate collaterals in the left MCA territory, D – good collaterals in left MCA ischemic circulation.

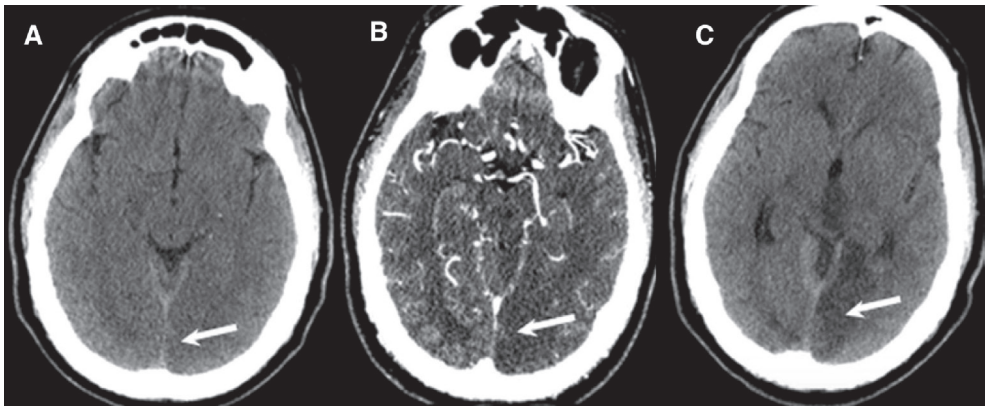

Fig. 6. Hyper-acute ischemic stroke in the left posterior cerebral artery circulation territory (arrow). A – NCCT, very difficult detectable early CT signs of ischemic lesion. B – CTA-SI, clearly detectable hypo-attenuation zone in the projection of left occipital lobe. C – follow up CT in subacute phase, confirming CTA-SI findings.

trast agent–exchanging blood pool. Results of pilot studies have suggested that hypoattenuation on CTA source images delineates ischemic tissue likely to be irreversibly infarcted and that lesion volume is seen on CTA source images. Focal hypoattenuation on CTA source images may reflect a profound degree of ischemia- induced endothelial injury with increased risk of reperfusion hemorrhage or inadequate leptomeningeal collateral blood flow. Data suggested that axial source images provide a visually detectable threshold of hypoattenuation without post-processing that is a powerful independent predictor of both HT and poor outcome [61]. Regions of abnormality on the NCCT usually cannot be segmented prospectively because of their ill-defined borders; areas of abnormality are more conspicuous on CTA source images. ASPECTS calculated on the CTA-SI have shown that CTA-SI is better at depicting early ischemic change than NCCT and therefore offers a more accurate score [59]. Investigators found that threshold at ASPECTS ≤5 on CTA-SI had high sensitivity (75.0%) and specificity (85.5%) for symptomatic HT [59]. Comparing CTA-SI and NCCT images Aviv et al., found that CTA-SI improved trainee accuracy by 9%, but had little impact on more experienced readers. The accuracy and sensitivity of stroke extent assessment was increased for all readers, but was greatest for the trainee (17% and 12%, respectively). Clinical history contributed little to CTA-SI accuracy. Observer resolution was higher for CTA-SI. NCCT could have resulted in the misclassification of more patients than CTA-SI [62].

Computed Tomography Perfusion (CTP). CT perfusion imaging uses dynamic contrast- enhanced data to produce parametric color overlay maps of cerebral blood flow (CBF), cerebral blood volume (CBV), mean transit time (MTT) and time to local peak enhancement (TTP) (fig. 7). These standard perfusion metrics are used to detect an acute ischemic stroke and the presence of "penumbra," the hypoperfused but potentially salvageable tissue at risk of infarction [63-65]. CTP, can not only provide the diagnostic information about ischemic region, but and the functional brain data about blood-brain barrier (BBB) physiology, especially its permeability. HT is generally thought to arise from ischemic damage to the blood-brain barrier (BBB), expressed in elevated permeability. Microvascular permeability (expressed as the transendothelial transfer of constant or permeability surface area product [PS]) is a metric of BBB integrity. Increased blood–brain barrier permeability, one of the pathological changes following ischemic stroke, is believed to predispose to complications such as hemorrhagic transformation [79], massive vasogenic edema, infarct expansion [80] and poor clinical outcome [81, 78]. Like the standard perfusion metrics, PS can also be calculated using dynamic imaging by measuring the leakage of an intravascular tracer (contrast agent) into the extravascular (interstitial) space [66, 67]. BBB permeability imaging provides a physiologic individualized measurement intimately connected to the underlying pathophysiology of PH-2 (ischemia-induced vascular damage followed by reperfusion) and may, therefore, offer excellent sensitivity and specificity (fig. 8).

Recent study [68] showed, that there was complete separation of PS in HT group and PS and in non-HT group measurements; any PS between 6.0 and 9.8 mL/100 mg per minute can be used as a threshold value to predict HT with a sensitivity and specificity of 100%. Investigators showed that elevated PS can be detected during hyper-acute period using first-pass dynamic CT data. Patients with high PS who were not treated with recombinant tissue plasminogen activator (rtPA) tended to develop smaller petechial hemorrhagic infarctions that were asymptomatic. Christopher D. d'Esterre (2013) demonstrated, that the patients with HT had a significantly higher PS than did those without haemorrhage. A PS threshold of 0.23 $ml^{-1} \bullet min^{-1} \bullet (100g)^{-1}$ also enabled the differentiation of patients with HT from those without [69]. Results showed that besides the PS parameter and ASPECT score, no other factors (age, sex, or baseline NIHSS score) were associated with HT. Hom J et al., investigated the opportunities of perfusion CT in prediction of HT and malignant edema in patients with acute ischemic stroke [76]. They reported that using admission BBB perfusion imaging above a volume threshold as the sole predictor yields a sen-

