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Welcome to the scientific and medical journal Curierul Medical!

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.

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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.

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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.

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Address of the Editorial Office
192, Stefan cel Mare Avenue, Chisinau, MD-2004
the Republic of Moldova
Phone: (+37322) 244751, (+37322) 205209
Fax: (+38322) 295384
www.curierulmedical.org
editor@curierulmedical.org, secretary@curierulmedical.org
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CONTENTS • CUPRINS • СОДЕРЖАНИЕ

RESEARCH STUDIES

REVIEW ARTICLES

PAGES OF HISTORY

CHRONICLE

RESEARCH STUDIES

The role of the heat factor in the pathogenesis of burns

V. Babiuc

Department of Orthopedics and Traumatology
Nicolae Testemitsanu State University of Medicine and Pharmacy, Chisinau, the Republic of Moldova
Corresponding author: babiucv@yahoo.fr. Received June 08, 2015; accepted September 15, 2015

Abstract

Background: Under the action of the increasing temperature and multiple stress factors, the quantity of catalyzing hormones suddenly increases, as well as the hydrolysis of carbohydrates, lipids and proteins. A large amount of energy is liberated in the body. A large surplus of energy is formed, which cannot be used by the body. It must be liberated in the surrounding environment. But, in tropical conditions, where atmosphere is extremely loaded with thermal energy, its liberation is very limited.
Material and methods: The investigations were performed on 427 patients in tropical climatic conditions and on 80 patients in continental climatic conditions. The hyperthermia state was determined immediately after the trauma, in its dynamics during the transportation in a shock state, at hospitalization and in the evolution of the combustions. At the same time, the catabolic reaction in the vascular system was determined by means of establishing the capillary structure, ascertained histologically.
Results: The obtained results showed that the tropical climate exerts a very big influence on the organism of the patient with combustions. Among all the patients with combustions in the tropical area, in 71% of them a surplus of thermal energy took place, which manifested from the first minutes after the trauma. Body temperature in these patients increased up to 38-39°C. It maintained during the first 24 hours and, only after intensive treatment in conditioned air, it decreased. At the same time, it was established that catabolism was very advanced, especially in the structure of the capillary wall, which was manifested by aggravated plasmorrhea, ascertained histologically and according to the hemoconcentration degree, the function of the respiratory and cardiovascular system, and the excretion of mineralocorticoids.
Conclusions: In tropical conditions, accumulation of thermal energy in the organism of the patients with combustions is very accelerated, even when up to 10% of the body surface is affected, body temperature can reach 39-40°C from the first hours after the trauma. The method of providing first aid by using hypothermia in patients with combustions is very necessary in order to decrease both locally, and generally the catabolic processes.
Key words: thermodynamics, heat production, heat release, combustion disease.

Introduction

The influence of tropical climate on the human body is very complex and widely varied. In some cases, it is favorable and can be used for treatment purposes (climatotherapy of asthma, bronchitis, tuberculosis, cardiovascular diseases etc.) [1,2,6,7]. In other cases, it is very negative. Thus, the increased temperature of the surrounding environment with a high humidity can lead to hyperthermia and severe hemodynamic disturbances even in healthy and well-adapted persons. However, it is not justified to affirm that increased temperature of the surrounding environment is harmful. The moderate action of this climate is always favorable. The scientific researches confirmed that even the increased temperature of the surrounding environment (for example, in Tashkent) has a favorable influence on many pathological conditions and provides a longer life to healthy individuals.

In hyperthermic climatic conditions, over time, the human body developed the ability of physiological adaptation. Even at considerable variations of temperature of the surrounding environment, the temperature balance of the body remains quite stable.

The basic functional processes employed in this adaptation of the body are represented by heat production and elimination. In these conditions, a very important role is attributed to the skin. Up to 80% of the excess heat accumulated in the body is eliminated through skin by evaporation of fluid from its surface, the secretion of which is ensured by the structural elements of the skin (dermis) [3,8,11].

On the other hand, when excessive accumulation of heat occurs, the thermoregulation may also be accomplished by inhibition of the biochemical processes of heat production [4,5,10].

These extremely subtle mechanisms in the skin structure and in the biochemical processes of thermal production by increasing or decreasing its activity ensure a constant temperature conditions in the body, which is extremely important for its normal functioning [9].

When the living or activity conditions change, a certain change of thermal production increase or decrease, the so-called slow adaptation or acclimatization process takes place. In such cases, an especial role is attributed also to the dietary pattern and consumption of fluids. For each liter of fluid evaporated from the skin surface to the surrounding environment, it is required 580 kcal of energy.

But all these functional changes are programmed by nature in a healthy body, which can easily adjust to different conditions. In case of any pathology, an imbalance of the adaptation system takes place in the organism. In such a case,

adaptation is either very slow or impossible. Otherwise, the sudden climate change from continental to tropical can lead to heat exhaustion associated with water-electrolyte imbalance with changes in hemodynamics, dysfunctions of the internal organs and often death.

This frequently happens with elderly people rapidly changing the climates and making efforts.

The researches performed by J. A. Kassirsky, N. N. Plotnikov [12] proved that when a severe pathology is involved, a state of disadaptation takes place in the body. As a result, in case of changes of the water-electrolyte balance, of the metabolism, internal organs' functioning and of the structure of the adaptation mechanisms, the organism cannot be provided with a normal regimen of thermoregulation. In such a case, the biochemical processes of heat production and heat liberation, or its preservation in the organism are also reversed.

This fact is strongly expressed in burn patients, whose most important mechanism of adaptation and adjustment – the skin – is not functioning.

Material and methods

The investigations were performed on 427 patients in tropical climatic conditions and on 80 patients in continental climatic conditions. The hyperthermia was determined immediately after the trauma, in its dynamic evolution during the transportation in a state of shock, at hospitalization and in the dynamic evolution of burns. At the same time, the catabolic reaction in the vascular system was determined by means of establishing the capillary structure, ascertained histologically.

Results and discussion

In severe burns, a part of the skin is removed as an organ, and in the intact part, as a result of capillary hypotonia and the increase of its permeability, the thermoregulation function decreases significantly. More than that, in case of burns, the catabolic processes are clearly accelerated and the metabolic processes are affected, this is why thermal production is increased.

And, if in a healthy organism, the biochemical processes in the thermoregulation system don't prevail, then, in case of burns, an insignificant change of thermal production can severely influence the functioning of the entire organism and can worsen the evolution of the pathology. The body temperature suddenly increases and the functions of the internal organs are disturbed. In all the patients with burns covering more than 10-15% of the body surface area and who were overheated in tropical conditions, the temperature of the organism suddenly increased, and the evolution of the general condition was aggravated, which complicated the treatment even more.

The general condition of aggravation as a result of overheating was determined at the patients transported at large distances, beginning from the first hours after the trauma. They also had general hyperthermia from the first hours after the trauma, associated with disorders of hemodynamics and of the water-electrolyte metabolism. A part of the patients, even with lesions of up to 10% of the body surface, but with hyperthermia, were in a state of shock. And, if in continental conditions, temperature increases up to 39-40°C on the 5^{th}-7^{th}-9^{th} day after the accident (the reaction of the organism to the antigen), when a marked autolysis takes place in the affected area, then, in tropical conditions, as a result of thermal production disturbances, body temperature is increased immediately after the trauma or on the 2^{nd}-3^{rd} day after the burn.

Among the 427 patients clinically investigated at the moment of hospitalization, 182 (42%) had a body temperature of up to 39°C, 97 (23%) of the patients – up to 38°C, 28 (7%) of the patients – up to 37°C and only 120 (28%) of the patients had a normal temperature. The majority of these patients were transported from a distance of 100-150-200 km. On the cartogram (fig. 1) this group of patients corresponds to the peak point "a". All these patients were hospitalized in rooms with conditioned air, where, besides cooling environment, all of them were applied cold antiseptic dressings with a temperature of up to 15-18°C and, at the same time, all received intensive anti-shock therapy. Two-three days later, the body temperature of all patients reduced to low-grade fever or normal temperature. On the cartogram, the temperature dynamics is represented in "M" sector. In dynamic evolution, their progress is completely conditioned by the affected area, burn depth, and age and burn consequences.

On the temperature cartogram, their dynamic evolution is varied. But from the 6^{th}-9^{th} day, four areas can be differentiated (marked with different colors on the cartogram). Considering the dynamic evolution of the pathological process, we can conclude as follows: Zone no.1 (green) – burn evolution with full recovery without any danger. Zone no.2 (yellow) moderate severity burns – alternative evolution with short-term low-grade or higher fever. Zone no. 3 (red-grey) – high risk, with fever, but without mental disturbances or internal organs decompensation, although there is a risk to develop such conditions. Zone no. 4 (black) – very high-risk evolution with very high fever – 39-40°C or higher, with mental disturbances, internal organs subcompensation or decompensation and, frequently, with fatal outcome. The more time the patient spends in this zone, the less chances of recovery he has. And vice versa, the quicker the patient is assigned to a lower zone, the bigger are his chances of survival.

If we compare the temperature cartogram of the patients in continental conditions of Moldova with the tropical one, we will see that in continental conditions starting from the first 24 hours and then in dynamic evolution, almost all patients move into the same direction. The point "a" on the cartogram of the continental climate is missing completely (fig. 2).

In case of hyperthermia in tropical conditions, mental disturbances, adynamia, headache, feelings of thoracic spasms, accelerated and superficial respiration, rarely general agitation, nausea associated with vomiting and hypotonia appear first of all. Body temperature rises up to 38-39°C.

These symptoms evolve rapidly, frequently right from the onset of burns, when the body intoxication mechanisms were not unleashed yet. Hyperthermia, which appears later on the 5^{th}-7^{th} day after the trauma, is mostly the body response to

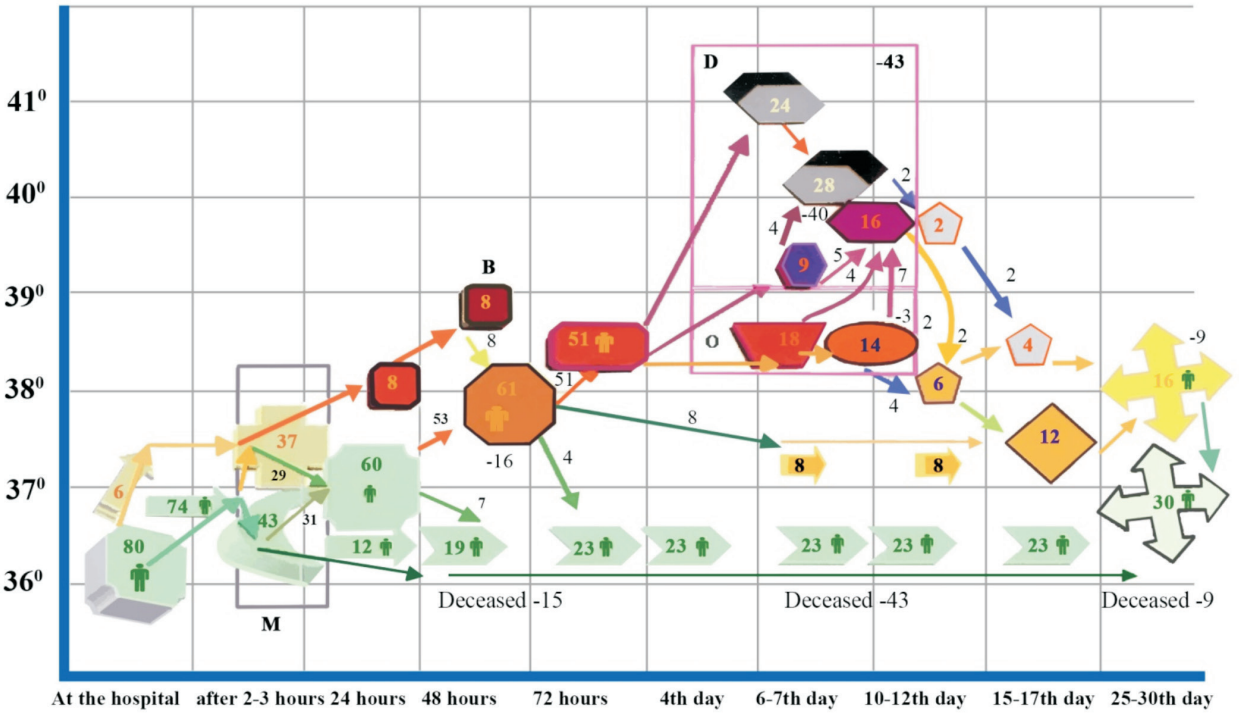

Fig. 1. Temperature cartogram in patients with burns in continental conditions (Moldova-Russia, Moscow).

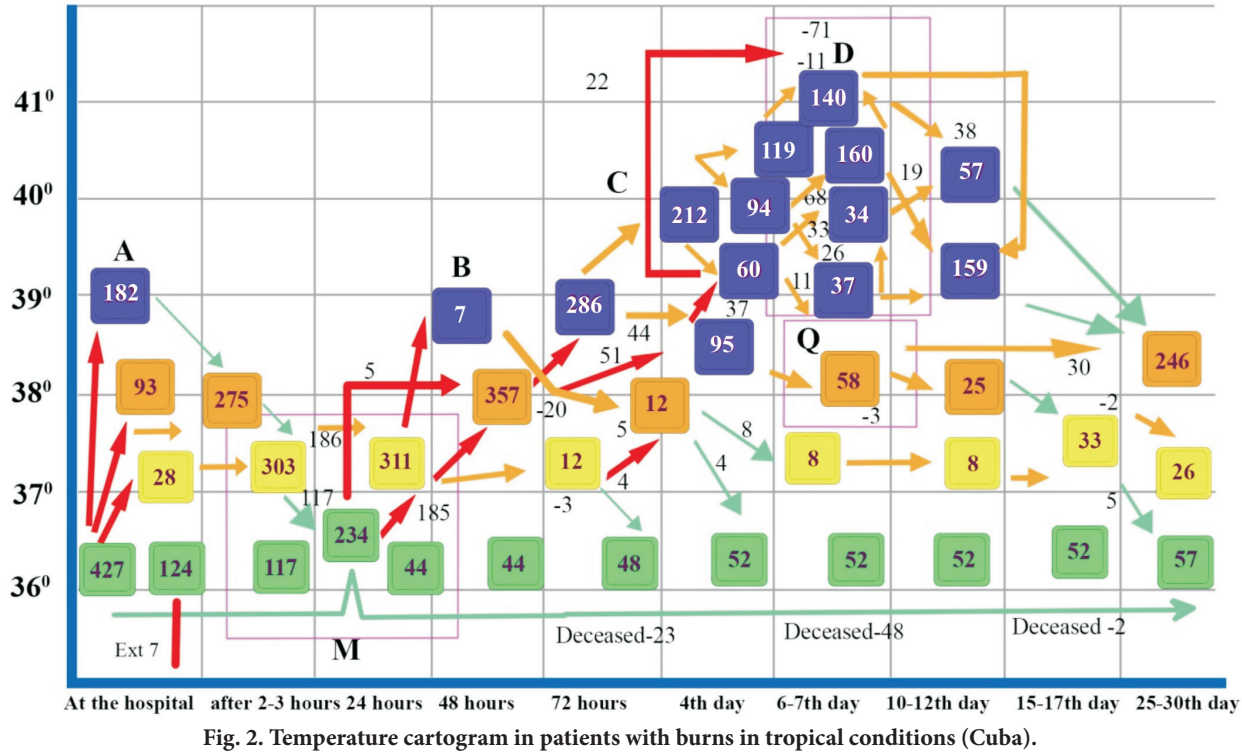

Fig. 2. Temperature cartogram in patients with burns in tropical conditions (Cuba).

toxic antigens from the affected area as a result of the autolytic process and microbial action.

Symptoms of intoxication are clinically present. At this moment, functional and organic changes occur in internal organs. And if no measures were taken from the start to treat the shock as quickly as possible, then the patient's condition very rapidly aggravates and can progress to death.

The clinical picture for hyperthermia in tropical conditions can be easily confounded with the one manifested in case of sepsis, except that in hyperemia we observe a constantly high temperature, while in sepsis, it varies from morning to evening. Besides that, the neurological syndrome, the clinical and laboratory investigation data allow to differentiate between them with more certainty.

However, in continental conditions there are also reported cases (rare) when the temperature remains constantly at 39-40°C and does not decrease despite the intensive treatment. But this clinical form of hyperthermia can be differentiated from the one in tropical conditions at the onset of the pathology. First of all, this form of hyperthermia, called "central" by many authors, occurs on a background of marked toxemia symptoms and, in most cases, the patient is unconscious and in extremely severe condition.

In all the patients with hyperthermia, especially in the tropical area and less in the continental area, major disturbances of the water-electrolyte balance took place. Their predominant clinical picture was similar to fluid disorder disease in a desert in scorching heat – a marked thirst, a confused general condition, increased weakness, tremor and often mental disturbances.

These disorders were, first of all, manifested by the increased permeability of the capillary membrane, which led to great plasma losses.

Considering the results of these studies, we can affirm that the action of the heat factor on the organism of the patient with severe burns, in tropical conditions, represents one of the main factors in the pathogenesis of burns. It plays a very important role in the long-distance transportation of patients. Ignoring this fact leads to the aggravation of the general condition of the patient and even to death.

Conclusions

1. In tropical conditions, the process of thermal energy accumulation in the body of burn patients is much accelerated.

2. In tropical conditions, even if less than 10% of the body surface is affected, the body temperature can reach 39-45°C and higher within the first hours after the trauma.

3. The tropical climate severely inhibits the release of excess heat accumulated as a result of advanced catabolism in the body of burn patients.

4. Hyperthermia, which appears during the first hours after the trauma in burn patients, requires changes in the first aid method and treatment employing local and general hypothermia.

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Dynamics of proinflammatory (TNF-α) and anti-inflammatory (IL-10) cytokines in different clinical forms and variants of children chickenpox

N. M. Prykuda

Department of Infectious Diseases, Danylo Halytskyi National Medical University of Lviv, Ukraine
Corresponding author: n.prykuda@gmail.com. Received October 02, 2015; accepted 07 December, 2015

Abstract

Background: Research of the levels of tumor necrosis factor -α and interleukin-10 in serum of children with chickenpox in different clinical forms and variants of the disease.

Material and methods: 84 children aged from 5 months to 14 years were tested to determine cytokine concentration. The dynamics of the content characteristics of necrosis factor tumor-α and interleukin-10 in serum of children with different clinical forms and variants of children chickenpox was studied. Statistical analysis was performed using the statistical software package "STATISTICA FOR WINDOWS 5,0" (StatSoft, USA, 1998).

Results: It was determined that the dynamics of the content characteristics of studied cytokines varied depending on the clinical form and variant of the disease. For uncomplicated varicella only necrosis factor tumor-α increase is typical. Significant increase of both studied cytokines was found in groups of children with complicated chickenpox. The imbalance in the system of cytokines with bacterial complications of varicella reflects the high activity of the inflammatory process.

Conclusions: These results confirm the feasibility of using indicators of TNF-α and IL-10 not only to assess the severity of chickenpox, but to predict the development of bacterial complications.

Key words: chickenpox, varicella-zoster virus, tumor necrosis factor-α, interleukin-10, children.

Introduction

Chickenpox is one of the most common highly-contagious airborne infectious children diseases [1]. The data of many clinical and epidemiological observations in recent years refute traditional idea of chickenpox as the classic "children infection" characterized mainly by mild course of the disease and full recovery. Not only a significant increase in the incidence of varicella [2], but also frequent severe and complicated variants of the disease draw attention [3]. Besides the specific complications of chickenpox caused directly by varicella-zoster virus, the bacterial complications developed as a result of penetration of pathogenic bacteria through damaged skin and mucous membranes require special attention. Children with immune system disorders suffer the most severe course and complications. For these children chickenpox is particularly dangerous, because the rate of complications among them reaches 30-50% [4, 5].

Considering the urgency of varicella issue in modern medicine, a detailed study of this disease represents not only academic interest, but also has important practical value. It is important to pay careful attention to still unexplored immunopathogenic mechanisms of different courses of varicella, including the development of complications.

Accumulated over recent years studies confirm the role of cytokines in the regulation of immune response, because the cytokines are responsible for the interaction between innate and adaptive immunity, acting in both directions. Clinical features and peculiarities of many infectious diseases directly depend on the level of production of proinflammatory and anti-inflammatory cytokines and their effect on immune effector and immunoregulatory mechanisms [6].

It is natural to expect that the development of severe and complicated forms of chickenpox is largely dependent on violations in intercellular connections that develop as a result of maladjustment of immune processes [7, 8].

One of the triggering mediators and cytokines of the immune response is the tumor necrosis factor-α (TNF-α), which has a key role in the inflammatory response and its progression [9, 10]. The TNF-α is directly involved in mobilizing cells in the source of infection [10], and the increase in the concentration of this cytokine is a sign of the adverse course of the disease [11]. Interleukin-10 (IL-10) plays an important role in blocking the production of inflammatory cytokines, including TNF-α [12, 13]. It also plays an important role in limiting the immune response to pathogens and their input with minimal immunopathological changes to the body [14]. In turn, it was proved that hyperproduction of IL-10 leads to reduced resistance to infectious factors [15, 16] and the development of potential complications.

Objective: research of the levels of tumor necrosis factor-α and anti-inflammatory cytokine – IL-10 in serum of children with chickenpox in different clinical forms and variants of the disease.

Material and methods

84 children aged from 5 months to 14 years were tested to determine cytokine concentration. All patients were hospitalized in the Lviv Oblast Infectious Clinical Hospital (LOICH) and/or in the surgical department of the City Clinical Hospital for Children (CCHC) during 2006-2012. Four comparison groups were formed, considering the clinical form (mild, moderate) and course of the disease (uncomplicated, complicated). The first group included 16 children with mild chickenpox and uncomplicated course of the disease, the second group – 23 children with moderate form of chickenpox, uncomplicated course, the third group – 26 children with superficial complications of varicella with skin lesions of subordinated soft tissue and oral mucosa, the fourth group – 19 patients with chickenpox with deep abscess lesions in different parts of the body.

The diagnosis of varicella was based on typical clinical manifestations of the disease, epidemiological history data and laboratory results. The changes in cytokine profile were studied by determining the levels of TNF-α and IL-10 in serum of children with chickenpox.

To study the levels of cytokines the following diagnostic kits of reagents were used: ELISA TNF-alpha (TNF-α) kit of "Orgenium Laboratories" (Finland) and "IL-10 - ELISA BEST" (Vector-Best CJSC, Koltsovo, Novosibirsk Region, Russian Federation). Determining the level of cytokines was performed in dynamics – with patient's admission to hospital (2-6 days of illness) and in early convalescence period (8-12 days of illness). The control group contained 14 healthy children of the same age, with the average values of TNF-α and IL-10 that were respectively 11.59 (8,48-14,24) [9,68–12,82] and 27,32 (19,42–29,88) [22,56–29,02] pg/ml. Statistical analysis was performed using the statistical software package «STATISTICA FOR WINDOWS 5,0» (StatSoft, USA, 1998). Since the Shapiro-Wilks test testified their non-Gaussian distribution, all the obtained during the study data were processed by calculating the median (Me), minimum and maximum (Min-max), and interquartile scale (Lq – bottom quartile; Uq – top quartile). To detect statistical significance of differences between groups of indicators they used the non-parametric U-Mann-Whitney test, the comparison of ranked characteristics within individual groups at different stages of research (at the time of admission of the patient to the hospital, which coincided with 2-6 days of illness, and on 8-12 days of the disease) was performed using paired Wilcoxon test. The differences of parameters were compared for the two points that were considered statistically significant at $p < 0.05$.

Results and discussion

Depending on the intensity of the manifestations of intoxication and the nature of lesions, 16 patients were diagnosed with mild (Group 1), and 23 with moderate (Group 2) form of chickenpox. The chickenpox of 45 patients was accompanied by the development of bacterial complications, among which the most frequently encountered complication that developed as a result of direct penetration of pathogenic bacteria through damaged skin rashes and mucous membranes. These complications are: abscesses, phlegnons, bullous streptoderma, boils, gingivostomatitis,

Table 1

Dynamics of cytokines in serum of children with uncomplicated chickenpox

Comparison groups	Content of TNF-α, pg/ml., [Lq–Uq]		Content of IL-10, pg/ml., [Lq–Uq]	
	2–6 day	8–12 day	2–6 day	8–12 day
Group 1, n=16	16,195 (12,06–4,15) [14,39–21,43]* ‡	11,195 (8,24–18,02) [10,01–13,4]Δ	27,68 (18,02–2,06) [22,54–29,375]	28,715 (18,04–30,92) [24,7–29,5]
Group 2, n=23	74,11 (24,26–93,82) [44,12–148,11]*‡	22,06 (9,16–124,28) [12,04–42,75]* Δ	28,76 (19,88–34,01) [26,52–29,99]	28,04 (20,18–32,96) [24,96–29,73] Δ
Control group, n=14	11,59 (8,48–14,24) [9,68–12,82]		27,32 (19,42–29,88) [22,56–29,02]	

Notes: * - significant difference compared with those of the control group (p <0,05); ‡ - significant difference between groups comparing values; Δ - significant difference between the two time points.

etc. Considering the localization of the pathological process, patients were divided into groups with surface and deep complications of varicella (third and fourth group).

The group of surface complications included 26 patients with children chickenpox with bacterial complications (Group 3), accompanied by the development of purulent inflammatory lesions of skin surface or oral mucosa: 11 children with pyoderma, 7 patients with gingivostomatitis, 5 patients with subcutaneous abscesses and 3 patients with isolated severe boils with perifocal infiltration of tissues.

The group with deep varicella complications included 19 children with abscess lesions in different parts of the body (Group 4). The first clinical symptoms of abscess lesions of various parts of the body evolved on 3-6 days of illness when set against a sudden deterioration of general condition and fever febrile numbers appeared to intense pain in various parts of the body: chest (7 patients), abdominal (6), lumbar (4) and hips (2 patients). On examination of these areas, they observed redness, cyanotic skin tone, and significant infiltration of soft tissues. With some children the pathological process quickly spread to adjacent parts of the body.

In order to study the role of cytokine profile imbalance in shaping variant of the disease course, we determined the level of cytokines in serum of children. In conducting this study, it was found that the patients had multi-directional changes in the concentrations of TNF-α and IL-10, depending on the clinical form of the disease and its course.

In the process of studying the content of cytokines in the group of children with mild chickenpox, it was revealed that at the time of hospitalization only increase of the TNF-α level was observed, which was 1.4 times higher than the corresponding figures in the control group (p <0.001). In the dynamics of disease, the level of TNF-α was normalized and normal value did not differ from the indicators in the control group. The values of IL-10 in serum with mild form did not differ from the values in the control group.

In the group with moderate uncomplicated form of chickenpox early in the disease course, it was marked the increase in TNF-α, which was 6.4 times higher than the corresponding figures in the control group (p <0.001). Within 8-12 days of illness, the cytokine concentration in serum decreased 3.4 times in comparison with baseline, although its values continued to differ from the values in the control group (p <0.001). In the study of IL-10 values in this group of children, only a tendency of values excess at the time of hospitalization compared to the control group was observed. The research results are presented in table 1.

In the group with surface complications of varicella early in the illness, the level of TNF-α in serum by 6.7 times exceeded the values in the control group (p <0.001). A similar pattern was observed in the study of IL-10, because its concentration in the serum at the beginning of the disease was 2.9 times higher than the values in the control group (p <0.001). On the 8-12 days of illness, the increase of TNF-α and excess of values of the control group by 7.5 times was noticed. Values

Table 2

Dynamics of cytokines in serum of children with complicated chickenpox

Comparison groups	Content of TNF-α, pg/ml., [Lq–Uq]		Content of IL-10, pg/ml., [Lq–Uq]	
	2–6 day	8–12 day	2–6 day	8–12 day
Group 3, n=26	77,415 (7,08–590,62) [29,04–112,05]*	86,7 (6,92–403,46) [43,21–132,42]* ‡	81,73 (36,54–214,19) [72,11–92,43]*	89,19 (49,16–208,72) [79,96–96,61]* ‡ Δ
Group 4, n=19	96,89 (19,46–740,39) [70,13–216,14]*	214,92 (80,92–775,55) [120,52–270,96]* ‡ Δ	124,73 (58,34–246,54) [91,02–185,46]*	162,42 (96,43–284,16) [132,96–248,14]* ‡ Δ
Control group, n=14	11,59 (8,48–14,24) [9,68–12,82]		27,32 (19,42–29,88) [22,56–29,02]	

Notes: * - significant difference compared with those of the control group (p <0,05); ‡ - significant difference between groups comparing values; Δ - significant difference between the two time points.

of IL-10 content were 3.3 times higher than the values of the control group (p <0.001).

In the group with deep complications of chickenpox in early disease, the high level of two cytokines was found: TNF-α level was 8.4 times (p <0.001) higher than the values in the control group, and the level of IL-10 – by 4.6 times (p <0.001). In the dynamics of the disease, an increase in cytokine production compared to baseline was noticed. The level of TNF-α was in 18.5 times higher that the values in the control group (p <0.001), and the level of IL-10 – by 5.9 times (p <0.001). The research results are presented in Table 2.

The analysis of the received data showed that the IL-10 level in serum did not change in groups of children with mild to moderate forms of varicella with uncomplicated course, and its values did not differ from that of children from the control group. The increase of IL-10 level in early disease was observed only in the group with moderate form of varicella without complications. But the dynamics of TNF-α in the groups of children with uncomplicated course of varicella (the first and the second group) was more expressed. The children with mild chickenpox early in the illness were observed to have a slight increase in the level of TNF-α. In moderate form of chickenpox at the time of admission to hospital it was noted a high level of TNF-α with subsequent lowering of the level of the dynamics of the disease. High rates of proinflammatory cytokine TNF-α at the onset of the disease indicate the activation of cellular factors of immunity and antiviral protection of the inflammatory reaction in response to the presence of the virus.

The changes in cytokine status observed in the groups of children with complicated course of chickenpox deserve particular attention. It was revealed a high content of both studied cytokines in early disease with increasing of their concentrations in blood serum during the second study on the 8-12 days of illness. The highest content of TNF-α and IL-10 were observed in the group of children with deep complications of chickenpox. High concentration of TNF-α in early disease with a tendency to increase its dynamics is not only an indicator of inflammatory activity, but also a factor in determining the severity of the disease. Increased level of IL-10 indicates the activation of immune antibody factor, suppression of monocyte-macrophage system, which plays a key role in the development and regulation of innate and adaptive immunity.

Conclusions

1. The study of cytokines content in blood serum of children with chickenpox detected the increased levels of proinflammatory cytokine TNF-α and anti-inflammatory cytokine IL-10 relative to the indicators in the control group.

2. The dynamics of the values of content of the studied cytokines varied depending on the clinical form of the disease and its course. In groups of children with uncomplicated course of varicella, only increase of the level of TNF-α was discovered. Instead, in complicated varicella course, the high rates of both cytokines in early disease with increasing concentration in the dynamics of the disease were observed. The highest content of TNF-α and IL-10 was registered in the group of children with deep complications of chickenpox.

3. The results prove the feasibility of studying the values of TNF-α and IL-10 as an additional criterion for assessing not only the degree of severity of the disease, but for the prediction of complications.

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A six-year evaluation of antibiotics consumption in the defined daily doses in the septic orthopedic-traumotology department

E. P. Bernaz

Business Administration Office, Emergency Medicine Institute, Chisinau, the Republic of Moldova

Corresponding author: bernaz_e@yahoo.com. Received October 26, 2015; accepted December 12, 2015

Abstract

Background: Septic orthopedic and traumatology treatment permanently required a great spectrum of antibiotics to be used. Evaluation of consumption in defined daily doses is one of principal methods for improving rational usage and good planning of hospitals' necessities of antiinfectives for systemic use.
Material and methods: For this study we used data of a six-year (2009-2014) period, in septic orthopedic-traumotology department of the Emergency Medicine Institute, which show the consumption dynamics of anti-infectives for systemic use of drugs in grams and value indexes.
Results: The defined daily doses (DDD)/1000 occupied-bed days (OBD) of antibiotics in septic orthopedic-traumotology department from 578 in 2009 increased to 675 in 2014 or by 16.78% and is 14.05% lower than medium consumption of 769.83 in 152 international hospitals with the similar activity. The value of 5741 lei per DDD/1000 OBD in 2009 recorded a slow decline to 5447 lei or by 5.12%. The cost of one medium DDD from 9.94 lei in 2009 decreased to 8.07 lei in 2014 or 18.81%. The rate of anti-infectives for systemic use in 2014 presented 50169.00 lei or a share of 32% from the total departmental value of consumption; the same data in 2009 were 78054.84 lei or 34.75%. The share from the total antibiotics institutional consumption in 2014 was recorded 3.34% and 5.00% in 2009 respectively. The average antibiotics annual institution consumption constituting 464.1 in 2014 is higher by 1.06% comparatively with medium consumption of 459.20 registered in 1706 international hospitals, and by 35.31% in comparison with global consumption of 343 defined daily doses per 1000 patient-days.
Conclusions: The increase of DDD/1000 OBD took place as a result of worldwide increasing pathogenic microbes resistance to antibiotics. Nevertheless, decrease value indexes and cost of one DDD show in the best way the capacity of departmental management to cope with institutional budget deficiency and maintain qualitative antimicrobial treatment of hospitalized patients.
Key words: antibiotics, defined daily dose, consumption, rational use, hospitals.

Introduction

"The doorstep to the temple of wisdom is knowledge of our own ignorance" – Benjamin Franklin. The battle against infection is as old as human civilization. During the last few centuries, great scholars such as Louis Pasteur, Ignaz Philipp Semmelweis, Alexander Fleming, and Joseph Lister have transformed the practice of medicine through their extraordinary discoveries. Despite the progress made and strides gained, our mission to prevent infection following surgery remains unaccomplished. It is not an exaggeration to claim that fear of infection lives in the heart of every surgeon who steps into the operating room daily [1]. Septic orthopedic and traumatology treatment permanently requires a great spectrum of antibiotics to be used. Evaluation of consumption in defined daily doses is one of principal methods for improving rational usage and good planning of hospitals necessities of anti-infectives for systemic use.

The World Health Organization "European strategic action plan on antibiotic resistance 2011–2016" mentioned that "Antimicrobial resistance is not a new phenomenon, but it is increasing and new resistant strains continue to emerge". One of the main aims of the plan includes to promote prudent use of antibiotics and other drugs [2]. An important source of information is "DRUG CONSUMPTION DATABASES IN EUROPE" published by a European Consortium in 2015 [3]. We must recognize that in the Republic of Moldova drugs consumption analysis in defined daily doses (DDD) per 1000 occupied-bed days (OBD), DDD/1000), as an important indicator for optimization of rational use of drug remedies in hospitals as all and the society are not addressed enough and highlighted by scientific research literature.

The primary aim of the study was to evaluate institutional representative data on antibiotics' utilization in accordance with World Health Organization (WHO) requirements, for six-year (2009-2014) period in septic orthopedic and traumatology institutional department, and to determine value of DDD/1000. Based on the obtained data, it aimed to make conclusions on the use of anti-infectives for systemic use in department and to propose recommendations for ensuring their optimization.

Emergency Medicine Institute of the Republic of Moldova (EMI) was founded in 1959. EMI consists of 9 clinical services with 600 beds overall including orthopedic-traumatology for 150 beds, municipal center with 8 seats of hemodialysis and 9 beds. There are 4 outpatient departments of traumatology and orthopedics as well [4].

Material and methods

For this study we used the data of a six-year (2009-2014) period, in septic orthopedic-traumotology department of EMI for 40 beds, which show the dynamics of consumption of anti-infectives for systemic use drugs, as classified by Anatomical Therapeutic Chemical (ATC), classification system of World Health Organization indicated in grams and value indexes. Statistical, analytical, mathematical, compararative, logical and descriptive were used as the methods of study.

Results and discussion

Total institutional antibiotic consumption in value indexes was 1562575 lei in 2009 and 1500888 lei in 2014 that represents respectively 17% and 14% from the whole amount of drugs [5].

In figure 1 it is shown the consumption rate of anti-infectives for systemic use in lei in comparison with other pharmacotherapeutic groups in orthopedic-traumatology department in 2014.

As can be observed the rate of anti-infectives for systemic use present 50169.00 lei or a share of 32% from the total value amount of 156821 lei drugs consumption in 2014. In 2009 the same data were 78054.84 lei or 34.75% from total of 224644 lei. The share of departmental from the total antibiotics institutional consumption in 2014 recorded 3.34% and 5.00% in 2009 respectively.

For evaluating the consumption of anti-infectives for systemic use drugs in the department during 2009-2014 were followed 10 steps of determining DDD/1000 [6, 7, 8] and the statistics data concerning the number of treated patients (for only patients with health insurance and other free treated by the state categories of citizens), the number of bed/days (2009 =10664; 2010 = 10017; 2011 = 9540; 2012 = 10178; 2013 = 9701; 2014 = 9535) and data about total annual consumption of antibiotics were used.

All in all 48 antimicrobial remedies (both for parenteral and enteral use) for treating assistance of hospitalized patients in the evaluated period were used, from which with only enteral form 22 names, with only parenteral form 26 names and with both forms 10 names, which represents 37 active antimicrobial substances.

Parenteral forms consumption rate of antibiotic subgroups evaluated in DDD/1000 during 2009-2014 is shown in figure2.

As can be observed from figure 2 in the evaluated period the average consumption annual rate of all antibiotic subgroups records a decline from 543 in 2009 to 490 DDD/1000 in 2014 or by 9.76%. The main consumption of 461.65 DDD/1000 or 85.02% from the total in 2009 to 458.41 or 93.55% in 2014 with a slow decrease of 0.70% during mentioned years was registered for aminoglycoside antibacterials (Streptomycinum 1.0, Gentamycinum 0.2, Kanamycinum 1.0, Amikacinum 1.0), other beta-lactam antibacterials (Cefazolinum 3.0, Cefuroximum 3.0, Cefotaximum4.0, Ceftazidimum 4.0, Ceftriaxonum 2.0, Cefoperazonum 4.0) and macrolides, lincosamides and streptogramins (Clarithromycinum 0.5, Azithromycinum 0.5, Lincomycinum 1.8).

In figure 3 consumption rate of enteral forms of antibacterials for systemic use subgroups in DDD/1000 during 2009-2014 is shown.

