PROCEEDINGS

ALLERGY, ASTHMA, COPD, IMMUNOPHYSIOLOGY & NOREHABILITOLOGY: INNOVATIVE TECHNOLOGIES

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FILODIRITTO
INTERNATIONAL PROCEEDINGS

FiLO diritto
dal 2008 editore

ISBN 978-88-95922-83-6

First Edition April 2017

filodirittoeditore.com
inFOROmatica srl, Via Castiglione, 81, 40124 Bologna (Italy)
inforomatica.it
tel. 051 9843125 - Fax 051 9843529 - commerciale@filodiritto.com

Printed by Rabbi S.r.l., Via del Chiù, 74, Bologna (Italy)

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Dear Colleagues and Friends, Ladies and Gentlemen,

We are happy to welcome you in New York on April 29 – May 1, 2017 at the X WORLD, ASTHMA, ALLERGY & COPD FORUM that will be concurrently held with the XXIII World Congress on Clinical Medicine and Immunorehabilitation.

This Volume of "Allergy, Asthma, COPD, Immunophysiology & Immunorehabilitology: Innovative Technologies" presents a selection of reports delivered at this Forum, organized by the World Immunopathology Organization. The lectures and discussions during this Congress will cover every aspect of basic research in the field of asthma, COPD, respiratory allergy, and immunophysiology, and will also bring forth the latest innovations in immunorehabilitation. The prevalence of asthma and COPD around the world, their severity and mortality rates allow us to consider them as the most socially significant diseases of today and the nearest future. The problem of allergy, asthma, COPD and immunopathology is of global character. Every year the prevalence rate of this pathology is increasing both in adult and children population. The life longevity is diminishing while the quantity of chronic cases and recurrence rate rise sharply. The same is true for the mortality rate of patients with such pathology. That is why, development and implementation of new diagnostics and immunorehabilitation innovative technologies methods is crucial in this situation. They would not only reduce the recurrence rate, these

novel innovative methods would also increase the remission duration.

It is one of the most vital problems of contemporary medicine. Advances of modern medical science as well as concerted efforts of various specialists – allergologists, immunologists, pediatricians, pulmonologists, dermatologists and other physicians, specialists in cellular and molecular biology etc. – would contribute a lot to solve these problems.

The world's leading experts in the field of allergy, asthma and COPD, immunopathology and immunophysiology accepted our invitation and attended this scientific Forum. They represent the World Immunopathology Organization (WIPO), World Allergy Organization (WAO), American College of Allergy, Asthma & Immunology (ACAAI), American Academy of Allergy, Asthma & Immunology (AAAAI), American Thoracic Society (ATS), European Academy of Allergy and Clinical Immunology (EAACI), European Respiratory Society (ERS), Asian Pacific Association of Allergy, Asthma & Clinical Immunology (APAAACI), Asian Pacific Respirology Society (APRS). Some of their reports presented at the Congress are published in this volume.

I sincerely hope that these articles will allow you to obtain new information on the recent advances in the basic knowledge and new innovative technologies in clinical management of allergy, asthma, COPD, immunopathology and immunorehabilitology.

This is the main goal of all meetings organized by World Immunopathology Organization (WIPO) and this is the main goal of the materials published in this volume.

Professor Revaz SEPIASHVILI, MD, FAAAAI, FACAAI, FACCP
President, World Immunopathology Organization (WIPO)

Immunorehabilitology: Sources, Present and Perspectives. From Immunotherapy to Personalized Targeted Immunorehabilitation

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B428R9050

Abstract

Development and introduction of modern clinical diagnostic tests (that allow to evaluate the functional system of immune homeostasis) into medical practice, a huge body of evidence on the leading role of the immune system in pathogenesis most acute and chronic diseases and even identification of specific nosological forms of immune-mediated diseases forced the scientists to search and develop new tools and techniques that have therapeutic effects on the impaired immune homeostasis and restore it to the normal state.

The introduction of a novel concept – immunorehabilitation – was an impetus for the accumulation of new knowledge and a catalyst for research in clinical immunology. The first papers on this topic were published over 30 years ago [6, 8–11, 15, 17]. It was Revaz Sepiashvili who breathed life into the concept of immunorehabilitation. He was lucky to be at its origin. He became not only the founder of the brand new scientific field – immunorehabilitation, but also the founder of a new medical science – immunorehablitology.