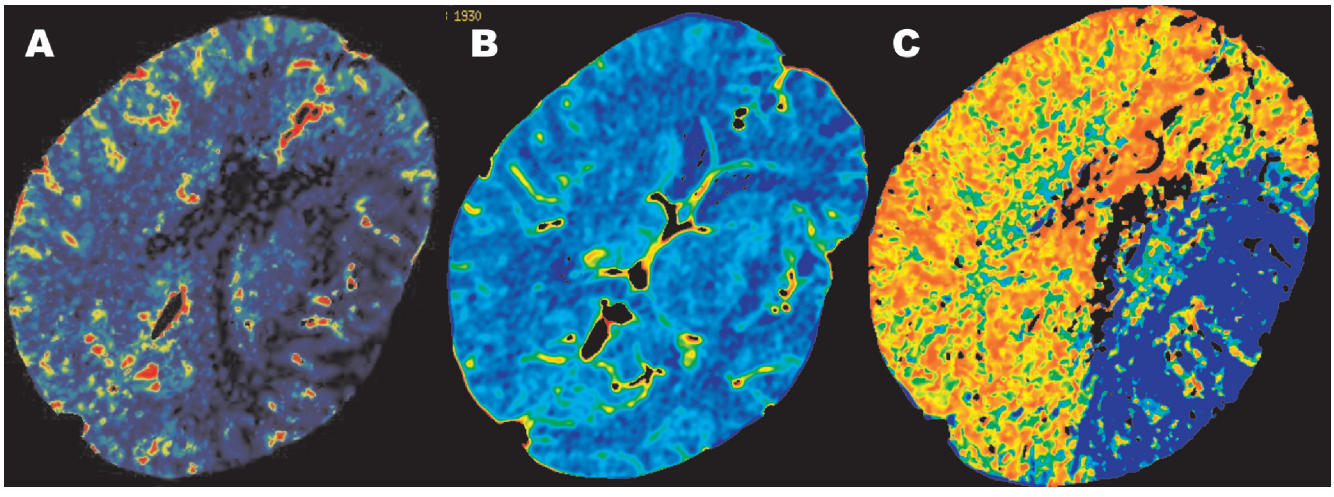

Fig. 7. Female, 84 years, acute (1 hour from the symptoms onset) ischemic stroke in left middle cerebral artery circulation territory. Standard CT perfusion maps. A – CBF. B – CBV. C – MTT.

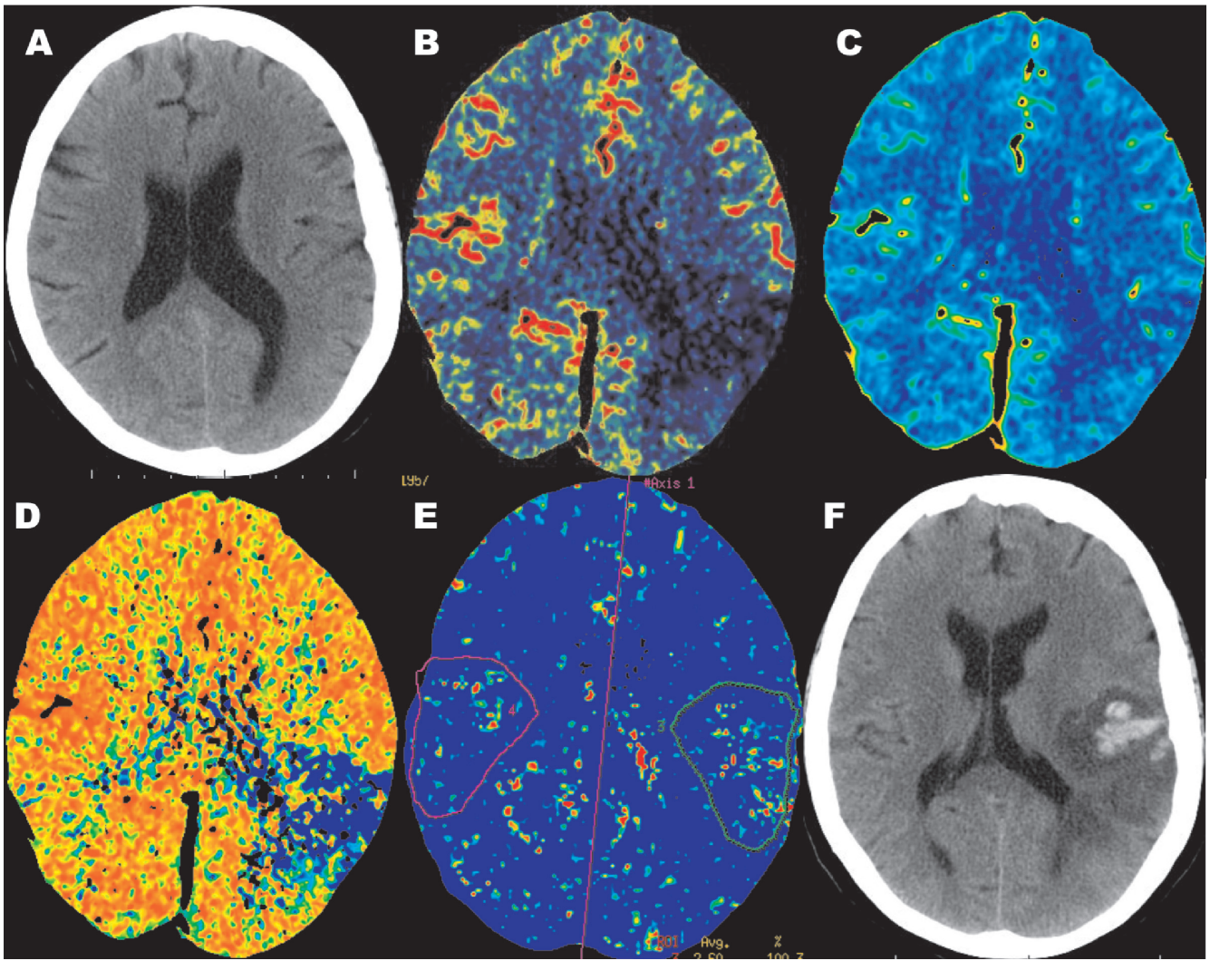

Fig. 8. Female, 57 years, acute (2 hour from the symptoms onset) ischemic stroke in left middle cerebral artery circulation territory. CT perfusion functional maps. A – non-contrast CT, there is no evidence of abnormalities, B – CBF, C – CBV, D – MTT, E – permeability surface area product (PS), F – non-contrast CT 26 hours after symptoms onset, hemorrhagic transformation, parenchymatous hematoma type 2.

sitivity of 100% and specificity of 79% (5 false-positives from 32). Reperfusion was a statistically significant predictor of SHT in the univariate analysis, but the reperfusion scores in patients with SHT were lower than those in other patients, not higher as we would have expected on the basis of the role of reperfusion in SHT [77].