As it is seen from figure 2 the average consumption annual rate of antibiotics for oral usage increased from 35 in 2009 to 185 DDD/1000 OBD in 2014 or by 5.29 times. The highest consumption from 19.4 DDD/1000 or 55.43% of the total in 2009 to 169.9 or 91.84% of the total in 2014 and an increase by 8.76 times during the evaluated period was registered for quinolone antibacterials (Gatifloxacinum 0.4, Acidum pipemidicum 0.8),other beta-lactam antibacterials (Cefalexinum 2.0, Cefuroximum 0.5, Cefaclorum 1.0 gram and Cefixim 0.4) and beta-lactam antibacterials, penicillins.

In figure 4 the total (parenteral and enteral forms) antibiotic subgroups used rates are demonstrated.

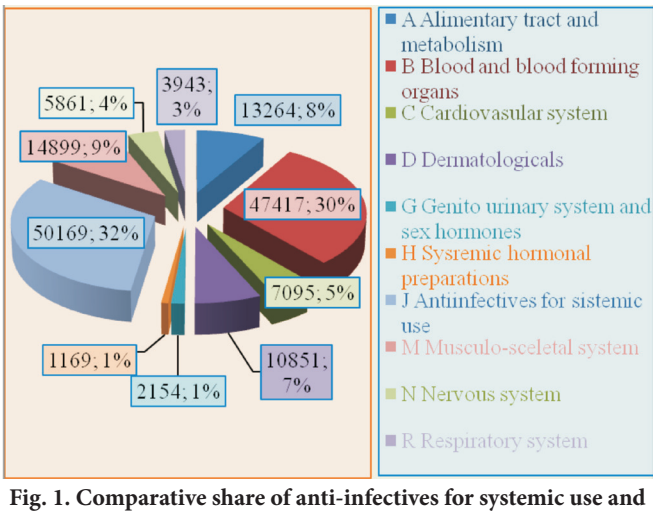

Fig. 1. Comparative share of anti-infectives for systemic use and other pharmacotherapeutic groups from total consumption.

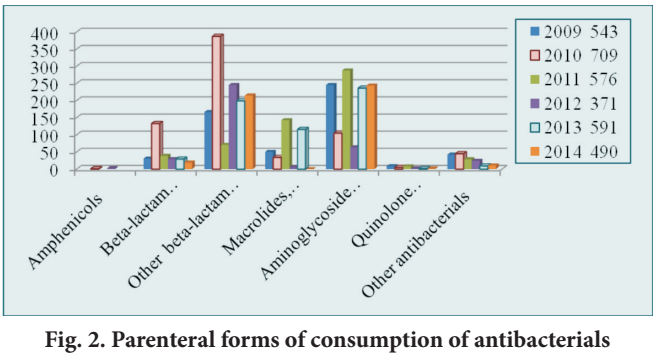

Fig. 2. Parenteral forms of consumption of antibacterials for systemic use in DDD/1000.

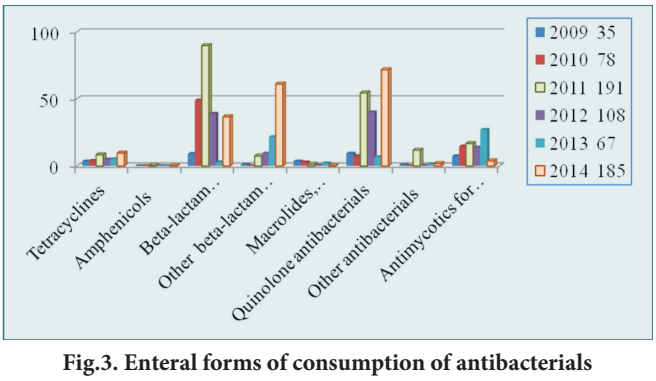

Fig.3. Enteral forms of consumption of antibacterials for systemic use in DDD/1000.

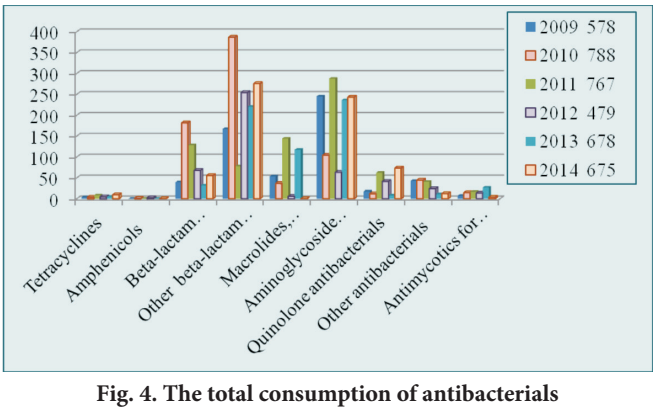

Fig. 4. The total consumption of antibacterials for systemic use in DDD/1000.

Table 1

The ratio between DDD/1000 for parenteral to enteral forms of antibiotics and percentage from the total

The ratio DDD/1000 of parenteral to enteral use and percentage from the total							
Years		2009	2 010	2011	2012	2013	2014
Parenteral		543	709	576	371	590	490
Enteral		35	78	191	108	67	185
The ratio of parenteral to oral		15.74	9.09	3.02	3.43	8.81	2.65
Total		578	788	767	479	657	675
Percentage from total	Parenteral	93.94%	89.97%	75.10%	77.45%	89.80%	72.59%
	Enteral	6.06%	9.90%	24.90%	22.55%	10.20%	27.41%

As it can be observed from figure 3 the average aggregated annual rate for total antibiotics consumption in the evaluated period increased from 578 in 2009 to 675 DDD/1000 in 2014 or by 16.78%.

The first 4 subgroups with the highest yearly consumption from 451,97 DDD/1000 or 78.20% of the total in 2009 to 575,42 DDD/1000 or 85.20% of the total in 2014 respectively and an increase of 27.31% during the evaluated period were registered for other beta-lactam antibacterials, aminoglycoside antibacterials, beta-lactam antibacterials, penicillins.

In table 1 the ratio DDD/1000 of parenteral to enteral use forms and percentage from the total is shown.

From table 1 it can be seen that in the evaluated period the ratio between antibiotics DDD/1000 parenteral to enteral forms decreased from 15.74 to 2.65 times. The percentage of parenteral forms from the total antibiotics DDD/1000 decreased from 93.94% in 2009 to 72.59% in 2014 and vice versa enteral forms increased the ratio from 6.06% to 27.41% respectively. Similar data for the entire institution were published early [9].

Comparison of total consumption data of anti-infectives for systemic use evaluated in DDD among 1576 European hospitals and surgeries, orthopedics and traumotplogy departments with the similar data of EMI and department is presented in figure 2.

As we can see from table 2 the average annual rate for total-hospital antibiotics utilization period in EMI decreased from 662.4 in 2009 to 464.1 DDD/1000 in 2014 or by 30%. That result was higher by 67.65 DDD/1000 or by 14.58% than the medium consumption of 396.45 DDD/1000 registered in case of 1256 international hospitals [(1115x393 + 34x395 + 43x422 + 55x448 + 1x400 + 1x403 + 7x390):1256] and lower by 112.66 DDD/1000 or by 36.68%

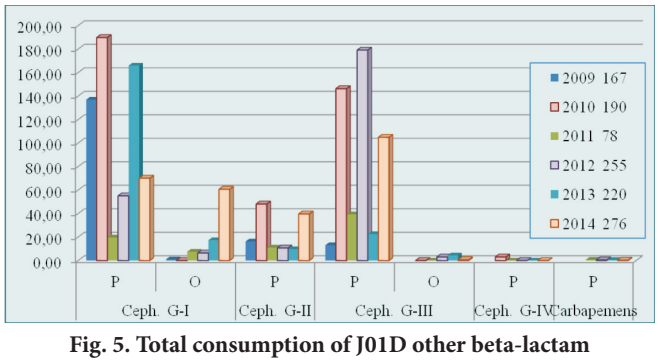

Fig. 5. Total consumption of J01D other beta-lactam antibacterials for parenteral and enteral use in DDD/1000.

in case of 450 international hospitals where the mentioned medium was 634.34 DDD/1000 [(8x601 + 54x547 + 1x595 + 40x499 + 195x583 + 7x570 + 7x1610+ 8x724 +776x130) : 450] respectivly.

Other all medium consumption in 1706 (1256+450) international hospitals constituing 459.20 DDD/1000 was lower than consumption of 464.1 DDD/1000 in EMI in 2014 by 4.90 DDD/1000 or by 1.06% and lower by 121.1 comparatively to global antibiotic consumption of 343 defined daily doses per 1000 patient-days or by 20.09%.

In septic orthopedic-traumotology department of EMI in the evaluated period was registered an increase from 578 in 2009 to 675 DDD/1000 in 2014 respectively or by 16.78%. In case of 152 international hospitals with similar surgery and orthopedic-traumotology activities medium consumption was (8x793 + 7x570 + 8x724 + 776x130) : 152= 769.83 DDD/1000, that was by 94.83 DDD/1000 more than results recorded in EMI in 2014 or by 14.05%.

In figure 5 the total consumption of other beta-lactam antibacterials for parenteral and enteral use in DDD/1000 is demonstrated.

From this chart as one can see in the evaluated period the total consumption of parenteral and enteral forms of cephalosporin's G-I (Cefalexinum and Cefazolinum) decreased slowly from 137.83 to 130.68 or by 5.20%, cephalosporin's G-II (Cefuroximum, Cefaclorum) and G-III (Cefotaximum, Ceftazidimum, Ceftriaxonum, Cefixim, Cefoperazonum, Cefoperazonum + Sulbactamum) demonstrate a considerable increment of consumption from 29.29 (16.25+13.04) in 2009 to 145.15 DDD/1000(39.64+105.5) or by 4.85 times, cephalosporins G-IV and carbapemens (Meropenemum, Imipenemum+Cilastatinum) had a low consumption only during 2010 to 2014. The total consumption of other beta-lactam antibacterials shows a considerable increment from 167 to 276 DDD/1000 or by 65.27%. It can be mentioned a visible decrement of consumption of cephalosporin's G-I with parenteral forms from 136.92 DDD/1000 in 2009 to 70.56 in 2014 or by 48.47% and vice versa a considerable growth of consumption of cephalosporin's G-I with enteral use forms from 0.93 in 2009 to 70.1 in 2014 or by 75.38 times. Similar data for the entire institution were published early [23].

The cost of DDD/1000 in lei for parenteral forms of antibacterials for systemc use during 2009-2014 is shown in figure 6.

Table 2

Surveillance studies of antibiotic use in international hospitals in comparison with the similar data in Emergency Medicine Institute and department

Setting	Surveillance time-period	Data source	Frequency of data collection	Use of antibiotics in DDD/1000 bed-days over the study period
Emergency Medicine Institute	6 years (2009– 2014)	Pharmacy dispensing records (PDR)	Annual	662.4, hospital-wide in 2009; 464.1, hospital-wide in 2014
Septic orthopedic-traumotology department of EMI	6 years (2009–2014)	(PDR)	Annual	578, department-wide in 2009; 675, department-wide in 2014.
1115 hospitals in France [10]	3 years (2008–2010)	(PDR)	Annual	370.0, hospital-wide in 2008; 393.0, hospital-wide in 2010.
34 public hospitals and 43 private hospitals located in south-western France [11]	2005	(PDR)	Annual	395, hospital-wide; 422, hospital-wide.
49-59 hospitals in the Netherlands [12] medium 54 hospitals	5 years (1997–2001)	(PDR)	Annual	472.0, hospital-wide in 1997; 547.0, hospital-wide in 2001.
55 public hospitals in Denmark [13]	5 years (1997–2001)	Danish Medicines Agency	Annual	380.0, hospital-wide in 1997; 448.0, hospital-wide in 2001.
1 university hospital in Switzerland [14]	5 years (1996–2000)	(PDR)	Not specified	400.0, hospital-wide;
Military Medical Academy, Sofia, Bulgaria [15]	1 year (2011)	(PDR)	Annual	403.0, hospital-wide.
1 general hospital in Spain [16]	5 years (1996–2000)	(PDR)	Annual	595.0, hospital-wide.
8 university hospitals in Germany [17]	3 years (1998–2000)	(PDR)	Annual	601.0, medical wards; 793.0, surgical wards.
40 non-university regional acute care general hospitals in south-western Germany, 2001-2002 [18]	2 years (2001–2002)	(PDR)	Annual	499.0, with a mean in internal medicine; 434.0, with a mean in surgery.
530 French hospitals in 2007 from which 195 in general hospitals and 357 for hospitals detailed clinical surgery activity [19]	1 year (2007)	(PDR)	Annual	557.0, in medicine; 553.0, in surgery.
7 hospitals in Stockholm [20]	1 year (2000)	(PDR)	Annual	390 to 570 internal medicine; 1020 to 1610 infectious disease.
8 Norwegian hospitals serving 36% of the nation's population [21]	from 2002 to 2007	(PDR)	Annual	increased from 617 to 724 DDDs/1000 bed-days.
130 US hospitals[22]	August 2002 1–31 July 2003	(PDR)	Annual	792 776
The global antibiotic consumption [23]	varied little between 2006 and 2008	(PDR)	Annual	343 defined daily doses (DDD) per 1000 patient-days (PD).

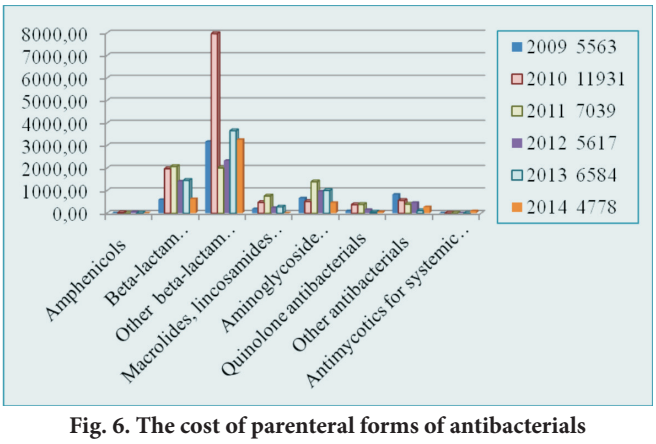

Fig. 6. The cost of parenteral forms of antibacterials for systemic use per DDD/1000 in lei.

As we can see from figure 5 the average consumption annual rate per DDD/1000 in value indexes (lei) of all parenteral antibiotic subgroups records a decline from 5563 in 2009 to 4778 lei in 2014 or by 14.11%. The medium yearly consumption for the evaluated period with more than 1000 lei per DDD/1000 was registered for other beta-lactam antibacterials (3735.62 leis) and for beta-lactam antibacterials (1358.73 lei), more than 500 lei recorded aminoglycoside antibacterials (838.3 lei). Other subgrups as other antibacterials, macrolides, lincosamides and streptogramins, quinolone antibacterials, antimycotics for systemic use registered less than 500 leis per DDD/1000.

Consumption rate in value indexes in lei for enteral forms of antibiotics subgroups per DDD/1000 during 2009-2014 is shown in figure 7.

From figure 7 it can be found that the average consumption annual rate in value indexes of all antibiotic subgroups records an increase from 177 in 2009 to 669 lei per DDD/1000 in 2014 or by 3.78 times. The medium yearly consumption for the evaluated period with more than 100 lei per DDD/1000 was registered for beta-lactam antibacterials, penicillins, other beta-lactam antibacterials

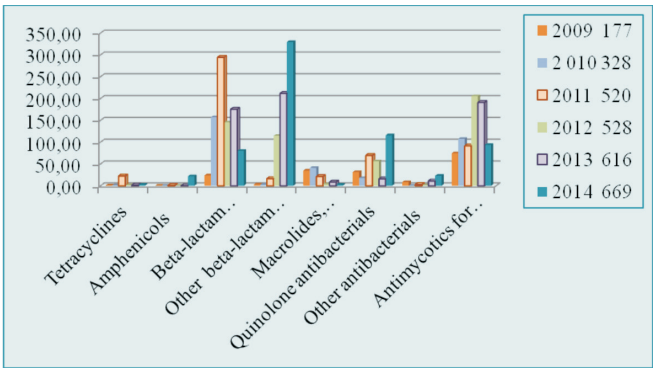

Fig. 7. The cost in lei for enteral forms of antibacterials for systemc use per DDD/1000.

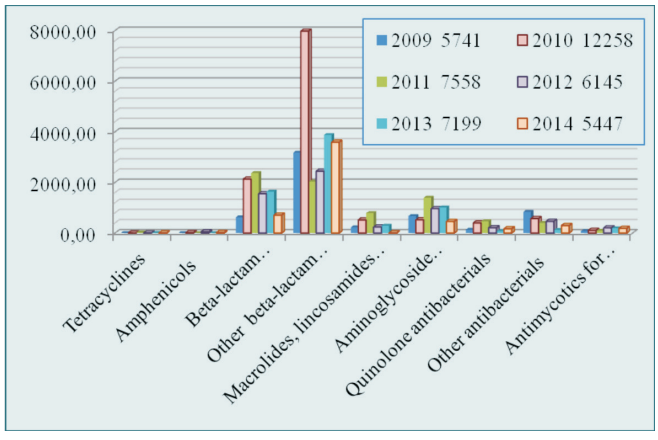

Fig. 8. Total cost of antibacterials for systemc use per DDD/1000 in lei.

and antimycotics for systemic use. All other subgroups recorded a consumption from 3 to 51 DDD/1000.

Consumption rate in value indexes (lei) of parenteral and enteral forms of antibiotics subgroups in DDD/1000 during 2009-2014 is shown in figure 8.

In this chart the presented data demonstrate that the average consumption annual rate in value indexes (lei) of total antibiotics record a decline from 5741 in 2009 to 5447 lei per DDD/1000 in 2014 or by 5.12%. The medium yearly cost of DDD/1000 was registered more than: 3000 lei for other beta-lactam antibacterials, 1500 lei for beta-lactam antibacterials, penicillins, 500 lei for aminoglycoside antibacterials, between 100 – 500 lei for macrolides, lincosamides and streptogramins, quinolone antibacterials, other antibacterials and antimycotics for systemic use, and between 2 – 20 lei for tetracyclines and amphenicols.

Table 3

Cost of one medium DDD antibacterial for systemic use of parenteral, enteral forms and total in lei

Septic orthopedic-traumatology department						
Data for determining and cost of 1(one) DDD	2009	2010	2011	2012	2013	2014
Parenteral cost (lei) DDD/1000	5563.38	11930.48	7039.12	5616.86	6583.6	4778
Enteral cost (lei) DDD/1000	177.27	327.53	519.6	528.1	615.67	669.24
Total (Parenteral and enteral cost (lei) DDD/1000)	5741	12258	7558	6145	7199	5447
Parenteral DDD/1000	543.12	709.39	576.33	371.17	590.46	490.06
Enteral DDD/1000	34.5	78.07	190.67	108.17	67	185.21
Total (Parenteral and enteral DDD/1000)	577.62	787.46	767	479.34	657.46	675.27
Total (Parenteral and enteral cost (lei) 1 (one) DDD)	9.94	15.57	9.85	12.82	10.95	8.07
Parenteral cost (lei) 1 (one)DDD	10.24	16.82	12.21	15.13	11.15	9.75
Enteral cost (lei) 1(one) DDD	5.14	4.20	2.73	4.88	9.19	3.61

Table 4

The medium cost per one DDD in lei of other beta-lactamantibacterials for parenteral and enteral forms and total

Septic orthopedic-traumotology department						
Data for determining and cost of 1(one) DDD	2009	2010	2011	2012	2013	2014
Parenteral cost (lei) DDD/1000	3174.22	7974.98	2012.62	2328.99	3662.1	3260.84
Enteral cost (lei) DDD/1000	3.02	2.88	16.52	114.6	211.81	329.52
Parenteral and enteral cost (lei) DDD/1000	3177.24	7977.86	2029.14	2443.59	3873.9	3590.4
Parenteral DDD/1000	166.21	387.24	70.55	245.24	198.74	214.89
Enteral DDD/1000	0.93	0	7.44	19.73	21.65	61.25
Parenteral and enteral DDD/1000	167.14	387.24	77.99	254.57	220.39	276.14
Total (Parenteral and enteral cost (lei) 1 (one) DDD)	19.01	20.60	26.02	9.60	17.58	13.00
Parenteral cost (lei) 1 (one) DDD	19.10	20.59	28.53	9.50	18.43	15.17
Enteral cost (lei) 1 (one) DDD	3.25	0.00	2.22	5.81	9.78	5.38

To determine the cost of one medium DDD of antibacterials for systemic use separately for parenteral and enteral pharmaceutical forms were divided by the cost of DDD/1000 to DDD/1000 respectively. The cost of one medium DDD antibiotics in lei for parenteral and enteral forms and total is shown in table 3 and 4.

As we can see from table 3 in the evaluated period the cost of one medium DDD decreased from 10.24 lei in 2009 to 9.75 lei in 2014 or by 4.79% for parenteral forms, from 5.14 to 3.61 lei or by 29.77% for enteral forms and from 9.94 to 8.07 lei or by 18.81% for one total DDD.

In chronological way for the evaluated years the ratio between the cost of one medium DDD of parenteral and enteral forms was respectively 1.99:1; 4:1; 4.47:1; 3.1:1; 1.2:1 and 2.7:1.

Calculation of the cost in lei per one medium DDD for parenteral, enteral forms and total for other beta-lactam antibacterials is shown in table 4. As we can see from this table in the evaluated period total cost of one medium DDD decreased from 19.01 in 2009 to 13.00 leis in 2014 or by 31.94%, for parenteral from 19.10 to 15.17 lei or by 20.58% and for enteral use increased from 3.32 to 5.38 lei or by 59.64%.

The ratio between the cost of parenteral and enteral forms per one medium DDD in the evaluated years was respectivly 5.88:1; 20:1; 12.87:1; 1.64:1; 1.88:1 and 2:82:1.

Conclusions

1. DDD/1000 OBD in the septic orthopedic-traumotology department from 578 in 2009 increased to 675 in 2014 or by 16.78% of which other beta-lactam antibacterials from 167 or 28.89% and 276 or 40.89% respectively. The consumption of parenteral forms constituing 543 DDD/1000 or 93.95% from the total in 2009 decreased to 490 DDD/1000 OBD or by 9.76% in 2014 and vice versa use of enteral forms constituing 35 or 6.06% from the total in 2009, increased to 185 DDD/1000 OBD in 2014 or by 5.29 times respectively.

2. The cost of 5741 lei per DDD/1000 OBD in 2009 recorded a slow decline to 5447 lei or by 5.12%. The cost of one medium DDD from 9.94 lei in 2009 decreased to 8.07 lei in 2014 or by 18.81%. The rate of anti-infectives for systemic use in 2014 presented 50169.00 lei or a share of 32% from the total departmental value consumption; the same data in 2009 were 78054.84 lei or 34.75%. The share from the total antibiotics institutional consumption in 2014 recorded 3.34% and 5.00% in 2009 respectively.

3. The average annual rate for total-institution antibiotics utilization period in EMI decreased from 662.4 in 2009 to 464.1 DDD/1000 OBD in 2014 or by 30%. Obtained record is higher by 6.69% comparatively with medium consumption of 433.06 DDD/1000 registered in 1576 international hospitals, and by 35.31% than global antibiotic consumption of 343 defined daily doses per 1000 patient-days.

4. There were evaluated 48 antimicrobial remedies (both for parenteral and enteral use) for hospitalized patients, from which with only enteral form 22 names, with only parenteral form 26 names and with both forms 10 names, which represent 37 active antimicrobial substances.

5. The ratio between the cost of one medium DDD of parenteral and enteral forms of antibacterials for systemic use was 1.99:1; 4:1; 4.47:1; 3.1:1; 1.2:1 and 2.7:1 for the evaluated years. For other beta-lactam antibacterials this ratio was 5.88:1; 20:1; 12.87:1; 1.64:1; 1.88:1 and 2:82:1 respectivly.

6. The increase of DDD/1000 OBD during the evaluated period took place as a result of worldwide increasing pathogenic microbes' resistance to antibiotics. Nevertheless, decrease value indexes and cost of one DDD show in the best way the capacity of departmental management to cope with institutional budget deficiency and maintain qualitative antimicrobial treatment of hospitalized patients.

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Antibiotic therapy in the treatment of acute otitis media

***S. Cazacu[1], C. Georgescu[2], A-M. Pelin[2], L. Brunchi[1], P. Cazacu[1], L. Dodon[1]**

[1]Department of Otorhinolaryngology, District Hospital of Orhei, the Republic of Moldova
[2]Department of Pharmacology, Dunarea de Jos University of Galati, Romania
*Corresponding author: cazacusimion@mail.ru. Received October 12, 2015; accepted December 07, 2015

Abstract

Background: Acute otitis media is an infection of abrupt onset that usually presents with ear pain. Worldwide acute otitis media affects about 8-11% of people a year. In acute otitis media, antibiotics may speed recovery but may result in side effects. Antibiotics are often recommended in those with severe disease or under two years old.
Material and methods: Our study included a retrospective analysis of 117 patients with acute otitis media, whose medical records were examined. The information was analyzed statistically.
Results: The results demonstrated an increased involvement of older people and women. The use of antibiotics to treat acute otitis media is determined by Cefazolin in 49-50% cases, Ceftriaxone - 49%, Cefotaxime – 48%, Cefoperazone – 14%, Amoxicillini+Clavulanic acid in 16% of cases. Antibiogram was released for 53 patients, and according to it: Cefazolin – 23%, Amoxicilini+Clavulanic acid – 15% and the rest were treated with cephalosporin third generation.
Conclusions: Depending on severity, the treatment requires the use of antibiotics with broad spectrum. Antibacterial therapy according to antibiogram is contemporary and has the advantage of assessing the appropriate antibiotic.
Key words: otitis media, antibiotics, cephalosporin generation.

Introduction

Acute otitis media represents the inflammation of the middle ear [3, 4]. The frequency of acute otitis media in people of any age and complications that follow it set them first in otorhinolaryngology practice [1, 6].

According to data from literature, about 8-11% of the population of the globe suffers from acute otitis media [4]. In Moldova the figure constitutes 8-10% with the upward trend in the mature population and children [1].

Acute inflammation of the middle ear with its clinical course manifests itself through otalgii, leaks from the middle ear, fever, illness severity and subsequently reveals complications, more often endocranian [11].

The treatment should be ample, appropriate, and lead to a cure both morphologically and functionally, without having to admit the evolution of acute otitis in a chronic form. The treatment is applied depending on the phase of the development of otitis [2, 5, 12, 13, 14, 16].

Material and methods

It was realized a retrospective statistical study for a period of 2 years in Otorhinolaryngology Department of Orhei District Hospital, which describes an incidence of about 60-70 patients per year interned in the hospital, about 10% of the total number of patients.

The study included 117 hospitalized patients with diagnosis of acute otitis media. They were treated empirically but also according to antibiogram. Data were collected from the patients' observational records and processed statistically.

Results and discussion

From the analysis of the data used in the study, it was observed that most affected by acute otitis are elderly people (mostly female), due to deficient immune system, and the children, aged 6-18 years. They are prone to fall sick because the body and the immune system are incompletely developed.

The empirical treatment is more often used in children aged up to 5 years and teenagers between the ages of 6 to 18 years, the underlying cause being limited possibilities to take ear secretion considering the age and the fear of the child. In adults and elderly it is easily carried out.

The majority of patients affected by acute otitis media present pathology of average gravity (48% – in 57 patients),

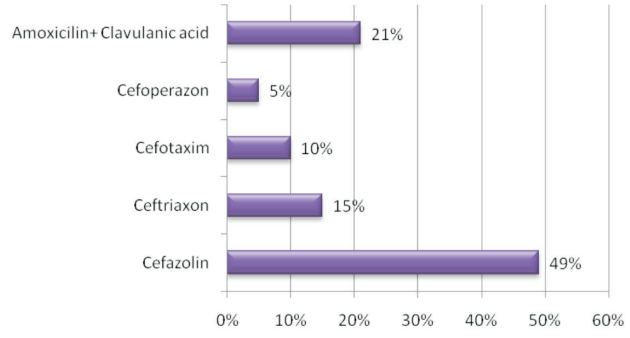

Fig. 1. Antibiotic therapy of light acute medium otitis.

which confirms the fact that people do not address the physician at the first symptoms of the disease, but they go to see the doctor when symptoms do not allow them to carry on activities normally, the rest of the patients have severe (17%) and mild (33%) forms.

There were 39 hospital patients with diagnosis of acute otitis media. Cefalosporin of generation I was the base antibiotic given to 19 patients (49%). Third generation of cephalosporins have been used less frequently, Ceftriaxone at 6 patients (15%), at 4 patients – Cefotaxim (10%), at 2 patients – Cefoperazon (5%), while Penicilin, Amoxicillin + Clavulanic Acid at 8 patients (21%) (fig. 1).

The antibiotic prevailing in the treatment of acute otitis media is the average form Ceftriaxone, 28 patients (47%) which is a semi-synthetic cephalosporin of the beta-lacto masses and is resistant to most beta-lacto masses. It is used widely in the treatment of otitis due to long half-life which makes it very convenient treatment, the patient is administered a single daily dose. With almost equal frequency we returned Cephazolin to 8 patients (14%), Cefoperazon to 9 patients (16%) and Cefotaxim to 11 patients (19%) (fig. 2).

The least used was Amoxicillin + Clavulanic, 2 patients (4%), most likely due to the presence of amoxicillin in almost all kinds of meat on the market, and its effect could be significantly diminished.

16 patients (81%) were treated with generation III Cephalosporins, predominantly Cefotaxim – 9 patients (48%), ceftriaxone – 4 patients (19%) and Cefoperazon – 3 patients (14%). Of all the 20 patients hospitalized with diagnosis of acute otitis media only 4 patients received Cefazolin (19%) (fig. 3).

According to the severity of patients' pathologies, 51 (44%) were hospitalized for less than 6 days, 46 patients (39%) were placed for 7 days, and 20 patients (17%) – more than 7 days.

Hospital patients for less than 6 days are considered those with light and medium otitis media. In this case the treatment was performed according to antibiogram. Prescribed treatment without enterotoxin was administered: Cefazolin to 25 patients (50%), Cefoperazon to 2 patients (4%), Cefotaxim to 6 patients (12%), Amoxicillin + Clavulanic Acid – 8 patients (15%) and Ceftriaxon to 10 patients (19%) (fig. 4).

In this case, for hospital patients of 7 day staying Ceftriaxon has been recommended for 22 patients (48%). For 11 patients (24%) was administered Cefazolin, but Penicilin for 2 patients (4%) (fig. 5).

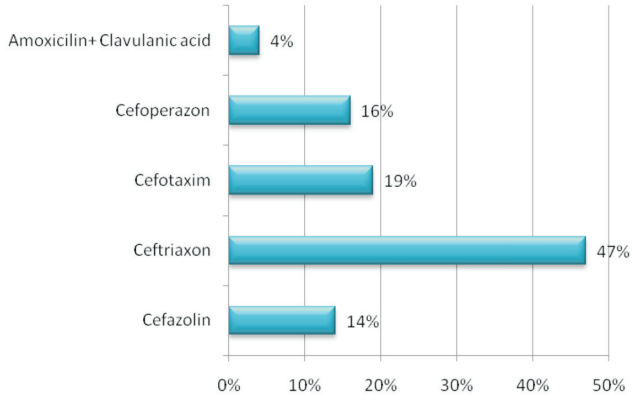

Fig. 2. Antibiotic therapy of moderate otitis media.

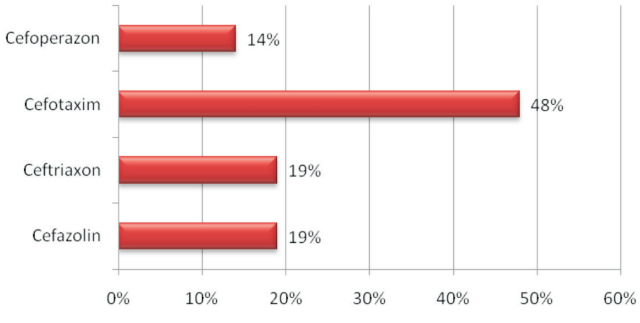

Fig. 3. Therapy with antibiotics of severely acute moderate otitis media.

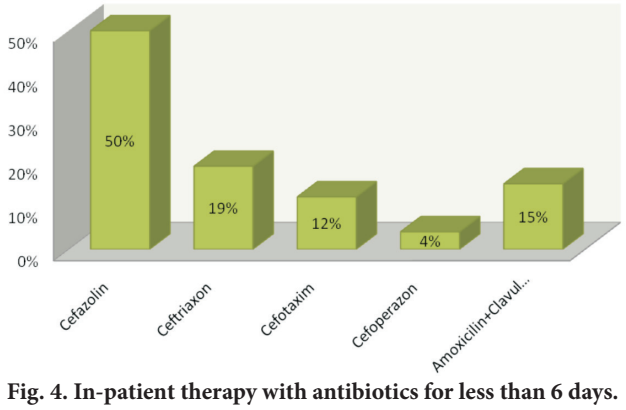

Fig. 4. In-patient therapy with antibiotics for less than 6 days.

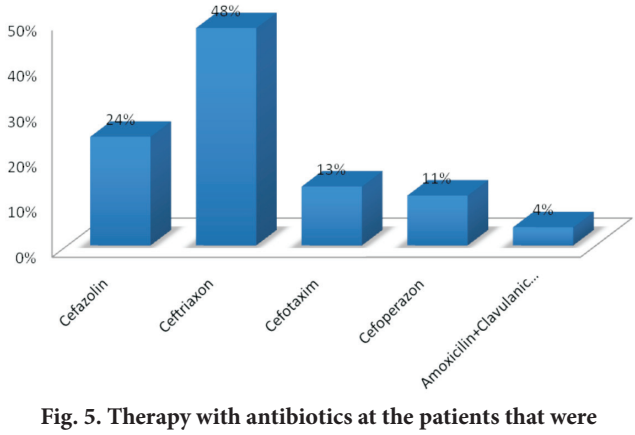

Fig. 5. Therapy with antibiotics at the patients that were hospitalized for more than 7 days.

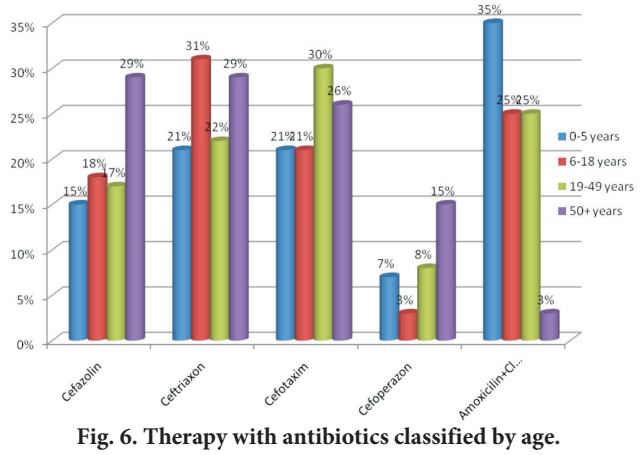

Fig. 6. Therapy with antibiotics classified by age.

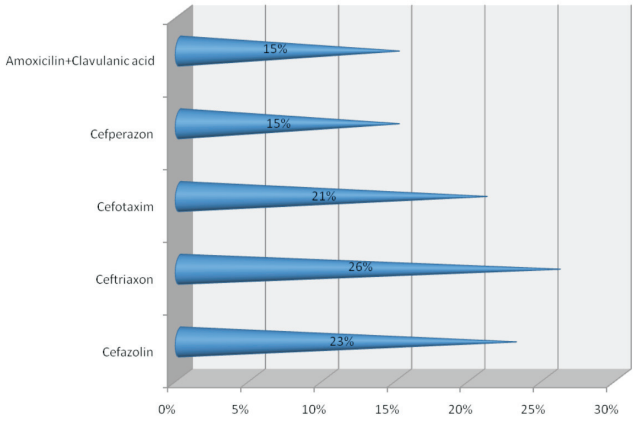

Fig. 7. Therapy with antibiotics after antibiogram.

For all the studied patients prevailed Cephalosporins. Cefazolin was used by 15-30% more in the elderly patients, but ceftriaxon was administered with a frequency of 21-31%. A similar percentage has Cefotaxim only with a minimum insignificant difference. Amoxicillin+Clavulanic Acid prevails in the treatment of children in 35%, due to the slight possibility of being used in the treatment of acute otitis media, it is also used in the form of syrup or suspension (fig. 6).

14 patients (26%) of all for whom antibiogram was carried out were elected to use Ceftriaxon, Cefotaxim was prescribed for 11 patients (21%) and Cefazolin – slightly more used. Cefoperazon and Amoxicillin + Clavulanic acid have an equal percentage (as there were 8 patients) (fig. 7).

According to literature, Cephalosporin has the best therapeutic effect in the treatment of acute otitis media [5, 7, 8]. This scheme of antibiotic therapy in acute otitis media is well tolerated by patients and has a maximum effect for treating patients [5, 7].

Pharmacological data in our study demonstrated that Ceftriaxon, Cefazolin and Cefotaxim showed superior efficacy in most cases, irrespective of the studied aspects depending on age, gender, or duration of treatment [5, 7, 8].

Conclusions

1. The use of antibiotics in the treatment of acute otitis media is determined by exposed and visible effects in the analysis with high efficacy: Cefazolin – 49-50%; Ceftriaxon – 49%; Cefotaxim – 48%; Cefoperazon – 14%; Amoxicillin + Clavulanic Acid – 16%.

2. With the degree of advancement of the severity of otitis media it is required to use antibiotics with a broad spectrum of action.

3. Antibiogram was performed in 53 patients, for 23% of them were prescribed Cefazolin – a broad-spectrum generation I cephalosporin, in 15% – Amoxicillin + Clavulanic Acid and in the most cases (62%) were administrated generation III Cefalosporins: Cefotaxim, Ceftriaxone and Cefoperazone.

4. Antibacterial treatment according to antibiogram is contemporary and it has the advantage of assessing the right antibiotic.

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Aspects of the population morbidity in some regions of the Republic of Moldova

***E.Ciobanu[1], C. Croitoru[1], S. Cebanu[1], M. Mogorean[1], V. Bernic[2], O. Burduniuc[2]**

[1]General Hygiene Department, Nicolae Testemitsanu State University of Medicine and Pharmacy
[2]National Centre of Public Health, Chisinau, the Republic of Moldova

*Corresponding author: elena.ciobanu@usmf.md. Received November 09, 2015; December 07, 2015

Abstract

Background: The health of the Moldovan population requires special attention, due to direct and indirect biological, environmental, behavioural and health factors.

Material and methods: We studied population morbidity from the following localities: town Cupcini, village Bratuseni, village Ruseni (Edinet district); town Vadul lui Voda, town Cricova, village Condrita (municipality Chisinău); village Rosu, village Moscovei, village Huluboaia (Cahul district). Statistical data were taken from the statistical report No 12, for the period from 2012 to 2014.