In this paper, the author returns to the roots and recalls the way that medical science has gone before coming to understand immunorehablitology and tells readers about current successes and its development prospects.

Keywords: immunorehabilitotogy, immunorehabilitation, immunomodulators, immunotherapy, immunosuppression, immunocorrection, classification of immunomodulators, immunotropic therapy, clinical immunology, rehabilitation, immune system physiology, immunophysiology, balneologic rehabilitation, allergy, asthma, COPD, immunopathology

Recent advances in clinical immunology made possible to identify abnormalities of immune system functions in various conditions. However, treatment tools and modalities did not always demonstrate adequate therapeutic

efficacy or desired outcomes. Other treatment strategies and approaches were required to restore immune system dysfunctions. The introduction of a novel concept, immunorehabilitation, was an impetus for the accumulation of new knowledge and a catalyst for researches in clinical immunology [1–10].

Immunorehabilitation implies not only the restoration of dysfunctional parts of immune system but the recovery from acute disease or stable remission in chronic conditions as well [14–23].

I was lucky to be at its origin and to become not only the founder of a novel scientific brand, immunorehabilitation, but also **the founder of a new medical science, immunorehabilitology**.

Today, one may speak with confidence that immunorehabilitology made the way from a simple term, "Eastern fairy tale", to worldwide recognition. I would like to return to the roots and to recall the way that medical science has gone before coming to understand immunorehablitology and to tell readers about current successes and its development prospects.

Historically, the term "immunorehabilitation" has appeared against a background of preexisted certain success in the treatment of immune dysfunctions. Initially, the conception of **immunotherapy** was introduced, i.e., some conditions were treated with immunological methods (e.g., diphtheria toxoid for diphtheria etc.).

Next, we faced with the problem of tissue and organ transplantation and autoimmune disorders which required to inhibit normal functions of immune system. The conception of **immunosuppression** exactly illustrated this aim.

The identification of secondary immunodeficiencies as a result of immune deficiency has raised the issue of **immune stimulation**, at first meaningful term. However, it was demonstrated that immune system functions are mediated by at least twenty various components. Therefore, one may ask, which components should be stimulated? If we stimulate suppressor cells, patient's condition will be aggravated instead of being improved. Hence, the conception of immunostimulation did not explain which component should be stimulated and which component should be inhibited and did not provide a basis for differentiated approach to each component.

The adoption of **immunocorrection** into clinical practice was an objective process which helps to correct abnormal immune parameters and to bring them to a new level which will correspond to normal parameters.

However, the question has often arisen as to whether immunocorrection was always required? Should we bring the reduced parameter back to normal? Considering this, the conception of immunocorrection has left unanswered some questions. That's why the introduction of a novel term,

immunomodulation, in the middle 1970s was an objective process in clinical immunology. Immunomodulators provided immunotropic effect to normalize reduced or elevated parameters.

Many authors have demonstrated stimulating and suppressive effects of various exogenous and endogenous factors (various organic and non-organic agents, biopolymers, components of foreign organs and tissues, microbial cells etc.) on immune system. The agents which provide benefit effects on immune system were referred to as adjuvants.

Many investigators uphold a broad interpretation of the concept of immunomodulators and reckon among them adjuvants, immunosuppressants etc. If we accept this viewpoint, we have to say that all approved immunobiological preparations (vaccines, immunoglobulins, bacteriophages, normal flora preparations, allergens, cytokines etc. – more than a thousand) are immunomodulators. Annually, 20 to 30 new immunobiological agents undergo clinical trials in Russia, and one-third of them are human preparations [12].

Such wide interpretation could be acceptedprovided the immune response would not be a specific reaction to antigen.

To comprehend immunomodulators, we introduced the following definition:

Immunomodulators are agents which affect mainly the functional system of immune homeostasis and demonstrate tropism and specificity to immune system.