Other (not PS) functional Perfusion CT parameters were studied to determine their correlation with HT and results failed to show any difference with statistical importance, with only exception for the relative cerebral blood volume (rCBV) map. Patients with HT had higher median total volume at risk of ischemia, longer median relative mean transit time (rMTT), and lower median relative cerebral blood flow (rCBF) compared with controls, though this did not reach statistical significance. Cases with HT did have a significantly lower median rCBV compared with controls (p = 0.01). For each 0.1-U decrease in rCBV, the odds of developing an HT increases by 14% [70]. Prediction of HT by reduced pretherapeutic CBF, an inherent characteristic of the stroke itself, was first suggested by Ueda et al., by using SPECT [71]. A reduction in CBF to <50% of normal was considered as the critical value for developing HT [72]. Gupta et al., in their study of 23 patients with symptomatic stroke or carotid stenosis concluded that mean ipsilateral CBF <13 mL/100 g per minute was the cutoff for developing HT [73]. They found that rCBV rather than rCBF was the strongest predictor of HT. This is similar to reports of rCBV (rather than rCBF) being a stronger predictor of penumbra viability in patients with acute ischemic stroke [74], with similar [75] or even lower [59] values reported by authors for rCBV in patients with HT. In other words, indicators of penumbra viability may also indirectly predict HT, which would be expected to occur more frequently in infarcted core rather than salvageable penumbra regions. Jain et al., found that a cutoff rCBV of at least 0.98 could predict development of HT in patients with AIS with 72% specificity [70].

Multimodality Computed Tomography. Different CT modalities showed different accuracy in prediction of hemorrhagic transformation, but most of studies reported better sensitivity and specificity for the Perfusion CT. Darius G. Nabavi et al., published a novel imaging CT algorithm using all 3 CT methods and developed a new scoring system – MOSAIC: Multimodal Stroke Assessment Using Computed Tomography [82]. With 3 main CT modalities (NCCT, CTA, PCT), profound knowledge about brain anatomy, vessel status, and tissue hemodynamics can be acquired in acute stroke patients within several minutes. The calculation is made by adding scores from every CT modality: 1) Early signs of infarction on NCCT: 0 = No infarction signs, 1 = 1–3 ASPECTS regions infarcted, 2 = >4 ASPECTS regions infarcted. 2) Ipsilateral pathology on CTA: 0= Normal or stenosis <50%, 1= ICA/MCA stenosis >50%, 2= ICA/MCA occlusion. 3) PCT_{CBF} slice-1: 0 = No perfusion deficit, 1= Perfusion deficit <20%, 2= Perfusion deficit > 20%. 4) PCT_{CBF} slice-2: 0= No perfusion deficit, 1= Perfusion deficit <20%, 2= Perfusion deficit >20%. The range of possible points consists from 0 to 8, with the lowest MOSAIC score of 0 representing normal findings in all 3 modalities. Linear regression analysis between CT results and outcome measures revealed consistent differences among the 4 CT components for all target parameters: of the 3 single CT components, the ASPECTS score on NCCT showed the lowest correlation coefficients (r =0.42 to 0.58), whereas PCT_{CBF} consistently showed the highest values (r =0.52 to 0.75) with respect to clinical status and infarction size. With respect to the correlation coefficients on linear regression analysis, MOSAIC was superior to all single CT components (r=0.59 to 0.78): sensitivity values were the lowest for NCCT (58.9%), followed by CTA (66.7%) and PCT_{CBF} (71.8%), whereas the MOSAIC score showed the highest values (87.1%); the specificity with respect to occurrence of infarction was 100% for all parameters. The combination of 3 CT modalities was superior to all single CT components with respect to the various outcome measures and showed a strong correlation to the size of infarction (r=0.78) [82].

Conclusions

Hemorrhagic transformation (HT), which refers to a spectrum of ischemia-related brain hemorrhage, is a frequent spontaneous complication of ischemic stroke and still represents the most feared complication. HT is a complex and multifactorial phenomenon and it is important to understand its underlying mechanism and identify its predictors. Different CT modalities are largely used worldwide for the ischemic stroke diagnosis and evaluation of their possibilities in HT prediction has been intensely studied in recent years. Non-contrast CT standard investigation showed high sensitivity and specificity values in assessment of early ischemic signs (dense MCA, loss of basal ganglia outline, loss of insular ribbon, hypodensity, effacement of sulci or ventricles, dense cortical sulci, loss of gray and white matter differentiation) in correlation with HT prediction. The most useful methods in HT prediction on CT were reported to be low The Alberta Stroke Program Early CT score (ASPECTS), presence of dense MCA, leukoaraiosis and early CT signs in 1/3 of middle cerebral artery territory. Another application of NCCT in relation to HT – is timely diagnosis and classification of its various types (hemorrhagic infarction type 1 and 2, parenchymal hematoma type 1 and 2) and dynamics of low-up. Next CT modality is Angiography, which showed the potential in HT prediction by the assessment of the collateral flow grade; presence, localization and extent of intra-arterial occlusion and ASPECTS score calculated from the ACT source images. Last CT modality is dynamic functional Perfusion imaging, which includes large spectrum of quantitative parameters (cerebral blood flow, cerebral blood volume, mean transit time, time to peak enhancement and permeability surface area product). Microvascular permeability (expressed as the transendothelial transfer of constant agent) expressed in color PS (permeability surface) map calculated from the Perfusion CT data showed the best results in HT prediction. The combination of 3 CT modalities

(MOSAIC scoring system: Multimodal Stroke Assessment Using Computed Tomography) was superior to all single CT components and showed a strong correlation to the size of infarction, clinical outcome and HT prediction.

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Transcranial magnetic brain stimulation in post-stroke motor recovery

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Abstract

Background: Repetitive transcranial magnetic stimulation (rTMS) is a noninvasive brain stimulation method that can modulate excitability of the human cortex. It has been assumed by different research groups that suppressing the undamaged contralesional motor cortex by repetitive low-frequency rTMS or increasing the excitability of the damaged hemisphere cortex by high-frequency rTMS will promote function recovery after stroke. Thus, repetitive TMS can be an adjuvant therapy for developed neurorehabilitation strategies for stroke patients. The purpose of this brief review was to provide an overview of the methods, physiologic basis and future views of the use of inhibitory and excitatory repetitive rTMS. Recent studies have reported that rTMS can effectively facilitate neural plasticity and induce motor recovery after stroke. The best rTMS pattern has not been established, a stronger evidence for the potential use of rTMS as clinical rehabilitative tool should be found.