Results: Morbidity analysis showed that the overall prevalence of increased average values was recorded in the village Bratuseni, Edinet district – 16119.0±247.70/000. In town Vadul lui Voda, Cricova, village Condrița (municipality Chisinau), overall prevalence values ranged within 6967.0±813.0 – 8721.3±375.90/000. Meanwhile, village Rosu, village Moscovei, village Huluboaia (Cahul district), mean values of overall prevalence were included within 2379.8±574.8 and 3219.8±152.70/000. Analysis of the general incidence emphasized that the community in the North recorded mean values within 1370.5±0.7 – 11162.4±190.40/000, localities in the center and south - 1877.8±246.3 – 3149.0±187.80/000 and respectively 899.3±95.0 – 1244.8±339.80/000.

Conclusions: Population morbidity was growing during 2012-2014. Prevalence of diseases of the circulatory, digestive and urogenital systems was higher in central areas and the osteoarticular system diseases – in northern communities. The incidence of cardiovascular, urogenital and osteoarticular diseases was higher in cities from North, and digestive system diseases – in central areas.

Key words: morbidity, incidence, prevalence.

Introduction

The public health problem is increasingly critical in the Republic of Moldova. Health is not a goal itself but a condition for life quality and a condition by which people can participate in economic and social development. The health of the population is an important indicator, both of quality of life and standard of living, and environmental quality also. Health is conditioned by biological factors (genetics, human reproduction), environmental (physical, chemical and social), behavioural and health [1]. The influence of these factors in determining a certain level of health of the population varies: 40% (lifestyle), 20% (environment), 30% (biological factors) and health care sector directly affects with only 10% [2].

The hygienic-epidemiological aspect of problem requires a comprehensive and integrated approach to population health indices. One of the important indices of population health is morbidity that highlights the underlying causal relationships between risk factors and disease. The World Health Organization encourages continuous monitoring of the health status of the population in each state, but also globally, simply because it provides the scientific and economic decisions of intervention to healthcare, disease prevention and control.

Material and methods

We analyzed population morbidity from the selected localities: town Cupcini, village Bratuseni, village Ruseni (Edinet district); town Vadul lui Voda, town Cricova, village Condrita (municipality Chisinău); village Rosu, village Moscovei, village Huluboaia (Cahul district). Morbidity data was taken from the statistical report No 12 "Concerning the number of registered diseases in patients domiciled on the territory of the servicing curative institution" for 2012-2014 period, concentrated at the National Center for Health Management, local health centers. In order to collect, process and interpret the data we used analytical, descriptive, selective and statistic-mathematical methods.

Results and discussion

For this study we set the major objective to determine the most widespread nosologic forms typical for some localities.

Out of all studied disease classes, according to the International Classification of Diseases, 10th WHO revision, we selected those with a greater likelihood of susceptibility to different environmental factors: biological factors (heredity, demographic characteristics of the population), environmental factors (the physical, chemical and social environment), behavioural factors and health services [3]. Based on multiple studies and arguments in the special literature we highlighted the disease classes, that have a leading position in the structure of morbidity, both of urban and rural population [4, 5, 6]. Based on previous research we selected and studied four classes of diseases: cardiovascular, digestive, urogenital and osteoarticular system diseases. Prior to studying these classes we analysed the disease prevalence and general incidence research.

General prevalence data (table 1) reveal greater values of morbidity averages in the northern localities: town Cupcini, village Brătușeni, village Ruseni (Edinet district). The maximum morbidity was registered in the village Bratuseni – 16119.0±247.7 cases per 10 000 inhabitants. Followed by localities from the center: town Vadul lui Voda, town Cricova, village Condrița (municipality Chisinau), with mean morbidity values included within 6967.0±813.0 – 8721.3±375.9 cases per 10 000 inhabitants. This area is followed by cities in the south: village Rosu, village Moscovei, village Huluboaia (Cahul district), with values between 2379.8±574.8 - 3219.8±152.7 cases per 10 000 inhabitants.

Table 1

General prevalence rate of the population in studied areas (per 10 thousand inhabitants)

Areas		2012	2013	2014	M±ES
North district Edinet	town Cupcini	6708.7	6735.4	6743.1	6729.1±10.43
	village Brătușeni	15780.8	15974.9	16601.6	16119.0±247.7
	village Ruseni	9044.5	10438.8	9189.2	9557.5±442.6
Centre municipality Chisinau	town Vadul lui Vodă	8153.5	9432.1	8578.2	8721.3±375.9
	town Cricova	7879.0	7601.9	7308.7	7596.6±164.7
	village Condrița	6042.9	6270.4	8587.8	6967.0±813.0
South district Cahul	village Roșu	3101.8	3035.0	3522.7	3219.8±152.7
	village Moscovei	2111.9	2372.3	2903.1	2462.4±232.8
	village Huluboaia	3529.4	1810.0	1800.0	2379.8±574.8

General morbidity analysis has shown that prevalences of the circulatory, digestive, urogenital and osteoarticular diseases were more common. A higher average of the circulatory diseases was detected in northern localities, within 1831.9±72.2 – 1985.6±64.0 cases per 10 000 inhabitants, in central area 1591.6±97.1 – 2610.7±128.2 cases per 10 000 inhabitants and towns in the south – 1062.5±116.9 – 1445.0±178.0 cases per 10 000 inhabitants.

Digestive system diseases prevailed in areas of the central zone, with values within 1467.4±205.4 – 849.9±16.6 cases per 10 000 inhabitants, while in cities in the north and south, the prevalence was 602.9±124.6 – 1157.8±0.7$^0/_{000}$ and 155.8±11.7 – 403.8±17.4$^0/_{000}$ respectively.

The frequency of the urogenital diseases recorded raised indices in the central localities, within 618.0±4.7 – 936.5±116.2 cases per 10 000 inhabitants, and the northern and southern localities, between 600.9±1.9 – 631.9±37.5$^0/_{000}$ and 83.2±31.7 – 152.4±35.2$^0/_{000}$ respectively.

Morbid conditions caused by osteoarticular disease proved to be more common in the northern areas accounting for262.4±0.7 – 1059.7±196.1$^0/_{000}$, while in cities in the south and centre, the values were respectively 75.5±28.1 – 396.2±48.2 and 209.2±25.8 – 477.3±64.6$^0/_{000}$.

The average values of general incidence (table 2) have revealed that on the first place we have the studied localities situated in the north, with average values within 1370.5±0.7 – 11162.4±190.4 cases per 10 000 inhabitants. On the second place we have the central area localities with 1877.8±246.3 – 3149.0±187.8 cases per 10 000 inhabitants, and the third place the southern localities with 899.3±95.0 – 1244.8±339.8 cases per 10 000 inhabitants.

The overall incidence is different from the overall prevalence. On the top, in the development and registration of new cases of the disease, were located the diseases of the osteoarticular system with values between 26.7±0.7 – 900.5±217.9 new cases per 10 000 inhabitants in the northern localities, in central area localities 82.6±21.5 – 156.3±30.3 new cases per 10 000 inhabitants, and south – 22.6±6.5 – 236.3±86.5 new cases per 10 000 inhabitants. This class of diseases is followed by circulatory, digestive and urogenital diseases. The highest values of cardiovascular diseases incidence were recorded in village Ruseni (Edinet district, north) – 342.3±998.6$^0/_{000}$, in town Vadul lui Voda (municipality Chisinau, central zone) – 147.2±20.5$^0/_{000}$ and village Huluboaia (Cahul district, southern zone) – 296.3±93.5$^0/_{000}$.

Regarding the incidence of digestive diseases, the highest values were recorded in village Brătușeni (Edinet district, north) - 168.6±21.6$^0/_{000,}$ in town Vadul lui Voda (municipality Chisinau, central zone) – 486.7±373.7$^0/_{000}$ village Huluboaia (Cahul district, south) – 76.5±25.9$^0/_{000}$.

Average incidence values of urogenital diseases were higher in village Ruseni (Edinet district, north) – 245.3±0.4$^0/_{000}$ in village Condrita (municipality Chisinau, central area) – 300.1±42.7$^0/_{000}$ and village Huluboaia (Cahul district, south) - 56.6±24.0$^0/_{000}$.

Table 2

General incidence rate of the population in studied areas (per 10 thousand inhabitants)

Areas		2012	2013	2014	M±ES
North district Edinet	town Cupcini	1370.5	1371.8	1369.3	1370.5±0.7
	village Bratuseni	11380.7	10783.0	11323.4	11162.0±190.4
	village Ruseni	5615.7	6406.8	5341.8	5788.1±319.3
Centre municipality Chisinau	town Vadul lui Voda	2490.6	2904.5	2444.1	2613.1±146.3
	town Cricova	3501.7	3084.7	2860.7	3149.0±187.8
	village Condrita	1674.3	1371.9	1763.9	1585.4±136.2
South district Cahul	village Rosu	740.4	888.7	1068.9	899.3±95.0
	village Moscovei	814.0	771.0	1149.0	911.3±119.5
	village Huluboaia	1854.4	680.0	1200.0	1244.8±339.8

Accessibility to medical care allowed to research and follow the evolution of the population morbidity from the studied localities. As a result of this research, we obtained the structure of the nosological forms that reflect the territory specific morbidity. The obtained information form collected data from the statistical report No 12 "Concerning the number of registered diseases in patients domiciled on the territory of the servicing curative institution" allowed to highlight and distribute the risk factors that determine such health situation.

Results of the analysis also contributed to the study of distribution of diseases and risk factors in the population, depending on the time, space and health of the person. Regarding the morbidity of the most priority classes of diseases, it was established that during 2012-2014 diseases of the circulatory, digestive, urogenital and osteoarticular systems certainly retain their positions among the groups with the highest disease morbidity rates, even by incidence; these groups of diseases tend to increase. The morbidity structure depends on the age too.

With decreasing age the incidence of infectious, parasitic, respiratory diseases decreases and, conversely, increases the number of cases of circulatory, digestive and osteoarticular diseases. The obtained results show a difference between morbidity of nosologic forms in three areas of Moldova.

Conclusions

1. Analysis of the general population morbidity showed that in investigated localities of three geographical areas of the country diseases of the circulatory, respiratory, digestive, urogenital and osteoarticular systems, prevail compared to other classes of diseases, including seasonal ones.

2. Population morbidity in studied areas was steadily increasing in the years 2012-2014.

3. Prevalence of circulatory, digestive and urogenital diseases was higher in localities from central area, but that of the osteoarticular system – in the north. The incidence of cardiovascular, urogenital and osteoarticular diseases was higher in northern localities and digestive system - in the central areas.

4. This study has offered the possibility to conclude that there are some conditions that determine some features of population morbidity, such as biological, environmental, behavioural factors etc.

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Conflict of interest

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Study on Vitamin D and nutritional status in children and adolescents with helminthiases in central Moldova

A. Voloc

Department of Pediatrics, Nicolae Testemitsanu State University of Medicine and Pharmacy
Chisinau, the Republic of Moldova

Corresponding author: voloc@mail.ru. Received November 05, 2015; accepted December 07, 2015

Abstract

Background: Vitamin D deficiency has a high prevalence among schoolchildren worldwide. The effects of deficiency include impaired immune response and increased risk of varios infections whose record is poor. Currently, scientists have the opinion that vitamin D status can be rightly considered as an indicator of health in a given country as well as a socio-economic indicator of each country. Unfortunately, very few researches are devoted to the role of vitamin D in parasitic invasions in children and associations between them, to be able to compare the resultants of our research with similar results in other countries.

Material and methods: The study included two groups: group 1 (control) comprised 34 girls and 37 boys from Chisinau, group 2 (study) included 97 girls and 129 boys from Orhei. The children in both groups were examined physically and anthropometrically. Vitamin D status was determined and copro-parasitological tests were carried out. There were determined hemoglobin levels and some biochemical markers in relation to their nutritional status.

Results: It was determined a poor vitamin D status in both cohorts and a high level of infestation with helminths without establishing any correlation between vitamin D status and the level of infestation. The prevalence of helminthiases varies depending on the type of helminth detected in each cohort. Intestinal parasitosis does not influence body weight, waist and body mass index.

Seven children with poly-invasion had a lower height, but their number was too small to validate the data. Children with parasitosis, regardless of its type, had some digestive signs more frequently than children without infestation and the incidence of pulmonary, hepatobiliary and dental diseases, as well as bone deformity was not influenced by the presence or absence of intestinal parasitosis.

Conclusions: Intestinal parasitoses do not influence body weight, waist and body mass index. Children with parasitosis, regardless of its type, have some digestive signs more frequently than children without infestation and the incidence of pulmonary, hepatobiliary and dental diseases as well as bone deformities was not influenced by the presence or absence of intestinal parasitosis. The children and adolescents from the boarding school in Orhei had a poor nutritional status due to an unbalanced diet with a low intake of milk/dairy products and meat and high in fiber.

Key words: Vitamin D status, helminth infestation, nutritional status.

Introduction

In the buletin of the World Health Organisation Experts Committee [1, 2] it is stated that intestinal parasitoses are prevalent worldwide, with a very high prevalence in some regions of the world. It was estimated, for example, that the global prevalence of soil-borne nematodes constitutes 100 million cases of Ascaris lumbricoides, 900 million cases of Ankylostome and 500 million cases of Trichuris trichiura. In some countries, the average global prevalence for Ascariasis may vary from 16 to 48% and in some places it may exceed 70%. Ascaris lumbricoides infestation is more prevalent in children under 17 years than in adults over 18 years [3]. In a questionnaire-based study conducted in Malaysia (Crompton D.W.T., 1985), on a representative sample of 25,000 children and adults, their age ranging from the neonatal age and up to 60 years, it was estimated that the global prevalence of intestinal parasitosis increases up to 39.6%, reaching 89% in children aged between 6 and 12 years.

In the last five years a series of publications have appeared which are entirely dedicated to the study of Giardia intestinalis infestation [4-15], which describe the role of giardiasis in the immune process [16], as well as the carcinogenic role of the pancreas [17, 18, 19, 20], the pathophysiological mechanism of giardiasis [21], giardiasis association with a diet poor in proteins [22], infestation influence on the nervous system of the host organism [23] and its prevalence in the world [24]. Publications certify the incidence of infestations in some European countries, the prevalence of giardiasis reaches 4.0% in Belgium [25], 1.5% in Germany [26], 0.4% - 6.2% in Italy [27, 28], 3.7% in Portugal [29], 5.4% in Spain [30], 1.3% in the UK [31], 1.4% in the US [32], 1.1 - 6.6% in Saudi Arabia [33]. In South Korea it is 2.5% [34], in Australia it ranges from 1.6% to 7.6% [35, 36] and in some regions in New Zealand it reaches 7.6 % [37].

Purpose

The aim of the study was to assess vitamin D status in children and adolescents in the boarding school of Orhei and those hospitalized in the public medical-sanitary institution, Municipal Clinical Children Hospital No. 1 in Chisinau, establishing its relationship with the level of helminth invasion, along with the evaluation of biochemical markers and calcium metabolism, comparing the prevalence in both cohorts.

Material and methods

The study included 2 lots: Lot 1: 226 children and adolescents, of whom 97 (43%) girls and 129 (57%) boys aged 7-16 years, observed at a regular medical and biological examination during the winter-spring months (from January to April) during the study process of schoolchildren at the boarding school in Orhei (latitude, 47 ° N). The children permanently lived in the boarding school during the study year, while in the summer they lived in their families in the rural area. There were excluded from the study only children with known chronic diseases and/or anthropometry data off the limits +/- 2 SD; Lot 2: 71 children of whom 33 (48.0%) girls and 38 (52.0%)

boys adjusted by age, being subjected to an identical clinical and biochemical examination while they were hospitalized for acute respiratory diseases in the same period in the Municipal Clinical Children Hospital No 1 (MCCH No 1) in Chisinau (latitude 47°21'N). These children lived in families in urban areas and did not have any endocrine, renal or neurological diseases, or any other known chronic diseases.

The presence of intestinal parasitoses, especially Enterobius vermicularis, Giardia lamblia, Ascaris lumbricoides and Trichuris trichiura, was determined by standard coprologic methods. Serum 25 (OH)D levels were determined using the international external scheme of quality assessment for vitamin D metabolite (DEQAS) in order to estimate the optimal levels of 25 (OH)D for all subjects included in the current cohort study.

The biochemical analyses included determination of the levels of serum calcium, phosphorus, magnesium, blood glucose, total protein, thymol probe, aminotransferases, urea and creatinine in the clinical-biochemical laboratory of MCCH No 1.

Given the objectives of the study, the children in both groups were investigated for helminths in the parasitological laboratory of the National Scientific and Practical Centre of Preventive Medicine. For this purpose, faeces and perianal scraping were used as biological material, applying coproparasitological methods, simple macroscopic examination, as well as dark-field examination of faeces or examination with a magnifying glass and microscopic method.

Statistical analysis. The level of serum 25 (OH) D was measured in serum samples stored at -20° C in Saint Vincent de Paul Hospital (Paris, France), using chromatographic assays of competitive protein binding with continuous DEQAS external quality assessment of 25 (OH) D tests. The samples were analyzed together with 20 DEQAS controls (range: 9-79 nmol/l). The values constituted – 0.11 ± 0.72 SD units (mean ± SD) of all laboratory media for each control. The normally distributed variables are reported as mean ± SD. The t-student test was used to compare the continuous variables normally distributed in pairs. The significant differences between groups <30 subjects were verified, applying the non-parametric test and variance analysis (ANOVA). The prevalence between groups was compared using the Chi-square test. All the analyses were performed using the Statview 5.0 (Abacus Concepts Inc., Berkeley, CA, USA). The value of P <0.05 was considered statistically significant.

Results

Nutritional intake. The children from the boarding school in Orhei were nourished according to a special and different menu, including at weekends. They had a grain-based diet with less consumption of meat, milk and dairy products. The food intake according to one-week menus for children in the boarding school during the study period was assessed on the basis of the table CIQUAL of nutritional composition of foods (http://www.afssa.fr/TableCIQUAL).

The daily intakes of the children of the public medical-sanitary institution, MCCH No 1 were calculated depending on the daily menu prepared by the dietitian of the setting. The comparative information on the nutrition of children in both groups is shown in table 1.

Using standard coprologic methods the presence of intestinal parasitosis was determined, namely *Enterobius vermicularis, Giardia lamblia, Ascaris lumbricoides and Trichuris trichiura.*

Values are expressed as mean ± ES or as a percentage of subjects with clinical symptoms or intestinal parasitoses in the study and control groups. They were compared using the unpaired t-student test (mean values) or the Chi square test (incidence).

The biochemical laboratory parameters were analyzed at the MCCH No1. They included serum calcium, phosphates, magnesium, total protein, creatinine, hemoglobin, glucose, alkaline phosphatase and transaminase activity ALT/AST.

226 participants studied in the boarding school and 71 children in the age-matched control group had comparable anthropometric parameters at birth and before puberty (table 2). However, the adolescents of both sexes from the boarding school had a smaller waist compared to the participants in the control group and according to the WHO growth reference curves. The two cohorts had poor dental health and an increased prevalence of gastrointestinal symptoms, mostly multiple, nausea (17%) and isolated abdominal pain (20%) in some cases and isolatedly.

Table 1

The average daily nutrient intakes in the research and control groups

Assessed parameters	Research group (Orhei)	Control group (Chișinău)	t	P
	M ± SD	M ± SD		
Total proteins (children) on body weight per day (g/kg)	2,5±0,41	2,6±0,12	0.2326	>0.05
Total proteins (adolescents) on body weight per day (g/kg)	1,8±0,32	2,3±0,43	0.9259	>0.05
Animal proteins (g)	24.1±0.41	48,6±1.24	18.7023	<0.001
Energy (kcal)	2842.6±46.74	3162.8±52.43	4.5587	<0.001
Phosphates (mg)	0,921 ±0165	1,03±0,22		
Magnesium (mg)	255.9±2.45	273.8±1.12	6.6543	<0.001
Calcium per day (mg)	493.5±8.47	930.6±12.02	29.7347	<0.001
Inclusive of dairy products (mg)	69.4±7.66	550.3±14.39	29.5031	<0.001
Vitamin D (mg)	2 ±0,12	2,01±0,05		

Table 2

Clinical characteristics of the two cohorts (m±DS and prevalence)

Population	Boarding school of Orhei	PMSI MCCH No 1	P
N	226	71	
Age (years)	11,4±1,8	11,1± 2,4	>0.05
Prepubertal (%)	52.0±3.32	46.0±5.91	>0.05
Sex % boys	57.0	52.0	>0.05
Sex % girls	43.0	48	>0.05
Parameters at birth			
Term (weeks)	39,4±0,8	39,2±1,6	>0.05
Weight at birth (kg)	3,11±0,42	3,08± 0,56	>0.05
Waist (cm)	50,5±2,3	50,4±2,1	>0.05
Anthropometry			
BMI (z-score by WHO)	- 0,205±1,041	- 0,471±1,141	<0.001
-Children (n)	- 0,325±1,107 (118)	- 0,261±1,228 (33)	>0.05
-Adolescents (n)	- 0,440±0,898 (108)	- 0,682±1,023 (38)	>0.05
Waist (z-score by WHO)	- 0,721±1,054	- 0,024±1,051	<0,001
-Children	- 0,088±0,964	- 0,155±1,101	>0.05
-Adolescents	- 1,009±1,126	0,106±1,000	<0,001
X2	X2 =12.1 GL=6 p>0.05		
Intestinal parasitosis (%)	49.0	63.0	
Enterobius vermicularis	40.0	20.0	0,0017
Ascaris lumbricoides	4.0	14.0	0,0104
Trichuris trichiuria	2.0	14.0	<0,0001
Giarda lamblia	-	15.0	
Poly-infestation	3.0	-	
X2	X2 =18.6 GL=5 p<0.01		

Table 3

Biochemical profile (m±ES) of children in the boarding school in Orhei (study group) and Municipal Clinical Children Hospital No 1 (control group)

	study group, n=226		control group, n=71		P
Children	118	52.0±4.59	44	46.0±8.68	>0.05
Adolescents	108	48.0±4.81	37	54.0±8.09	>0.05
Protein (g/l)	69.0±11.0		69.0±8.0		
Hb (g/l)	11,2±1,1		11,9±0,4		
Glucose (mmol/l)	4,2±0,8		4,3±0,7		<0,0001
Creatinine (µmol/l)					
children	41.0±12.0		54.0±10.0		
adolescents	58.0±13.0		60.0±11.0		<0,0001
Total calcium (mmol/l)	2,14±0,31		2,29±0, 23		0,0007
Corrected Ca (mmol/l)*	2,22±0,31		2,36±0,24		0,0025
Phosphate (mmol/l)	1,36±0,38		1,22±0,23		0,0072
Magnesium (mmol/l)	0,70±0,14		0,83±0,18		<0,001
PA (UI/l)					
children	453.0±202.0		407.0±123.0		
adolescents	596.0±227.0		384.0±136.0		<0,001
25(OH)D (nmol/l)	44.0±16.0		36.0±12.0		<0,001
25(OH)D (ng/ml)	18.0±6.0		14.0±5.0		<0,001

* The values of total serum calcium were corrected for protidemia. Significant differences were determined in the boarding school group and control group (unpaired t-student test and U Mann-Whitney).

The parasitic infestations were common, with significant differences concerning the identified parasites (table 2). Compared with the age-matched control group, the participants from the boarding school had lower values of calcium, serum magnesium and hemoglobin, and a higher average level of serum phosphate (table 3). In addition, the children in the study group from the boarding school were recorded lower serum creatinine values before puberty and higher alkaline phosphatase activity during puberty compared to the age-matched control group (table 4).

Assessment of vitamin D status and its clinical impact

The circulating values of 25 (OH) D and, thus, vitamin D reserves have been rated as "satisfactory" values so far, if they were within the mean values ± 1 or ± 2 standard deviations in the adult population. Sun exposure influences the reserves of vitamin D, "normal" values of 25-hydroxyvitamin D, being different depending on the season - summer/autumn or winter/spring, the geographical location of the country and its policy of enriching foods with vitamin D.

Table 4

Clinical characteristics in the cohort of Orhei boarding school according to the level of 25 (OH) D (m ± SD)

25-(OH)D	≤30 nmol/l	31-40 nmol/l	41-50 nmol/l	51-≥75 nmol/l
N 25-(OH)D (nmol/l) 25-(OH)D (ng/ml)	47 26,5±3,5 10,6±1,4	67 35,6±2,8 14,2±1,1	53 45,9±2,7 18,4±1,1	59 65,2±12,0 26,0±4,8
Age (years) -Children (n) -Adolescents (n)	11,8±1,9 9,9±1,1 (20) 13,2±1,0 (27)	11,5±1,6 10,2±0,9 (33) 12,7±1,0 (34)	11,3±2,0 9,7±1,2 (29) 13,1±1,1 (24)	11,2±1,7 10,2±1,1 (36) 12,9±0,9 (23)
BMI (z-score): -Children -Adolescents	 - 0,10±0,83 - 0,18±1,04	 -0,24±1,00 -0,44±1,30	 - 0,07±1,08 - 0,083±0,95	 0,07±0,88 -0,64±1,05
Height (z-score): -Children -Adolescents	 - 0,20±0,91 - 0,74±1,10	 - 0,62±0,86 - 1,04±1,04	 - 0,50±0,97 - 0,92±1,46	 - 0,42±0,87 -1,36±0,88
Prevalence: Digestive disorders Parasitoses	 71% 55%	 64% 43%	 68% 53%	 46% 47%

Implementation of a tailored prophylaxis has helped significantly reduce the incidence of severe vitamin D deficiency in all the Western countries, except the elderly, the clinical trials being focused on the effects of long-term moderate deficit.

Over time, scientists have gradually replaced the concept of "normal values" of 25 (OH) D with the term "required values" and with the notion of "threshold" below which some medium and long-term pathological changes may occur in apparently healthy individuals. The clinical consequences of this limit are taken into account when defining the value of this level, however, to date, there is no consensus on this level. However, from a practical perspective, it is useful to note: a) the level of "deficiency" below which the short-term pathological deficiency risk is significant and requires immediate correction. The multiple dosage of 25 (OH) D performed in neonates, infants and children with clinical signs of deficiency rickets (skeletal deformities and / or neurological signs as a result of hypocalcaemia) demonstrates eloquently the close correlation of these signs with values under 10 to 12 ng/ml (25-30 nnmol/l) of 25 (OH) D; b) the level of "insufficiency " of vitamin D status under which there is a risk of developing some long-term changes and may involve specific vitamin D prevention. The "insufficiency" threshold values vary in different authors from 20 to 52 ng/ml (50 - 130 nmol/l).

The children and adolescents examined in the rural boarding school in the period between January and April had a mean value ± SD of serum 25 (OH) D of 44 ± 16 nmol/l, with a prevalence of values of 25 (OH) D ≤ 30 ≤ 50 ≤ 75 nmol / l respectively equal to 21, 53 and 26%. The status of vitamin D was higher than the status measured in the children and adolescents in the control group who lived in Chisinau, either in flat (35.6 ± 1.8 nmol / l) or house (37.0 ± 2 3 nmol / l). It was not influenced by the pubertal maturation, but it varied by gender: girls from the boarding school had average values of 25 (OH) D less than boys (40.2 ± 14.8 versus 46.5 ± 16.0 nmol / l, p = 0.0031).

There are marked differences compared to the prevalence values observed in the case of 25 (OH) D ≤30 nmol/l. The statistical Chi-square test for prevalence and unpaired t-Student test were applied, as well as U Mann-Whitney test for age and anthropometry was used. The subgroups of sex and pubertal maturation were too small (less than 30 people) for the Chi-square analysis of prevalence.

Finally, the serum levels of 25 (OH) D were positively associated with serum calcium levels (r = 0.202, P = 0.0024)

Table 5

Biochemical indices depending on 25 (OH) D level in Orhei boarding school (m ± SD)

25-(OH)D (nmol/l)	≤30 nmol/l (n=47)	31-40 nmol/l (n=67)	41-50 nmol/l (n=53)	51-≥75 nmol/l (n=59)
Protein (g/l)	70±10	67±10	71±10	69±12
Hb (g/l)	11,0±1,1	11,4±1,0	11,4±1,0	11,2±1,1
Glucose (mmol/l)	4,2±0,8	4,0±0,8	4,2±0,8	4,2±0,8
Creatinine (µmol/l) -Children (n) -Adolescents (n)	 44±14 (20) 60±14 (27)	 40±12 (33) 56±13 (34)	 41±12 (29) 59±12 (24)	 41±9 (36) 61±14 (23)
Total Ca (mmol/l)	2,10±0,26 0,0205	2,12±0,30 0,0340	2,11±0,33 0,0259	2,24±0,32
Corrected Ca (mmol/l)	2,15±0,28 0,0083	2,22±0,31	2,16±0,35 0,0100	2,32±0,35
Phosphates (mmol/l)	1,33±0,34	1,35±0,41	1,37±0,38	1,39±0,37
Magnesium (mmol/l)	0,70±0,14	0,67±0,12	0,71±0,14	0,72±0,15
PA (UI/l) -Children -Adolescents	 534±1480,0138 596±228	 439±231 593±219	 432±187 633±244	 437±211 573±228

Table 6

Frequency of parasitoses in both groups

Type of parasitosis	<30				30-50				51 >			
	Chișinău n=37	Orhei n=46	T	P	Chișinău n=25	Orhei n=112	T	p	Chișinău n=9	Orhei n=68	T	p
	P±ES, %	P±ES, %			P±ES, %	P±ES, %			P±ES, %	P±ES, %		
1. Ascariasis	10,0±2,91	43,0±3,25	7,5	••••	6,0±2,31	5,0±1,43	0,4	•	6,0±2,31	3,0±1,12	1,2	•
2. Enterobiasis	24,0±4,15	44,0±3,26	3,8	••••	43,0±4,81	38,0±3,19	0,9	•	29,0±4,41	40,0±3,22	2,0	••
3. Giardiasis	15,0±3,47	-	4,3	••••	-	-			18,0±3,73	-	4,8	••••
4. Trichuriasis	12,0±3,16	4,0±1,29	2,4	••	1,0±0,97	1,0±0,65	0	•	12,0±3,16	1,0±0,65	3,4	••••

• p>0,05; •• p<0,05; •••• p<0,001

Table 7

Vitamin D status and calcium metabolism in relation to intestinal parasitoses

Parazitosis	Age, years	Corrected Calcium, mmol/l	Phosphates, mmol/l	Magnesium, mmol/l	25(OH)D, ng/ml
Absent (N=161)	10,8±2,9	2,25±0,31	1,33±0,34	0,74±0,16	18,5±6,3 (86)
Enterobiasis (N=114)	10,6±2,4	2,21±0,35	1,32±0,38	0,71±0,15	18,0±5,3 (70)
Ascariasis (N=19)	11,1±2,2	2,39±0,25	1,37±0,25	0,71±0,12	16,1±6,5 (9)
Trichuriasis (N=18)	11,1±3,3	2,31±0,27	1,26±0,20	0,75±0,16	15,1±4,2 (13)
Giardiasis (N=13)	10,4±4,0	2,51±0,15 (0,007)	1,32±0,18	0,94±0,23 (p<0,0001)	15,0±8,0 (6)
Poly-invasion (N=7)	11,5±2,0	2,23±0,32	1,34±0,49	0,77±0,11	18,0±4,7 (4)

Values represent the mean ± SD. Determination of the 25 (OH) D level was not possible in all children. The number of samples in which 25 (OH) D was determined is noted in parentheses. The significantly different values observed in uninfected children are noted by indicating the p value.

Table 8

Anthropometric and biochemical data depending on the infestation with intestinal parasites

	Weight (m±DS)	Waist (m±DS)	BMI (kg/m2)	Protein	Glucose	Creatinine	Hb
Absence of parasitosis (N=161)	-0,12±1,16	-0,12±1,34	16,9±2,4	69,2±9,5	4,17±0,87	54,2±14,2	118,2±9,0
Enterobiasis (N=114)	-0,09±1,13	-0,30±1,28	17,1±2,2	69,2±11,3	4,33±0,79	46,1±13,1 (p<0,0001)	111,8±9,1 (p<0,0001)
Ascariasis (N=19)	0,00±1,24	0,26±1,35	17,2±2,8	66,4±8,6	4,32±0,66	54,2±15,5	108,6±10,8 (p<0,0001)
Trichuriasis (N=18)	-0,09±1,32	0,54±1,09 (p=0,04)	16,6±2,6	69,6±8,4	4,04±0,85	56,5±11,6	113,6±13,6 (p=0,047)
Giardiasis (N=13)	-0,46±1,27	0,18±1,22	16,7±2,6	72,3±6,8	4,28±0,67	61,1±12,3	117,5±2,5
Poly-invasion (N=7)	-0,21±1,44	-1,18±1,17 (p=0,036)	18,1±2,1	68,4±10,2	4,29±0,77	47,1±8,6	105,1±7,7 (p=0,0003)

The mean values ± SD. Values significantly different from those found in uninfected children are **noted** (ANOVA).

in the study group: in participants with values of 25 (OH) D ≤ 30 nmol / l and even in those with intermediate values (30-50 nmol / l) serum calcium level was lower than in those with 25 (OH) D> 51 nmol / l (table 5).

On the contrary, serum calcium was not associated with the level of 25 (OH) D in the control cohort and the participants in the study group had significantly lower serum calcium levels than those in the control group when their level of 25 (OH) D was ≤ 30 nmol / l (2.15 ± 0.28 versus 2.41 ± 0.16 mmol / l, P <0.0001), but not in the case when the values of 25 (OH) D were greater than 51 nmol / l (2.32 ± 0.35 versus 2.50 ± 0.12 mmol / l).

The observed values are significantly different from those observed in the case of 25 (OH) D values greater than 50 nmol/l (using paired and unpaired t-student test and U Mann-Whitney test).

The lack of association with serum aminotransferase activity suggests that high alkaline phosphatase activity detected in children in the study group during puberty, reflects an increased turnover of bone tissue, possibly due to calcium deficiency.

Finally, 25 (OH) D level was positively associated with serum calcium level, with optimum 25 (OH) D values greater than 50 nmol/l. There was established no association of vitamin D level with clinical calcium-dependent symptoms, especially with hypocalcemia, probably because the participants in the study group were older and / or because their level of hypocalcemia and vitamin D deficit was moderate compared to previously reported cases in the literature [38, 39].

The status of vitamin D varies with the season in countries with temperate climate, being lower during the winter-spring period, when the ultraviolet solar spectrum energy is insufficient to produce vitamin D in the skin. But, a diet based on grains with restricted access to meat products, milk and dairy products had probably a major importance, an unusual diet for children in the Republic of Moldova, including those in the control group in urban areas, who theoretically receive an adequate intake of calcium / milk.

Helminthiases, vitamin D status, anthropometric and biochemical parameters and clinical manifestations

Comparing the frequency of helminthiases in both cohorts, we found an equivalent frequency (60% in Chisinau and 52% in Orhei). At the same time, their frequency varies depending on the parasite found (table 6).

Thus,the frequency of ascariasis, trichuriasis and giardiasis was higher in Chisinau than in Orhei, and, conversely, the frequency of enterobiasis was lower in Chisinau than in Orhei. We found no association between 25 (OH) D level and the presence or absence of intestinal parasitosis or its type.

Performing an ample analysis of the possible association between infestation with intestinal parasites and vitamin D status and the level of calcium, serum magnesium and alkaline phosphatase activity, we have observed a significant difference in some biochemical indices (table 7, 8).

Intestinal parasitoses do not influence body weight, waist and body mass index (BMI). Seven children with poly-invasion had a lower height, but their number was too small to validate the data. In contrast, lower concentrations of serum hemoglobin were detected in children with all types of parasitoses.

The other biochemical parameters did not show any difference in infected or uninfected children, with some exceptions:

- lower serum creatinine values were recorded in children with enterobiasis;
- higher values of serum calcium and magnesium were recorded in 13 children infected with giardia.

These findings remain unexplained and would require further study for confirmation.

Children with parasitosis, regardless of its type, had some digestive signs more frequently than children without infestation (table 9). However, the frequency of pulmonary, hepatobiliary and dental diseases as well as bone deformities was not influenced by the presence or absence of intestinal parasitoses.

Discussion

Despite the tense parasitic epidemiological status in all countries of the world and the large number of researches on studying just parasites alone, few researches are devoted to the study of vitamin D status and its correlation with intestinal parasitoses. The study to which we had access [40], conducted in a rural school in Mexico on a sample of 284 children (8 ± 1.6 years) and which researched the impact of Ascaris lumbricoides and Entamoeba coli invasion on the micronutritional status through rapid diagnostic methods, determined that 20% of the students involved in the study were infected with Echerichia coli and 16% with Ascaris lumbricoides. The prevalence of vitamin D deficiency was 28% and zinc deficiency constituted 18%. Perhaps the poor sanitary and hygienic conditions in which the children from metropolis lived in made researches detect such a prevalence. In one relatively recent publication [41] it is stated that vitamin D deficiency has a high prevalence among students worldwide. The deficiency effects include alterations in the immune response and an increased risk to various infections, the records of which are

Table 9

Prevalence of digestive signs and other clinical signs depending on the presence of intestinal parasitoses

	Absent	Enterobiasis	Ascariasis	Trichuriasis	Giardiasis	Poly-invasion
N / Digestive signs	172	118	21	20	14	7
Nausea	21 (12%)	10 (8%)	0	4	0	0
Pain	24 (14%)	9 (8%)	1	1	0	1
Some signs	25 (14%)	89 (76%)	18	14	14	6
Hepatic	8 (5%)	7 (6%)	2	0	0	0
Respiratory	56 (32%)	29 (24%)	12	16	14	0
Dental alterations	125 (73%)	103 (87%)	18	18	14	7
Bone deformations	67 (39%)	53 (45%)	14	9	4	4

Values represent the number of children. In addition, the percentage of children with any clinical signs considered were calculated in populations of more than 100 people (infected and uninfected children with enterobiasis).

low [1]. According to some ample studies made in Panama, it was pointed out a low digestion of lactose and intolerance to carbohydrates in preschool age children infected with Ascaris lumbricoides, which led, in turn, to accelerated intestinal transit [42] and probably a lower absorption of vitamin D and calcium in the diet, which in our view, has worsened the uptake of micronutrients in children of both groups involved in our study compromising the bioavailability of calcium from the diet low in milk/dairy products and rich in fiber mainly in children from the boarding school in Orhei.