It was all perfectly logical that in the middle 1980s, a novel branch of modern medicine, **immunorehabilitation**, has emerged. Immunorehabilitation considers the relations between immune and other systems (i.e., nervous, endocrine, respiratory, circulatory etc.). This conception implied not only the restoration of dysfunctional parts of immune system but the recovery from acute disease or stable remission in chronic conditions as well. Immunorehabilitation includes reconstructive, medical, physiotherapeutic, and balneologic restoration of immune system capacity to provide protective and regulatory functions [7, 8, 11, 17, 19]. First studies on immunorehabilitation in some internal diseases, autoimmune conditions, and secondary immunodeficiencies [3–21] demonstrated high efficacy of its principles.

It all started in 1984 when I have had to leave Krasnodar for Tskaltubo where we have founded resort immunological center. In 1989, Tskaltubo Research Institute of Clinical Immunology and Allergy was founded. The Institute is generally recognized as a worldwide leader in the development and scientific basing of major principles and methods of immunorehabilitation both in clinical

and resort settings which determine the prescription of medicines, balneologic, and preformed physical factors for immune system disorders. Additionally, we developed indications and contraindications for their use depending on the age and disease stage and introduced clinical and laboratory (including immunological) tests to evaluate the effect of natural factors which might be used in combination. Optimal conditions for their prescription (single doses and courses, methods of administration, compatibility with each other and with pharmacological immunomodulators and other medicines, complications) were specified as well. We also developed preventive and anti-recurrence measures.

The association between disease course and immune reactivity was established. In patients with relatively stable remission (6 to 12 months), normal, close to normal, or insignificant changes in immunological parameters were revealed. On the contrary, relatively early recurrences (1 to 2 months) were mainly seen in patients with stable and long-term immune dysfunctions.

Sanatorium and resort immunorehabilitative factors can be combined with medicinal immunomodulators. Administration of immunomodulators by physiotherapeutic methods (electrophoresis, phonophoresis) was introduced.

This technique demonstrated high efficacy and safety.

We developed and introduced step-by-step approach to the rehabilitation of patients of immune system dysfunction, i.e., institutional hospital → sanatorium (immunorehabilitative center). Immune system was assessed using similar methods at every stage. It was demonstrated that this strategy is superior to classic therapeutic approaches.

Clinical immunological characteristics of patients with immune dysfunction should be the essential basis for rehabilitative strategy choice.

Immunorehabilitative methods are harmless and provide neither adverse side effects nor complications. They are easy to assess for use in younger persons as well as in elderly patients who have contraindications to certain medicines or therapeutic methods and high risk of complications.

Our studies demonstrated that immunorehabilitation is as essential step of immune dysfunction treatment which is characterized by high efficacy.

This is illustrated by the inhibition of progressive course of a pathological condition, reduced treatment period, decreased rate of recurrences, significant prolongation of remission or complete recovery and disability restoration.

These findings laid the foundation for a novel line of investigations, immunorehabilitation. Immunorehabilitation is a complex multifactorial process which includes medical, professional and psychological aspects.

Its signatures and principles in various pathological conditions are the basis for a novel branch of medical science in general and, in particular, immunology.

Currently, significant progress has been achieved in the hospital treatment of immune dysfunction. Therapeutic efficacy of various immunomodulators (Tactivin, Thymalin, Myelopid, Splenin, Thymogen, sodium nucleinate, interferons, Prodigiosan, Dimephosphan, Vilosen, Thymonox, Thymulin, Imunox, Sandimmun, Gamimun N, Licopid, Polyoxidonium etc.) was examined in large patient populations. Additionally, several methods of immune dysfunction correction – immunoadsorption, plasmapheresis, administration of autologous macrophages, extracorporeal immunopharmacotherapy etc. – were introduced into clinical practice [12].

Numerous examples from the experience of the past two decades demonstrate the efficacy of sanatorium-resort rehabilitation measures. Correctly organized treatment to restore ability to work results in complete return to work in most cases.

A great experience of hospital immunorehabilitation has been accumulated, however, sanatorium-resort immunomodulation therapy is still challenging.

By addressing these issues, we will be able to use this principle in applied medicine.

An inevitable question is, do we as immunologists know how preexisted resort and physical factors affect immune system under normal and pathological conditions?

We say, "**This is a cardiac resort**" or "**This is a neurological resort**" or "**This is a gynecological resort**" etc. Nowadays, we can also say, "**This is an immunological resort**". This is a great step forward since immunological mechanisms underlie many disorders which require sanatorium-resort treatment [1, 11, 12, 17, 19-23].