Conclusions: Cumulative rTMS has been shown to be important for continuous motor improvement in patients with stroke. The results of the studies indicate that neural plasticity is consolidated by rTMS intervention. Therefore, rTMS induces a more suitable environment for neural plasticity by artificially modulating the ipsilesional motor cortex, thus counteracting use-dependent plasticity impairment by facilitating plasticity in the affected hemisphere. Further well-designed studies in larger populations are required to determine whether rTMS in stroke can improve motor function and to identify the most effective rTMS protocols for stroke treatment.

Key words: neural plasticity, neurorehabilitation, transcranial magnetic stimulation.

Stroke is the leading cause of adult disability in the world and the burden of stroke is expected to increase in the next 20 years [1]. At present, there are limited effective interventions for patients with acute stroke [2]. Consequently, the management of most patients with stroke remains primarily focused on secondary prevention and rehabilitation [3]. In addition, brain recovery and rehabilitation will also be a prioritised field in future stroke research [4].

Transcranial magnetic stimulation (TMS) is a focal non-invasive brain stimulation technique that can modulate excitability of the brain cortex [5]. TMS is based on the principle of electromagnetic induction. A TMS stimulator device consists of capacitors that store large electrical charges. The capacitor is connected to a casing with coil, made of copper wires. The coil is held tangentially to the scalp during a TMS procedure. A brief and time-varying magnetic field is produced when the stored charge is discharged to the coil. This magnetic field penetrates through the head tissues, and generates an electrical current in the cortical neurons under the coil. The generated current is sufficient to produce depolarization of the neuronal membranes and generate action potentials (fig. 1). TMS can be delivered in two main modalities: via single pulses regime or repetitively at a set number of pulses per second (repetitive TMS or rTMS). Typically, low-frequency rTMS (<5 Hz) is characterized by decreased cortical excitability, whereas high-frequency rTMS (≥5 Hz) is characterized by enhanced excitability [6]. Recently, also a new rTMS protocol, theta burst stimulation (TBS), was introduced which can produce longer-lasting and more stable changes in cortical excitability compared to standard rTMS [7]. Standard rTMS consists of single pulses of stimulation delivered repeatedly over a unit of time, while TBS consists of very rapidly delivered 3 pulses (at 50 Hz) every 200 ms. This stimulation can either be interrupted every few seconds [intermittent TBS (iTBS)] or can be uninterrupted [continuous TBS (cTBS)]. Intermittent TBS typically increases cortical excitability. Continuous TBS decreases cortical excitability. These changes in excitability over the motor cortex have shown to last for about an hour with more intense TBS methods [7].

Repetitive TMS for motor recovery following stroke aims to augment neural plasticity and improve motor function. The phenomenon is based on the so-called *interhemispheric competition model*. This concept proposes that motor deficits in patients with stroke are caused by reduced output from the affected hemisphere and excessive interhemispheric inhibition from the unaffected hemisphere to the affected hemisphere [8]. According to *interhemispheric competition model* a competitive relation is assumed to exist between each cerebral hemisphere regarding cognitive, motor and sensory function. The rightward bias elicited by the left hemisphere is naturally stronger than that elicited by the right hemisphere. By this account, interhemispheric inhibitory connections that normally modulate and effectively suppress right hemispheric activity are disturbed due to damage in the left hemisphere, enabling cortical sectors in the opposite (contralesional) right hemisphere to turn increasingly involved through disinhibition.

Therefore, rTMS method achieves improvement in motor deficits by either increasing the excitability of the affected hemisphere or decreasing the excitability of the unaffected hemisphere [9]. Inhibitory noninvasive brain stimulation (NBIS) increases excitability in the ipsilesional motor cortex by reducing excessive interhemispheric inhibition from the contralesional motor cortex [10]. Excitatory NIBS over the affected hemisphere directly increases the excitability of the ipsilesional motor cortex [11].

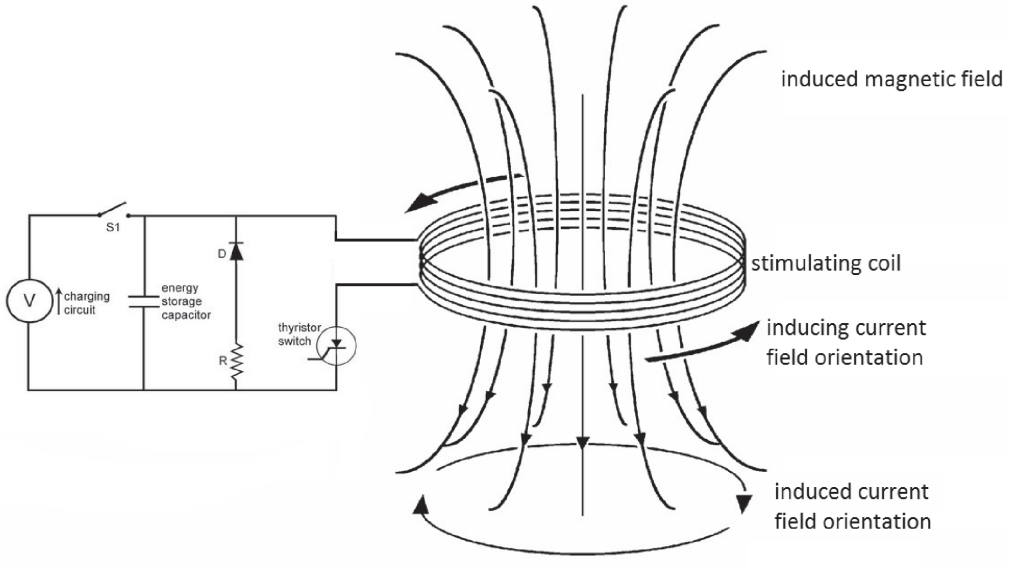

Fig. 1. The principle of the magnetic stimulator.