Some authors (Issenman 1987) have mentioned that in case of giardiasis, parasites compete with enterocytes, causing malabsorption, including vitamin D malabsorption. It might be assumed in our study that one of the causes of anemia in children of both groups may be a deficiency of vitamin D. Some authors hypothesize that iron deficiency causes malabsorption of fats and vitamin D as a fat soluble molecule, such as in the case of giardiasis, or vice versa, induction of anemia as a result of intense inflammatory processes in the gut and bone marrow myelofibrosis associated with vitamin D deficiency [43, 44] The obtained results require an analytical confrontation with the results reported in different parts of the world, to try to find an explanation for a very high proportion of anemia, deficiency of micronutrients, including deficiency of vitamin D.

The literature reports that in case of giardiasis, the malabsorption syndrome is present in 90% of cases in children and 30% in adults; the malabsorption in this population being characterized by weight loss and biological malabsorption, it being most commonly partial, especially fats malabsorption (steatorrhea), carbohydrates (D-xylose), folic acid and vitamins A and B12, accompanied by villous (total, partial or subtotal) atrophy and megaloblastic anemia by faulty absorption of folic acid and vitamin B12 [45].

It might be assumed that infestation with Trichuris trichiura, which lives mainly at the colon level and, most frequently, without any clinical manifestations, might, along with other causative factors, be at the origin of anemia in children and adolescents in both groups. Therefore, anemia in these subjects can be accounted for the fact that the adult parasite penetrates the intestinal mucosa causing an inflammation with lymphoplasmacytic and eosinophilic infiltration. It was estimated that a worm can consume about 0,005 ml of blood a day, while a helminth invading a person, can amount on average over 1,000 worms [46]. Iron deficiency is the most common cause of anemia, but the deficiency in other micronutrients is also involved (folic acid, vitamin B12, vitamin A). Regardless of acute or chronic inflammation, intestinal parasitoses are equally involved in the development of anemia [47].

The prevalence of anemia in our study was 100%. This value is higher than the 40% threshold set by WHO for a severe endemic anemia in a population group [48]. In fact, it does not reflect the prevalence of anemia among the general population and accounts for the study carried out on children in a hospital and school environment where there are specific predisposing factors (acute respiratory infection in children hospitalized in MCCH No 1, basic hygiene and poor nutrition along with a high degree of helminth infestation both in Chisinau and Orhei).

Worldwide around the globe, children are very susceptible to Enterobius vermicularis infestation. However, it seems that the disease is more present in temperate regions and highly developed countries than in tropical and subtropical regions [49]. It was certified that Enterobis vermicularis is the only parasite that is most frequently encountered in developed countries. However, this worm is sensed by the population as a "shameful" disease, constituting a social stigma in prosperous and highly developed societies [2].

Conclusions

Our study has not found any close relationship between the level of vitamin D deficiency and helminth infestation and vice versa.

The study results show that Giardiasis, Ascariasis, Enterobiasis and Trichuriasis are the most common intestinal parasites in children and adolescents in central Moldova, at least in the subjects involved in the study of both settings. The study allowed us to conclude that intestinal parasitoses do not influence body weight, waist and BMI. Seven children with poly invasion had a lower height, but their number was too small to validate the data.

Children with parasitosis, regardless of its type, have some digestive signs more frequently than children without infestation and the incidence of pulmonary, hepatobiliary and dental diseases as well as bone deformities was not influenced by the presence or absence of intestinal parasitosis. The children and adolescents from the boarding school in Orhei had a poor nutritional status due to an unbalanced diet with a low intake of milk/dairy products and meat and high in fiber.

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Representation of Biologische Heilmittel Heel GmbH in the Republic of Moldova
65, Tighina Street, Office 309, Chisinau, the Republic of Moldova
www.heelmd.org. Telephone: +(373)22507780

REVIEW ARTICLES

Engystol – possibility of bioregulation approach in viral diseases

S. V. Popovich[1], V. Savca[3], *V. V. Kolodko[2]

[1]Ukrainian Academy of Biological Medicine, Kiev, Ukraine, [2]Medical Center TerraMed, Chisinau, Moldova
[3]Representation of Biologische Heilmittel Heel GmbH in the Republic of Moldova

*Corresponding author: vkolodko@yahoo.com. Received October 20, 2015; accepted December 07, 2015

Abstract

Background: Acute viral infections due to widespread, high contagiousness and rapid development of viruses resistance to used drugs (medicines) is one of the greatest challenges of modern medicine. Therefore, the search for new approaches and methods for treatment and prevention of viral etiology is very relevant. The aim of this article was to review one such approach called bio-regulatory, which allows us to solve the above problem. It is carried out through the use of complex bio-regulatory medications. In this article was reviewed complex bio-regulatory medications for viral diseases – Engystol, it is antiviral immunomodulator of German pharmaceutical company "Biologische Heilmittel Heel GmbH" which is widely used in a variety of viral diseases in case of children and adults thanks to its favorable safety profile and proven efficacy.

Conclusions: National and foreign doctors in case of many viral diseases have experience of successful long-term use of complex bio-regulatory medications Engystol. Results of scientific and clinical studies show that Engystol is universal antiviral immune-modulator with proven efficacy in a wide range of viral diseases in children and adults. It has a high safety profile. It is used both independently and as part of complex treatment regimens. It goes well with any medicines, reducing their side effects on the body.

Key words: Engystol, bio-regulatory medications.

Introduction

According to statistics, most people at least once during the year suffer of acute respiratory viral infections (ARVI). But incidence of ARVI in paediatric population varies, according to data of the World Health Organization, from 5 up to 12 episodes per year that is more than 2.5-4 times higher than among adults. Because of the absence of specific immunity in human, respiratory viruses spread rapidly in population, causing not only increased morbidity, often with complicated course, but also lead to huge economic costs [1, 2, 3].

The so-called childhood diseases (measles, varicella, epidemic parotitis, etc.) besides high contagiousness, in case of incorrect treatment are dangerous with their complications [4, 5, 6].

Current approaches to treatment and prevention of ARVI and other viral infections are imperfect: patients receive a large amount of medicines, mainly symptomatic, which often cause side effects. Widely used today antiviral agents often do not provide sufficient effect, since there is a rapid development of resistance of viruses to used medicines [7, 8]. Also, there are difficulties due to age restrictions, inability to use medicines at various concomitant pathologies, pregnancy, lactation, early childhood.

In this connection it is important to expand the use of pathogenic agents and approaches that will improve both efficiency and safety profile of therapy, and reduce duration of reception of symptomatic medications with side effects.

What is Engystol?

One such approach, which allows us to solve the above problem, is bio-regulatory. It is carried out through the use of complex bio-regulatory medications (CBM). Earlier in the literature was used the term "antihomotoxic medications" (AHTM). CBM include ultralow doses of active ingredients that activate drainage and detoxification processes in the body that help to restore self-regulation and self-healing. They also do not have pharmacokinetics and therefore are not metabolized in organism and do not require additional energy, that is, do not exert pharmacological stress [9-11].

Let us consider experience of successful long-term use of CBM Engystol by national and foreign doctors in case of many viral diseases.

Engystol for many decades demonstrates its high efficiency at various viral infections. It has immune-modulatory effect on the body – activates production of endogenous interferon, as well as other antiviral immune mechanisms. Vincetoxin and asclepias acid (components of Vincetoxicum) have impact on blood vessels and sympathetic nervous system.

Different dilutions of colloidal sulfur (Sulphur) allow to release (recover) disturbed enzymatic intracellular processes (-SH-group of enzymes), activate elimination of toxins from loose connective tissue of intercellular space (drainage and detoxification effect). Results of experiments and clinical studies have shown also direct antiviral effect of Engystol [12, 13].

Action mechanisms of Engystol

Studies in vitro showed that Engystol enhances phagocytic activity of granulocytes in the human body by 33.5% compared to control cultures. Another study in vitro showed that Engystol leads to an increase of phagocytic activity by 20-40% (depending on the degree of dilution – 1: 10 or 1: 100) in three

different immunoassays: granulocyte test, test for removing coal dust and granulocytes bioluminescence test [12].

Further studies in vitro have shown that Engystol considerably increases expression of T-lymphocytes which produce interferon-α. This effect was observed in all degrees of dissolution with no apparent dose-dependent manner. Other studies confirmed the effect of Engystol in the increased activity of granulocytes, phagocytes and neutrophils: destruction of virus-infected and tumor cells [12].

Direct antiviral effect of Engystol

The study in vitro defined percentage of viral activity inhibition with various solutions of Engystol at testing of various DNA and RNA viruses [13]. Engystol demonstrated a dose-dependent antiviral activity against DNA viruses: inhibition of adenovirus type 5 by 73%, and herpes simplex virus type 1 (HSV-1) – by 80%, and in the case of RNA viruses: inhibition of respiratory-syncytial virus (RSV) – by 37% and of human rhinovirus (HRV) – by 20%. Thus, cytotoxic effects and other toxic effects of investigated doses of Engystol were not observed. The antiviral effect was independent of the activation of the cellular interferon system, which, according to the authors, indicates a direct antiviral effect of Engystol [12].

Engystol is produced in two medicinal forms – pills and injectable solution, which adds flexibility at prescription to various categories of patients [14].

Tolerability and efficacy of CBM Engystol had been studied for years by doctors of various specialties in many countries.

Thus, on the basis of Bogomolets National Medical University with the head of Department of childhood diseases in charge, major infectious diseases specialist of the Ministry of Health of Ukraine Professor S. A. Kramarev were developed methodical recommendations approved by the Ministry of Health of Ukraine "Alternative methods of treatment and prevention of influenza and ARVI in case of children". They set out both traditional and alternative schemes of treatment and prevention of the most common ARVI in children (influenza, parainfluenza, adenoviral infection, and others). In detail authors analyze bio-regulation approach to the treatment of ARVI and influenza, as well as provide a detailed description of the used CBM. Engystol as the basic CBM is included in all schemes of ARVI, respiratory-syncytial virus infection treatment, is included in the scheme of treatment of acute viral conjunctivitis, keratitis, adenoviral tonsillitis, pharyngitis, bronchiolitis, and others. In schemes of treatment are included also other CBM: antiphlogistic – Traumeel S (3), lymphatic-drainage – Lymphomyosot (4), detoxification – Echinacea compositum S (5), mucolytic, etc. Detailed algorithm on use of Engystol for prophylaxis of viral diseases is described in methodological recommendations of the Ministry of Health of Ukraine [15].

German doctor Ulrich Vemmer, based on years of experience of CBM using describes in his work schemes of measles and varicella treatment, in which is widely used Engystol as basic antiviral medicine [4, 5]. He also suggests using Engystol in complex treatment of infectious mononucleosis in order to improve overall immunity and as a non-specific antiviral agent [17].

Doctor of Medicine Anders Horst (Germany) describes patterns of CBM use in treatment of epidemic parotiditis, arguing that combination of medicines Engystol and Traumeel S reduces duration of illness by about half [6].

Within multicenter study conducted by German doctors Gabriella Gertsberger, Michael Weiser "Homeopathic treatment of infections of various origin", were analyzed data on use, therapeutic efficacy and tolerability of the medicine Engystol [18]. Totally were analyzed 1479 case studies of the practice of 154 physicians in three European countries. The main indications for use of CBM Engystol were influenza, infection causing fever and prevention of infections by activation of immune system. Additional indications for use of medicine have included a variety of acute and chronic diseases of the upper respiratory tract, as well as other infectious diseases. Medicine Engystol has obvious therapeutic effect when used alone or in combination with any other form of therapy. There were no adverse effects even in cases where the medicine Engystol was used in combination with allopathic drugs [18].

In methodical recommendations "Methods of traditional medicine in preventive, rehabilitation and complex therapy of patients with influenza and ARVI" of the Ministry of Health of Ukraine are presented alternative methods and approaches to treatment and prevention of ARVI and influenza, including homeopathy, herbal medicine, Su-Jok therapy [16]. A separate section is devoted to use of CBM, anti-inflammatory, immune-modulatory and antiviral medicines, including Engystol.

It is concluded that main advantages of CBM are ease of use, appointment according to nosological principle, raw materials and finished products quality control. Efficacy, safety, no side effects, economic accessibility, possibility of combination with allopathic medicines make CBM indispensable for treatment and prevention of influenza in children, pregnant women, the elderly, patients with tendency to allergic reactions. Simultaneous use of CBM and allopathic medicines allows reduced dose of the latter taking by 50% [16].

Engystol usage experience convincingly demonstrated its efficiency both in the treatment of ARVI, other viral infections and in the cases when viral infection becomes a complication factor in injuries and degenerative-inflammatory processes of locomotor apparatus.

In our practice Engystol in combination with Traumeel S is efficiently used for pain treating in patients whose illness preceded, caused or associated viral infection.

Conclusions

Based on the results of scientific and clinical studies, it can be concluded that Engystol is universal antiviral immune-modulator with proven efficacy in a wide range of viral diseases in children and adults. It has a high safety profile. It is used both independently and as part of complex treatment regimens. It goes well with any medicines, reducing their side effects on the body.

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Immunogenic aspect of the chronic tonsillitis associated with articular syndrome

C. Andronachi

Department of Otorhinolaryngology, Nicolae Testemitsanu State University of Medicine and Pharmacy
Chisinau, the Republic of Moldova

Corresponding author: cezglav@gmail.com.

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Abstract

Background: There are proposed various methods of treatment of articular syndrome associated with chronic tonsillitis (CT), however, in most cases the desired effect is not reached. The insufficient effectiveness of the treatment measures is, to a certain extent, subject to the underestimation of the immunogenetic role of the etiologic and pathogenetic character of these diseases. Recent years yielded obvious results in studying the correlation between the disease and the histocompatibility complex antigens – HLA (Human Leukocyte Antigens). At the foundation of the complex is the phenomenon of its predisposition to various diseases. In the curative approach of chronic tonsillitis, the presence of certain classes of HLA complex genes indicate the need for an early aggressive therapy. It deeply involves into the disease pathogenesis, coding the therapy evolution, prognosis and effects. In order to evaluate the impact of HLA class I antigenic determinants (A and B) on the clinical presentation, evolution and treatment strategy in patients with decompensated chronic tonsillitis associated with the articular syndrome (PSRA or ARF), we observed 101 adult patients aged 16-60 years, clinically and instrumentally diagnosed with decompensated chronic tonsillitis (CT): 50 patients had received conservative treatment and 51 patients were treated surgically.
Conclusions: The study of the major antigens has not found an association of HLA class I (A, B) with ARF and rheumatic heart disease (RHD).

Key words: chronic tonsillitis, articular syndrome, arthritis, antigens.

According to The American Academy of Otolaryngology - Head and Neck Surgery, chronic tonsillitis (CT) is: an entity unresponsive to medical therapy; which is associated with halitosis; with clinical presentation of recurrent tonsillitis (RT) at persons carrying beta hemolytic streptococcus class A (BHSA); with absence of adequate response to antimicrobial therapy and with surgery indication (tonsillectomy) [10].

Chronic inflammation of the tonsils is one of the most common otorhinolaryngology diseases. About 10-50% of people complain of symptoms of chronic tonsillitis [2, 3].

The study conducted among the population of the Republic of Moldova in 2008-2009 (1500 people were examined in 16 districts and 38 villages), found the most common disorders of the pharynx: CT and chronic pharyngitis. The authors introduced the term chronic amigdalo-pharyngitis in the daily use of doctors, which contributes to a better selection of patients for those treatments [1].

Determining the prevalence of CT was performed by examining a group of 1371 children (967 children from urban area and 404 children from rural area of the Republic of Moldova). CT prevalence in children is 7.7%, does not depend on sex, and is more common at the age of 12-13 years and in urban areas [4, 5].

BHSA infection complications are classified as non-suppurative and suppurative. Acute rheumatic fever (ARF), post-streptococcal reactive arthritis (PSRA) and acute glomerulonephritis are major non-suppurative complications

occurring generally after 1-3 weeks after the beginning of BHSA infection.

PSRA is a clinical syndrome without diagnostic criteria and clear treatment recommendations [34]. PSRA was defined as a non-suppurative inflammatory arthritis in two or more joints, which develops during or shortly after a streptococcal infection, located remotely, at a patient without Jones criteria for ARF diagnosis [18, 34, 40].

Studies conducted among both children and adults postulated the relationship between streptococcal tonsillitis and PSRA [11,14]. According to some studies, PSRA occurs most often in young adults [13]. Recurrent, severe and prolonged arthritis are important PSRA characteristics at adults [11].

ARF is an autoimmune disease caused by gram-positive bacteria Streptococcus pyogenes after an untreated oropharyngeal infection at children genetically susceptible. This multi-system disorder is characterized by involvment of heart, joints, central nervous system, subcutaneous tissue and skin, but, except the heart, the other organs are affected transiently [39, 41, 44, 45].

There is no "gold standard" and no specific test to diagnose ARF, therefore, the diagnosis is arbitrary and empirical, especially in adults over the age of 25 years [22, 33, 37]. The diagnosis criteria of ARF were developed and published by Jones TD in 1944 [27, 32, 37, 44, 45], and till now are extremely important in the diagnosis, study and management of this injury [37]. The changes and subsequent updates of ARF criteria, published in 1965, 1984, 1992 and 2002, have concretized and completed major manifestations, have simplified minor occurences, have removed the ambiguity and have detailed all criteria, have underlined the importance of the preceding streptococcal infections with the purpose of appropriate diagnosis of the initial stroke, the recurrent stroke, and minimizing the overdiagnosis of this condition, especially in countries with reduced incidence of ARF [22, 27, 32, 33, 37].

PSRA treatment represents the impovement of arthritis symptoms and eradication of streptococcal infection by administration of non-steroidal anti-inflammatories and antibiotics [41]. Early tonsillectomy is a viable treatment for patients with PSRA after strep throat [13]. According to the AHA, patients with PSRA are recommended to be under surveillance for several months and under echocardiographic monitoring in order to detect a possible further development of carditis, which may be atypical ("quiet"). One of the recommendations is the secondary prophylactic administration of antibiotics to patients with PSRA for up to 1 year and, if the carditis is not determined, prophylaxis may be interrupted. If the carditis is diagnosed, the patient is considered to have ARF and he must continue getting the long-drawn secondary prophylaxis treatment with antibiotics [14, 17, 18].

ARF treatment is performed in several directions, the most commonly used are [29, 41, 44]: the treatment of streptococcal infection with penicillin G, a first-line anti-inflammatory treatment with acetyl salicylic acid in uncomplicated cases and with corticosteroids in more severe cases.

ARF prophylaxis comprises several aspects: elimination of risk factors associated with BHSA, detection and proper treatment of throat infections with BHSA with penicillin G (primary prevention), detection of healthy carriers of strep that are treated as symptomatic patients (primary prevention), prevention of complications, in particular of carditis, in patients with ARF (secondary prevention) [30, 31].

Effective treatment of tonsillitis with BHSA reduces the risk of ARF by approximately 80-90%, but BHSA remains present in the pharynx in about 10% of cases, even after a proper treatment [29].

There are proposed various methods of treatment of CT: conservative treatment (local, general, prophylactic) and surgery (tonsillectomy). But in most cases the desired effect is not achieved. In addition, at this stage doctors demonstrate an explainable prudence regarding tonsillectomy, especially in children, when the physiological function of these lymphoid organs is maximal [2, 3, 19, 46].

The low efficiency of treatment measures is conditioned, to some extent, by the underestimation of the immunogenetic role of the etiopathogenic appearance of CT. In recent decades there were obtained obvious results in studying the correlation between CT and MHC- HLA antigen [38].

According to several recent studies, CT pathogenesis is complicated and diverse. Ethiopatogenic links between CT and other intercurrent diseases are not established. Studying the level of correlation between local immunological disorders and systemic immune processes, phenomena that are at the base of the immune response in the development zone of an isolated inflammatory process is important, at least from two points of view. On the one hand, these results will complement the vision of CT pathogenesis, and on the other hand, would give new impetus for the pathogenic treatment, serving also as a basis to form a fair outcome [6].

CT diagnosis is extremely difficult because both the onset and progression of the disease do not show specific clinical signs. The most common clinical manifestations of CT are obstructive hypertrophy of the tonsils (HT) and / or RT [37]. CT symptoms (painful and discrete sensations in the throat, non-disclosed sore throat, dysphagia, dry cough, bad breath, pharyngeal discomfort, sensation of a foreign body and discomfort in the throat, burning and dryness in the throat, low-grade fever, pain in the lymphatic submandibular nodes, pain in the joints) are also met in other diseases (pharyngitis, laryngitis, esophagitis, gastritis, sinusitis etc.) [6, 10, 37].

Therefore, CT diagnosis is based on a combination of clinical and laboratory data, and instrumental examinations.

The general algorithm of conduct in the diagnosis of CT includes: 1) collection of anamnesis (patient accusations, disease duration, angina incurred in the past, associated diseases), 2) clinical objective examination (local signs of CT, changes in the joints, changes in the cardiovascular, kidney systems), 3) paraclinic laboratory tests: general analysis of blood, urine summary, electrocardiogram, biochemical examination of the blood (bilirubin, alanine aminotransferase, aspartate aminotransferase, thymol test), inflammation samples (ESR, fibrinogen, C-reactive protein (CRP)), serology tests (ASLO), the examination of pharyngeal superficial exsudate, obtained from pads or fine needle aspiration (being significant

the presence of BHSA) and antibioticograma, consultation of an ENT doctor, pediatrician, rheumatologist, nephrologist and / or urologist, stomatologist [3, 7, 12, 16, 19 32].

Although the risk of infection depends on environmental conditions (exposure, season, geographical area) and individual variables (age, strength, immunity), the identification of the specific agent is of a significant importance for selecting the treatment, ensuring a rapid recovery and prevention of complications [19]. CT more often (42% of cases) is caused by infection with respiratory viruses (adenovirus, influenza virus, para-influenza virus, rhinovirus or respiratory syncytial virus), and in 30-40% of cases the cause is a bacterial infection [9, 10, 19]. Of all the microorganisms, the main ethiopatogenic agents in CT are BHSA (15 to 55.5% of cases), followed by Staphylococcus aureus [8, 12, 16, 40].

In the recent years, Staphylococcus aureus is considered the main pathogen responsible for CT [35]. According to the results of studies, the most common pathogen was Staphylococcus aureus (33%), followed by BHSA (30%), Streptococcus pneumoniae, Haemophilus influenzae, Escherichia coli, Pseudomonas aeruginosa and Streptococcus viridans [35]. According to another study, Staphylococcus aureus (30.3%), Haemophilus influenzae (15.5%) and Streptococcus pyogenes (14.4%) were the most frequently isolated from patients with CA [9].

Although the cultures of tonsil core compared with isolated cultures from superficial pharyngeal smears, provide a representative picture of the bacterial content in patients with CT and RT [15], in our study, the bacteriological analysis from the pharynx detected Streptococcus viridans in 56 (55.4%) cases, Staphylococcus aureus in 51 (50.5%) cases, Streptococcus pyogenes in 45 (44.6%) cases, BHSA in 17 (16.8%) cases, Streptococcus pneumoniae in 8 (7.9%) and other flora in 22 (21.8%) cases. It should be underlined that different combinations of determined pathogen agents were found in most cases – in 87 (86.1%) patients.

Several researchers have tried to determine the genetic susceptibility and protective immune responses for PSRA and ARF [21, 25, 28, 30]. Validation of HLA associations observed in various populations of the world can contribute to the development of cost-effective primary prevention strategies of these lesions [28]. It was observed a heterogeneity in terms of HLA alleles class I and II of susceptibility and/or protection in different studies in different geographical regions and ethnic groups, although associations with specific antigens have been reported [21, 25, 28, 30].

The study of major antigens did not find an association of HLA Class I (A, B and C) with ARF and RHD [23, 24, 31, 42]. In other studies in patients with CT, including decompensated CT, statistically significantly more often were found the antigens HLA-A2 and HLA-B12 [46]. Among children with mitral valve disease (MVD), compared to children in the control group, there was noted a significant increase in HLA-B5, and HLA-B49, HLA-B51 and HLA-B52 were found only in the control group and they have a protective role for these conditions [28].

Among mature people with ARF was also found a large increase, but statistically non-significant, of HLA-B5, compared to the control group, a statistically significant increase in HLA-A10 and HLA-B35 [36].

In different populations, the frequency of HLA-A10, HLA-Aw33 and HLA-B35 antigens is significantly higher in adult patients with ARF and / or RHD ($p<0.05$ and $P<0.01$, respectively). The frequency of HLA-A10 and HLA-DRw11 antigens in patients with RHD is significantly higher than in those with non-cardiac involvement ($p<0.05$ and $p<0.01$, respectively). On the other hand, the frequency of HLA-CH2 antigen is significantly higher in patients without RHD compared to those with RHD ($p<0.05$) [36].

The absence of a hereditary marker HLA class I in patients with RHD underlines the multiple and important factorial complexity in ARF pathogenesis, but does not exclude the role of genetic factors [42]. However, the susceptibility of RHD is mediated by HLA class II [43].

At patients in our study with chronic tonsillitis and articular syndrome, most frequently were diagnosed the following HLA Class A antigens (HLA-A2 – 44,6%, HLA-A28 – 41,6%, HLA-A24 – 23,8%, HLA-AX – 20,0%) and HLA class B (HLA-B35 – 31,7%, HLA-B44 – 17,8%, HLA-BY – 14,9%, HLA-B18 – 12,9%).

Therefore, the relationship between ARF and PSRA with HLA is contradictory and heterogeneous, and the sensitivity to ARF (and PSRA) is polygenic. Some diseases with poor initial association with the antigens HLA-A and HLA-B were found to have strong association with HLA-DR antigens. The strongest and most frequent association of ARF was proved to be with HLA-DR2, HLA-DR4 and HLA-DR7 phenotypes, and the PSRA – with HLA-DRB1 * 01 [14, 17, 23, 24, 39]. The relatively small number of patients tested and the differences in genetic background may partly explain the different results and the difficulty in the study of HLA alleles in identifying the same genetic susceptibility in different population. In order to understand completely the overall mechanism of genetic susceptibility, such studies need to be duplicated and validated in different ethnic populations, using a wider range of appropriate methods of analysis [28].

Currently, there are very few studies that estimate the role of the genetic system in the development of CT. Most studies provide additional information on genetic predisposition for MVD and protection genotypes in RHD. The results of several studies conducted in concordance to the hypothesis that the susceptibility to RHD is genetically determined, have found a possible association with antigens HLA class II (HLA-DR) and a weak one with antigens HLA class I (HLA-A, HLA-B and HLA-C) [26]. Estimation of HLA class I antigens found a statistically significant increase in HLA-B5 alleles in patients with RHD ($p=0.03$) compared to the control group, whereas the alleles HLA-B49 ($p=0.004$) and the HLA- B52 ($p=0.02$) were found only in the control group [28]. A recent case-control study compared the frequency of CHM class II, HLA-DR alleles between patients with and without RHD. The authors found a low genetic susceptibility of HLA-DR1 with RHD, while HLA-DR11 was associated with an increased risk for RHD [20].

According to specialized literature, the impact of tonsillectomy versus non-surgical treatment is modest [48]. However,

tonsillectomy reduces the symptoms of CT or RT in adults with remarkable effectiveness, resulting in a reduction in the number of episodes of sore throat and days with sore throat in children in the first year after surgery [48].

Conclusions

The study of the major antigens has not found an association of HLA class I (A, B) with ARF and rheumatic heart disease (RHD).

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Treatment of portal hypertension in the light of the Baveno VI Consensus Conference

E. Tcaciuc

Department of Internal Medicine, Medical Clinic No 3
Nicolae Testemitsanu State University of Medicine and Pharmacy, Chisinau, the Republic of Moldova
Corresponding author: eugentcaciuc@yahoo.com. Received October 15, 2015; accepted November 27, 2015

Abstract

Background: Portal hypertension is the haemodynamic abnormality associated with the most severe complications of cirrhosis, including ascites, hepatic encephalopathy and bleeding from gastro-oesophageal varices. Pharmacological and endoscopic treatment of portal hypertension has played an increasing clinical role in the past 30 years. Despite the progress achieved over the last decades, the 6-week mortality associated with variceal bleeding is still in the order of 10–20%. In the setting of acute variceal bleeding, drug and endoscopic therapy should be considered the initial treatment of choice and can be administered as soon as possible. Management of treatment of portal hypertension and variceal hemorrhage is based on the clinical stage of portal hypertension. Prevention of first variceal hemorrhage depends on the size of varices. In patients with small varices and high risk of bleeding, non-selective β-blockers are recommended, while patients with medium/large varices can be treated with either β-blockers or oesophageal band ligation. Standard of care for acute variceal hemorrhage consists of vasoactive drugs, endoscopic band ligation and antibiotics prophylaxis. Patients who had failed this therapy should be considered for transjugular intrahepatic portosystemic shunt or shunt surgery. Prevention of recurrent variceal hemorrhage consists of the combination of β-blockers ± isosorbide 5-mononitrate and endoscopic band ligation. Patients with recurrent variceal hemorrhage are in a category of "further decompensation" of cirrhosis and, as such, should be evaluated for liver transplantation.

Conclusions: In the last decades significant advances in the field of portal hypertension have improved the clinical care and survival of patients with cirrhosis and portal hypertension. Further research is necessary to explore new pharmacological options that would allow to get a positive hemodynamic response in most patients.

Key words: portal hypertension, liver cirrhosis, variceal hemorrhage, treatment.

History consensus conferences dedicated to the treatment of portal hypertension

The management of portal hypertension is linked with so-called "Baveno consensus" that serves as an important basis for developing guidelines and clinical protocols in this area. Baveno is a small town in northern Italy situated on the west Shore of Maggiore Lake. It became the epicenter of consensus workshops dedicated to portal hypertension, which aim is to reach a common denominator about definitions and the most important events associated with portal hypertension and variceal bleeding. The first workshop dedicated to Baveno consensus was held in April 1990 [1], in which were evaluated significant progress in terms of diagnosis and management of eso-gastric varices and variceal bleeding, including the administration of vasoactive drugs and the use of endoscopic sclerotherapy. Additionally, were defined certain features and complications of portal hypertension including the varices sizes, the bleeding and its recurrences, the recommendations about diagnosis methods and were traced new directions for future clinical studies. Therapeutic recommendations included the following: β-blockers for primary prophylaxis of bleeding from large varices, endoscopic sclerotherapy and vasoactive drugs for acute variceal bleeding, and to prevent recurrent bleeding – endoscopic sclerotherapy, β-blockers or surgical shunt.

The Baveno II workshop was held in April 1995 [2]. Were revised definitions of the most important clinical syndromes in portal hypertension and new definitions were proposed. After analyzing several randomized controlled trials, nonselective β-blockers were recommended as the elective treatment for primary prevention of variceal bleeding, while isosorbide-5 mononitrate has been recommended in patients who were intolerant or had contraindications to β-blockers. Endoscopic sclerotherapy was not recommended for the prevention of the first episode of bleeding. While the treatment of acute bleeding was based on endoscopic therapy, on terlipressin administration (which was considered one of the most effective vasoactive agents) and somatostatin analogues. TIPS has been recommended in case of failure of endoscopic and pharmacological treatment. The recommendations related to the prevention of recurrent bleeding include the administration of β-blockers or endoscopic variceal ligation, which it has shown to be more effective and safer than sclerotherapy [3]. TIPS and surgical shunt followed to be used only for patients with frequent repeated episodes of variceal bleeding.

The Baveno III Conference was held in April 2000 [4], where it was introduced the concept of portal hypertension with significant clinical manifestations, which is determined if hepatic venous pressure gradient (HVPG) ≥ 10 mmHg. The presence of varices, variceal bleeding or ascites indicates the portal hypertension with significant clinical manifestations. Nonselective β-blockers were kept in elective treatment for preventing the first episode of bleeding from large/medium varices, while the endoscopic variceal ligation was considered necessary for an additional evaluation. Were defined the targets of β-blockers therapy (25% reduction in heart rate from baseline or establishing a heart rate of 55 beats/min). It was not proposed the using of isosorbide-5 mononitrate as an alternative treatment, which was previously recommended [5]. For the acute bleeding treatment, immediate administration of vasoactive medications and the continuation over 5 days together with endoscopic therapy (endoscopic variceal ligation or sclero-

therapy) were considered as the standard. Also, were proposed some additional measures: antibiotic use to prevent bacterial infection [6] and the lactulose for the treatment of hepatic encephalopathy. About the prevention of repeated bleeding, β-blockers were considered first-line therapy [7], in parallel with endoscopic variceal ligation, and TIPS was reserved in the case of treatment failure. Also, were clearly defined the complications of portal hypertension and were proposed targets for further research.

The Baveno IV Conference was held in April 2005 [8], there were revised some key criteria (inability to control bleeding, the failure of secondary prevention). For primary prevention, β-blockers were kept as an elective treatment, but endoscopic ligation was placed as an excellent alternative for patients with large and medium varices and contraindications or intolerance to β-blockers [9, 10]. Isosorbide 5-mononitrate, was not recommended either in monotherapy, or even in a combination of pharmacological therapies [11]. Primary prevention of small varices can be considered only if they are at high risk for hemorrhage ("red signs" or Child-Pugh Class C) [12]. The recommendations for acute variceal bleeding were kept the same as in the Baveno III Consensus. Along with vasoactive drugs administration for at least 5 days, it was recommended the use of tamponade with balloon and only in massive bleeding as a temporary bridge until the establishment of defenitive treatment. The endoscopic variceal ligation was declared superior to sclerotherapy and was considered the elective endoscopic procedure for acute bleeding control [10, 13]. It is recommended that secondary prophylaxis should be initiated by the 6th day after variceal hemorrhage, which must include a combination of endoscopic ligation and nonselective β-blockers [14, 15]. As in previous consensuses, TIPS and surgical shunts were reserved for patients with secondary prevention failure.

The Baveno V Conference of May 2010 revised the definitions relating to the failure of variceal bleeding control and secondary prophylaxis [16]. Primary prophylaxis recommendations for small varices were the same as in the previous consensuses. There was no significant change in the recommendations for primary prevention of medium and large varices (β-blockers or endoscopic variceal ligation). The choice of therapy was dictated by local resources, the experience of specialists and patient preference [17]. The recommendations for treatment of acute variceal bleeding were unchanged, except a recommendation, which involves the use of TIPS in the early period (in 72 hours) in patients with increased risk of pharmacological or endoscopic treatment failure [18]. For preventing recurrent bleeding was proposed the combination therapy of β-blockers and endoscopic variceal ligation.

The Baveno VI Consensus Conference took place in April 2015. Below are outlined the basic principles of modern management of portal hypertension.

Standard modern treatment of portal hypertension in adults

The therapy of esophageal varices and variceal bleeding in adult patients with cirrhosis should be differentiated according to clinical stages of the natural history of portal hypertension which were divided into 4 stages:

1. Patients with cirrhosis and portal hypertension, which has not yet developed varices and the therapy purpose is to prevent the formation of varices (pre-primary prophylaxis).

2. Patients with esogastric varices which are not bleeding and the therapy purpose is to prevent their rupture (primary prevention).

3. Patients with acute variceal hemorrhage for which the objective of treatment is to stop the bleeding and prevent its recurrence in the early period.

4. Patients who had survived an acute variceal bleeding and the goal of therapy is to prevent recurrence of bleeding in the late period (secondary prevention).

The prevention of the esogastric varices formation (pre-primary prophylaxis)

Each new patient diagnosed with cirrhosis requires the effectuation of upper gastrointestinal endoscopy for the identification of varices presence and their degree. Large, multicenter, randomized and controlled trials found no difference between placebo and β-blockers in preventing the formation of varices in patients who have not developed esogastric varices [19]. Therefore, no specific treatment for portal hypertension is recommended in these patients. The main focus at this stage is to treat the underlying cause of cirrhosis, which will reduce portal hypertension and therefore will prevent the development of clinical complications.

The prevention of the first variceal bleeding (primary prevention)

The first variceal hemorrhage occurs with an annual rate about 15%, although currently mortality after a variceal bleeding is lower than in the last two decades, however, remains significant (7% – 15%) [20 – 22] and it is associated with significant morbidity and high costs for treatment. The prevention of the first episode of bleeding, therefore, is an important part of the portal hypertension treatment. The size of varices, "red signs" on varices (endoscopic view) and severity of liver disease (Child-Pugh C class) identifies patients with the highest risk of variceal hemorrhage [12]. Therefore, at this stage, patients must be differentiated depending on the risk of bleeding:

- high-risk patients (those with medium/large varices or those with small varices, but with "red signs" or evolutionary stage Child-Pugh C).
- low-risk patients (those with small varices without "red signs" or which appear in patient with Child-Pugh A or B class).

Several studies had demonstrated that for patients with medium/large varices, the nonselective β-blockers (propranolol, nadolol) are as effective as endoscopic variceal ligation in the prevention of first variceal bleeding [23, 24], but the author's recommendations are based on using the therapy according to existing local resources, the specialist experience in this area and patient preference.

For patients with increased risk of bleeding and a low degree of varices the principal therapeutic options is nonselective β-blockers administration, and the application of endoscopic variceal ligation is difficult.

For patients with low risk of hemorrhage and small varices there is limited evidence demonstrating that their growth can be slowed by using nonselective β-blockers [25]. Therefore, the use of nonselective β-blockers in this case is considered optional and should be discussed with the patient.

For primary prevention the starting dose of propranolol administration is 20 mg orally 2 times a day. Dosage adjustment is made every 2–3 days until the target dose is reached, which decreases the heart beats by 25% from the initial, but must not fall below 50 – 55 beats/minutes. The maximum dose does not exceed 320 mg. At each ambulatory visit it is necessary to adjust the dose of β-blocker. In the absence of contraindications the treatment is indefinite. Nadolol is administered in a 40 mg dose in a single dose and the maximum dose should not exceed 160 mg. Dose adjustments are performed as in the case of propranolol administration. Endoscopic variceal ligation is performed every 2 – 4 weeks until the final variceal obliteration. The repeating of endoscopy is made over 1 – 3 months after obliteration and later over every 6 – 12 months.

For secondary prophylaxis, the management of nonselective β-blockers administration and endoscopic procedures is the same as in primary prevention. Isosorbide-5-mononitrate may be associated with non-selective β-blockers initially in a dose of 10 mg orally at night, and then 10 mg orally twice a day with a maximum dose of 20 mg twice a day. Systolic blood pressure should not fall below 95 mmHg. The duration of therapy with isosorbide-5-mononitrate is also indefinite.