Millions of patients with various immune abnormalities are referred to sanatorium resorts. As a rule, neither health care professionals nor patients know about these abnormalities. Complex treatment does not provide desired outcomes as many physicians are not aware of the effects of various natural and physical factors on major and/or supplementary pathogenic mechanism of the disease, i.e., human immune homeostasis. There are many natural healing factors which provide certain effects on human immune status under normal and pathological conditions. This problem is an important issue of modern medicine which solution will allow physicians to control immune homeostasis with medicines or in combination with non-medical, balneologic, and physical factors depending on the clinical situation.

In addition, most patients are in remission when they arrive at the resorts.

In remission, immune abnormalities are not so significant. However, that's not to say that immune system of these patients functions normally. Considering

this, methods which objectively assess immune system under balneologic conditions should be improved.

The mechanisms of immunomodulating action of certain preexisted factors (i.e., radon, hydrogen sulfide or iodide-bromide baths, drinking mineral water, climate, speleotherapy, sea salt inhalations etc.) at every resort should be studied independently considering local specificity.

In inpatient care departments (irrespective of its geographical situation), certain medicinal immunomodulator provides similar effect on patients.

However, similar preexisted balneologic factors in various regions of the country may provide totally opposite effects. Therefore, precise criteria which determine the prescription of certain balneologic and physical factors in immune disorders should be developed and the mechanisms of their immunomodulating action should be investigated. Indications and contraindications to their use depending on the age and disease stage should be established as well.

Clinical and laboratory (including immunological) tests to assess the efficacy of natural factors should be introduced. Optimal conditions for their prescription (single doses and courses, methods of administration, compatibility with each other and with pharmacological immunomodulators and other medicines, complications) should be also specified. Preventive and anti-recurrence measures should be developed as well. The recognition of these aspects will help the physicians to provide pathogenically based treatment of immune abnormalities.

When establishing modern conception of immunorehabilitation, we defined its two major areas, i.e., ***specialized and applied immunorehabilitation***.

Specialized immunorehabilitation measures are indicated when immune disorder signs prevail in the pathogenesis of the condition.

In addition, novel findings provided a different view of immunorehabilitation of patients with immune dysfunction. We attempted to establish ***a strategy and an approach to complex immunorehabilitation*** of patients with immune dysfunction predisposed to chronic and recurrent course as well as to develop major principles, approaches, and methods of immunorehabilitation regarding immunopathogenic features of the disease. The basis is the general philosophy of immunorehabilitation and the analysis of my factual scientific matter, the data of my followers, and published data.

Therefore, ***complex program of immunorehabilitation*** of patients with immune dysfunction should include several components and approaches which provide targeted correction of identified quantitative and functional immune abnormalities at cellular and subcellular levels using multifactorial medical and non-medical methods of immunocorrection promoting restoration.

Considering long-term achievement of clinical immunological remission, we should also prolong beneficial effects and consolidate them in the course of immunorehabilitation.

Patients with immune dysfunction require long-term systematic differential pathogenic immunorehabilitation according to the severity of clinical immunological abnormalities and using immunocorrecting medicines or methods which normalize immune imbalance at every treatment stage.

Complex immunorehabilitation should be performed in a stepwise manner under immunological monitoring. In recurrent disease course, step-by-step therapeutic approach promotes regression or prevents disease progression and maintains clinical effects of immunorehabilitation. Recovery of immune dysfunction in recurrent immunopathological conditions should include basic, restorative, and maintenance immunorehabilitation as well as the following steps [15]:

First (clinical) step (14 to 45 days) is generally performed in inpatient department to verify clinical diagnosis and to specify the severity of immune abnormalities or in the exacerbations of underlying disease. At this stage, early rehabilitation involves the prescription of traditional basic etiotropic and symptomatic treatment as well as the administration of customized targeted immunocorrecting therapy (basic immunorehabilitation). When prescribing the treatment, comorbidities should be considered. Basic immunorehabilitation is a therapeutic diagnostic process to produce essential conditions for the recovery of abnormal immune homeostasis which includes the diagnosis and treatment of the conditions closely associated with the development and progression of immune imbalance. The nature and severity of immune abnormalities are evaluated by the results of clinical immunological examination while initial agent for targeted immunocorrecting therapy is determined by the defect of immune system. The development and adoption of complex strategy which accelerates the recovery and maintains the effect is an important aspect as well.