During the recent years there have been made some important researches. In 2009 Khedr et al., reported a therapeutic effect of rTMS in patients with post-stroke dysphagia [12]. Real and sham rTMS were compared in a group of 26 patients with mono-hemispheric stroke and post-stroke dysphagia. There were no significant differences at the baseline assessment between patients who received real rTMS and the sham group. The parameters were of 300 rTMS pulses at an intensity of 120 % hand motor threshold for 5 consecutive days for each patient. Dysphagia and motor disability were assessed four times: before and immediately after the last session and then again after 1 and 2 months. Real rTMS led to a significantly greater improvement compared with sham in dysphagia and motor disability that was maintained over 2 months of follow-up. The amplitude of the motor-evoked potential (MEP) evoked by single-pulse TMS was also assessed before and after 1 month in 16 patients. A significant increase in the amplitude of the esophageal MEP evoked from either the stroke or non-stroke hemisphere. The authors concluded that rTMS may be a useful adjunct to conventional therapy for post-stroke dysphagia. These results need to be validated by well-designed studies.

In another study the long-term effects of combined time-locked rTMS and physical therapy (PT) intervention in chronic-stroke patients with mild motor disabilities were studied (Avenanti et al. 2012) [13]. A double-blind, randomized, single-center clinical trial included a total of 30 patients. Patients received 10 daily sessions of 1 Hz rTMS over the intact motor cortex. Patients were randomly assessed to real (rTMS(R)) or sham (rTMS(S)) groups. TMS session was administered either immediately before or after PT session. Clinical assessment included dexterity, force, inter-hemispheric inhibition, and corticospinal excitability for the time of 3 months after the end of treatment. Treatment consisted of cumulative rebalance of excitability in 2 hemispheres and a reduction of inter-hemispheric inhibition in the real TMS group. In all groups there were detected use-dependent improvements in trained abilities. These were small and transitory in sham TMS group. Greater behavioral and neurophysiologic outcomes were detected in the group with real TMS. Amongst the latter the improvements in the group receiving TMS before PT were robust and stable and in the other group (PT before TMS) the improvements showed a decline over time. The authors concluded that priming PT with inhibitory rTMS is optimal to boost use-dependent plasticity and rebalance motor excitability and suggest that time-locked rTMS is a valid and promising approach for chronic stroke patients with mild motor impairment. Furthermore, the authors stated that further studies are needed to evaluate the effect of intervention order of time-locked rTMS in the same patients.

In 2012 Corti et al., investigated the concurrent effects of rTMS on the excitability of corticospinal pathways and upper-limb motor function in adults after stroke, they stated that conceptually rTMS could be used therapeutically to restore the balance of inter-hemispheric inhibition after stroke [14]. In this publication rTMS has been used in 2 ways: (i) low-frequency stimulation (less than or equal to 1 Hz) to the motor cortex of the unaffected hemisphere to reduce the excitability of the contralesional hemisphere or (ii) high-frequency stimulation (greater than 1 Hz) to the motor cortex of the affected hemisphere (AH) to increase excitability of the ipsilesional hemisphere. The evidence regarding the safety and effectiveness of high-frequency rTMS to the motor cortex of the AH was reviewed. The findings of this review suggested that rTMS applied to the AH is a safe technique and could be considered an effective approach for modulating brain function and contributing to motor recovery after stroke. The authors concluded that double-blinded and sham-controlled clinical trials with larger samples are needed to validate this approach.

Kakuda et al., (2012) in a pilot study examined the safety

and feasibility of the inpatient protocol of low-frequency rTMS (LF-rTMS) and intensive occupational therapy (OT) for post-stroke patients with upper limb hemiparesis [15, 16]. The study subjects were 204 post-stroke patients with upper limb hemiparesis (mean age at admission of 58.5 +/- 13.4 years, mean time after stroke of 5.0 +/- 4.5 years). During 15-day hospitalization, each patient received 22 combined sessions of 20-min LF-rTMS (1 Hz to the contralesional hemisphere over the primary motor area) and 120-min intensive OT daily. The OT was provided after TMS session. Fugl-Meyer Assessment and Wolf Motor Function Test were performed serially. There were no adverse effects. The FMA score increased and WMFT log performance time decreased significantly at discharge. This decline was relative to the respective values at admission (change in FMA score: median at admission, 47 points; median at discharge, 51 points; $p < 0.001$. change in WMFT log performance time: median at admission, 3.23; median at discharge, 2.51; $p < 0.001$). In 79 patients these changes were constant up to four weeks after discharge. Linear regression analysis found no significant relationship between baseline parameters and indexes of improvement in motor function. The authors concluded that this combined protocol was safe and clinically useful in patients with post-stroke upper limb hemiparesis. They stated that the effectiveness of the intervention should be confirmed in a randomized controlled study including a control group.

In a meta-analysis, Hsu et al., (2012) investigated the effects of rTMS on upper limb motor function in patients with stroke [17]. These investigators searched for RCTs published between January 1990 and October 2011 in PubMed, Medline, Cochrane, and CINAHL. The following key words were used: "stroke", "cerebrovascular accident", and "repetitive transcranial magnetic stimulation". The mean effect size and a 95 % CI were estimated for the motor outcome and motor threshold using fixed and random effect models. Eighteen of 34 candidate articles were included in meta-analysis. These studies involved 392 patients. A significant effect size of 0.55 was found for motor outcome (95 % CI: 0.37 to 0.72). Further sub-group analyses demonstrated more prominent effects for subcortical stroke (mean effect size, 0.73; 95 % CI: 0.44 to 1.02) or studies applying low-frequency rTMS (mean effect size, 0.69; 95 % CI: 0.42 to 0.95). Only 4 patients of 18 articles included in this analysis reported adverse effects from rTMS. The authors concluded that rTMS has a positive effect on motor recovery in post-stroke patients (especially sub-cortically localized stroke). Low-frequency rTMS over the unaffected hemisphere may be more beneficial than high-frequency rTMS over the affected hemisphere.

Conclusions

Thus, pairing of rehabilitative training with NIBS results in more enduring performance improvements and functional plasticity in the affected hemisphere compared with motor training or stimulation alone in patients with chronic stroke [18]. Cumulative rTMS has been shown to be important for continuous motor improvement in patients with stroke. The results of the studies indicate that neural plasticity is consolidated by rTMS intervention. Therefore, rTMS induces a more suitable environment for neural plasticity by artificially modulating the ipsilesional motor areas of the cortex. This facilitates the phenomenon of plasticity in the affected hemisphere.