Nonselective β-blockers decrease portal pressure by reducing portal blood flow. Their mechanism of action involves the decreasing of cardiac output by blocking β_1 receptors, but splanchnic vasoconstriction is achieved by blocking β_2 receptors. The latter is the most important effect of nonselective β-blockers in portal hypotensive therapy (unlike the selective β-blockers). Nonselective β-blockers advantages include low cost and simplicity of administration. Because nonselective β-blockers decrease the degree of portal hypertension, their use may also reduce other complications of cirrhosis, such as bleeding from eso-gastric varices and portal-hypertensive gastropathy, ascites and spontaneous bacterial peritonitis [26, 27]. It was found that, in fact, a significant reduction in portal pressure was associated with the improvement of survival in patients with liver cirrhosis [28]. In addition, in the opinion of some authors, once the patient administers nonselective β-blockers it is not necessary to repeat endoscopy.

Endoscopic variceal ligation has some advantages: during the procedure can be examined the whole upper digestive tract mucosa, has relatively few contraindications and a lower incidence of side effects compared to nonselective β-blockers [9].

The main disadvantage of nonselective β-blockers is that approximately 15% of patients may have absolute or relative contraindications to the therapy administration and the other 15% require dose reduction or discontinuation of therapy due to frequent side effects (eg, fatigue, weakness, bronchospasm) that disappear after stopping these medicines [17].

Endoscopic variceal ligation risks include those related to endoscopic procedures and sedation (bleeding, aspiration, perforation and reaction to medications), along with the risk of bleeding from ulcers induced by the ligation. In fact, although the number of side effects is higher in nonselective β-blockers administration than after endoscopic variceal ligation [9], the severity of side effects is higher in performing the latter. Fatal side effects after using the non-selective β-blockers have been not reported, but have been reported deaths resulting from endoscopy procedures (e.g., bleeding from ulcers induced by varices ligation) [9, 10].

It is certain that the ideal portal hypotensive therapy has not been established. There are medical centers where is preferred endoscopic variceal ligation, while in other centers prefer to start with nonselective β-blockers, and then, if necessary, the use of endoscopic variceal ligation.

Carvedilol is a nonselective β-blocker, possessing additional vasodilating effect through anti-α_1-adrenergic activity. There is evidence that carvedilol administration in portal hypotensive purpose is more effective than endoscopic variceal ligation in preventing first variceal bleeding [29]. Although the use of carvedilol is considered a promising alternative, it is necessary to perform further research before it can be widely recommended.

The management of acute variceal hemorrhage

Acute variceal hemorrhage is a major medical emergency requiring intensive care. First, the basic treatment is directed to achieve hemodynamic stability. Blood transfusion is done to raise hemoglobin levels between 70 – 80 g/l [30], because the return of excessive blood volume can increase portal pressure [31, 32]. The survival is higher in patients with variceal hemorrhage submitted to restrictive transfusion policy. Restrictive transfusions significantly reduce mortality, particularly in Child-Pugh A and B cirrohosis [33].

It is necessary to correct coagulation disorders, although, currently, there are no clear recommendations on the management of coagulopathy and thrombocytopenia [34, 35].

Antibiotic prophylaxis is an integral part of therapy for patients with liver cirrhosis and upper gastrointestinal bleeding since the admission. Antibiotic prophylaxis is provided by quinolones and/or ceftriaxone in i/v administration [6, 36]. The risk of bacterial infections and mortality is reduced in patients Child-Pugh A, but are necessary prospective studies that can demonstrate that antibiotico-prophylaxis may be excluded from this group of patients.

Vasoactive medication needs to be started as soon as possible, even before diagnostic endoscopy. Endoscopy also is made as soon as possible and not later than 12 hours after addressing. If the bleeding source is identified, the elective procedure is endoscopic variceal ligation, but sclerotherapy is an option when ligation is technically difficult. TIPS is recommended in patients failing standard therapy (a combination of endoscopic and pharmacological treatment).

However, TIPS has a high mortality. The predictive factors of standard therapy failure are Child-Pugh C class, HVPG > 20 mmHg, and active bleeding on endoscopy [37]. Using the TIPS in the early period (approximately 48 hours of onset) in patients with increased risk of failure to standard therapy, significantly reduces mortality [18]. It occurs in patients with evolutionary stage Child-Pugh C (score 10 – 13 points) or evolutionary stage Child-Pugh B with active bleeding (at the moment of diagnostic endoscopy) and represents <20% of patients with variceal bleeding. In these patients it is recommended to consider the possibility of TIPS effectuation. The rest of the patients need to continue the standard therapy with vasoactive drugs for 2 – 5 days continually, depending on the bleeding control and the severity of liver disease. Vasoactive drugs may be discontinued once the patient had no bleeding for at least 24 hours. The tamponade with balloon is used only as a temporary measure (balloons are inflated for 12 hours or less) to control bleeding, while is planned the definitive treatment (TIPS or endoscopic therapy). A new esophageal stent was proposed last year that can replace the tamponade with ballon [38].

Although there are pros and cons for each of these first-line therapies, current recommendations are for the combination of pharmacological and endoscopic methods to effectively control acute hemorrhage.

Vasoactive agents improve the control of variceal bleeding when are combined with endoscopic therapy in comparison with application of endoscopic therapy exclusively [39]. However, there is a significant difference between various vasoactive agents on controlling bleeding and early rebleeding. Vasopressin is a powerful vasoconstrictor, but because it is associated with more side effects [40] it is not considered as vasoactive drug for first-line. Its use is limited because of many side effects associated with splanchnic vasoconstriction (for example, intestinal ischemia) and systemic vasoconstriction (e.g., hypertension, myocardial ischemia). However, in case of using vasopressin, it must be taken with nitroglycerin.Terlipressin is an analogue of vassopresin and represents a pharmacological agent which demonstrated in comparative studies the improvement of survival in patients with variceal hemorrhage [40]. It possess splanchnic vasoconstrictor effect. The active metabolite of terlipressin, lysine-vasopressin, is released gradually over several hours thus reducing typical side effects of vasopressin. Terlipressin is administered 2 mg i/v in bolus, and 2 mg every 4 hours during a bleeding episode. For the prevention of rebleeding the maintenance doses are 1 mg/4 hours i/v bolus up to 5 days.

Somatostatin inhibits the vasodilatory substances, such as glucagon, causing splanchnic vasoconstriction and the decrease of portal blood flow. Initially, is administered 250 pg i/v in bolus followed by continuous infusion from 250 pg to 500 pg/hour up to 5 days.

Octreotide is a somatostatin analogue and has the same mechanism of action as somatostatin, but a longer duration of action. Initially, is administered 50 μg i/v in bolus, then each 50 μg/hour in continuous infusion up to 5 days.

Vapreotide is also an analogue of somatostatin with the same mechanism of action but with a higher metabolic stability. Initially it is administered 50 μg i/v in bolus, then each 50 μg/hour in continuous infusion up to 5 days. The main side effects of somatostatin analogues (octreotide and vapreotide) are sinus bradycardia, hypertension, arrhythmias and abdominal pain.

In practice, the choice of a pharmacological agent is based usually on availability and cost.

So, the preferable treatment of acute variceal hemorrhage is combined: vasoactive drugs administered before the effectuation of endoscopy and emergency endoscopic therapy. Pharmacological elective therapy is represented by terlipressin (lower mortality in placebo-controlled trials) or somatostatin and octreotide (fewer side effects). The elective endoscopic therapy is endoscopic variceal ligation.

Recommendations can vary according to the severity of liver disease. In patients who are in evolutionary stage of cirrhosis Child-Pugh C (or Child-Pugh B with active bleeding), the risk of standard therapy failure (vasoactive drugs plus endoscopic variceal ligation) is large and therefore it is appropriate to pass to "rescue" therapy (i.e., TIPS) before we get a failure of standard therapy. Patients in Child-Pugh A class, the mortality after standard treatment is around zero [20, 22], and these patients may respond to vasoactive treatment in monotherapy, although this requires further researches.

The prevention of variceal hemorrhage recurrence (secondary prevention)

The risk of rebleeding in patients who have suffered a variceal bleeding is high (average rate of rebleeding is 60%), with a mortality of up to 33%. The prevention of rebleeding is therefore an essential part of the management in patients with variceal bleeding. Patients in the acute episode of bleeding who benefited from TIPS, do not require a specific hypotensive therapy or endoscopic surgery on varices, but should be cautious for transplant. TIPS's permeability should be checked by Doppler ultrasonography every 6 months. For most patients (who have not been effectuated TIPS during an acute episode of bleeding), the secondary prophylaxis with nonselective β-blockers should be started as soon as possible, but after intravenous vasoactive drug administration is discontinued. Nonselective β-blockers significantly reduce the risk of recurrent bleeding [7]. Although, according to several studies, the addition of isosorbide 5-mononitrate with nonselective β-blockers has a greater effect on reducing portal pressure [41], in clinical trials the combination of these groups is not different from monotherapy with nonselective β-blockers in terms of rate rebleeding or death, but has a higher rate of side effects [42].

The sclerotherapy decreases the rate of bleeding and the mortality, but is associated with serious complications (eg., esophageal stricture, bleeding from ulcer). Sclerotherapy has been replaced with endoscopic variceal ligation because the ligature showed significantly better results in comparison with sclerotherapy about rebleeding, mortality and side effects. Several studies have compared pharmacological treatment

(nonselective β-blockers in addition with isosorbide 5-mononitrate) versus variceal endoscopic ligation and found that there are no significant differences in the occurrence of recurrent haemorrhage, but long-term administration of pharmacological treatment has a beneficial effect on patient survival [43]. It was found that the combination of pharmacological treatment (nonselective β-blockers in monotherapy or nonselective β-blockers + isosorbide 5-mononitrate) plus endoscopic variceal ligation is associated with lower rates of rebleeding than pharmacological or endoscopic monotherapy [44, 45] and it is an elective option.

If patients have and recurrent variceal hemorrhage despite of combined pharmacological and endoscopic treatment, is indicated the application of TIPS with polytetrafluoroethylene-covered stents [46] or, if necessary, surgical shunts [47].

Pharmacologic agents give protection against rebleeding both in the period up to the first variceal bleeding and in the period of recurrence prevention, with or without the effectuation of endoscopic variceal ligation. It is considered useful to give nonselective β-blockers in monotherapy or in combination with isosorbide 5-mononitrate. The choice of treatment tactics depends on patient tolerability. Patients who are not candidates for endoscopic variceal ligation should administrate the combined drug therapy (nonselective β-blockers + isosorbide 5-mononitrate).

The lowest rates of variceal bleeding recurrences (about 10%) are observed in people who have a positive hemodynamic response to pharmacological treatment, defined as a decrease in HVPG under 12 mmHg or a decrease of more than 20% from baseline of HVPG [28, 48]. It is reasonable to guide pharmacological therapy according to hemodynamic response and, therefore, patients who achieve a positive hemodynamic response do not require endoscopic therapy. Patients who are intolerant or have contraindications to pharmacological therapy should receive endoscopic variceal ligation in monotherapy.

Some researchers have observed that the administration of non-selective β-blockers in patients with refractory ascites is associated with a lower survival than patients without refractory ascites [49]. However, it is noted that patients with refractory ascites have a higher prevalence of varices and, particularly, those with an increased risk of bleeding, which leads to a higher mortality. So now, even these patients may benefit from treatment with nonselective β-blockers [7, 50] and therefore nonselective β-blockers are not contraindicated in patients with refractory ascites. It is necessary to note that combined treatment with nonselective β-blockers and isosorbide 5-mononitrate has a higher incidence of superimposed side effects caused by isosorbide 5-mononitrate association, usually manifested by headaches and dizziness. As mentioned above, the lowest rate of rebleeding is in patients with a positive hemodynamic response. On the other hand, while HVPG guiding therapy seems to be rational, a small study showed that HVPG guiding therapy results are not different from combined endoscopic and pharmacological treatment [51]. Although HVPG determination is standardized and it is performed widely in large clinical centers, HVPG guiding therapy has not yet been introduced in the guidelines recommendations[52].

As mentioned above, endoscopic variceal ligation is associated with bleeding from ulcer induced by this procedure. Treatment with proton pump inhibitors after variceal ligation significantly reduces the size of these ulcers and, ultimately, decreases the risk of bleeding [53].

The current standard treatment of portal hypertension in children

The most common causes of portal hypertension in children are biliary atresia and portal vein thrombosis. The data about prevalence of esophageal varices in children with portal hypertension are very limited and till now there have been no randomized controlled trials that would compare different methods of treatment for primary and secondary prevention [54].

About primary prevention, currently there are no clear treatment recommendations [55, 56]. Gathering of experts at the annual meeting of the American Association for Study of Liver Disease concluded that before getting the results of a randomized study in children, the pediatric research should focus on approach to the natural history and diagnosis of varices, the predictive factors of variceal bleeding, optimal therapy with β-blockers and endoscopic variceal ligation and alternative methods of treatment to assess therapeutic efficacy in children [57]. The management of acute variceal bleeding in children is based on the use of vasoactive agents, antibiotic prophylaxis and endoscopic variceal ligation. In children with portal vein thrombosis, the meso-rex by-pass seems to be the best option for secondary prophylaxis [55, 56, 58].

Conclusions

The elective treatment for the prevention of variceal hemorrhage is a combination of pharmacologic therapy (nonselective β-blockers ± isosorbide 5-mononitrate) and endoscopic variceal ligation. The assessment of bleeding risk is difficult, but the most important predictor of recurrent bleeding is evolutionary stage of cirrhosis Child-Pugh. The patients with Child-Pugh A class cirrhosis need to apply a single method of treatment, while patients with advanced liver disease require a combined therapy. Patients who have failed this therapy should be considered for TIPS placement, or surgical shunt. Patients with recurrent variceal bleeding are in a state of "continuous decompensation" of liver cirrhosis and, as such, should be evaluated for liver transplantation.

In the last two decades significant advances in the management of portal hypertension have improved the survival of patients with liver cirrhosis and portal hypertension. Were developed treatment strategies and well-established therapeutic options for each clinical stage and were identified different subpopulations of patients who require differentiated management.

It is evident the necessity to perform new research directed towards identifying new pharmacological options that would

allow to get a positive hemodynamic response in most patients and thus would give up on the necessity to determine HVPG and may even give up on endoscopic therapy.

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Ursolic acid: do we need other derivates?

A. Fulga

Department of General Chemistry, Nicolae Testemitsanu State University of Medicine and Pharmacy
Chisinau, the Republic of Moldova

Corresponding author: ala.fulga@usmf.md. Received October 16, 2015; accepted December 06, 2015

Abstract

Background: The nature is a fascinating source of biologic active substances, many of which are showing promising antitumor activities [1, 2]. The triterpenes are an important class of phytochemicals, classified in accordance with isoprene units [3, 4]. These chemicals are synthesized by the plants through cyclic processing of squalen. About 20000 of triterpens, such as cucurbitanes, cycloartanes, friedelanes, holostanes, hopanes, lanostanes, lupanes, oleananes, dammaranes, euphanes, tirucallanes, isomalabaricanes, ursanes and others are identified at the moment. From a wide diversity of triterpenes, the pentacyclic derivatives were the most frequent studied chemicals, due to their anti-inflammatory, analgesic, hepatoprotective, cardiotonic, anti-alergic, anti-microbial and anti-tumor properties [5-9]. Ursolic acid or 3β-hydroxi-urs-12-en-28-oic acid is a triterpen's pentacyclic acid was discovered in plants, such as Ocimum sanctum L.(*Holy Basil*), Prunus laurocerasus L. (*Cherry laurel leaves*), Vaccinum myrtillus L. (*Bilberry*), Crataegus laevigata (*Hawthorn*), Harpagophytum procumbens DC (*Devil's Claw*), Thymus vulgaris L. (*Thyme*), Sambucus nigra L. (*Elder Flowers*), Origanum vulgare L. (*Oregano*), Lavandula augustifolia Mill. (*Lavender*), Vinca minor L. (*Periwinkle*), as well as in the wax from apples, plums and pears peels [10]. It is a pentacyclic triterpenoid which belongs to cyclosqualenoid family [11]. This acid can be determined free or as aglicon of saponins. The recent results are supporting the anti-inflammatory, anti-proliferative, pro-apoptotic, anti-metastatic, anti-angiogenic and anti-parasite functions of this chemical [7,12]. The aim of this study was to highlight in details the anti-tumor activity of ursolic acid, by pointing out its influence on cells proliferating, apoptosis and metastatic property.

Conclusions: Ursolic acid is a promising compound in tumor prevention and treatment, with many mechanisms of action on cell's proliferation. Its derivatives usually are more biologically effective than initial compound, so obtaining of new ursolic derivatives makes further investigations in this field have a particular relevance.

Key words: triterpenes, ursolic acid, cancer.

Ursolic acid isolation

This chemical was isolated through different methods [13]. Generally, plants are extracted by two solvents with increasing polarity, hexan and ethyl acetate in Soxhlet. The obtained extract of ethyl acetate is concentrated in rotary evaporator. Until now were purposed many isolation methods in organic solvents by using high pressure liquid chromatography (HPLC), thin layer chromatography (TLC) and gas-chromatography after silylation and methylation [14-16]. Kontogianni et al. (2009) have demonstrated that combination of ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC NMR spectroscopies is a fast analytical method which clarifies and quantifies triterpenic acids in plants' extracts [17]. However, today the most frequently used method is bioassay-guided fractionation, based on physico-chemical differences. But this method anyway implies chromatographic techniques.

Finally, a clean isolated ursolic scid (UA) looks as glossy prisms after purification in absolute alcohol or as long threads, hair-like from diluted alcohol. The melting point of this chemical reaches 284-288 ^{0}C. Ursolic acid is soluble in organic solvents such as ethanol, hot glacial acetic acid, in 2% alcoholic NaOH, dimethyl sulfoxide and dimethyl formamide,

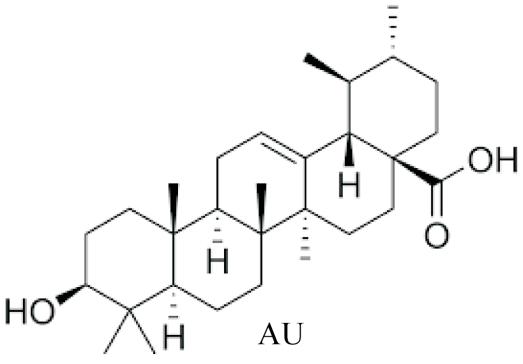

Fig. 1. Chemical structure of ursolic acid.

which should be purged with an inert gas. It is insoluble in water (fig. 1).

Antitumor activity of ursolic acid

Multiple studies have confirmed that tumor progression is stimulated by pro-inflammatory factors: nuclear factor NF-kB, transcriptor activator 3 (STAT3), protein kinase B (AKT), cyclooxygenase-2 (COX-2) [18-20] among them. All these factors showed a pro-tumor activity, by stimulating cell proliferation, angiogenesis and metastatic properties.

Nuclear factor NF-kB is a regulating key implicated almost in all cell's processes [22]. Activation of this factor is often associated with chronic inflammation, tumorigenesys and resistance to chemo/radiotherapy [22, 23]. Many studies in the field support the role of chronic inflammation in tumor formation. Its presence is represented as a high risk in cancer development. Oeckinghaus et al. (2011) demonstrated that phosphorylation of IkB proteins by IkB kinases is a key process finalized with NF-kB coupling DNA and transcription activation of certain genes [24]. Until now have been purposed many agents targeted at this mechanism [7, 25]. Studies in vitro demonstrated the UA ability to block NF-kB activation induced by carcinogenic agents, such as TNF, okadaic acid, H2O2 and tobacco smock. By Shishodia et al. (2003) this action of UA is realized through IkBα kinases suppression and blocking of p65/Re1A phosphorilation [26]. Authors have reported that NF-kB inhibition was supplemented and by NF-KB dependent enzymes blocking, as cyclin D1, COX-2 and MMP-9 (matrix metalloproteinase-9).

Ursolic acid showed a promising activity against multiple types of tumors. Pathak et al. (2007) demonstrated a cytostatic activity of UA in case of multiple myeloma [27]. This action was realized through suppression of a wide series of kinases, such as c-SRC, Janus-activated kinases 1 and 2 (JAK ½ kinases). Doudican et al. (2014) by using a predictive simulation technology demonstrated that UA is very effective in case of multiple myeloma, especially in combination with other anti-cancer agents, such as pan-JNK inhibitor SP600125 [28]. Authors showed that such combination synergistically inhibited proliferation and induced apoptosis, evidenced by an increase in the percentage sub-G1 phase cells, cleavage of caspase 3 and poly-ADP-ribose-polymerase, as well a significant reduction in the expression of cyclin D1 and c-Myc.

Recently, Shanmugam et al. (2011) examined UA action on prostate carcinoma cell lines. This chemical was effective in androgen-independent tumors (DU145), as well androgen-dependent (LNCaP) [29]. This action was realized by suppression of genes regulated by STAT3 and NF-kB [30]. More, Shin et al. (2012) demonstrated that this triterpene has a stimulatory effect on LC3-II (microtubule-associated protein 1A/1B-light chain 3) resulted with activation of autophagy process in PC3 cells [31]. Zhang et al. (2010) consider that UA is beneficial in prostate cancer by its implication in signaling pathway mediated PI3/Akt/mTOR and Beclin-1, finally stimulating apoptosis [32].

Wang et al. (2011) tested UA and its cis-, trans-3-O-p-hydroxycinnamoyl derivatives on prostatic cells clone DU145 [33]. Authors presented an increased inhibitory capacity of UA on metalloproteinases MMP-2 and MMP-9.

Ursolic acid showed anti-tumor effect and in vivo experiments. Shanmugam et al. (2012) tested UA action during 4-36 weeks on mice with DU145 cells implant and with a transgenic prostate adenocarcinoma [7, 34]. Authors demonstrated that UA had an inhibitory action on tumor progression after 8 weeks of UA administration, effect supplemented at 12 weeks with a significant tumor size diminishing. Therewith UA inhibited in prostate a series of pro-inflammatory mediators, as NF-kB, STAT3, IKK α/β and AKT. Systemic effect of UA was expressed by diminishing the TNFα (tumor necrosis factor alpha, cachexin or cachectin) and IL-6 (interleukin 6) levels in peripheral blood.

In accordance with data reported by Teicher et al. (2010), UA has ability to block metastasis development [35]. The mechanisms of action suppose blocking signaling pathway CXCR4/CXCL12 (C-X-C chemokine receptor type 4/C-X-C motif chemokine 12). In Shanmugam et al. (2011) opinion this acid can suppress expression of CXCR4 in prostatic tumor cells, regardless of HER2 (human epidermal growth factor receptor 2) status [30]. Therewith authors support the implication of this natural compound in transcription regulation and blocking of NF-kB activation.

The anti-proliferative effect of UA was confirmed by Zheng et al. (2012) on T24 urinary bladder cancer cells line [36]. Authors confirmed that UA can induce organization of an intracellular signaling complex IRE1-TRAF2-ASK1 (the serine/threonine-protein kinase/endoribonuclease inositol-requiring enzyme 1- TNF receptor-associated factor 2- Apoptosis signal-regulating kinase 1) with pro-apoptotic function.

In accordance with data published by Liu et al. (2012) this triterpene has capacity to improve bronchial epithelium status affected by tobacco extract. More is a promising prophylactic agent able to prevent pulmonary cancer development [37]. Huang's et al. (2011) results demonstrated that UA can induce apoptosis in tumor cells A549, H3255 and Calu-6 [38].

Lung cancer is one the most frequent tumor among smokers [39]. Ursolic acid demonstrated its activity in treating this cancer by blocking invasive properties of series of tumor clones as A549, H3255 and Calu-6. Moreover, this triterpene was able to initiate apoptosis in cancer cells at quite small dosage, of 2μmol/L [38, 40].

Prasad et al. (2012) presented results which denote the efficacy of UA in colorectal cancer [41]. In authors opinion, pro-apoptotic effect is realized by NF-kB inhibition and suppression of proteins with anti-apoptotic function (cFlip (FLICE-like inhibitory protein), survivin, Bcl-2 (B-cell lymphoma 2), Bcl-xl (B-cell lymphoma-extra large)), proliferative (cyclin D1) and pro-metastatic (ICAM-1 (Intercellular Adhesion Molecule 1), VEGF (vascular endothelial growth factor), MMP-9 (Matrix metallopeptidase 9)). This chemical at digestive tract level could stop growing and induce apoptosis in pancreatic tumor cells (PANC-1 (human pancreatic carcinoma, epithelial-like cell line), CAPAN-1 (human pancreatic ductal adenocarcinoma cell line)). In opinion of Li et al. (2012) this action is realized through UA implication in signaling pathways JNK and PI3K/Akt/NF-kB [42].

The anti-tumor activity of UA was demonstrated in vivo mouse models of colorectal cancer [41]. Prasad et al. (2012) reported a considerable decreasing of tumor, ascites, as well diminishing of metastatic properties of cancer cells. Authors support that UA realizes this activity through inhibition of Ki-67 marker of proliferation and CD31. These effects were attended by NF-kB, STAT3 and β-catenin suppression. Andersson et al. (2008) have reported a diminishing of aberrant crypts in colorectal adenoma, after UA oral administration [43].

Ursolic acid presented a promising action in vitro experiment on K562 clone of leukemia cells. Wu et al. (2012) have demonstrated that UA induces apoptosis by stimulating PTEN (Phosphatase and tensin homolog) expression, blocking activation of Akt kinases, alteration of mitochondrial membrane potential, reducing of cytochrome C releasing, stimulating a series of caspases [44]. These data were supplemented by Zhang et al. (2011) who consider that UA can induce differentiation of HL60 promyelocytic leukemia cells to monocytes and stimulate expression of CEBPβ (CCAAT/enhancer-binding protein beta) [45]. Gao et al. (2012) presented promising results in vivo experiments [46]. Ursolic acid, 50 mg/kg administrated 20 days to NOD/SCID mice with U937 implant concluded with impressive blocking of tumor proliferation. These results are in line with Chiang et al. (2003) data, which reported that UA is very effective against P3HR1 cells (2.5 μg/ml) and human immortalised myelogenous *leukemia* line K562 (17.79 μg/ml) [47]. Lauthier et al. (2000) demonstrated that ursolic acid can decrease cell viability in human lymphoma Daudi cells (human Burkitt's *lymphoma* cell line) in a dose-dependent manner [48]. Authors showed that UA also induced morphological changes in cells such as loss of membrane asymmetry, DNA fragmentation and nuclei condensation. In their opinion these changes indicating that the mechanism by which UA induced cell death was through apoptosis. More, authors hypothesized that the binding of UA to glucocorticoid receptors and the Ca2+ currents constituted the first steps of apoptosis. Ovesná et al. (2006) investigated protective effects of UA against H2O2 -induced DNA damage in leukemic L1210, K562 and HL-60 cells [49]. Authors demonstrated that after 24h pre-treatment of cells with UA (2.5-10μmol/l) the incidence of DNA single strand breaks induced by H2O2 decreased significantly.

In mammary carcinoma cells MDA-MB-231 this triterpene initiated apoptosis by stimulating Fas receptor, cleavage of caspases 3, 8 and PARP (*Poly (ADP-ribose) polymerase*), stimulating pro-apoptotic protein Bax and releasing of cytocrome C from mitochondrion in cytoplasm, blocking anti-apoptotic BCL-2 receptor [50]. Subbaramiah et al. (2000) investigated the influence of UA on COX-2 expression in mammary cells treated with PMA (phorbol 12-myristate 13-acetate) [51]. The results attested a long-standing blockage of COX-2, protein-kinases C, c-Jun N-terminal kinases, inhibition of prostaglandin E2 synthesis. An antitumor action presented and UA derivative, 2α-hydroxiursolic acid. This one could block tumor cells MCF-7 proliferation at 20μM concentration, function realized through TNF-α and NF-kB [52]. Plus, De Angel et al. (2010) presented a promising result of UA action on C57BL/6 mice, ovariectomized with transgenic breast carcinoma [53]. Ursolic acid administered during 5 weeks resulted in a significant diminishing of tumor size, effect in authors' opinion exercised by UA involvement in Akt/mTOR signaling pathway and apoptosis inducing. But, this results are contested by Singletary et al. (1996), who did not establish any therapeutic effects after UA administration to the rats with breast tumors induced by 7,12-dimethyl-benz(a)-antracene [54].

A promising, anti-angiogenic action of UA was described after its testing on hepatic cancer cells, as Hep3B, Huh7 and HA22T. Lin et al. (2011) consider, that this function is realized by inhibition of a series of factors, such as HIF-1α (hypoxia inducible factor-1α), bFGF (basic fibroblast growth factor), VEGF (Vascular endothelial growth factor), interleukin 8, urokinazic plasminogen activator (uPA), supplemented by diminishing the levels of reactive oxygen (ROS) and nitric oxide (NO) [55]. Tian et al. (2006) reported that UA has ability to block both hepatic tumor cells HepG2 and their derivatives R-HepG2, resistant to chemotherapy, supplemented by a minor inhibitory effect on normal hepatocites [56]. Authors also demonstrated that COX-2 blocking and HSP (heat shock protein) stimulating, correlated with apoptosis enhance in HepG2 cells. This, pro-apoptotic effect was further determined on other hepatic tumor cells, such as Hep3B, Huh7 and HA22T. But this action was dependent on UA concentration: at high dosage a DNA fragmentation and cells' viability decreasing was attested. Similar results were reported by Ramos et al. (2008), who consider that ursolic acid can prevent DNA damage and has antiproliferative properties applied on HepG2 cells [57].

In accordance with results reported by Yan et al. (2010), treating of hepatic tumor cell with UA lead to Na+, K+-ATP-ase blocking and VEGF reduction [58]. Gayathri et al. (2000) have investigated COX-2 expression in mammary cells, treated with phorbol (PMA), a carcinogenic agent (phorbol 12-myristate 13-acetate). Ursolic acid suppressed effectively the PMA action by blocking COX-2 protein and diminution of E2 prostaglandin synthesis. Likewise, the tumorigenic action of PMA was diminished by blockage of a series of kinases, such protein kinase C, c-Jun-N-terminal-kinase and proten kinase p38 mitogen activated. Administration of

20 mg/kg UA per os during 6 weeks resulted in a significant reducing of oxidative stress markers in hepatic cancer DENA (diethylnitrosamine) induced to Wistar rats [59]. In accordance with authors' opinion, these data support the UA role as prophylactic drug in cancer prevention. The UA activity was examined also in vivo on hepatic tumor H22 [60]. Shao et al. (2011) demonstrated that UA usage at 100 mg/kg could lead to a significant inhibition of tumor growing.

A promising result of UA action was reported and in case of neural origin tumor. Wang et al. (2012) studied the behavior of glioma cells U251 after treating with this triterpene [61]. Authors realized that UA activated caspase-3 and suppressed miR-21(microRNA-21) at concentrations of 5-20 μM. The final effect was expressed by blocking tumor cells proliferation and apoptosis inducing.

The wide chain of UA function is supplemented by Tokuda's et al. (1986) research on skin tumors induced by TPA (tetradecanoyl-phorbol-13-acetate) [62]. Authors concluded that this triterpene could inhibit tumors growing, in a manner similar to retinoic acid, well-known for its anti-tumor activity. Kowalczyk et al. (2009) reported that UA is a very effective agent to prevent skin cancer, because it can block mutation occurrence in 61 codon of Ha-ras oncogene [63].

The ursolic acid utilization to prevent chemoresistance development

The resistance developed by the tumors to specific therapy, chemo or radio, is one of the main reasons of recurrences and neoplastic progression. In drug resistance development were involved a series of MDR mediators (multi-drug resistance proteins), as well factors with anti-apoptotic function [64]. Shan et al. (2011) demonstrated that UA has ability to block MDR proteins in case of intestine tumor clones (SW480, SW620), leukemia cells HL60, HL60/ADR, K562, K562/ADR and breast carcinoma cell lines (MCF7 şi MCF7/ADR) [48]. Moreover, this compound was very effective and in case of very aggressive HepG2, doxorubicin-resistant clones [65].

The biological activity of ursolic acid derivatives

A promising biological activity manifest and UA derivatives, frequently more emphasized as incipient chemicals. As we mentioned above, a remarkable anti-proliferative effect demonstrated 2α-hydroxyursolic acid in breast carcinoma cell lines [52]. A series of new derivatives were synthesized on acyl piperazin base. In accordance with Liu et al. (2012) opinion, these chemicals showed an inhibitory action significantly higher than clean UA in case of gastric carcinoma cells (MGC-803) and breast cancer (Bcap-37) [66]. These results are a confirmation of previous data published by Ma et al. (2005), who highlighted the cytotoxic activity of 2α-hydroxyursolic acid on 4 tumor cell lines, as HL-60 (*human promyelocytic leukemia* cells), BGC (*gastric cell line*), Bel-7402 (*hepatic carcinoma* cell *line*) and HeLa (*cervical cancer cell line*) [67].

The recent presentation of Chen et al. (2011) argues the development of new derivatives of UA [68]. Authors demonstrated that UA derivatives obtained on the furoxan (or 1,2,5-oxadiazole 2-oxide) base have a higher cytotoxic potential than native chemical, applied on HepG2 tumors. Tanaka et al. (2012) support the idea that UA derivatives obtained through oxidation with dioxoruthenium-VI- tetraphenylporphyrine had an enhanced cytotoxicity (in comparison to UA) on glioma C6 and skin carcinoma A431 cell lines [69].

A new possibility of UA derivatives obtaining was recently presented by Leipold et al. (2010) [70]. Authors have metabolized UA with 3 clones of gram-positive *Nocardia* bacteria (NRRL 44000, 44822 and 5646). As a result of these biotechnological assays, researches obtained a mixture of UA derivatives: ursolic acid methyl ester, ursonic acid, ursonic acid methyl ester, 3-oxoursa-1,12-dien-28-oic acid and its methyl ester. The acetylating of UA at C-3 position, combined with amino alcohol acetate coupling at C-28 had increased significantly its anti-proliferative activity. In accordance with Meng et al. (2009) these compounds were very effectively applied on different tumor cell lines, such as HeLa, SKOV3 and BGC-823 [71].

A recent study presented by Bai et al. (2012) emphasizes 2 groups of UA derivatives, in accordance with their electrical properties: group I, negatively charged and group II, with positive charge [72]. These derivatives had ability to block cell cycle and stimulate apoptosis in several tumor cell lines: HepG2, AGS, HT-29 and PC-3. It is necessary to mention that cytotoxic effect of group II was more pronounced than group I and UA. Plus, authors synthesized 3β-acetoxy-urs-12-en-28-oyl-1-mono-glicerid derivative, able to induce apoptosis in BGC-823 cells [73].

Shao et al. (2011) tested another UA derivative, N-[3β-acetoxy-urs-12-en-28-oyl]-2-aminodiethanol [60]. This compound showed remarkable pro-apoptotic activities applied on HepG2, BGC-823, SH-SY5Y, HeLa and HELF tumor cells.

Promising effects demonstrated heterocyclic derivatives of UA, obtained by Leal et al. (2012) [74]. New compounds could induce the p53, p21waf1 and NOXA synthesis, effects summarized as anti-proliferative activity in pancreatic carcinoma cells AsPC-1.

An innovative method was purposed by Zhang et al. (2013), which consists in using nanoparticles UA charged (UA-NPs) [75]. In their assays, authors transported effectively this complex into gastric carcinoma cells SGC-7901. The results pointed out a strong inhibition of COX-2 and caspase-3 activation, effects which lead to apoptosis and cytotoxicity.

Conclusion: ursolic acid is a promising compound in tumor prevention and treatment, with many mechanisms of action on cell's proliferation. Its derivatives usually are more biologically effective than initial compound, so obtaining of new ursolic derivatives makes further investigations in this field have a particular relevance.

Conflict of interests

The author declares that there is no conflict of interests regarding the publication of this paper.

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Matrix metalloproteinases in the development of varicose disease

I. Mahovici, *M. Gaina

Nicolae Anestiadi Department of Surgery, Nicolae Testemitsanu State University of Medicine and Pharmacy Chisinau, the Republic of Moldova

*Corresponding author: gaina.maryna@gmail.com. Received October 19, 2015; accepted December 07, 2015

Abstract

Background: The imbalance between extracellular matrix components is a consequence of MMPs activity. Increased expression of matrix metalloproteinases (MMPs), in particular type 2 and 9, has been identified in varicose veins. MMP-2 and -9 have acute venodilatory effect in addition to their known effects on the extracellular matrix. The data suggest that protracted increases in venous pressure and wall tension increase MMPs expression, which in turn reduce venous contraction and lead to progressive venous dilation. Increases in magnitude and duration of wall tension are associated with reduced contraction and overexpression of MMP-2 and -9. MMP-2 and -9 promote inferior vena cava relaxation. MMP-2 did not inhibit venous contraction during membrane depolarization by high KCl, suggesting that MMP-2 induced relaxation likely involves hyperpolarization and activation of a K^+ channel. MMPs cause hyperpolarization of the smooth muscle cells of the vein wall, leading to prolonged opening of Ca^{2+}-dependent K^+ channel (BK_{Ca}).
Conclusions: MMP-2 causes significant inhibition of Phenylephrine and Angiotensin II-induced IVC contraction likely through a post-receptor mechanism involving activation of plasmalemmal K^+ channels, membrane hyperpolarization, and inhibition of Ca^{2+}influx. MMP-2 induced inhibition of the Ca^{2+} entry mechanism of venous smooth muscle contraction may play a role in the venous dilation associated with varicose vein formation. Studying the mechanisms of action of MMPs is an important step in the development of new treatment methods of varicose veins, such as synthetic inhibitors of matrix metalloproteinases.
Key words: varicose, dilatation, MMPs, extracellular matrix, hyperpolarization.

Introduction

Throughout time phlebology has been the subject of much research and workings, and today the theme is more actual than ever due to numerous progresses in research, to the development of diagnosis and treatment technologies, and the people's medico-sanitary conscience evolution [1]. Varicose veins affect up to 25 percent of women and 15 percent of men. By the age of 50, nearly 40 percent of women and 20 percent of men have significant leg vein problems [2].

Studying this theme is too important because currently there are a number of treatments: sclerotherapy, laser therapy and surgical treatment, all accompanied by administering of phlebotonics and wearing bandages compression, but none of them resolves the problem, and recurrent varicose veins is an evidence of that. Despite medical and socio-economic consequences of varicose veins, the pathophysiological mechanisms involved are not fully understood. Although there were identified risk factors for varicose veins, such as female gender, pregnancy, obesity, aging, family history, the molecular mechanisms underlying pathogenicity and progressive varicose veins remain unclear. The tendency of most studies is hereditary component orientation and the genetic pathogenesis [1,3,4].