Further well-designed clinical trials with larger samples are required to determine whether rTMS in stroke can improve motor function and to identify the most effective rTMS protocols for stroke treatment.

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Evidence-based medicine – an innovative approach to clinical medicine

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Abstract

Background: Evidence-based medicine (EBM) represents an approach that integrates clinical experience and patient's values with the best available research information. It involves a process of lifelong problem-based learning that requires a variety of new skills for medical practitioners such as efficient literature-searching, familiarity with the types of research evidence, clinical literature evaluation and evidence appraisal. The key elements of EBM such as common EBM steps that have been developed to provide an efficient framework for medical practitioners, the types of evidence and how the evidence is appraised for validity, the types of research studies and study designs as well as their corresponding levels of evidence and the "*evidence pyramid*". It is necessary to increase the awareness of EBM principles among medical practitioners and pave the way to an evidence-based clinical practice.
Conclusions: 1. EBM integrates the best available information with patient's values, clinical expertise and latest research evidence; 2. A significant progress in the practical implementation of EBM has been made by the increasing availability of EBM resources able to search, appraise and summarize the literature on a given topic; 3. An important element of external evidence and EBM are represented by the systematic reviews and meta-analyses, designed to provide relevant summaries about the best available evidence on a specific topic while minimizing the bias; 4. As a result of increasing demands for an evidence-based approach and a wider availability of information resources, EBM is gaining increasing support among the medical practitioners, optimizing their clinical decision-making, increasing their confidence and facilitating their clinical practice.

Key words: evidence-based medicine, evidence pyramid, research designs, level of evidence, patient's values.

Introduction

Evidence-Based Medicine (EBM) is a form of medicine aiming at transforming the delivery of healthcare based on a systematic and detailed approach that integrates patient's values with clinical expertise and current best research evidence. According to the definition given by David Sackett, who is considered the founding father of EBM, "*Evidence-based medicine is the conscientious, explicit, and judicious use of current best evidence in making decisions about the care of individual patients. The practice of evidence-based medicine means integrating individual clinical expertise with the best available external clinical evidence from systematic research*"[1]. The concept of EBM was introduced in 1991 by Gordon Guyatt [2], even though many underlying principles have been developed earlier by the Scottish epidemiologist Archi Cochrane, who is also considered to be the originator of the idea of evidence-based medicine in our era [3, 4]. For his distinguished services towards EBM, Archi Cochrane has been honored through the naming of evidence-based medical research centers as Cochrane Centers and an international organization as the Cochrane Collaboration. Many programs have been developed lately to make EBM more accessible to medical practitioners. Among relevant EBM publications can be listed *EBM reviews* published by the Cochrane Center, the periodical *Clinical Evidence* launched by the BMJ Publishing Group, the *Journal of Evidence-Based Medicine* published by the Wiley Editing Services, etc. In many countries, EBM courses are also included in the standard curriculum of the medical schools. Furthermore, nowadays evidence-based practice is becoming a goal for health care institutions and often an accreditation requirement [5].

Evidence-Based Medicine – a six step approach

The practice of EBM provides a unique and enriching experience for medical practitioners, creating an environment of problem-based learning and identifying current information to support care decisions for individual patients. Such an approach based on identifying the best evidence with which to answer a clinical question correlated with a critical appraisal of the existing evidence for validity and clinical usefulness provides a foreground for effective evidence-based interventions that are responsive to patient's needs and priorities, avoiding uncritical acceptance of "usual practice". The integration of EBM into clinical practice is a complex process and several steps have been developed to provide an efficient framework for medical practitioners.

The *First Step* relates to converting the clinical information into focused questions. Thus, when a clinical problem arises during the care of a patient, it is very important to construct a well-built clinical question that can address such key points as identifying the problem, considering standard and alternative management strategies as well as potential outcomes. A commonly used mnemonic in this regard is PICO: P = Patient or Problem (identifying the most important characteristics, similarities and particularities compared to other patients), I = Intervention (i.e. what are the options, risks and benefits, etc.), C = Comparison (i.e. comparing the existing alternatives for the chosen intervention), O = Outcomes (i.e. what are we trying to reach with the chosen interventions, possible complications and prognostic factors).

The *Second Step* requires answering the focused questions based on "internal evidence", which includes acquired knowledge through formal education and professional training, personal experience accumulated from daily practice, specific experience gained from this particular patient, etc. [6]. Common questions that have to be answered during this step include, "How to proceed and what are the pros and cons based on the internal evidence?", "How much the expertise in the field has evolved since the internal evidence

was created?", "Are there any alternatives to reach this goal and is the internal evidence sufficient"?

The *Third Step* extends the search from internal evidence to "external evidence", which is represented by the available information from research studies performed on the topic of interest. This is frequently accomplished by searching medical literature databases created for this purpose. Selecting the appropriate resource and conducting a proper search represents an important step in the decision-making process and can be performed at several levels: *formal*, that involves searching the sources using formal terminology and key words; *cognitive*, that involves appraising the content; and *analytical*, when the practitioner understands the study design and potential implications on the presented data, being able to critically assess the available external resources. An important resource for external evidence can serve the Cochrane Library, which represents a collection of databases of systematic reviews and meta-analyses that summarize and interpret the results of medical research. The Cochrane Library is supported by the Cochrane Collaboration and a number of other organizations, representing a key resource in evidence-based medicine worldwide.

The *Fourth Step* involves appraising the evidence for validity and applicability to clinical practice. Examples of key issues requiring critical evaluation include randomization, blinding, concealed allocation, completed follow-up etc. The common questions that have to be answered during this step are as follows: "Are the results of the study and the available evidence valid?" and "Will the results help for answering the questions related to this patient?". Commonly used mnemonics in this step include DOE (Disease Oriented Evidence), POE (Patient Oriented Evidence) and POEM (Patient Oriented Evidence that Matters) [7-11]. DOEs are very common in the medical literature, being frequently brought to our attention by pharmaceutical representatives eager to promote their medication brands. This kind of evidence needs to be approached with caution as it may be misleading and should not be used to change practice, especially when other types of evidence such as POEM are available [10]. POEs use patient-oriented outcomes, however, most such studies confirm what we already do and the findings don't have the potential to change practice[11]. POEMs, on the other hand, use patient-oriented outcomes that have the potential to change our practice if the results are valid and applicable to our patient or clinical setting. The concept of "patient-oriented evidence that matters" was developed by David Slawson and Allen Shaughnessy in 1994 [8]. Apart from being patient-oriented and having the potential to change practice, the POEM approach allows medical practitioners to focus only on what's important for individual patients, significantly simplifying EBM. Today, about 30 to 40 POEMs in the form of short synopses of research focusing on patient-oriented evidence that matters are published monthly in such clinical journals as American Family Physician, the British Medical Journal, the Journal of Family Practice, etc. [9]. This helps physicians to find useful information and assists them in caring for their patients during their clinical practice.