For example, the genetic mutations in iron metabolism genes may play a role in varicose veins (VVs). Prolonged venous reflux is associated with iron overload and dermal hemosiderin deposition that is directly correlated with clinical symptoms of chronic venous insufficiency (CVI) including skin changes and lipodermatosclerosis. Iron deposition may induce the formation of free radicals which can cause further tissue injury, and progression to advanced forms of CVI and leg ulcers. Also, Factor XIII is a cross-linking protein that plays a key role in ulcer healing. Mutations in hemochromatosis C282Y (HFE) gene and Factor XIII V34L gene variants have been identified in patients with chronic venous disease (CVD) and have been associated with increased risk of severe forms of CVI, skin changes and the size of venous ulcers. Some clinical conditions support a genetic component of VVs. Patients with Klippel-Trenaunay Syndrome have congenital venous anomalies in the form of atresia, agenesis of the deep venous system, valve insufficiency, venous aneurysms, and embryonic veins [4].

The primary cause of varicose vein formation is not clear; however, both vein valve dysfunction and hydrostatic venous pressure appear to play a critical role in the initiation and progression of the disease. Although valve-reflux may precede vein-dilatation,there is a significant body of evidence supporting the view that vein dilation can precede venous reflux, and that valvular dysfunction may be an epiphenomenon of vein wall dilation. Clinical studies have demonstrated that venous insufficiency can occur in varicosities without axial reflux of the superficial, deep or perforated veins, and that an imbalance in extracellular matrix proteins may cause connective tissue changes prior to valvular insufficiency [5].

Recent studies suggest that the balance between vascular proliferation, extracellular matrix deposition and degradation can be disturbed leading to loss of mechanical strength of the wall, venous dilatation and elongation. Fundamental changes in varicose dilatation involving cross linking and structural changes in the vein wall, inclusive fragmentation and disorganization of elastin smooth muscle [5].

Advanced stages of varicose veins are characterized by degeneration of the extracellular matrix, the endothelium and of smooth muscle cells of the vein wall. Histologically is modified extracellular matrix that shows the fragmentation of elastic blades, loss of circular and longitudinal fiber of smooth muscle and deterioration of endothelium [6,7]. Venturi [8, 9] and his co-workers have shown a decrease in desmosine, isodesomsinei and elastin collagen ratio in varicose veins compared to normal veins. C. Michiels [9] has shown that hypoxia induced leukocyte activation and generation of free radicals, activation of proteases, and therefore, the degradation of the extracellular matrix. Additionally, they have demonstrated that hypoxia activates endothelial cells to secrete growth factors and stimulate smooth muscle cell (SMC) proliferation and synthesis of extracellular matrix. These studies suggest that the disturbance in the synthesis and degradation of structural elements appear in the segments of varicose veins. The degradation of extracellular matrix triggeres the leukocyte infiltration and activates the inflammation which damages the vein wall. Vascular wall remodeling depends on the activity of macrophages, SMC, endothelial cells and fibroblasts. The extracellular matrix is made of SMC and fibroblasts and is degraded by enzymes secreted by the macrophages, such as matrix metalloproteinases (MMPs). The change in collagen-elastin ratio, which is observed in varicose veins, indicates an imbalance in the connective tissue matrix. Also, there are observed significant variations in the ratio of collagen type I and type II and increasing the activity of MMP, in particular 2 to 9 [4, 7]. All this has determined the shift towards the new direction of study. The research is based on the treatemet methods discovery which is focused physiopathologically at cellular and molecular level. The article is based in particular on the work of West Roxbury vascular surgeon and researcher Joseph D. Raffetto [3,5,7], professor at the University of Massachusetts.

Recent studies are based on the research of MMPs in the development and progression of varicose veins. MMPs were described initially by Jerome Grossand and Charles Lapiere [10, 11], who observed enzymatic activity (collagen triple helixdegradation) during tadpole tail metamorphosis (by placing a tadpole tail in a collagen matrix plate). Therefore, the enzyme was named interstitial collagenase (MMP-1). Later, it was purified from human skin [10]. Since then, the MMP family has grown to include at least 28 members in vertebrates, 23 in humans, and 14 in blood vessels [4].

MMPs are also called matrixins, they are multidomain zinc metalloproteinases that degrade various components of extracellular matrix (ECM) and belong to the larger superfamily of proteases called metzincins, which also includes adamalysins, serralysins, and astacins. Sequence homology with the catalytic domain of MMP-1 (collagenase 1) is a common feature of all members of the MMP family. To classify

MMPs there have been employed several methods. MMPs are classified as the matrixin subfamily of zinc metalloprotease family. The most common methods of classification MMPs are based on organization domain and substrate preference into collagenases, gelatinases, stromelysins, matrilysins, membrane-type (MT)-MMPs and others [4]. From the evolutionary point of view MMPs have been classified depending on their primary sequence into 6 subgroups (A–F): subgroup A (MMP-19, -26, -28), B (MMP -11, -21, -23), C (MMP-17, -25), D (MMP-1, -3, -8, -10, -12, -13, -27), E (MMP-14, -15, -16, -24), and F (MMP-2, -7, -9, -20) [13]. Thus MMP 1 is interstitial collagenase and its substrate is fibrillar collagen, MMP 2 and 9 are the gelatinases which acts on collagen type IV, V, collagen degraded and elastin, and MMP3 is stromelysin and has the substrate proteoglycans, fibronectin, laminin, pro MMP-1 9. Other subgroups include: matrilysin, enamelysin and metalloelastase macrophagal [13].

Matrix metalloproteinases are excreted by connective tissue and a variety of pro-inflammatory cells including fibroblasts, osteoblasts, endothelial cells, macrophages, neutrophils, and lymphocytes. These enzymes are expressed as zymogens, which are subsequently processed by other proteolytic enzymes (such as serine proteases, furin, plasmin, and others) to generate the active forms. Typically MMPs consist of a propeptide of about 80 amino acids, a catalytic metalloproteinase domain of about 170 amino acids, a linker peptide (hinge region) of variable lengths and a hemopexin domain of about 200 amino acids. The catalytic domain contains the Zn^{2+} binding motif HEXXHXXGXXH and a conserved methionine [11].

Most of the matrix metalloproteinases consist of four distinct domains, which are N-terminal pro-domain, catalytic domain, hinge region, and C-terminal hemopexin-like domain. This may be responsible for the macromolecular substrate recognition as well as for interaction with tissue inhibitors of metalloproteinases (TIMPs). The membrane-type MMPs contain an additional transmembrane domain that anchors them in the cell surface [14].

MMPs are synthesized as pre-proenzymes. The signal peptide is removed during translation and proMMPs are generated. Activation of these zymogens is therefore an important regulatory step of MMP activity.

Thirteen MMPs are secreted from the cell as proMMPs. The presence of a proteinase susceptible "bait" region in the propeptide allows tissue and plasma proteinases or opportunistic bacterial proteinases to activate proMMPs. Cleavage of the bait region removes only a part of the propeptide and complete removal of the propeptide is often conducted in trans by the action of the MMP intermediate or by other active MMPs [15].

MMPs degrade different components of ECM including collagen, casein and laminin. MMPs also modulate many bioactive molecules at the cell surface, and may regulate the cellular environment via interaction with G-protein coupled receptors. MMPs may play a role in cell proliferation, migration (adhesion/dispersion), differentiation and apoptosis, as well as physiological processes such as immune function, tissue healing, and angiogenesis. Matrixins participate in many normal biological processes (embryonic development, blastocyst implantation, organ morphogenesis, nerve growth, ovulation, cervical dilatation, postpartum uterine involution, endometrial cycling, hair follicle cycling, bone remodeling, wound healing, angiogenesis, apoptosis, etc.) and pathological processes (arthritis, cancer, cardiovascular disease, nephritis, neurological disease, breakdown of blood brain barrier, periodontal disease, skin ulceration, gastric ulcer, corneal ulceration, liver fibrosis, emphysema, fibrotic lung disease, etc.). Although the main function of matrixins is the removal of ECM during tissue resorption and progression of many diseases, it is notable that MMPs also alter biological functions of ECM macromolecules by specific proteolysis. For example, MMP-2 released by growth cones promotes neurite outgrowth by inactivating neurite-inhibitory chondroitin sulfate proteoglycans, thereby unmasking the neurite-promoting activity of laminin [11]. Among lung injuries, MMPs have been implicated in acute respiratory distress syndrome and chronic obstructive pulmonary disease, which encompasses both emphysema and chronic bronchitis. There has been considerable interest in the role that MMPs play on the progression of tumours to metastatic neoplasms. In neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease, MMPs have been shown to play an important and complex role. MMPs have been shown to increase blood-brain barrier permeability that can cause oedema, haemorrhage and cell death and it is thought to play a role in white matter damage [16].

MMPs are regulated at multiple levels including transcription, secretion, and activation of the zymogen forms, extracellular inhibition and internalization by endocytosis. MMP activity is positively modulated by ions and reagents that induce MMP cleavage and activation. Zn^{2+}chelators suppress MMP activity by depriving MMPs from the Zn^{2+}critical for their activity. Cu^{2+}ions may decrease MMP-2 secretion. MMPs are also inhibited by both endogenous and exogenous inhibitors. Under normal physiological conditions, the proteolytic activity of the MMPs is controlled at any of the following three known stages: activation of the zymogens, transcription, and inhibition of the active forms by various TIMPs. In pathological conditions this equilibrium is shifted toward increased MMP activity leading to tissue degradation [14].

The role of MMPs in VV has been largely attributed to their proteolytic effects on ECM, degradation of the valve leaflets and weakening of vein wall structure. The localization of MMPs in the VV wall adventitia and fibroblasts is consistent with a role in ECM degradation [11].

The last two decades have made a great progress in understanding the role of MMPs in the development and progression of varicose veins. Although MMP effects are generally attributed to the degradation of extracellular matrix, their effects on the mechanisms of contraction / relaxation vein are unclear. In order to loose this enigma Joseph D. Raffetto and his team conducted a series of research.

The purpose of the first study was to test the hypothesis that prolonged increases in vein wall tension cause overexpression of MMPs and decreased contractility promotes

venous dilation. The purpose of the second study was to test whether MMP-2 induced venous relaxation involves an endothelium-dependent mechanism, or an endothelium-independent inhibition of venous smooth muscle contraction. The task was to test this novel hypothesis, on evaluated effects of MMP-2 in inferior vena cava in the presence and absence of endothelium, in the presence and absence of inhibitors of endothelium-derived vasodilators, and during modulation of K^+channel activity by depolarizing solutions and by activators and blockers of K^+channels. Another study determined the mechanism which inhibits MMPs Ca+2 channels and generates venous dilation.

For this were used inferior vena cava obtained from rats of 12 weeks, weighing 250-300g, housed in the animal facility and fed standard rat chow and tap water in 12 hours / 12 h light / dark, then they were euthanized by CO2 inhalation. Inferior vena cava (IVC) were quickly excised, placed in Krebs solution, carefully dissected and cleaned of connective tissue under the microscope. IVC has been divided proportionally in four rings of 3 mm.

For the first experiment 4 vein segments were mounted on the wire hooks in different tissue baths at no specific order to minimize the effects of variability in tissue size on the observed contractile response. Unless indicated otherwise, vein segments were stretched under 0.5g of basal tension and allowed to equilibrate for 1 hr in a temperature controlled, water-jacketed tissue bath, filled with 50 mL Krebs solution continuously bubbled with 95% O_2, 5% CO_2 at 37°C. The changes in isometric contraction were recorded on a Grass polygraph. To determine the control contraction, IVC segments were stimulated twice with 96 mmol/L KCl solution. To test the IVC relaxation function, the tissues were stimulated with phenylephrine (Phe 10^{-5}mol/L) to achieve a steady-state contraction, and then treated with acetylcholine (Ach, 1^{-5}mol/L) to confirm the presence of intact endothelium. The veins were frozen to determine the expression and localization of MMPs using immunoblots and immunohistochemistry. These MMP-2 and -9 concentrations are physiologically consistent with the plasma and vein tissue levels in human which range between 1000 ng/g tissue (~1 mcg/ml) and 100 mcg/g tissue (~0.1 mcg/ml) [5].

In the second experiment there were measured the effects of MMP-2 on Phe- and KCl-induced contraction. To study the role of endothelium-derived vasodilators, the experiments were performed in the presence and absence of endothelium, L-nitro-arginine methyl ester (L-NAME), inhibitor of nitric oxide (NO) synthesis, indomethacin, inhibitor of prostacyclin (PGI_2) synthesis, cromakalim, activator of ATP-sensitive K^+channel (K_{ATP}); and iberiotoxin, blocker of large conductance Ca^{2+}-dependent K^+channel (BK_{Ca}) and smooth muscle hyperpolarization [3].

To evaluate the effects of MMP-2 on Ca^{2+} entry, IVC segments nontreated or pretreated with MMP-2 were incubated in Ca^{2+}-free Krebs solution for 5 min, stimulated with Phe (10^{-5}M), then increasing extracellular $CaCl_2$ concentrations ($[Ca^{2+}]_e$) were added and the contractile response was measured after 5 min in each $[Ca^{2+}]_e$. To further test the effect of MMP-2 on Ca^{2+} entry, the effect of MMP-2 on Phe contraction was tested in IVC segments treated with the Ca^{2+} channel blocker diltiazem (10^{-5}M) [7].

Later it was tested if the prolonged increases in vein wall tension affect venous contraction. Phe activates α-adrenergic receptors, and angiotensin II (AngII) activates angiotensin type 1 receptors. It was found that the prolonged increases in basal tension are associated with reduction in Phe-induced IVC contraction, which may not be specific to α-adrenergic mediated responses as AngII-induced contraction was similarly reduced under these conditions. The observation that the reduced IVC contraction in tissues subjected to prolonged basal tension was reversed in tissues treated with TIMP-1, suggested possible involvement of MMP-2 and -9. The immunoblot analysis in IVC suggested increased expression of MMP-2 and -9 during prolonged increases in basal tension. Also, immunohistochemical staining suggested localization of MMP-2 and -9 in the three layers of the vein wall. Additionally, prolonged increases in wall tension were associated with relative increases in MMP-2 and -9 in the vicinity of the smooth muscle layer, suggesting an effect on the contractile cells. Also, the observation that prolonged tension was associated with reduced contraction not only to Phe but also to AngII indicates that the reduction in contraction is not specific to a particular receptor. Furthermore, the Phe and AngII contraction were restored in veins treated with TIMP-1, suggesting that the reduction in contraction was not due to reduction in the Phe or AngII receptors, but rather it was due to increased MMP. It was also shown that MMP-2 causes significant inhibition of Phe- and AngII-induced IVC contraction likely through a post-receptor mechanism involving activation of plasmalemmal K+channels, membrane hyperpolarization, and inhibition of Ca2+influx. However, these studies do not exclude the possibility that the presence/activation of MMP-2 may lead to activation of other MMPs. MMP-2 and -9 have acute venodilatory effect in addition to their known effects on the extracellular matrix. MMP-2 and MMP-9 induced inhibition of Phe contraction in vascular segments is dose-dependent. Further, it was demonstrated that the venodilator effects of MMP-2 and -9 are time-dependent. MMP-2 could cleave big endothelin-1 yielding a novel vasoconstrictor and thereby enhance vascular contraction, an effect that will not be manifested in endothelium-denuded tissues. MMP-2 induced venous relaxation could also be due to the enhanced release of nitric oxide (NO), prostacyclin (PGI2) or endothelium-derived hyperpolarizing factor (EDHF). It was established that the NO synthesis (NOS) inhibitor L-NAME did not attenuate, and instead, enhanced MMP-2 induced IVC relaxation. MMP-2 did not increase NO production. Although the data suggest that MMP-2 induced venous relaxation may not involve increased NO production, they do not rule out possible interaction between the NO pathway and MMP. NO, being a major vasodilator may downregulate the effects of MMP-2 on other endothelium-derived vasodilators, and NOS inhibition may unmask these effects. As well, the studies have shown that NO inhibition may increase MMP-9 expression in rat vascular smooth muscle cells. MMP-2 may also directly or indirectly through increased endothelin-1 production, induce NO synthesis (NOS) uncoupling and lead to

increased superoxide production and decreased NO bioactivity. It has also detected that the MMP-2 induced venous relaxation was not affected by the cyclooxygenase inhibitor indomethacin, suggesting that it does not involve increased PGI2synthesis and activation of PGI2-cAMP relaxation pathway. The inability of NOS and cyclooxygenase inhibition to block the MMP-2 induced venous relaxation raises the possibility of MMP-2 mediated release of EDHF leading to enhanced K+ efflux via K+ channels and venous smooth muscle hyperpolarization and relaxation. The high extracellular KCl creates a K+ concentration gradient that does not favor K+ efflux through plasma membrane K+ channels. MMP-2 did not inhibit venous contraction during membrane depolarization by high KCl, suggesting that MMP-2 induced relaxation likely involves hyperpolarization and activation of a K+channel. K+ channels include the ATP-sensitive K+ channel (KATP), large conductance Ca2+-activated K+channel (BKCa), intermediate and small conductance Ca2+-activated K+ channel, voltage-gated K+ channels, and inward rectifier K+ channels. Activation of K+ channels likely causes smooth muscle hyperpolarization, and leads to decreased Ca2+influx through voltage-gated channels. MMP-2 can also activate directly the K+ channels. MMP may facilitate a conformational change in the BKCa channel from a closed state to an open state, during changes in voltage and intracellular Ca2+. As well the MMP-2 may activate K+ channels through specific protease-activated receptors (PARs). PARs are activated by serine proteases such as thrombin, trypsin, and tryptase. The trypsin-induced PARs-mediated relaxation was inhibited by KCl-precontraction, or pretreatment with apamin or charybdotoxin, blockers of small and intermediate Ca2+-activated K+channels, respectively. These facts suggest that PARs-mediated relaxation of the vasculature involves hyperpolarization of vascular smooth muscle and activation of Ca2+-activated K+ channels. In vascular smooth muscle, agonist-receptor interaction is coupled to increased release of inositol-1,3,5-trisphosphate (IP3) and activation of plasma membrane Ca2+ channels. IP3 induces Ca2+ release from the intracellular Ca2+ stores. Parallel activation of Ca2+ channels promotes Ca2+ influx from the extracellular space. In Ca2+-free Krebs solution, Phe and AngII produced transient contraction, suggesting that they activate IP3-induced Ca2+ release. Diltiazem inhibited Phe-induced contraction, suggesting the presence of functional voltage-gated Ca2+ channels in IVC. Moreover, diltiazem did not cause any further relaxation in MMP-2 treated IVC, indicating that the L-type channels are already inhibited by MMP-2. Importantly, MMP-2 caused further relaxation in diltiazem treated IVC, suggesting that during blockade of L-type Ca2+ channels, MMP-2 may inhibit other subclasses of voltage-gated channels (T- or N-type) or perhaps ligand-gated, store-operated Ca2+ channels or non-specific cation channels [3, 5, 7].

Conclusions

While inherent differences in the physiologic behavior and structure of rat veins and human veins make it difficult to extrapolate the response of rat IVC to human veins, the results indicate that the magnitude and duration of vein wall tension have significant impact on venous function and MMP expression. It was established that increases in basal tension cause a significant and irreversible reduction in IVC contraction, suggesting structural changes and irreversible vein damage. Increases in magnitude and duration of wall tension are associated with reduced contraction and overexpression of MMP-2 and -9. MMP-2 causes relaxation of Phe contraction in IVC segments by a mechanism involving hyperpolarization and activation of BKCa. Large amounts of MMP-2 have been detected in the plasma and venous tissues of patients with varicose veins. In addition, it is possible that some of the effects of MMP-2 can be due to activation of other endogenous MMPs. However, MMP-1, -3, -9, and -13 are also expressed in human varicose veins. The obtained results are the first step in the establishment of a new phlebology development direction. Understanding the molecular basis of VVs formation and MMPs-induced changes in endothelial cells (Ecs) and vascular smooth muscle (VSM) function and vein wall remodeling will provide valuable information on the mechanisms involved in CVD development and progression. Using human veins, obtained by minimal surgical manipulation can reveal not only the effects of MMPs on the veins, but can also divert treatment for use of MMPs inhibitor in the treatment of varicose veins. Previous attempts to study the effects of MMP inhibitors, such as doxycycline, batimostat and marimostat have been carried out, but they have significant side effects, especially on the musculoskeletal system. A deeper study of MMPs and their effects can lead to the creation of new synthetic inhibitors and their potential use. In the context of accelerated technological progress all they may become phlebology targets tomorrow.

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Update on type 2 cardiorenal syndrome

***E. Bivol[1], L. Grib[1], B. Sasu[2]**

Department of Internal Medicine, [1]Clinic of Cardiology, [2]Clinic of Rheumatology and Nephrology
Nicolae Testemitsanu State University of Medicine and Pharmacy, Chisinau, the Republic of Moldova
*Corresponding author: bivol.e@gmail.com. Received October 30, 2015; accepted December 07, 2015

Abstract

Background: Cardiorenal syndrome type 2 is an "umbrella" term used to describe clinical conditions in which chronic cardiac failure through a chronological and causal relationship leads to renal dysfunction. The syndrome is associated with a significant morbidity and mortality, that is why it has recently become a matter of growing debate related to pathogenesis, diagnosis, treatment effectiveness and safety. Our aim was to review epidemiological and pathological mechanisms underlying cardiorenal syndrome, to focus on up-to-date diagnosis and treatment strategies. We performed literature search in the Pubmed database in July 2015. The 1st key word used for search was "cardiorenal syndrome type 2"; and the 2nd key word was "cardiorenal syndrome in heart failure".

Conclusions: Over the last decade, a significant advance in the understanding of the cardiorenal syndrome has been achieved. However, precise pathways remain to be clarified. Clinical management of these patients include diuretics, vasodilatators, ultrafiltration, all these modalities promise more rapid volume removal, but their ultimate impact on survival and renal function is unknown. Future research is necessary to improve diagnosis, severity grading, to differentiate type 2 and type 4 cardiorenal syndrome and to determine efficient treatment strategies. Because of the syndrome's complexity and poor outcome, it is important that cardiologists, nephrologists and internists work together for a unique goal – protecting the patient with cardiorenal syndrome.
Key words: cardiorenal syndrome type 2, heart failure, biomarkers.

Introduction

Many organ systems are tightly connected. In normal state, this connection helps maintain optimal homeostasis and function of the human body. In pathology, however, an affected organ may initiate and perpetuate structural and functional dysfunction in other connected organs [1, 2]. Thus, acute or chronic heart and kidney diseases often coexist in the same patient. Observational studies and clinical trials have proven that acute / chronic heart disease can directly contribute to acute / chronic kidney disease worsening and vice versa. Considering the close and bidirectional relationship between these two organs, Acute Dialysis Quality Initiative recently proposed a consensus definition and classification. The term cardiorenal syndrome (CRS) is used to identify cardiac and renal disorders as "a complex pathophysiological condition in which acute or chronic dysfunction in one organ can cause acute or chronic dysfunction in the other" [1].

The prevalence of both heart failure and chronic kidney disease in Europe is continuously increasing [2,4,5]. In any case / any genesis the association of heart and kidney dysfunction is accompanied by an increased risk of morbidity and mortality [4,5].

Classification [1]

Acute cardiorenal syndrome (type 1)

Acute worsening of heart function leading to kidney dysfunction and /or damage. This type of injury occurs more frequently as a complication of acute heart failure and / or acute coronary syndrome. It occurs in 27-40% of patients hospitalized with acute heart failure and 70% of patients with cardiogenic shock. In these patients morbidity, length of stay and mortality increases.

Chronic cardiorenal syndrome (type 2)

Chronic heart disease leading to renal dysfunction or injury. This syndrome is frequently encountered, occurs in 63% of hospitalized patients with congestive heart failure. A meta-analytic study focused on the heart failure (IC) - renal dysfunction interrelationship reported a prevalence of 63% mild and 20% moderate renal impairment. In addition, there was a 7% increase in mortality for every decrease in glomerular filtration rate (GFR) of 10 mL / min [4].

Acute renocardiac syndrome (type 3)

Acute worsening of renal function leading to cardiac dysfunction or damage. The incidence is 10-53%.

Chronic renocardiac syndrome (type 4)

Chronic kidney disease (CKD) leading to heart dysfunction, injury and/or disease, dysfunction or heart damage. The

incidence is unknown and difficult to appreciate (depending on primary renal disease incidence), but was noted an increases by 50% in cardiovascular (CV) mortality.

Secondary cardiorenal syndrome (type 5)

Systemic diseases leading simultaneously to renal and cardiac dysfunction/damage (ex. sepsis, diabetes, lupus erythematosus). The incidence and severity depend on systemic disease incidence and severity.

Cardiorenal syndrome (CRS) can be acute, chronic or secondary, of cardiac or renal genesis. However, the classification is not static, it is generally accepted that patients suffer various types of CRS during disease (ex. 1↔2; 3↔4; 2,4↔5) [3] and all types are associated with increased mortality and morbidity, having a significant impact on health care costs [2, 3].

Epidemiology

CRS syndrome (type 2) occurs when a chronic heart condition leads to chronic renal dysfunction. There are several observational studies describing the coexistence of chronic heart failure (CHF) and chronic kidney disease (CKD), but usually, studies enroll subjects based on the presence of a disease (ex.: HF) and describe the prevalence of the other (ex.: CKD) [2, 4].

A meta-analytic study focused on the heart failure (IC) - renal dysfunction interrelationship reported a prevalence of 63% mild and 20% moderate renal impairment. In addition, there was a 7% increase in mortality for every decrease in glomerular filtration rate (GFR) of 10 mL / min [1,2,4]. These types of studies are not able to identify which was the primary pathology in order to classify properly the CRS. In such cases, the use of the term SCR type 2/4 was suggested [5]. Another study focused on congestive heart failure outpatients, established severe renal impairment (creatinine clearance < 30 ml / min) in 39% of the HF patients with functional class (FC) IV and 31% of the HF patients with FC III NYHA [5].

A comparative analysis of studies focused on the renal dysfunctions prevalence in HF patients shows the following data:

- The SOLVD study conducted in 2000, on a cohort of 2161 patients with ejection fraction of 35.7%, recorded 24.7% cases of renal impairment (GFR <60 mL / min) [13];
- The PRIME II study conducted in 2000, on a cohort of 1906 patients with ejection fraction of 49%, recorded 26.2% cases of renal impairment (GFR <58ml / min) [13];
- The ANCHOR study conducted in 2006, on a cohort of 59 772 patients, reported 39.2% renal impairment cases (GFR 60 mL / min) [14];
- The JCARE-CARD study conducted in 2009, on a cohort of 2013 patients with ejection fraction of 70.3%, reported 44.8% cases with renal impairment (GFR 60 mL / min) [13,15];

In the recent years, the global prevalence of the moderate-severe renal dysfunction gradually increased, up to an epidemic state [9.13]. The HF "epidemic" is also increasing due to aging and post-myocardial infarction survival improvement [8.10]. The risk of CKD occurrence in heart failure is not well-established, but kidney dysfunction is very often encountered in HF patients and it is associated with a poor prognosis [5,13].

Renal function is a prognostic marker as important as ejection fraction and NYHA functional class [5.13].

Pathogenesis

In HF patients who develop renal dysfunction, there are intrinsic interactions between these two organs (organic cross

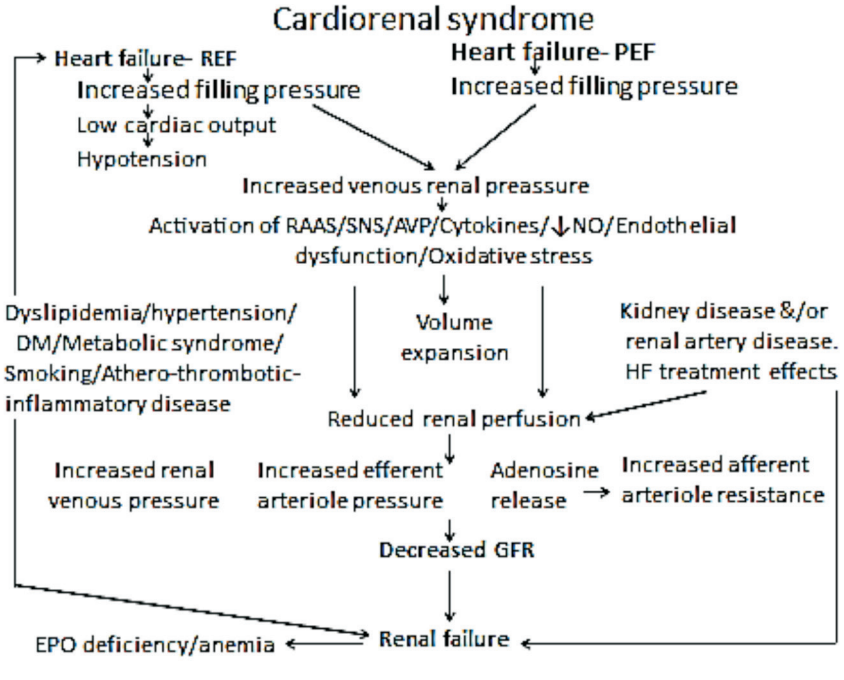

Fig. 1. Cardiorenal syndrome mechanisms.

talk) that could lead to severe complications [1,2,4]. Any HF underlying mechanisms, plus existing comorbidities, and / or their treatment affect renal function with subsequent renal failure development (fig. 1) [1,2,17].

The CRS pathogenesis is multifactorial including structural lesions caused by atherosclerosis, hemodynamic changes, neurohormonal and inflammatory components effects [1,5,17].

The hypothesis of low cardiac output. Over the past decade, progressive worsening of renal function in HF is considered as a direct result of the renal flow reduction caused by decreased cardiac output. Inadequate afferent renal flow activates RAAS - leading to volemic retention, increased preload and impaired pump function [17]. Recent studies state that, although it is correct, this mechanism does not fully explain the CRS features. The ESCAPE Study (Evaluation Study of Congestive The Failure and Pulmonary Artery Catheterization Heart Effectiveness) evaluated management through guided pulmonary artery catheterization in 400 patients; no correlations were found between renal function and cardiac index; and, at cardiac index improvement renal function has not changed [18], moreover, impaired renal function was proven despite the preserved ejection fraction.

The renin-angiotensin-aldosterone system (RAAS) activation - RAAS activation at renal perfusion depression is a protective mechanism in dangerous situations (ex. hemorrhage). The chronic stimulation - in heart or renal failure, has adverse consequences on both, heart or kidney. Angiotensin II has multiple negative effects on the cardiovascular system in HF patients, increases both pre- and afterload with a subsequent increase in myocardial oxygen demand [1.18]. Angiotensin II activates NADPH oxidase in endothelial cells, renal tubules and cardiomyocytes, releasing free radicals responsible for aging, inflammation and progressive organ dysfunction [1].

Sympathetic nervous system activation (SNS) – SNS activation has initially a protective character, overactivation, however, reduces the myocardial beta-adrenoreceptors density and adrenoreceptors sensitivity both in heart and renal failure [17,26]. SNS induces cardiomyocyte apoptosis and increases the neuropeptide Y release, which is a promoter of vascular growth, accelerates atherosclerosis, induces vasoconstriction and interferes with the normal immune system functions.

Intra-abdominal hypertension. HF patients have increased central venous pressure, which reduces the renal capillary perfusion gradient. It was established that HF patients with renal changes had higher central venous pressure than those without impaired renal function [17]; also, both high venous pressure and jugular pressure correlated with increased creatinine levels [16].

Cardiorenal syndrome anemic. Anemia occurs frequently in patients with HF and in 30% patients with CRS, being caused by renal and heart failure progression, but also by iron deficiency as pointed out the authors of the FAIR-HF study (Ferinject Assesmentin Patients with Iron deficiency and chronic Heart Failure) [26]. At this time the role and treatment of CRS anemia remains controversial.

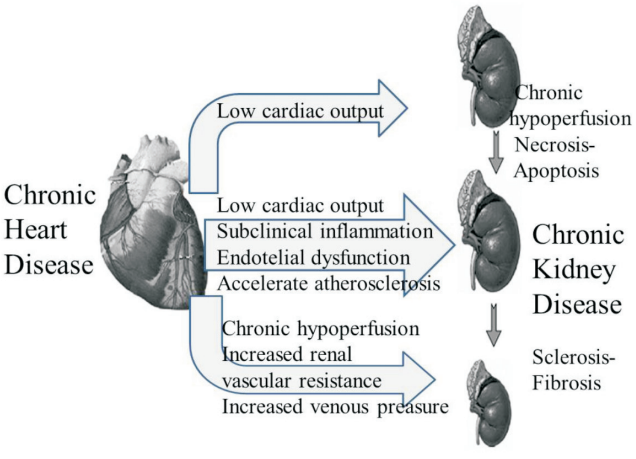

Fig. 2. CRS appearance assumptions. Reproduced from C. Ronco, 2010 [1].

Diagnosis

The prevalence of symptomatic CHF in the European population is 2-3% [8]. The mortality rate increases in concordance with the functional class (CF): 5-10% for CF II, 10-20% and 20-40% for CF III and CF IV respectively [10]. For the ability to evaluate and compare the patients, it is necessary to stratify / divide them by gravity; but because of the CRS complexity, until now there was no severity classification consensus and it is recommended to use specific classifications for HF (NYHA) and CKD (KDOQI). [1].

The diagnosis, prevention and treatment of this syndrome are usually fragmented, focused on a single organ and not on a multidisciplinary approach. In result, the timing and quality of treatment may be affected. In 2010, for the first time the ADQI (Acute Dialysis Quality Initiative) consensus group comes to define and classify the CRS, to provide standardized recommendations for diagnosis (Cystatin C, KIM-1, BNP, NT-proBNP, etc.), prevention and management of disease, and most importantly, they recommended the cardiologists and nephrologists collaboration for the optimization of the proposed outcome [1].

The mere coexistence of HF and chronic kidney disease is not sufficient for diagnosis. According to the working group of the 11th Conference of the Consensus ADQI (2013), to confirm CRS type 2 it is necessary: 1) the coexistence of HF and CKD in a patient; 2) temporal causality (documented or presumed onset of heart failure precedes the onset of kidney damage); and 3) pathophysiological plausibility (the manifestation and the degree of renal impairment could be explained by the preexisting cardiac pathology) [2].

Imaging investigations

Chest X-ray - assesses pulmonary congestion and fluid load for the HF severity assessment [19].

Echocardiography - provides information about the heart function and anatomy, differentiates preserved or reduced ejection fraction HF. Usually echocardiography is sufficient as routine imaging diagnostic [17,19].

Table 1

Proposed definition of CRS2 in stable chronic HF. Reproduced from D. Cruz, 2013 [2]

Chronic HF		EITHER: New onset of CKD
Symptoms typical of HF Signs typical of HF (HF-REF) Reduced LVEF OR: (HF-PEF) Normal or mildly reduced EF and LV not dilated, with relevant structural disease and/or diastolic dysfunction (according to ESC, ACC/AHA)	➡	Albuminuria and/or GFR <60 ml/min/1.73m2 (according to KDIGO/KDOQI) OR: Progression of CKD Sustained ↓ eGFR of >5 ml/min/1.73m2/year, or >10 ml/min/1.73m2/5 years* OR: sustained increase in albuminuria
Plus		
Temporal association: A documented or presumed onset of congestive heart failure precedes the occurrence or progression of CKD		
Plus		
Pathophysiological plausibility: The manifestation and degree of kidney disease is plausibly explained by the underlying heart condition.		

Stress echocardiography / PET CT (positron emission computed tomography) - assesses the ischemic extent and myocardial viability. Limited use because of the high cost [19].

Ultrasonography - assesses renal volume, echogenicity, vena cava; contributes to the proper syndrome classification; differentiates acute renal failure from chronic kidney disease; excludes renal structural pathologies.

Computed tomography and magnetic resonance imaging - study the heart function and structure in particular cases (usually echocardiography is sufficient), and examine the renal vessels.

Cardiac biomarkers

B-type natriuretic peptide (BNP) and the N-terminal proBNP (NT-proBNP) are secreted by the heart muscle as response to the parietal stress and play an important role in the fluid and sodium homeostasis. Volemic loading is the most powerful stimulus for the proBNP and BNP secretion [1, 2]. The RedHot study (Rapid Emergency Department Heart Failure Outpatient Trial) in 2004 showed that natriuretic peptides are independent predictors for cardiovascular events and mortality in patients with HF [17]. Moreover, their prognostic role was reported in patients with different stages of renal failure, demonstrating the potential application of these markers in type 2 and type 4 cardiorenal syndrome [1.2].

Cardiac troponins - in HF, there is a progressive loss of myocytes due to necrosis or apoptosis. Proof of myocyte death was obtained by histological studies and, more recently, by testing troponin (Tn) T and I [1, 25]. These markers can identify subclinical myocardial injury. The Vecchis et al. showed an increase of TnI in a group of severe non-ischemic HF patients; furthermore, they observed a decrease in troponin level at HF improvement [3]. Existing studies enrolled relatively small groups of patients and excluded patients with severe CKD, so the troponin clinical significance in patients with HF and severe CKD is not fully clear. Tsutamoto et al. after measuring the troponin level difference between caroticus sinus and basal aorta; assume that the troponin elevation may be caused by glomerular filtration reduction [25].

Highly sensitive troponins - allow HF patients risk stratification. In a cohort of 4053 HF patients TnT was found in 10.4%, while highly sensitive TnT in 92% patients [25].

Renal biomarkers

Most existing randomized clinical trials (Heywood, 2007 ADHERE; Cruz, 2010) have focused on mortality and cardiovascular events, and only few have examined the long-term renal changes occurrence (Capes, 2000; Testani, 2011) by dynamic evaluation of creatinine, GFR and some of inflammatory markers. The prognostic role of renal changes (increased creatinine level and / or decreased GFR) in HF was demonstrated, it is associated with increased hospitalization rate and CV mortality (Jackson, 2009).

Creatinine is an available marker, but may vary up to 5% throughout the day, has a latency of 2-3 days (changes with a delay of 2-3 days), it is influenced by the infections, inflammatory processes, meat intake, weight. Measured glomerular filtration rate (GFR) may decrease up to 50% until the creatinine level reaches the upper limit of normal (i.e. estimated GFR will be within normal range). In the recent period more sensitive and specific renal biomarkers appeared.

Cystatin C - a marker of proximal tubular damage, most commonly used for early detection of CKD [1, 2, 11]. It is freely filtered in the glomerulus, completely reabsorbed and degraded in the tubules, thus its level is considered an ideal marker for glomerular filtration rate assessment [3, 25]. Most studies suggest that cystatin is not influenced by age, sex, muscle mass or diet. It is superior to creatinine in early detection of renal damage, preclinical renal disease detection or in acute conditions. So far, however, the comparative role of cystatin and creatinine in diagnosis / treatment decision making in patients with chronic stable or relatively stable HF is not established.

KIM-1 (Kidney injury molecule 1) - it is detected in proximal tubule epithelial lesions and changes rapidly in acute chronic failure; predictor for patients at risk of renal function rapid deterioration; decreases after antihypertensive

treatment. There is limited evidence about the KIM-1 value in HF patients [1,2].

NAG (N acetyl-beta-D-glucosaminidase) - the enzyme formed in the proximal tubule in response to tubular damage. It is a sensitive marker of acute renal impairment or renal dysfunction worsening. Increases significantly in congestive HF, with an important prognostic role independent from the glomerular filtration rate [2,24,25].

NGAL (neutrophil-associated lipocalin gelatinase) is secreted in the lungs, kidney, trachea, stomach and in the colon, thus, it is less specific; it may increase in inflammatory processes, sepsis or cancer. It is freely filtered in the glomerulus and completely reabsorbed in the proximal tubules. NGAL is a marker of renal impairment or acute worsening of renal dysfunction. It increases in HF, without proven prognostic role.

Other markers

Albuminuria- assesses the glomerular permeability.

Interleukin 18 -proinflammatory cytokine, precedes creatinine elevations, but it is secreted less than NGAL. Increases in renal failure, but there are not enough studies to prove its predictive role in renal function worsening in HF.

High sensitive C-reactive protein - prognostic value in cardiovascular disease.

Copeptin- is C-terminal segment of the vasopressin prohormone - important prognostic biomarker in HF, but also in albuminuria and renal failure.

Insulin resistance, leptin, adiponectin, procalcitonin, adrenomodulin, interleukin 6, interleukin 1, tumor necrosis factor α - are markers with questionable role.

Because of high cost and limited access to CRS specific biomarkers; because of the serious damage they cause (increased post-myocardial infarction mortality) occurs the need to highlight predisposing factors (hypertension, diabetes, obesity and metabolic diseases, cachexia, renal disease, preexisting proteinuria, uremia, anemia, chemotherapy, mineral and bone deficiencies, electrolyte and acid-base imbalances, etc.)

Treatment

Treatment is complex and incompletely defined [2]. Accurate HF treatment is essential to reduce or eliminate the causes that led to the appearance / progression of renal dysfunction.

Diuretics

Hypervolaemia is the most pre-eminent CRS manifestation. Normalization of the fluid status may be achieved by sodium reduction or diuretics use. Although for long diuretics were considered the essential strategy in this syndrome, there is little data to confirm their beneficial effect on mortality. ADHERE registry data reported that 81% patients with acute HF, were receiving chronic diuretic treatment at admission. Other studies have shown a decreased glomerular filtration rate due to furosemide [4], and increased cardiovascular mortality [23]. Marker of poor prognosis in patients with HF can be considered diuretic resistance, most likely caused by inadequate doses of diuretics, high sodium intake, slowing diuretics intestinal absorption because of the intestinal mucosa edema, reduced diuretic clearance [25, 26] or concomitant NSAIDs administration by decreasing natriuretic and vasodilating prostaglandin synthesis [4]. In such cases: 1) furosemide dosage should be increased, not the administration frequency; 2) to avoid low absorption and bioavailability, diuretics will be administered intravenous. A Cochrane review article confirms that the diuresis obtained at intravenous continuously with furosemide administration is superior to that obtained at bolus administration; they also noted a reduction in the mortality and length of stay. Other options are thiazides or low salt content albumin supplementation to increase sodium excretion.

Vasodilators. Intravenous nitroglycerin or nesiritide (recombinant human type B atrial natriuretic peptide).

Kidney detrimental effects are lower than those of diuretics are. Vasodilators rapidly decline central venous pressure and decrease myocardial oxygen demand without blood pressure lowering (in small doses), may decrease systemic vascular resistance, left ventricular pressure, and improve cardiac output. Central venous pressure reduction may decrease renal perfusion pressure, but long-term effect on renal function and survival is not known [25].

Angiotensin-converting enzyme (ACE) inhibitors

ACE are known to reduce HF patients' mortality [13], but most of these studies have excluded moderate-severe renal impairment patients [21]. The CONSENSUS study (Cooperative North Scandinavian Enalapril Survival) demonstrated that in patients with moderate renal impairment upon the enalapril initiation creatinine levels substantially increased. Despite initial growth of creatinine in some of these patients improved long term prognosis was noted, therefore ACE should not be excluded, but should be administered with caution and with close renal function monitoring during the treatment initiation and titration[4].

Beta blockers

Although they have a role in HF by decreasing sympathetic activity, their use in CRS is limited because of the hemodynamic changes. When the patient is stabilized, it can re-initiate its administration in low doses [17].

Positive inotropic support - controversial

Albeit is known that Milrinone, Levosimendan and Dopamine improve cardiac index and in "renal" (small) doses increase renal perfusion, the OPTIME-HF trial (The Outcome Of A Prospective Trial of Intravenous Milrinone for exacerbations for Chronic Heart Failure) demonstrated their beneficial effects on renal flow and cardiac output, however they do not influence the mortality [4.26].

Statins

Are used for lipid lowering effect, but also for endothelial function improvement by increasing the nitric oxide availability, reducing vascular inflammation and oxidative stress [17].

Vasopressin antagonists

Vasopressin, by coupling to specific receptor V1a (vascular) and V2 (renal), induces vasoconstriction and water absorption. The selective antagonists V2 (Tolvaptan) activates the free water clearance. The EVEREST study (Efficacy of vasopressin antagonist Failure Outcome Study with Tolvaptan Heart) part of the

ACTIV research has confirmed the early beneficial effect in acute HF patients, although on long term there were not significant differences compared to placebo [26].

Adenosine antagonists

There are new agents that promote diuresis by coupling with A1 receptors, it may improve renal blood flow and increase sodium excretion [24, 26]. The efficacy and safety of the medication is being assessed.VVV

Ultrafiltration

It is a method increasingly used in HF patients. The amount of sodium and water removed by ultrafiltration is much higher than that eliminated by forced diuresis, it further decreases the hospitalization length, decreases mortality and rehospitalization rate [4.24]. The ultrafiltration decreases right atrial and pulmonary artery pressure, improves cardiac output and gas exchange. However, aggressive ultrafiltration, can convert a non- oliguric renal dysfunction in oliguric renal insufficiency.

The renal impairment worsening may require calcium, vitamin D agonists, iron or erythropoietin administration.

Unsolved problems

While remarkable progress has been made in the cardiorenal syndrome understanding, it is necessary to implement new biomarkers that would allow early diagnosis before the appearance of kidney irreversible changes, in order to slowdown the cardiorenal complications progression in CHF patients with adverse impact on length and quality of life. Also, there are no criteria for severity and evolution assessment in cardiorenal syndrome; in clinical practice renal impairment severity within cardiorenal syndrome is assessed according to the KDOQI classification and heart failure according to NYHA criteria.

There are multiple studies that assess the coexistence of HF and kidney disease, but there are no clear, objective (other than chronological) criteria that may differentiate type 2 from type 4 cardiorenal syndrome. Usually, such studies enroll patients admitted to a cardiology ward or who address the cardiologist and were conventionally considered as having primary cardiac pathology, i.e. type 2 CRS; if the same patient addressed the nephrologist he could be conventionally considered as having primary renal disease, respectively CRS type 4. Therefore, the study accuracy may be affected. A possible solution would be the start of a long-term prospective project with enrollment and monitoring of HF FC I-II NYHA patients, without other comorbidities or renal function impaired. From our best knowledge up to this moment, there are no such studies; the present studies used only chronological criteria.

CRS treatment involves the use of diuretics, vasodilators, ultrafiltration; all these options provide rapid volemic decline, but until now, their real impact on renal function and survival is not known.

It requires further research to determine effective, safe and cost-efficient therapeutic strategies.

It was determined that when the treatment is fragmented on pathologies, the patient's condition worsens: intensive treatment with loop diuretics for heart failure worsens renal dysfunction; angiotensin converting enzyme, spironolactone or vasodilators treatment, may also aggravate renal dysfunction; on the other hand, renal failure may affect the drugs clearance with the need of dosage review [1, 3]. To achieve a common goal – patient's safety, in clinical practice collaboration of cardiologists, nephrologists and internists is of utmost importance.

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Disability caused by ischemic stroke – a medico-social problem

D. Mereniuc

Department of Neurology, Nicolae Testemitsanu State University of Medicine and Pharmacy
Chisinau, the Republic of Moldova

Corresponding author: imereniuc@rambler. ru. Received November 02, 2015; accepted December 03, 2015

Abstract

Background: Stroke is an important health problem at a global level, being the third cause of mortality in the general hierarchy of pathologies (after cardio-vascular disease, neoplasm) and the first cause among neurological diseases. According to data from the National Statistical Office of the Republic of Moldova in 2012 11836 new cases of strokes were registered and 6125 persons passed away. The prevalence in 2012 was 33,3 for 10 000 people. In 2014, 853 persons were given disability levels after stroke. According to the American Stroke Association 87% of strokes are ischemic and the rest of hemorrhagic type. Stroke represents the main etiological factor of long term disability. Only 20% of people who have suffered from stroke will return to work, 40% have moderate deficiencies. The majority of secondary deficiencies are healed in a few months, but others can persist throughout life.

Conclusions: Even though the biggest progress in healing is achieved in early periods perspectives in recovery are over the course of months and even years (late period). Development of cooperation between medical and social services is necessary and has the aim of identification of common interest problems in the prophylaxis, treatment and adequate recuperation, the supreme objective being the reduction of the number of people with post-stroke disabilities and maintaining human potential. Improvement of the quality of medico-social services for people with a disability favors the achievement of objectives for their socio-professional inclusion.

Key words: stroke, disability, deficiency, socio-professional inclusion.

Introduction

Stroke is an acute neurological dysfunction of vascular origin with symptoms and signs implying cortical areas. It is an important health problem at a global level, being the third cause of mortality in the general hierarchy after cardio-vascular and neoplasm and the first cause among neurological diseases. According to the American Association of Stroke, every 45 seconds someone has a stroke, every 3 minutes someone dies of a stroke, 87% of strokes are ischemic and the rest hemorrhagic [1]. According to the American Journal Circulation of the Association of Cardiologists from 2013, the following data are presented: approximately 795.000 people have a stroke annually among which 610 000 cases are manifested for the first time, and 185 000 are repeated [2]. In the Republic of Moldova (RM) the incidence of stroke and mortality rate is a leader among European countries. According to data from the National Statistical Office of the RM in 2012 11836 new cases of stroke were registered.

Stroke is the main etiologic factor of long-term disability. About 75% of stroke victims have residual effects from stroke, and for some, these effects make it impossible to work. Only 20% of people who have suffered from stroke will return to work. A third of stroke victims are socially active. According to the World Report for disability 40% of those who have suffered from stroke have moderate deficiencies and 15-30% – severe deficiencies [3]. Furthermore, stroke patients are at high risk for future vascular events, including recurrent stroke, putting them at a greater risk of death and further disability [4]. With growing number of stroke survivors, there is an urgent need to improve our understanding of the long-term recovery process after stroke and to identify the ways for developing efficacious therapeutic strategies to enhance poststroke outcomes [5]. Handicap is the disadvantage resulting from poor health that limits fulfillment of societal roles: disability limits their ability to perform tasks. Handicap is common after stroke even in nondisabled patients, and reduction of handicap is a key aim of rehabilitation. Stroke may cause physical and cognitive impairments. Age, functional status and disease duration on admission, co-morbidities, and cognitive functions are known to be the predictors of functional outcome in stroke. Acute poststroke cognitive impairment is commonly seen. Cognitive impairment occurs in 35.2%-43.9% of the patients three months after stroke and may continue for a long time in approximately 1/3 of the patients. Cognitive impairment may decrease functional capacity, for it affects rehabilitation outcomes in stroke [6].

The brain is an organ with a great capacity for regeneration, but cannot regenerate wholly (ad integrum healing). The unaffected portions of the brain can successfully undertake the function of the destroyed cerebral tissue. Over time some previously lost functions can gradually restore their deficient cortical areas. Healing depends on the location and expansion of affected central tissue and also on the capacity of the functions of healthy tissues [7]. Also, the brain has a big capacity for adaptation and helps the body find new ways to carry out its normal activity. The first 36 months after stroke are the period of time in which the majority of disabilities are recovered, even so some problems are improved over years and depend on a series of factors (the expansion and place of central lesion, the pacients age, the associated pathology, treatment) [8]. The period of recovery differs from person to person and is in the most cases a process that continues for the remainder of life. The capacity of recovery and disability occurance after a stroke depends on many factors: affected brain areas (each area controls a certain function of the body), the affected central areas (dominant hemisphere), the surface and depth of the lesion, the general state of health and other diseases [9, 10, 11]. Evidence of the differences between the functional consequences of strokes in the left and right hemispheres is particularly interesting. The left hemisphere is more important for motor control, while the

right hemisphere is more important for spatial orientation [12]. Motor activities requiring planning and coordination are more dependent on the left hemisphere and are strongly affected in individuals with right side hemiparesis [13, 14]. Right hemisphere lesions are more likely to result in deficits in attention and contralateral perception and stabilization of the position in relation to lesion of the left side [14]. The right hemisphere integrates sensorimotor information which is critical for maintaining posture and maintaining sitting or standing positions [15]. In many cases despite recovery measures patients are left with deficiencies such as: paralysis of certain body parts, perception disorders, depression and other disorders of cognitive functions. This makes stroke a major health problem, because, of the high rate of mortality and squeals with a devastating effect on the life quality of the patient and his family. The economic and social costs of post-stroke disability are significant, but difficult to quantify. They include direct and indirect costs. Direct costs fall into two categories: additional costs that people with disabilities and their families incur to achieve a reasonable standard of living, and disability benefits in cash and kind, paid for by governments and delivered through various public programmers. Indirect cost of diminished work capacity and high mortality among young people, also the economic loss are related to the long recovery period and socio-professional inclusion of these patients. Non-economic costs include social isolation and stress and are difficult to quantify [16].

The notion of social inclusion is different from that of integration. If social integration implies the acceptance of a person with disability in society (work place, public areas), social inclusion means the dumping of society, of value, community rules for accepting diversity (establishing the service for determining of disability and the work capacity in the RM 30.03.2007).

The problem of post-stroke disability is a priority in contemporary approach of organizing the health services in countries with advanced economies. Because ischemic stroke is the number one cause of long-term disabilities the measures for prophylaxis and recovery have great importance. Rehabilitation is an active process of recovering completely or if that is not possible the achievement of an optimal social physical potential, followed by the integration in suitable environment. Recovery depends on the motivation of the patient, the ability to learn, family support, quality and intensity of treatment. The main goals of the program are: recognition the complications and their profilaxy, functional independence, improving quality of life, social and family reintegration. Also the education of families in which a member has suffered from stroke for support.

The notion of disability is interpreted in three distinct concepts: a person with disability or impairment, disability, handicap [16]. International Classification of Impairments, Disabilities and Handicap was developed and adopted as a document in 1980. Because the process of medical rehabilitation is guided by a bio-psycho-social approach of the disabled persons the International Classification of Functioning (ICF), Disabilities and Health was adopted, approved in 2001 (fig. 1). This conceptual framework is useful for individual of every rehabilitation program. The ICF was designed to classify not only limitations in functioning but also positive experiences for all body functions, activities, and participation in the environment. Examples of positive experiences include communicating, tending to personal hygiene, working, and studying. In summary, the ICF portrays health as a dynamic interaction between an individual's functioning and disability within a given context [16, 17].

Functioning is an umbrella term for body functions, body structures, activities and participation. It denotes the positive aspects of the interaction between an individual (with a health condition) and that individual's contextual factors (environmental and personal factors).

Disability is an umbrella term for impairments, activity limitations and participation restrictions. It denotes the negative aspects of the interaction between an individual (with a health condition) and that individual's contextual factors (environmental and personal factors).

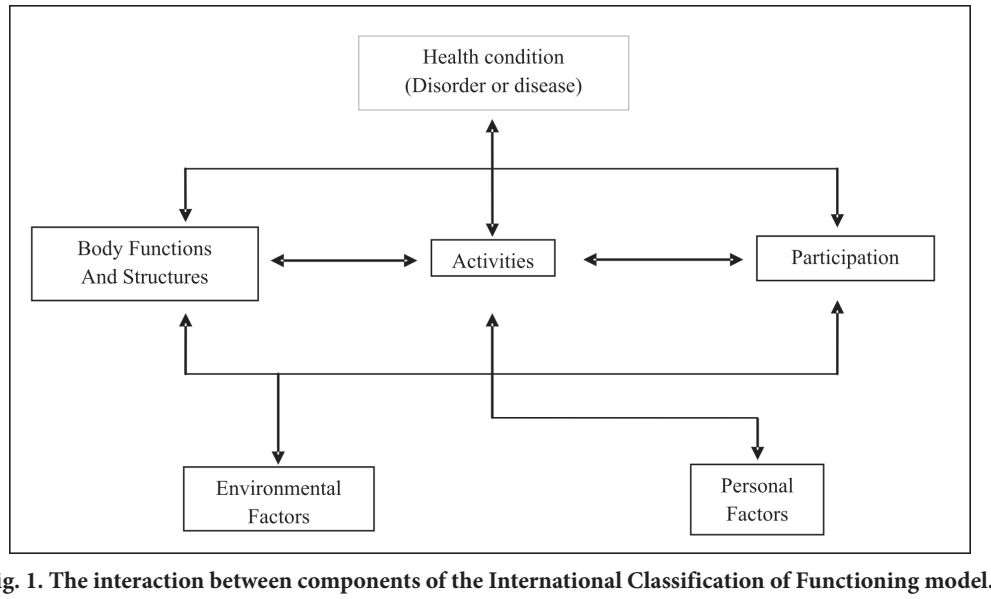

Fig. 1. The interaction between components of the International Classification of Functioning model.

Body functions - The physiological functions of body systems (including psychological functions).

Body structures - Anatomical parts of the body such as organs, limbs and their components.

Impairments - Problems in body function and structure such as significant deviation or loss.

Activity - The execution of a task or action by an individual.

Participation - Involvement in a life situation.

Activity limitations - Difficulties an individual may have in executing activities.

Participation restrictions - Problems an individual may experience in involvement in life situations.

Environmental factors - The physical, social and attitudinal environment in which people live and conduct their lives. These are either barriers to or facilitators of the person's functioning.

The Service for Determining of Disability and Work Capacity of the RM was subjected to radical changes in 2013 by the bases being established by the Government decision No 65 23.01.2013, according to which National Council of Determining Disability and Work Capacity was established. The term invalid was replaced by disabled [18].

The institution for determining disability and work capacity was approved. After the release of the 12/70 Order 28.01.2013 of the Ministry of Work and Social Protection of RM the medical criteria for determining structural and functional impairment is implemented. Also a big accent is made on the medio-social evaluation of the patient. The actual methodology of disability evaluation is oriented on rehabilitation and socio-professional inclusion of people. The notion of social inclusion is different from that of integration. If social integration assumes the acceptance of the person with a disability in society (work place, public place), social inclusion means the changes of society, attitudes, values, rules of community for acceptance of diversity.

Conclusions

Because ischemic stroke is the number one cause of long-term disability the measures for prophylaxis and recovery have a great importance.

It requires the development of cooperation between medical and social services which has the aim to identify common interest problems in the profilaxy, treatment and adequate recuperation, the supreme objective being the reduction of the number of people with poststroke impairments and maintaining the human potential. The big number of people with stroke, the disability, socio-professional inadequacy, high mortality, considerable economic costs cause the actuality of the problem.

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Therapeutic strategies of subclinical hypothyroidism, including statin therapy

***G. Bezer, L. Vudu**

Department of Endocrinology, Nicolae Testemitsanu State University of Medicine and Pharmacy Chisinau, the Republic of Moldova

*Corresponding author: ipgalina@mail.ru. Received November 02, 2015; accepted December 05, 2015

Abstract

Background: Thyroid disorders are an actual problem of contemporary medicine. Hypothyroidism represents the insufficiency of thyroid to secrete thyroid hormones in necessary quantities for human body. Primary hypothyroidism is the most common endocrine disease. Although the diagnosis and treatment of hypothyroidism is often considered simple, there are large numbers of people with this condition who are suboptimally treated. We are very concerned that some patients with and without thyroid disease are being inappropriately diagnosed and managed, using levothyroxine and other thyroid hormones, in ways which compromise patient safety. Subclinical hypothyroidism has multiple etiologies and manifestations. Appropriate treatment requires an accurate diagnosis and is influenced by coexisting medical conditions. Clinical symptoms of hypothyroidism are nonspecific and may be subtle, especially in older persons. Diagnosis and treatment of hypothyroidism is often considered simple and is mostly carried out in a primary care setting. The dangers of statin use in hypothyroid patients have been illustrated and the necessity for appropriate biochemical monitoring has been emphasized. Statin therapy is safe and effective when patients are appropriately diagnosed, educated, and followed up. Statins can be cautiously reinitiated once a euthyroid state has been established in patients who developed statin-induced myopathy while hypothyroid.

Conclusions: Despite the fact that nowadays problems persist in the management of subclinical hypothyroidism, administration of statins in secondary dyslipidemia will prevent cardiovascular diseases especially atherosclerosis thus enhancing the quality of life of patients with hypothyroidism.

Key words: subclinical hypothyroidism, thyroid, statin therapy.

Introduction

Hypothyroidism is the complex syndrome, caused by the lack of action of thyroid hormones of different metabolisms, devices and systems. Hypothyroidism is one of the most common thyroid disorders, predominantly in females. According to world literature data from 3 to 5% of the population suffer from hypothyroidism [3,4].

According to some epidemiological studies, the prevalence of subclinical hypothyroidism reaches 10-12%. Subclinical hypothyroidism, in most cases, does not have clinical manifestations, which would allow it to be suspected. Very often, hypothyroidism occurs being "disguised" along with numerous somatic diseases, gynecological and other endocrine diseases. Under many prospective studies, subclinical hypothyroidism has quite serious consequences [5.13].

A study conducted on a group of patients in Rotterdam (with subclinical hypothyroidism) revealed that the risk of atherosclerosis was 1.7 times greater, and that of acute myocardial infarction 2.3 times higher than in the general population [4].

With the emergence of highly sensitive methods for determining the hormones, subclinical thyroid dysfunction concept was formed. The term "subclinical" means that the level of thyroid stimulating hormone (TSH) is increased, while all the other thyroid functional parameters remain within the normal limits. The absence of clear symptoms of subclinical hypothyroidism arose the question whether subclinical hypothyroidism is a pathology or a laboratory phenomenon, that does not require hormone therapy and normalization of TSH. Subclinical hypothyroidism is the subject of several studies, which analysis showed that in subclinical hypothyroidism changes in various organs and systems occur, and thyroid hormone replacement therapy improves patient's well-being and normalizes many functional parameters [2].

Subclinical hypothyroidism frequency in the general population ranges from 1.3 up to 17.5%, depending on age and sex. Subclinical hypothyroidism prevalence is higher in women than in men and increases with age, reaching the peak of 21% of women and 16% men after 74 years. According to Framingham study, conducted for 10 years by monitoring elderly patients, showed that in studied 2139 patients over 60 years , subclinical hypothyroidism was detected in 126 patients (5.9 %) and among women almost 2 times more frequently [7]. Whickham study showed the risk of developing clinical hypothyroidism: 4.3% per year, if they show increased serum TSH and thyroid antibodies are present from the start, only 2.6% per year if serum TSH is increased, 2.1 % per year if only thyroid antibodies are present, and after 20 years the three examined groups have developed hypothyroidism in 55%, 33 %, 27 % and 4% of those without TSH increased serum and thyroid antibodies. The likelihood of developing hypothyroidism increases with TSH serum rise [24].

In Colorado population studies, which included 25862 people aged 18-91 years, manifested hypothyroid frequency increase and subclinical hypothyroidism with age were also observed. As a result of long-term monitoring of patients with subclinical hypothyroidism during 4-8 years, it was proved, that subclinical hypothyroidism in 20-50% passes into manifested hypothyroidism. In the presence of thyroid antibodies in patients aged over 65, the risk of developing manifested hypothyroidism in the next four years is 80% [5].

In subclinical hypothyroidism and in the presence of thyroid antibodies the risk of developing hypothyroidism manifested in the general population is 5% per year. In epidemiological studies subclinical hypothyroidism is more common, but in clinical practice is less diagnosed [13].

The etiology of subclinical hypothyroidism is the same as in manifested hypothyroidism. Subclinical hypothyroidism is most often caused by autoimmune thyroiditis. Other proces-

ses, underlying subclinical hypothyroidism development are destroying thyroid tissue with a reduction in its functional activity, impaired thyroid hormone synthesis in endemic, sporadic goiter in toxic effects on the thyroid gland.

The modern laboratory diagnosis of subclinical hypothyroidism is based on TSH and FT4 level determination. The manifested hypothyroidism is characterized through TSH increased level and FT4 low level and in subclinical hypothyroidism TSH level is increased and FT4 levels within the norm. Hormonal changes characteristic for primary hypothyroidism are based on the principle of negative feedback between the thyroid gland and the pituitary gland, in accordance with the reduction in T4 and T3 leading to increased synthesis of TSH.

The priority testing in diagnosis of primary hypothyroidism is assessing TSH.

It should be noted that subclinical hypothyroidism may be transient and it is not always possible to avoid technical errors in determining hormones. Therefore, for subclinical hypothyroidism diagnosis TSH levels and FT4 should be repeated within 3-6 months and if TSH increase is confirmed, a decision on replacement therapy will be taken.

The data from the specialty literature show that in patients with hypothyroidism various disorders of the cardiovascular system can develop, first of all the changes generated by the increase of cholesterol, of lipids with a low density and triglycerides. Protein metabolism is disturbed by the basic structures impairment of myocardial contractile proteins, as well as, by changes in the cardiovascular system and contribute to the composition of the fluid from the pericardial effusion, and fibrinolysis system disorders [2, 4].

In patients with subclinical hypothyroidism endothelial dysfunction is determined (early marker of atherosclerosis), which can be reversible on the background therapy with levothyroxine. Subclinical hypothyroidism is 2-3 times more often detected in patients with hypercholesterolemia. In individuals with subclinical hypothyroidism elevated levels of triglycerides, low density lipoproteins (LDL), apolipoprotein B and lipoprotein A are identified. The atherogenic changes of lipid profile diminish on levothyroxine replacement therapy background [6.14].

In patients with subclinical hypothyroidism, as in manifested hypothyroidism the myocardial hypertrophy, hypertrophy of interventricular septum, the maximum increase rate of atria blood flow, are determined reducing the average value of the acceleration of blood flow in the aorta, to extend the relaxation isovolumic period, inferior variation of systolic index.

Thyroid hormones exert different effects on the cardiovascular system and its hemodynamics. Cardiac activity indicators such as heart rate, cardiac output, blood flow, blood pressure, total peripheral vascular resistance, cardiac contractile function, are directly linked to thyroid function status. Changes of cardiovascular system in insufficiency of thyroid hormones are: coronary atherosclerosis, ischemic heart disease, arrhythmias and management disturbances, arterial hypertension.

According to the results of the Rotterdam study (a. 2000), subclinical hypothyroidism was detected in 10.8% of women aged 69 ± 7.5 years, which often have been associated with signs of aortic atherosclerosis and myocardial infarction. In subclinical hypothyroidism the level of LDL and total cholesterol in contrast with the total cholesterol is positively correlated with the level of TSH and negatively with FT4 level. Meanwhile, the level of high-density lipoprotein (HDL) decreases, and the ratio between total cholesterol / HDL increases. However, data on subclinical hypothyroidism lipid disorders are contradictory. In some studies there were neither increased cholesterol levels nor lipid metabolism dynamic parameters during treatment with levothyroxine [4].

In a study conducted in 2004, researchers examined and pursued the association between subclinical hypothyroidism and cardiovascular disease in a group of 212 men and women aged between 20 and 69 years without a known thyroid pathology, untreated with drugs that interfere with thyroid function or with TSH analysis. There have studied the clinical signs of cardiovascular disease based on the indices of lipid metabolism, atherosclerotic risk markers, C-reactive protein and TSH. It was found high incidence of subclinical hypothyroidism 19.7% in the investigated group. Subclinical hypothyroid was associated with higher concentrations of triglycerides and C-reactive protein. Cardiovascular disease was more common in men under the age of 50 years with subclinical hypothyroidism compared to the euthyroid ones. The probability report for cardiovascular disease was 3.4 (confidence interval (1.6 to 6.8) compared to euthyroid men of the same age. In the NHANES III study, where people Caucasian, African-American and Hispanic races were investigated the prevalence of hypothyroidism was 5.1%, 1.7% and 4.1% and the category the most susceptible included the woman in the "post-partum" and also subjects with a family history of autoimmune thyroid disorders [25 26].

Subclinical hypothyroidism in pregnant women has certain features. During pregnancy, a number of factors that have an impact on its functional activity are seen. These factors include: the excess synthesis of human chorionic gonadotropin (hCG), estrogens, which induce an increased synthesis of transport proteins, which cause a decrease in the free fraction of the thyroid hormones, the increase of renal iodine clearance and the change of the metabolism of thyroid hormones in correlation with active functioning of the fetus placental complex. These factors contribute to the increased synthesis of thyroid hormones during pregnancy by 30-50%. In first trimester of pregnancy placenta actively produces hCG. Increased hCG level leads to stimulation of the thyroid gland, increased levels of FT4, which after negative feedback mechanism reduces the level of TSH. In the second trimester of pregnancy the production of hCG reduces and TSH returns back to normal. So now, thanks to the variety of factors that act on the thyroid gland during pregnancy, altering thyroid function, in different trimesters of pregnancy, has its own characteristics. In the first trimester, due to overproduction of hCG, gestational transient thyrotoxicosis can develop, which should be distinguished from true thyrotoxicosis. The reduction of FT4 in the first trimester instead of the expected growth should alert the clinician for an increased risk of hypothyroidism. The presence

of both subclinical and manifested hypothyroidism can have irreversible consequences for the fetus development, primarily for the central nervous system. It is known that during the first 16 weeks of pregnancy, the thyroid gland of the fetus is in the process of formation and the fetal development is realized only formed under the action of thyroid hormones of the mother. If during pregnancy the lack of thyroid hormone is not adjusted, in women with hypothyroidism, the child may have malformations and decreased intelligence. Numerous studies have shown that children born from mothers who have not been treated for subclinical hypothyroidism have a low coefficient of intellect, of survival, Apgar score is worse compared with the children whose mothers had received appropriate dose of levothyroxine.

It is necessary to conduct immediately a screening for hypothyroidism, including the subclinical stage for all pregnant or planning a pregnancy women for a thyroid hormone replacement therapy.

In hypothyroidism associated with pregnancy, the determination of serum TSH in pregnant women should be done at the first visit, which is one of the most useful investigations, giving the high specificity of TSH, which can diagnose subclinical forms. The level of TSH> 4.0 mU / L, requires the determination of fT4, which also has a high sensitivity. The dosage of antithyroperoxydase - ATPO antibody is indicated, to exclude an autoimmune process because ATPO positive women with hypothyroidism have a higher risk of miscarriage or premature birth and to develop a portpartum thyroiditis. For hypothyroid screening dosage of T3 and T4 free levels will be done. Monitoring of pregnancies with hypothyroidism should be complex - clinical, biochemical and ultrasound, but thyroid parameters monitoring will be done dynamically. Thyroid insufficiency during pregnancy requires maintainance of TSH in the first trimester is less than 2.5 mIU / l, while the second and third trimesters is less than 3 mIU / l. The discovery of hypothyroidism during pregnancy requires the administration of an early treatment, since starting the follow up of a pregnant woman, ideally from 4-8 weeks of gestation to prevent maternal-fetal complications. The dose of the thyroid hormones should be adjusted to maintain TSH serum under 2.5 M / l, which requires control of TSH repeatedly at every 4-8 weeks of gestation. In pregnant women with hypothyroidism pre-existing to pregnancy it's recommended to increase the dose of L-thyroxine on an average by 50% compared with preconception dose. The collaboration between obstetrician and endocrinologist is particularly important for preventing complications associated with maternal-fetal hypothyroidism in pregnancy [1,13].

In women with subclinical hypothyroidism vaginal bleeding, infertility, failure during in vitro fertilization, preterm delivery, placental abruption, high blood pressure are more common, cesarean need arises. Pregnancy in hypothyroidism is accompanied by an increased incidence of anemia, preeclampsia, eclampsia and uteroplacental apoplexy, there is a tendency to swelling and weight gain due to fluid retention.

Subclinical hypothyroidism is often transient, which can develop as a result of destructive forms of thyroiditis (subacute, induced by amiodarone) and can be determined as options of autoimmune thyroiditis (postpartum), especially in women with personal or family history of autoimmune thyroiditis. Postpartum thyroiditis occurs in 3-6 months after birth and is manifested by hypothyroidism or hyperthyroidism. Hyperthyroidism lasts 1-3 months, after which most women return to normal thyroid function, or may have hypothyroidism. Thyroid dysfunction is due to a destructive thyroiditis associated with thyroid microsomal autoantibodies. Postpartum thyroiditis often tends to recur in subsequent pregnancies, which ultimately develop subclinical or manifested hypothyroidism.

Subclinical hypothyroidism can develop after surgery, antithyroid drug administration, potassium perchlorate, lithium preparations. TSH research is recommended in 3-6 months and if it is necessary, the replacement therapy with levothyroxine administration and TSH level will be repeated after 3 months [10,11,12].

Treatment

Until the mid-twentieth century hypothyroidism treatment was performed using animal thyroid extract. The emergence of synthetic thyroid hormone treatment has fundamentally changed the treatment of thyroid pathology. Levothyroxine therapy in hypothyroidism is considered the "gold standard" [13], there are several reasons for this statement:

- easy diagnosis of hypothyroidism (in most cases only by determining the level of TSH);
- the single vital function of the thyroid gland is to produce thyroid hormones;
- the circadian rhythm of secretion of thyroid hormone is almost absent and, therefore, the daily intake of levothyroxine, in the same dose is sufficient;
- high bioavailability for peroral administration of levothyroxine;
- breaking time of levothyroxine in plasma (approximately 7 days);
- availability of the exact test (TSH level), which fully reflects the quality of compensation of hypothyroidism;
- relatively low price of levothyroxine;
- Patients that administer sufficient doses of levothyroxine are recommended to determine TSH levels once in 6-12 months.
- The quality of life in patients with hypothyroidism, who permanently administer levothyroxine and are compensated, is no different from that of patients without hypothyroidism.

According to population surveys conducted by K. Peterson [8], which lasted for 12 years (1968-1969 up to 1980-1981), and that included 1462 middle-aged women, in 29 women aged up to 28 years levothyroxine replacement therapy was administered with the diagnosis of primary hypothyroidism. As a result, it was shown that the duration and the quality of life and the risk of major diseases, did not differ in patients with hypothyroidism treated with levothyroxine, and in the control group (n = 968). The treatment of hypothyroidism, regardless of its clinical form, is substituted by administering

thyroid hormones. The effectiveness of therapy is judged by clinical status, normalization of hormonal status. The treatment lasts a lifetime, and for the hypothyroidism replacement therapy levothyroxine is indicated.

In adults levothyroxine dose of 1.6 mcg / kg of body weight per day is indicated. The need for levothyroxine is significantly higher in children and can be from 2 micrograms / kg to 5 mcg / kg per day.

The need for levothyroxine decreases with age. Some elderly can manage no more than 1 mcg / kg per day of levothyroxine for hypothyroidism compensation. The need for levothyroxine increases during pregnancy. Evaluation of thyroid function in pregnant women with TSH and free T4 is recommended every trimester of pregnancy.

The starting dose of the drug is determined individually depending on the age, body weight, and the presence of concomitant cardiovascular diseases.

Subclinical hypothyroidism treatment is solved individually. Taking into consideration the high frequency of dyslipidemy and atherosclerosis and increased risk of heart attack justifies the prescription of levothyroxine for subclinical hypothyroidism. The indications for replacement therapy with levothyroxine TSH levels are ≥ 10 IU / L or TSH level between 5 and 10 mU / l and concomitant dyslipidemia. Typically, the starting dose is selected based on the age of the patient and the presence of concomitant heart pathology. The aim of replacement therapy is to maintain subclinical hypothyroidism TSH values in the range of 0.5-2.0 mIU / l [6,13].

TSH level varies slowly after a change in dose of levothyroxine. TSH level will be examined sooner than 6-8 weeks after changing the dose.

The treatment should be slowly progressive, with a gradual dose increase, especially in the elderly ones and in case of severe hypothyroidism. It begins with daily doses of 25 mcg levothyroxine and gradually rising every 7-14 days, at doses of 50, 75, 100, 125 mcg etc. until euthyroid state is achieved. For older people, 1 mcg/kg of levothyroxine per day is enough for compensation of hypothyroidism.

The treatment of subclinical hypothyroidism in patients with concomitant cardiac disease, particularly coronary artery disease and cardiac arrhythmias should begin with minimal doses of levothyroxine - from 12.5 to 25.0 mcg, by gradually increasing the dose with 12.5 to 25.0 mcg every 1-2 months to reduce TSH levels to normal levels. Replacement therapy is performed under ECG supervision or Holter ECG monitoring, avoiding decompensation of cardiac abnormalities or arrhythmias. After values normalization it is recommended to repeat TSH after 3-6 months [2.14].

In elderly and coronary subjects is recommended the concomitant use of β- blockers coronary dilators, calcium channel blockers.

The effectiveness of therapy is judged by clinical status, normalization of hormonal status and of parameters, indicating the action of thyroid hormone at the level of tissue receptor.

Ideally, levothyroxine is administered on an empty stomach, 30 minutes before a meal.

It will be taken into account the possible medicaments interactions: thyroid hormones intensify K antivitaminic actions, which are important in the blood clotting process, reduce the permeability of capillaries, promote tissue regeneration, enhance the action of tricyclic antidepressants, reduce hypoglycaemic action. Their action is reduced by cholestyramine.

Often, levothyroxine hormone replacement finally normalizes the lipid metabolism, nervous system and psycho-emotional sphere disorders.

If the hormone replacement therapy does not lead to normalization of plasma lipids, the patients with hypothyroidism are indicated a lipid-lowering therapy [15].

The most perspective drugs for the correction of lipid metabolism are in present inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the enzyme that catalyzes an early step in the biosynthesis of cholesterol.