The *Fifth Step* requires integrating "external evidence" that has been obtained through various resources into "internal evidence". As a whole, external and internal evidence may be mutually supportive, non-supportive, or even conflicting. When two sources of information (external and internal) are non-supportive or conflicting, medical practitioners may choose to use the external evidence to change their practice or to stick to their original opinion (i. e. to the "internal evidence"). As a third option, a practitioner may also decide to discuss the conflict between the internal and external evidence with the patient, offering him/her the possibility to take part in the decision-making process. Since by definition EBM integrates patient's values with clinical expertise and current best research evidence, the patient's preference is considered an essential part of EBM and, therefore, the last approach is recommended in this instance [6]. Because of this, some authors even call this stage as "returning to patient", when the acquired evidence is integrated with patient's preferences and clinical expertise before being applied to practice.

The *Sixth Step* relates to evaluating the decision-making process and final results. During this step the entire process is assessed, the outcome concerning the status of the patent is appreciated, the results are compared to those published in the literature as well as to the patient's expectations and potential options for improvement are identified. Furthermore, just because an intervention was effective in a rigorously controlled trial, it still doesn't mean it will show the same results in a clinical setting and potential negative consequences should be also identified. Familiarity with relevant parameters and a proper interpretation of the obtained results become indispensible for a valid evaluation.

The principles of EBM are not entirely new, as clinicians have always striven to combine their clinical expertise and their patients' values with the best available evidence. However, interest in EBM has grown tremendously after the term "evidence-based medicine" was introduced in the early 1990s and the subject was gradually included in the undergraduate and postgraduate curriculum in many schools [12, 13]. At the moment, the percentage of physicians who apply the EBM principles in their clinical practice remains largely unknown. Despite enlisted advantages of EBM, the actual implementation depends on many factors like institutional culture, lack of knowledge about EBM and familiarity with the basic skills, barriers to practicing high-quality medicine, busy schedule and lack of time, the need to develop new skills, lack of information resources in the spoken language, shortage of coherent, consistent scientific evidence, impediment of clinical freedom, difficulties in applying evidence to the care of individual patients, etc. [13-19]. Studies performed in Europe and Australia also indicate that textbooks are consulted more often than the Cochrane Library [17, 19, 20]. A lot of efforts have been directed to overcome these limitations and encountered difficulties at various levels. As a result of these efforts and a wider availability of information resources, EBM is gaining increasing support among the medical practitioners, facilitating their clinical activities in a variety of different ways such as:

- ✓ Keeping abreast with updated medical literature and the growing body of developments in the field;
- ✓ Active communication with specialty consultants and other medical practitioners;
- ✓ Effective use of available medical literature and information resources;
- ✓ Targeted and effective data collection and information processing related to patient's medical history, physical exam, laboratory and imaging findings;
- ✓ Optimized clinical decision-making and evidence-based clinical management;
- ✓ Helping clinicians to challenge dogma and to avoid uncritical acceptance of "usual practice";
- ✓ Teaching how to integrate the best available information with patient's values, clinical expertise and latest research evidence;
- ✓ Facilitating clinical judgment and allowing the physicians to individualize the information for every patient's situation;
- ✓ Stimulating a process of lifelong learning and an evidence-based up-to-date clinical practice.

It should be noted that EBM always begins (step 1) and ends (steps 5 and 6) with the patient, being usually triggered by a variety of patient's encounters that generate questions about the etiology of his/her condition, utility of diagnostic tests, the effects of therapy, expected outcome and overall prognosis. After identifying a problem, it is very important to formulate the question in such a way as to facilitate finding an answer applicable to patient's situation. At the same time, it is equally important at this stage to determine the most appropriate type of study able to provide a valid answer to our question. A brief summary of the main types of studies and study designs is provided by the so-called "*evidence pyramid*". The *evidence pyramid* also reflects the hierarchies of evidence that have been developed to help describe the quality of evidence that may be found to answer various questions. When viewed from its base to top, the pyramid consists of several main components such as *Animal Research °Case Reports °Case Control Studies °Cohort Studies °Randomized Control Trials °Systematic Reviews °Meta-Analysis*. Most information usually starts with an idea potentially leading to laboratory research, development of diagnostic tools or therapeutic interventions, which are subsequently tested in laboratory models, animals, and finally in humans.

Animal testing, or animal research, is the use of non-human animals in experiments, commonly being conducted inside universities, medical schools or pharmaceutical companies. It represents an initial step in the research hierarchy; therefore the range and reliability of research results obtained in animals are invariably restricted for being extended to clinical practice. Before a certain diagnostic tool or therapeutic agent can be authorized for use within general population, it should also undergo subsequent human testing on a limited number of volunteers, followed by extended testing within several phases of clinical trials according to the established standards.

Case reports and Case series represent published reports about the clinical features, diagnosis or treatment of individual patients. Because they report single cases and use no control groups with which to compare the outcome, case reports and case series have little statistical validity.

Case control studies represent studies in which patients who already have a certain condition are compared with people who do not have that condition. By definition, case-control studies are always retrospective since they start with patients who already have the outcome, while the researcher is trying to link the outcome with prior exposures and potential causative factors. Case control studies require fewer resources, but are often less reliable than cohort studies and randomized controlled trials because revealing a statistical relationship does not necessarily show a cause-and-effect relationship.

Cohort studies take a group of people who share a common characteristic or experience within a certain period (e. g., taking a particular treatment or have an exposure), follow them over time, and then compare the outcomes with a similar group that has not been affected by the treatment or exposed factor. Of note is that *Case control studies* and *Cohort studies* represent *Observational Studies*, where the researchers observe the effect of a risk factor, diagnostic test or treatment without trying to influence what happens. Observational studies are inherently not as reliable as experimental studies, since the studied groups may differ in ways other than in the variable under study. Sometimes, however, they may represent the only way to explore certain questions. For example, it would be unethical to design an experimental study to deliberately expose patients to certain harmful risk factors. Randomized controlled trials (RCTs) are carefully planned projects in which subjects are randomly assigned to two or more groups. One group receives the intervention (a certain drug or intervention) while the control group receives no intervention or placebo. This allows a formal comparison between intervention groups and control groups (no intervention), differences in the outcomes being directly linked to the intervention. Additional strengths include randomization and blinding that reduce the potential for bias. As a study design a randomized controlled trial represents an experiment and can provide scientific evidence related to the cause and effect. If the subjects are not randomly assigned to the treatment or control groups (i.e. the subject is allowed to choose which group to join or the investigator is assigning to a particular group for any other reason), the study is then called Controlled Clinical Trial (CCT) instead of Randomized Controlled Trial. Both *RCTs* and *CCTs* represent *Experimental Studies*, however, CCTs have a higher chance for "bias" compared to RCTs, since non-randomly assigned subjects have the potential to influence the final results. The RCTs are commonly considered the "gold standard" for producing reliable evidence because little is left to chance, even though such studies are usually time-consuming and expensive. A systematic review (also called systematic literature review or structured literature review) is a literature review focused on a research question that in-

volves a systematic search with a view to minimizing bias, followed by a formal appraisal and synthesis so that relevant conclusions can be drawn and decisions be made. The main difference from a traditional narrative review is that a narrative review is mainly descriptive and does not involve a systematic search of the literature for minimizing bias, while a systematic review typically involves a detailed plan and a search strategy derived as a priority, with the goal of reducing bias by identifying, appraising, and synthesizing all relevant studies on a particular topic [21, 22]. A systematic review may also include a quantitative statistical analysis of the collected data, called a meta-analysis. Thus, a meta-analysis will examine and select a number of valid studies on a topic and combine the results using formal statistical calculations. Many systematic reviews contain meta-analyses, but not all of them [22]. A well-known and respected international organization that promotes, supports, and disseminates systematic reviews and meta-analyses in the field of medicine is the Cochrane Collaboration (www.cochrane.org).

Familiarity with the hierarchy of study designs allows a practitioner to keep in mind the "*evidence pyramid*" when searching for the best evidence. However, the highest level of study to answer a particular question may not always be found or available. In such situation, a practitioner may consider moving down the pyramid. Furthermore, the highest ranked level of evidence might not always be applicable to a particular question. For example, although randomized clinical trials are considered the "gold standard" for establishing the effects of a treatment of intervention, they might not represent the best sources for answering questions about diagnosis, prognosis or harm [13]. Hence, familiarity with the types of study designs and their applicability to different clinical situations becomes indispensible when evaluating the evidence. Last, but not least, it should be also remembered that evidence, by itself, does not make a decision for a clinical practitioner, even though it represents an important variable in the process of EBM practicing. Therefore, the last two steps of EBM relate to integrating internal and external evidence with patient's preferences and clinical expertise before being applied to practice followed by a subsequent assessment of the decision-making process and final results. The integration of these EBM steps enables both the practitioner and patient to optimize their clinical decisions, so that optimal clinical outcomes can be achieved.

Conclusions

1. EBM integrates the best available information with patient's values, clinical expertise and latest research evidence.

2. A significant progress in the practical implementation of EBM has been made by the increasing availability of EBM resources able to search, appraise and summarize the literature on a given topic.

3. An important element of external evidence and EBM is represented by the systematic reviews and meta-analyses, designed to provide relevant summaries about the best available evidence on a specific topic while minimizing the bias.

4. As a result of increasing demands for an evidence-based approach and a wider availability of information resources, EBM is gaining increasing support among the medical practitioners, optimizing their clinical decision-making, increasing their confidence and facilitating their clinical practice.

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2. The title page should include the first and last names of all the authors, their academic degrees, the name of the department and institution from which the paper has arrived, the phone number and e-mail address of the corresponding author.

3. The abstract should be written on the title page in English and be limited trom 220 to 240 words. The abstract should end with 3 to 6 key words.

4. The text of clinical or experimental articles (has to be less than 16 pages long) should consist of an Introduction, Material and Methods, Results, Discussion, Conclusions and be followed by no more than 40 References. The **review articles** must not exceed 25 pages and contain no more than 100 references.

5. The tables and figures must be typed, consecutively numbered and followed by an explanatory text. The figures that have to emphasize a comparison or details are published in colour. If coloured figures are to be placed, the author must pay an additional fee of €100 per page (1-8 figures on a page).

6. The references are to be listed in order of their appearance in the text, and the appropriate numbers are to be inserted in the text [in square brackets] in proper places. The references must comply with the general format outlined in the Uniform Requirements for the Manuscripts Submitted to Biomedical Journals developed by the International Committee of Medical Journal Editors (www.icmje.org), chapter IV.A.9. The references in the Cyrillic script should be transliterated into Latin script as follows: А–A, Б–B, В–V, Г–G, Д–D, Е–E, Ё–E, Ж–ZH, З–Z, И–I, Й–Y, К–K, Л–L, М–M, Н–N, О–O, П–P, Р–R, С–S, Т–T, У–U, Ф–F, Х–KH, Ц–TS, Ч–CH, Ш–SH, Щ–SCH, Ы–Y, Э–E, Ю–YU, Я–YA, Ь and Ъ are omitted. Immediately after the transliteration the translation of the title in English [in the square brackets] should follow. For example: Ivanov IV, Sidorov VM, Kozlov NF. Transplantatsiya organov i tkaney [Transplantation of organs and tissues]. Vestnik Khirurgii [Messenger of Surgery]. 2010; 26(6):45-49.

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GHID PENTRU AUTORI

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3. Rezumatul în limba engleză (220-240 cuvinte) se expune consecutiv pe foaia de titlu, inclusiv cuvintecheie, de la 3 până la 6. În rezumat este obligat să fie expus scopul cercetării (dacă nu este clar din titlu), metodologia studiului, rezultatele obţinute şi concluziile.

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