The statins along with the lipid-lowering effect have a number of pleiotropic effects: anti-inflammatory, antioxidant, anti-thrombotic properties. Statins are the most effective cholesterol lowering drugs acting selectively on HMG-CoA reductase, the key enzyme in cholesterol synthesis; they decrease LDL-C by 20-40% triglycerides - by 10-20% and increase LDH-C by 5-10% [23]. Statins are the most successful cardiovascular drugs that have the ability to prolong life and to improve its quality. These data confirm the overriding importance and need for hypolipimiant treatment in hypothyroidism.

Complication of statin therapy is the myopathy induced by these drugs [15,16], which is manifested through spontaneous muscle pains, cramps and weakness that are typical clinical features, regardless of other factors. The risk of myopathy increases by the concomitant use of fibrates, inhibitors of hepatic cytochrome P-450, major trauma and surgery. The most servere complication is rhabdomyolysis [19] having a lethal potential [17,18]. Data from clinical trials show that the rate of statin-induced myopathy in the general population is 0.1% to 0.2%. Knowing this negative effect of statins is very important, the risk being possibility of myopathy appearance due to hypothyroidism. Hypothyroid myopathy was first of all described by Johann Hoffmann in 1887. In patients with primary hypothyroidism this syndrome may occur with a frequency of 25% - 60%. Hypothyroid myopathy manifests with muscle fatigue, myalgia, slowness in movements, muscle stiffness [18]. Sometimes muscle weakness, "rigidity" of muscles are accompanied by severe myalgia and a significant increase in the serum of creatine phosphokinase (CK). A number of publications report the association of myopathy with rhabdomyolysis complicated by acute muscle necrosis, due to the lack of diagnosis of hypothyroidism in time [17,18].

Although the biochemical mechanism both of myopathy in hypothyroidism and that induced by statins remains unclear, but hypothyroidism increases the risk of statin-induced myopathy [15,16]. However, some authors believe that for the mechanism of myopathy hypothyroid are responsible glycogenolysis defects or impairment of mitochondrial oxidation [17]. Presumably, these mechanisms are synergistic when statins are prescribed to patients

with hypothyroidism. Myopathy is more likely to occur after administering high doses of statins to patients with acute coronary syndrome who undergo coronary angioplasty [21]. Therefore the use of statins in patients with hypothyroidism should be carefully indicated. Biochemical testing is essential for patients with symptoms that can be attributed to myopathy on the background of statin therapy.

ATP III (Adult Panel III) guide of the National Cholesterol Education Program 13 and American College of Cardiology ACC / AHA 11 recommend that the determination of creatine phosphokinase (CK) is determined before initiating statin therapy and that will be re-evaluated in comparison with the initial if patients report any muscle symptoms. More frequent determinations of CK and transaminase are indicated to patients, that got maximum statin doses and those who receive a combination therapy, usually with fibrates [21,22]. Finally, statins are not absolutely contraindicated to patients who developed myopathy induced by statins [20,22]. Statin therapy should be performed with caution, with re-initiation of statins therapy in this context, and the patients should always be instructed that if muscle pains or cold-like symptoms develop, statins should be discontinued immediately and they should contact the doctor.

Conclusions

Hypothyroidism is a well-known cause of secondary dyslipidemia and the link with atherosclerosis has been known for 125 years. Elevated circulating levels of a very low density lipoprotein (VLDL) and low density lipoproteins (LDL) cholesterol are major lipid abnormalities observed in patients with hypothyroidism.

The reduction in circulating levels of atherogenic lipoproteins (VLDL and LDL) is the main goal of treatment with statins. In addition, the statins possess pleiotropic properties: improve endothelial dysfunction, have anti-inflammatory, antioxidant, antiplatelet, antiproliferative effect; stabilize and slow the progression of atherosclerotic plaque.

Thus the administration of statins in secondary dyslipidemy will help to prevent cardiovascular diseases, especially atherosclerosis, which will enhance the quality of life of patients with hypothyroidism.

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Psychological rehabilitation of patients with endogenous disease

T. Kryvonis

Department of Medical Psychology and Psychiatry
N. I. Pirogov National Medical University of Vinnitsa, Ukraine
Corresponding author: tamara.kryvonis@gmail.com. Received November 13, 2015; accepted December 07, 2015

Abstract

Background: Clinical picture of the mental illness „schizophrenia" includes numerous symptoms, described in detail in the literature on this subject. Diagnosis of the illness involves detection of the present signs and symptoms, which are closely connected with the impaired social and occupational functioning. Schizophrenia presents itself in a form of psychotic disorder. The term „psychotic" refers to a significant breach in the assessment of reality, or in psychodynamic terms, is defined as a loss of communication between the Ego and the reality, as well as a degree of functional impairment. The article provides the substantiation of early psychotherapeutic intervention in combination with psycho-pharmacotherapy in patients with endogenous disorders. It also describes the mechanisms of psychological defences to deal with traumatic experience, used by personalities functioning on a psychotic level. Characteristic behaviour patterns of extended family members in terms of emotional co-dependency are provided.
Conclusions: Individual pathopsychology is considered as a symptom of abnormal functioning of the family. The article places emphasis on the importance of inclusion of family members in psychotherapeutic interaction in order to correct interpersonal relations. Psychosocial rehabilitation of patients with endogenous disorders should be implemented in two stages: inpatient and outpatient.
Key words: psychological rehabilitation, emotional dysfunction, co-dependency.

Introduction

Today, schizophrenia is still one of the most common and severe mental illnesses. Despite the effective application of pharmacotherapy in relieving acute psychotic symptoms and attenuation of the negative ones, what remains unresolved is the achievement of sustained remission with resumption of social functioning and quality of life. The given problem is especially significant due to the high incidence of disability in such patients. Disability, in turn, includes the following adverse aspects such as the socio-economic burden for society and the degradation of the patient's personality, which significantly affects the quality of life not only of the patient but also of his inner circle. For this reason, psychosocial rehabilitation of patients with schizophrenia is one of the most urgent problems of modern psychiatry. In the spectrum of methods of psycho-social rehabilitation, the most common are individual and group psychotherapy, socio-therapy, occupational therapy in occupational therapy workshops, stimulation of social activity, and development of daily living skills, culture-therapy, and others.

Clinical picture of the mental illness "schizophrenia" includes numerous symptoms, described in detail in the literature on this subject. Diagnosis of the illness involves detection of the present signs and symptoms (productive and negative), which are closely connected with the impaired social and occupational functioning.

Schizophrenia presents itself in a form of psychotic disorder. The term "psychotic" refers to a significant breach in the assessment of reality, or in psychodynamic terms, is defined as a loss of communication between the Ego and the reality, as well as a degree of functional impairment.

Psycho-pharmacotherapy of any kind is only a part of the general approach to the treatment of schizophrenia. Medication affects only physiological symptoms, leaving unattended the psychological content of the problem.

It is important to point out so-called functional negative symptoms, which are often caused by the negative influence of the environment, for example - hyper stimulation in a hospital environment, on account of specific conditions and the system of relations in psychiatric hospitals.

Functional negative symptoms may also be initiated and supported by the adverse psychological atmosphere in the family, associated with excessive control, distrust, predisposition of relatives to the unfavorable development of the disease. The study on the patient's family influence should not be limited to observation of the parents only. It is necessary to take into account the role of other family members (in the so-called extended family).

Information about the patient, obtained from the family members, is important. Family members tend to report information about the patient that differs from the information provided by the patient, which is incomplete and insufficiently reliable, due to its passing through the prism of his mental state.

Individual psychopathology needs to be examined in a family context. Since any psychopathology developed by an individual member of the family is at the same time a symptom reflecting the mental problem of the family that may have existed before the emergence of the given abnormality, or a manifestation of the total psychopathology of the whole family. The diagnosis determines the rigidity of the relationship, chronic stress and conflicts within the family, double standards and difficulty in expressing emotions.

Investigation of the family structure of the mentally ill patients discovers symptoms of emotional co-dependency of "healthy" family members. Most often, it takes form of behavioral symptoms, such as the use of control strategies and protection, closely connected with control.

Control strategy takes the following form. "Healthy" family members assume the functions of control (sometimes quite tight) over the behavior of the mentally ill. The role of the controller gives a special meaning to the life of the co-

dependent. The pathological pattern of the behavior means that in case the patient's behavior does not correspond with the scheme existing in the controller's mind, the activities associated with conventional motivations start to be condemned and criticized.

Control involves suspicion, accompanied by questions like, "Where have you been? Who did you speak to? What did you do?" This behavior has negative consequences for all family members, provoking the emergence of negative feelings in the patient with respect to the controller, and vice versa. This vicious circle reinforces negativity.

Control leads to the aggravation of autism and behavioral passivity of the patient. It can also provoke aggressive patients, due to pathological restriction of life space and actualization of paranoid reactions.

Patients' inner circle preconceives any non-obedience as a form of a negative attitude, fixing their time and attention on it.

It is important to remember that the ambivalent interpersonal relations, perceived as pathological a priori, may well be within the normal range. "Exposing" pathology in ambivalence can significantly impair interpersonal relationships.

The second strategy, used with the emotional co-dependency, is protection, closely associated with control. The patient is protected from the consequences of his active behavior, so he does not communicate with others, as this may undermine the credibility and prestige of the family. In some cases, doctors and family members work together - for example, in reaching the decision that the patient should not work or study for some time, reinforcing passive subordinate behaviors. As a result, motivational-volitional problems of the patient, as one of the characteristics of endogenous disease, get only worse.

In terms of interpersonal relations, any mental illness should be viewed as a result of the whole complex of traumatic experiences that occur in the life of the patient.

With endogenous mental disorders, a patient has hidden negative experiences (topics) that he does not intend to discuss with random people or persons he (perhaps for painful reasons) does not trust [1].

The patient may have sufficient grounds for mistrust, as he can view a specialist not only as a person ready to come to his aid, but also as the one able to hurt him.

Disruption of information processing is aggravated by the impairment of the information conveyance. Patients with schizophrenia demonstrate significant difficulties in recognizing the value of emotional states and reactions of others, increasing their social exclusion. Impaired information processing is accompanied by adoption of inadequate response options, and the enhancement of pathological (rigid) patterns of behavior.

The psychotic patient operates on the preverbal or rather pre-object level. The illness involves his primary senses (pre-feelings) or an early ego state experienced in the first months of his life, before the child learns to differentiate I from not I [1]).

In the 1960s of the last century, Dr. Silvano Arieti in1962 [2] had already written: "If you can establish interpersonal situation with the patient, functioning on psychotic level, you can apply psychotherapeutic methods which will enable him to become aware at first of his psychotic intra-psychic mechanisms, and later, of the dynamic conflicts, interpersonal by origin, that are causing these intra-psychic mechanisms. These processes allow the patient to abandon his symptoms and to focus himself on the greater maturity and non-psychotic life. "

In accordance with modern views, psychosocial rehabilitation of patients with endogenous pathology should be carried out in two stages: inpatient and outpatient. It is obvious that each stage of psychosocial rehabilitation should have its own approaches and characteristics, based on the principles of continuity.

To address the problems of increase in efficiency of psychosocial rehabilitation of this group of patients, we conducted a series of studies on the use of individual and group psychotherapy as basic psychological methods of rehabilitation of patients with schizophrenia. The research task is to determine realistic and achievable therapeutic goals for each stage and to find the optimal forms and methods of psychotherapy.

As the result of the studies, carried out in the Academician Yuschenko Vinnytsia Regional Psychoneurological Hospital as part of the "primary psychotic episode" program, it was found that at the inpatient stage, it is advisable to use psychotherapy with the patients from the first days of arrival, despite the presence of acute psychotic symptoms.

The possibility of verbal contact with the patient is a prerequisite here. As practical experience has proven, at this stage, the most appropriate are the methods of individual psychotherapy, aimed primarily at reducing the emotional stress of the patient by releasing aggression without damaging the perception of reality. Amelioration and onset of restitutional symptoms indicate that the level of aggression has decreased enough to be neutralized to the extent necessary to resume contact with objects, bringing back the interest in the external world. Emotional stress is caused not only by the patient's psychotic experiences, but also by the behavior of others towards him, which is perceived as groundless violence; stressful conditions of staying in the closed ward, in particular connected to the perception of psychopathology of other patients; frustration of the individual behavioral patterns of the patient, such as limited communications with the family, etc.

Instillation of the thought that the patient must be less aggressive is a good way to make him more ill. Accumulated aggression must be adequately expressed. The process of expression is neutralizing aggression to some extent, and the task of the psychotherapist is to remove obstacles for the release of the accumulated aggression [3]. When this is done, psychotic symptoms decrease and the patient can learn a healthier way of dealing with aggression.

In this work process, realization of the transfer, occurring during communication with the therapist, helps the patient to observe him in dealing with other people, which increases the realistic assessment of reality and effectiveness of social behavior. The transfer, subjective to the patient, does not mean distorted perception, but believable point of view, based on his recurrent life experience. This does not mean a specific

retrieval, but the revitalization of the conflict attitudes and behavior patterns, integrated into the personality structure. The patient was forced to cope alone with the effects of traumatic experiences, sometimes for quite a long time, and he was able to survive only due to the presence of psychological defense mechanisms – denial, rejection and depersonalization.

It is important to note that in the process of psychotherapy with patients in acute condition, some methods proved to be ineffective and in some cases even provoked a short-term increase in symptoms. These are the methods, aimed at immersing the patient in his experience, activation of unconscious processes for their clearer identification and interpretation as a psychotherapeutic tool, as well as methods of severe confrontation of inadequacy of psychopathological experiences of the patient. In most cases, premature interpretation and reaction of the therapist is viewed by the patient as rejection, and the fact that the therapist is afraid or unable to bear the situation corresponds to the reaction of the patient's inner circle in his past experiences. Interpretation is more appropriate for emotional reactions that are a template of reactions from the past superimposed on actual behavior.

Recognition of maladaptive psychological defense mechanisms meets inevitable resistance from the patient, which is not limited to aggression, but can take forms of passivity and various forms of denial [4].

The patient usually appreciates the doctor's intervention to eliminate alien to his personality symptoms and direct manifestations of the disease, but has a negative attitude to attempts to eliminate the symptoms that take form of psychological defenses and feel like part of his personality (ego-syntonic).

Understanding the processes of psychodynamic state experienced by the patient and the analysis of rather severe countertransference reactions by the therapist should be an integral part of the therapy. Since it is the understanding of the deep essence of what is happening that allows the therapist to retain his psychotherapeutic position and constructively accompany the patient in the way of treatment [1].

The main thing is that countertransference feelings and emotions do not get involved into play to meet the infantile needs of the patient in dependence and aggression.

To non-specific means of overcoming resistance belong tools for increasing the motivation of the patient to treatment and his emotional support. The resistance decreases in proportion to increase in confidence in the interaction with the therapist.

Another important goal of psychotherapeutic interventions is the correction of the patient's behavior in the ward, increasing the level of conformity of the patient regarding the conditions of stay in a psychiatric hospital, and compliance with his treatment with psychotropic drugs, despite their side effects.

Therefore, the most reasonable and effective tools are containment of the material by the patient's psychotherapist, expression of empathy to the patient's experiences and support of all constructive manifestations of the patient ("mirroring" manifestations of the ego-functions).

With the reduction of psychotic symptoms, it is advisable to expand the arsenal of psychotherapeutic interventions and methods, ultimately resulting in the inclusion of the patient into the group therapy as a safe model of society.

Psychotherapeutic group creates a favorable, secure environment in which the patient learns to talk about what is bothering him, instead of keeping it to himself, to express his feelings, and learn from others how he looks, what impression he makes, to recognize what measure of responsibility he must bear for his own life, regardless of the support received from the others. Dynamics of the patient's behavior in the group is a clear indicator of the increasing adaptability of his social behavior [5].

In the course of group interaction, patients will inevitably begin to display rigid patterns of behavior. The group makes optimal correction of maladaptive attitudes possible, which then can be followed by the rehearsal of adaptive behavior.

Conclusions

When working with people with schizophrenia, it is not productive to use the models, only designed to work through global and general group phenomena. The main objective is the greatest possible individualization of tasks for each member of the group. An individual interview with each member of the group is advisable after each session of group psychotherapy to review the material. Such a combination of individual and group approaches is optimal, because it allows you to combine deep insight into intimate problems (in individual conversations with the doctor) with potential means to diagnose communicative disorders and correction of interpersonal conflicts, provided by the interaction in the group.

Psychosocial rehabilitation of patients with endogenous disorders should be implemented in two stages: inpatient and outpatient. The condition for a successful psychological recovery is the combined use of individual and group therapy at all stages, taking into account the severity of psychopathology: in the direction from support (holding, containment) to a balanced application of genetic reconstruction and analysis of interpersonal relations in individual therapy and solving individual problems in the context of the general group phenomena.

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PAGES OF HISTORY

History of Nicolae Testemitsanu State University of Medicine and Pharmacy through its rectors

Gh. Ghidirim[1], *R. Scerbina[1], Iu. Grossu[2], A. Muravca[1]

[1]Nicolae Anestiadi Department of Surgery
[2]Nicolae Testemitsanu Department of Social Medicine and Sanitary Management
Nicolae Testemitsanu State University of Medicine and Pharmacy, Chisinau, the Republic of Moldova
*Corresponding author: romeo.scerbina@yahoo.com. Received October 13, 2015; accepted December 03, 2015

Abstract

Background: The authors present the history of Nicolae Testemitsanu State University of Medicine and Pharmacy from the beginning, since the transfer of Leningrad Institute of Medicine No 1 in November 1945. Rectors: Ipatie Sorocean, Nicolai Harauzov, Leonid Ganul, Nicolae Starostenco, Nicolae Testemitsanu, Vasile Anestiadi, Leonid Cobaleanschi, Ion Ababii are presented with a broad feature of their work – teaching, scientific, managerial. A particular attention is given to rectors academicians: Nicolae Testemitsanu, Vasile Anestiadi and current rector Ion Ababii. A decisive role in the development of the university belongs to Nicolae Testemitsanu, who was the first local rector to promote national staff, contributed to the opening of the faculties of Dentistry, Continuous Medical Education, Preventive Medicine and Pharmacy. He sent local staff for advanced studies to different USSR centers, who after returning occupied the positions of heads of departments and laboratories. Vasile Anestiadi continued the promotion of the university, which became one of the most prestigious medical institutions in the former Soviet Union. Ion Ababii, the current rector, has promoted the university applying European ideas, liaising with other universities of the same type, a strategic partnership with future effects. Thus, Nicolae Testemitsanu State University of Medicine and Pharmacy reached the anniversary of 70 years, having an imposing history and a foreseeable future, occupying a deserved place among higher educational institutions of the Republic of Moldova.
Conclusions: The history of Nicolae Testemitsanu State University of Medicine and Pharmacy represents an important page of our country's history. The teaching team brings new achievements in the training process of medical staff of the Republic of Moldova.
Key words: history, university, rectors.

Motto: "Though history contemplates not hastily,
but substantially and irreproachably"

M. Eminescu

State University of Medicine in Chisinau (fig. 1) was inaugurated in the fall of 1945 by the Order No 427-899 of 31.08.1945, signed by the minister of health of the former USSR, George Miterev, who ordered the transfer of Leningrad Institute of Medicine No 1, temporarily deployed in Kislovodsk, to Chisinau. The reason for this transfer was Iosif Stalin's decision to punish the teaching staff of Leningrad Institute for continuing their activities during German occupation of the North Caucasus [1-4].

The first rector (1945-1948) was **Ipatie Sorocean** (fig. 2), native of the town Balta of the Moldovan Autonomic Soviet Socialist Republic, who received order directly from the hands of Veacheslav Molotov, first vice prime minister of the USSR, went to Kislovodsk, and came back to Chisinau in charge of an echelon, which included 15 heads of departments, eight university professors, 43 associate professors and lecturers, and 570 students.

The institute having a single faculty – General Medicine – began its activity on 20 November 1945. When asked, over some time, by the Minister of Health of the USSR if there were local teachers among the teaching staff, rector I. Sorocean responded – "Yes, we have one only". He was a single teacher

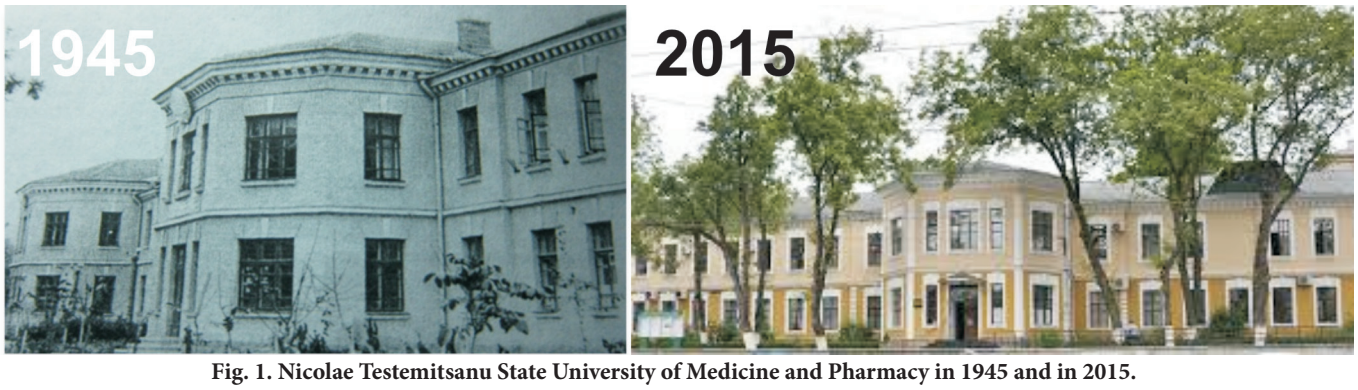

Fig. 1. Nicolae Testemitsanu State University of Medicine and Pharmacy in 1945 and in 2015.

Fig. 2. Ipatie Sorocean, the first rector.

Fig. 3. Nikolai Harauzov, the second rector.

Fig. 4. Leonid Ganul, the third rector.

Fig. 5. Nicolae Starostenco, the fourth rector.

Moldovan originated and made the faculty in Bucharest, in the interwar period. Ipatie Sorocean was rector for a period of the toughest three years – war, famine, the beginning of forced collectivization. He remained in the memory of students due to his parental care about local students, through sharing with the neediest food and clothing. In this context, students affectionately called him "dad Sorocean". During that period, there was presented one PhD thesis by Natalia Gheorghiu (1948) – a graduate of the University of Medicine in Bucharest.

The second rector (1948-1951) was the Associate Professor **Nikolai Harauzov** (fig. 3). In 1950 the first graduation of local physicians took place: Constantin Tabarna, Ion Vovc, Teodor Gatu, Valentina Halitov and George Neghina among them. Meanwhile, 12 PhD theses were presented, but none of local scholars.

The third rector (1951-1953) was **Leonid Ganul** (fig. 4). During his period of activity there were presented 12 PhD theses, and also none of the local scientists. The second graduation of local doctors was, rightly, the 'gold' graduation. Among them: Nicolae Testemitsanu – future rector, minister of health, professor, academician of Moldovan Academy of Sciences (MAS), Vasile Anestiadi – future rector, academician of MAS; George Paladi – future Professor, Academician of MAS; Diomid Gherman – future Professor, Academician of MAS, renowned surgeons – Pavel Batca, Eugen Semeniuc and Eugen Maloman, the famous oncologist Professor Gurie Cosciug, Professors Vasile Negrescu, Victor Gheteu, Teodor Chiticari and Alexandru Nacu, Honorary Member of MAS.

The fourth rector (1953-1959) was Professor **Nicolae Starostenko** (fig. 5). He was a very good specialist-therapist, who was the treating doctor of Leonid Brezhnev, when he was the First Secretary of the Communist Party of Moldova (1952-1953). During his activity, more active promotion began for supporting training of local staff and leadership positions: heads of departments, deans, vice deans. During that time there were presented 42 PhD theses and 2 medical dissertations, of which 19 (18 PhD theses and one medical dissertation) by local researchers. The Faculty of Pediatrics was inaugurated in 1954.

The fifth rector (1959-1963), appointed to this position at the age of only 32, was **Nicolae Testemitsanu** (fig. 6), the first of indigenous origin and the first who was not dismissed from the post of rector with administrative penalties and by the ruling party, later promoted to the post of Minister of Health of the Republic of Moldova (1963-1968). The merits of Testemitsanu are undeniable: he introduced the differentiation of specialties, opening new departments: traumatology and orthopedics, anaesthesiology, phtiziology, urology, ophthalmology and others. He stimulated by all means, post-graduate and doctoral studies, he founded faculties – Dentistry (1959), Advanced training of Doctors (1963), Preventive Medicine (1963), Pharmacy (1964). He assured the switching – over the language of instruction from Russian into Moldovan as well as with editing and reprinting textbooks promoting national staff at different positions. Being the son of a peasant, a child educated in the national spirit, he could not tolerate the situation that affected indigenous people. Having been appointed the rector of the Institute of Medicine in 1959, he remained displeased with the fact that during 10 years of activity of the Institute of Medicine of Chisinau, there had been prepared 1689 doctors, of whom only 168 – natives, and out of 33 PhD theses only 16 were presented by locals, and out of 8 medicine dissertations, only one belonged to a native scientist.

He remained a son of earth, a branch of nation deeply implanted in the native land. Often he said: "*We have a big debt to the people from villages, who grow our bread, do not forget that most of us are from the countryside and we need to give back our debt to the village, which inhabitants are the Soul of the Country.*" And indeed, being rector and minister, he directed to all the prestigious scientific centers of the former USSR sons of peasants, who were honest and smart. Remaining faithful to the debt to peasants, for the first time in the USSR and the world, he proved in a scientific way the need to strengthen the primary medicine branch, followed by the construction of hundreds of outpatients' clinics. This paper work was honored with the State Prize in 1974. Much later this conception became the notion of "family doctor".

The sixth rector (1963-1985) of the Institute of Medicine and Pharmacy becomes the **Vasile Anestiadi** (fig. 7) – the future professor, academician of SAM, Laureate of the State prize (1967). He was leading the institute for 23 years. He was a controversial person. Taking the baton from rector Nicolae

Fig. 6. Nicolae Testemitsanu, the fifth rector.

Fig. 7. Vasile Anestiadi, the sixth rector.

Fig. 8. Leonid Cobâleanschi, the seventh rector.

Fig. 9. Ion Ababii, the eighth rector.

Testemitsanu, following his proposal and insistence, but even if Vasile Anestiadi followed the way of Nicolae Testemitsanu, he did not remain faithful to Nicolae Testemitsanu's principles

He contributed to the construction and arrangement of: morphology block (1964), block for studies No 2 (1967), Pharmacy of the University (1982), Museum of the University (1975), aesthetic center (club) "Ion and Doina" (1985), houses for collaborators etc. During the time he was the head of the university there were presented 462 PhD theses and 61 dissertations.

The seventh rector (1986-1994) of the Institute was **Leonid Cobâleanschi** (fig. 8). He fortified the material and technical basis of the University required for improving the training process for the students and residents. He introduced the training for doctors through residency. During that time there were presented 173 PhD theses, and 73 dissertations of which 105 and 59 respectively by natives. In 1991 State Medical Institute in accordance with rector Leonid Cobileanschi's proposal supported by minister of health Gheorghe Ghidirim, becomes Nicolae Testemitsanu State University of Medicine and Pharmacy in acknowledgement of his honorable work to promote the national staff.

The eighth rector (1994 – present) is a University Professor, State Prize Laureate, Academician **Ion Ababii** (fig. 9). He is the Honorary Citizen of the City of Chisinau. Through diligent work in difficult socio-economic conditions, rector I. Ababii contributed to solving various urgent issues regarding the development of the university – through grants, international cooperation and partnership agreements. During his activity, rector I. Ababii acts as a true reformer of higher medical education, integrating the university in European and world educational structures.

He introduced residency and fellowship training; he began to enroll students from other countries to all faculties, implemented advanced methods and information technology in training process and medical research. He constantly develops material base of the University: there have been built dormitories, apartment buildings for university employees, and fitness complex, University Library and Museum, Alley of Great People of Indigenous Medicine etc. He created a prestigious school of otorhinolaryngology. For his work, Professor I. Ababii was awarded with the Albert Schweizer Great Gold Medal, "P. Elrich" Gold Medal, "Robert Koh" Medal, "N. Pirogov" medal, etc. He is a Doctor Honoris Causa of several universities, Member of the European Office of the World Health Organization, Honorary Member of the Academy of Sciences of Poland and Finland, member of the Russian and US Academy of Otorhinolaryngology. In January 2014, Steten Lindgren – the President of the World Federation for Medical Education noticed: "We visited several Universities, but I am sure that the University of Medicine and Pharmacy in Moldova has everything needed to be recognized internationally in medical education field".

During 2005-2008, the position of interim rector of Nicolae Testemitsanu State University of Medicine and Pharmacy was assured by Professor **Nicolae Esanu**, who has contributed a lot to directing the training process of the youth and residents.

An important contribution to local science has made all the teaching staff of Nicolae Testemitsanu State University of Medicine and Pharmacy. In the period of 1994-2011 there have been presented 544 PhD theses and 95 dissertations, after 2011 – till present, there have been presented 47 PhD theses and dissertations.

This autumn (2015), Nicolae Testemitsanu State University of Medicine and Pharmacy of the Republic of Moldova celebrates 70 years since its inauguration. Let's wish the prestigious Alma Mater and its rector – Ion Ababii, remarkable success in preparing future medical specialists needed so much for the Bassarabian nation, and let's wish the rectors gone into eternity a living and constant memory!

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CHRONICLE

Address to the Ceremony dedicated to the 70th Anniversary of Nicolae Testemitsanu State University of Medicine and Pharmacy

Nicolae Sulac National Palace Moldova Concert, Chisinau, October 08, 2015

Professor David Gordon
World Federation for Medical Education

State Secretary, Prime Minister, representatives of the government of the Republic of Moldova, friends, colleagues and students!

Above all, I would like to address what I say to my good friend and colleague, Rector Ion Ababii.

First, I must apologize that I cannot speak to you in the Romanian language, and I am sure you will understand if I speak in English. I am greatly honored to be here at this celebration of the seventieth anniversary of the University.

A seventieth birthday – and I know personally, because my own seventieth birthday is not far away – is an occasion to ask three questions: where did we come from; how are we doing now; and what will become of us?

To answer the first question, the boldness and vision in opening a new medical university during the dark and chaotic days of 1945 are remarkable. It was a gesture of optimism and hope for the future and recognition of the need of the people of Moldova for their own well-educated doctors, and other health professionals.

How is the University doing now? This speaks for itself; the evidence is here today – graduates from the University practicing medicine, not only in Moldova, but all over the world; the growth of the University and its facilities; the energetic, talented and enthusiastic student body; and the wide international collaborations of the University.

What will become of the University? I would ask the University just to remember this: a university is not buildings; or money; or facilities. It is a community of people; staff and students working together to understand what we know and to gain new knowledge of the world around us; and applying that knowledge for the benefit of our patients and the community in which we serve.

I have a fourth comment, and this is addressed to the representatives of the government of the Republic of Moldova. Your Medical University is a fine example of the strength of this beautiful country. It shows the vigor of academic life in Moldova. Its students and graduates are excellent examples of the high ability and commitment of your young people. The University is a strong ambassador for Moldova. Please, look after it well!

I would like to return to something I mentioned at the start of my talk.

We all know the University is strong, and will continue to thrive. A truly outstanding reason for this success is the exceptional leadership, over many years, of the rector.

I am sorry I cannot say this in Romanian, but – Ivan Ivanovich, spasibo! [Thank you!].

Address to the Ceremony dedicated to the 70th Anniversary of Nicolae Testemitsanu State University of Medicine and Pharmacy

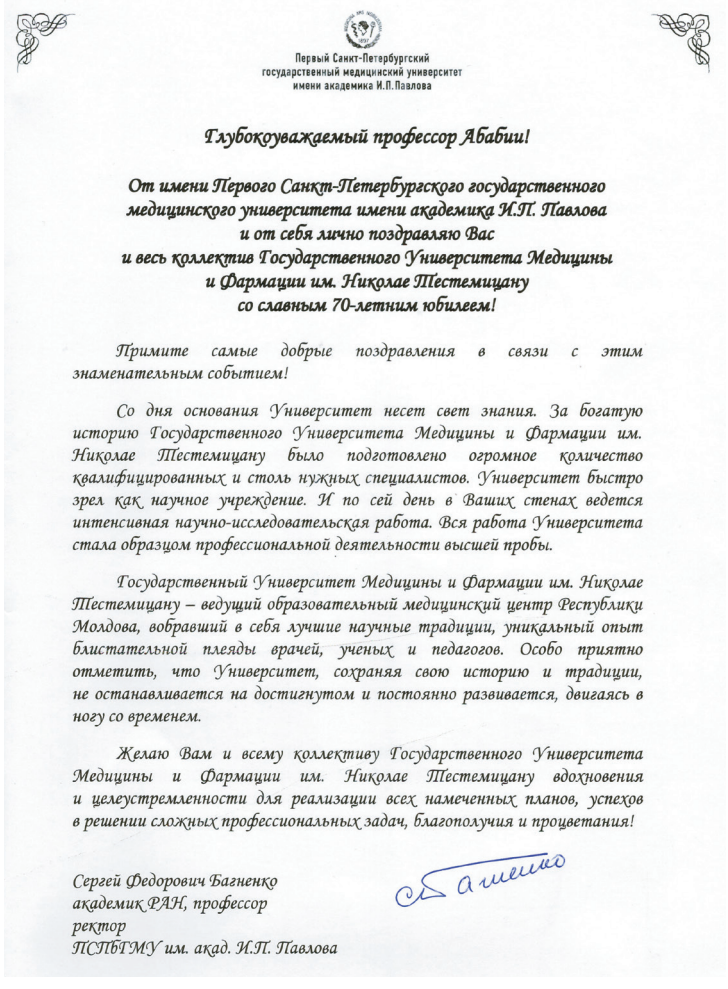
Nicolae Sulac National Palace Moldova Concert, Chisinau, October 08, 2015

Sergei Anatolievich Karpischenko
MD, PhD, Professor, Head of the Departament of Otorhinolaryngology
Secretary of the Academic Council of the First State Medical University of St Petersburg, Russia

Dear rector!

Dear honorable guests, colleagues, ladies and gentlemen!

First of all I would like to express my gratitude personally to you, Mr. Ababii, as a rector, for the great honor to be present here and participate in the memorable gathering, dedicated to the 70-th Anniversary of this renowned medical university!



Первый Санкт-Петербургский
государственный медицинский университет
имени академика И.П.Павлова

Глубокоуважаемый профессор Абабии!

От имени Первого Санкт-Петербургского государственного медицинского университета имени академика И.П. Павлова и от себя лично поздравляю Вас и весь коллектив Государственного Университета Медицины и Фармации им. Николае Тестемицану со славным 70-летним юбилеем!

Примите самые добрые поздравления в связи с этим знаменательным событием!

Со дня основания Университет несет свет знания. За богатую историю Государственного Университета Медицины и Фармации им. Николае Тестемицану было подготовлено огромное количество квалифицированных и столь нужных специалистов. Университет быстро зрел как научное учреждение. И по сей день в Ваших стенах ведется интенсивная научно-исследовательская работа. Вся работа Университета стала образцом профессиональной деятельности высшей пробы.

Государственный Университет Медицины и Фармации им. Николае Тестемицану – ведущий образовательный медицинский центр Республики Молдова, вобравший в себя лучшие научные традиции, уникальный опыт блистательной плеяды врачей, ученых и педагогов. Особо приятно отметить, что Университет, сохраняя свою историю и традиции, не останавливается на достигнутом и постоянно развивается, двигаясь в ногу со временем.

Желаю Вам и всему коллективу Государственного Университета Медицины и Фармации им. Николае Тестемицану вдохновения и целеустремленности для реализации всех намеченных планов, успехов в решении сложных профессиональных задач, благополучия и процветания!

Сергей Федорович Багненко
академик РАН, профессор
ректор
ПСПбГМУ им. акад. И.П. Павлова

No doubt, I am aware of the fact that in this way you demonstrate your respect to otorhinolaryngology, my favorite field of medical science. As the secretary of the Academic Council of the First St Petersburg State Medical University I have the great pleasure to read the official address of our rector personally to you and your university personnel. Let me do it…

Using this unique situation I can't help expressing the idea in which I am completely convinced. This idea emphasizes the fact that any traditions are valuable if they are continued. At my age of 45 it is not easy to look 70 years back and imagine that period of time when Mr. Ipatie Sorochean founded this University. Due to the fact that our Universities established useful and fruitful contacts 2 years ago, I had an opportunity to read the recollections of people who worked closely with Mr. Ipatie Sorochan and knew him personally. In this way I learned many interesting and important facts about that remarkable person and got ready for coming here. Now I know that Ipatie Sorochean was wounded twice, his life was rather hard, similar to lives of most people at those war and post-war periods. In spite of all hardships he managed to create a team of teaching staff and students with exceptionally warm and friendly atmosphere and he was even like a father for many of students. For that students called him "Dad Sorochean"! To my thinking such attitude is the key factor for setting up any team, especially the academic one.

Intellectuals are characterized by such sincere and friendly relations and this is the basis for any creative activities in higher schools and everywhere else, in any human society. At my age, of course, I can feel this atmosphere which was laid 70 years ago.

Nowadays, when I come to you, meet your doctors I feel the warmth of your hearts. We feel at home here and thanks for the warmth that you willingly share with us. We highly appreciate that you don't forget that Medical University of Moldova originated from the city on the banks of the Neva river.

We wish the Medical University of Moldova further great achievements in medical science and training of highly qualified doctors to contribute to the development and prosperity of your country.

Thank you!

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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.

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GHID PENTRU AUTORI

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4. Textul articolelor clinice, experimentale (până la 15 pagini) cuprinde: Introducere; Material şi metode; Rezultate obţinute; Discuţii; Concluzii şi Bibliografie până la 40 de referinţe. Altă structură se acceptă, dacă aceasta corespunde conţinutului materialului. **Articolele de sinteză** nu vor depăşi 25 de pagini şi bibliografia până la 100 de surse.

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Address of the Journal Office
192, Stefan cel Mare Avenue
Chisinau, MD-2004
Republic of Moldova

Telephone: +37322244751
Fax: +37322295384
www.curierulmedical.org
editor@curierulmedical.org
secretary@curierulmedical.org

Adresa redacţiei
Bd. Ştefan cel Mare, 192
Chişinău, MD-2004
Republica Moldova

Telefon: +37322244751
Fax: +37322295384
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editor@curierulmedical.org
secretary@curierulmedical.org

Адрес редакции
Пр. Штефан чел Маре, 192
Кишинёв, MD-2004
Республика Молдова

Телефон: +37322244751
Факс: +37322295384
www.curierulmedical.org
editor@curierulmedical.org
secretary@curierulmedical.org