Extracorporeal medical procedures (plasmapheresis or hemosorption, 3 to 5 procedures) which provide rapid effect in immune disorders may be performed at this step depending on the disease severity.

Second (ambulatory) step (up to 3 years). This component of immunorehabilitation is the most prolonged and implies restorative treatment in outpatient department (restorative immunorehabilitation). However, if the diagnosis was primarily verified in outpatient department and the patient does not require admission to the hospital, basic and restorative immunorehabilitation may be included into the complex immunorehabilitation. Ambulatory immunorehabilitation is responsible for the most favorable conditions for the

recovery of lost functions. Considering this, complex measures to recover the health are required. Restorative immunorehabilitation involves various combinations of immunocorrecting agents and methods of the same specificity but with different mechanisms of action. Therefore, complex measures to eliminate disability (as a result of the disease) should include both medical aspects as well as psychological, pedagogical, and social measures.

This step characterized by complex and multifactorial nature is very important. Medical strategy should include individualized approach and objective assessment of disease activity based on clinical immunological abnormalities and psychological status of the patient. A complex of treatment modalities should include both medical therapy (basic therapy as required and immunotropic agents depending on the severity of immune imbalance under immunological monitoring) and non-medical physical methods (adaptation and therapeutic exercises, medical massage, topical application of immunomodulators on major inflammatory locus by electrophoresis or phonophoresis) regarding disease severity and leading clinical syndromes.

Physical activity and physiotherapeutic procedures should be prescribed in a stepwise manner with intervals and in specific dosages (starting with small doses) and considering comorbidities.

Third (sanatorium-resort) step (annually, at least 24 days a year).

Maintenance immunorehabilitation is performed when the signs of disease exacerbation disappeared. This step may follow clinical or ambulatory immunorehabilitation and is an important and essential part of immunorehabilitation since it involves additional balneologic and pre-existed physical factors. Hyperbaric oxygenation, radon, hydrogen sulfide or bischofite baths, peat muds and natural peloids, adaptation and therapeutic exercises, magnetic laser therapy, and medical massage reduce the rate of exacerbations by 2 to 5 times, accelerate the improvement of immunological parameters, withdraw or minimize the doses of corticosteroids and non-specific anti-inflammatory agents, and provide long-term clinical remission.

We developed an algorithm of immunorehabilitation of patients with immune dysfunction based on the analysis of our studies [17, 19].

Complex strategy of immunorehabilitation of patients with immune dysfunction considers pathogenic features of the disease. **The tactics of immunorehabilitation** involves a set of methods and principles to achieve major goal, i.e., to restore immune dysfunction and (as a consequence) to improve health and quality of life. Comlex immunorehabilitation should include methods which directly affect immune system and methods which indirectly promote immune system recovery. These methods should be

complex, differential, rational, consistent, and gradual. They should not exceed patient's adaptation potentialities as well.

There are certain fundamental principles of complex immunorehabilitation of patients with immune dysfunction and chronic recurrent disease course:

- ***to verify clinical diagnosis and to establish immune disorder severity*** while considering comorbidities. Traditional basic pharmaceutical agents should be used in combination with specialized pathogenic immunocorrection;
- ***individual choice of immunotropic medications*** according to the severity of immune abnormalities. Initially, the drug is selected by the physician depending on immune system defects. In long-term and recurrent immunopathological conditions, several immunomodulators which target various immune cells should be used;
- ***combination of systemic and topical administration of immunomodulators*** is useful and significantly improves laboratory and clinical parameters. Topical administration of immunomodulators is pathogenically valid and improves clinical efficacy of immunorehabilitation;
- ***non-medical methods of immunorehabilitation*** (balneologic and pre-existed physical factors) are obligatory;
- ***individual choice, sequence, and dosing of medical and non-medical treatment***. Combined use of agents and methods of the same specificity but with different mechanisms of action is optimal;
- ***step-by-step, continuous, and backward patient management***. Immunorehabilitation measures should be performed consecutively and continuously at every stage of complex treatment (inpatient department – outpatient department – sanatorium). Improvements in clinical immunological parameters at previous stages of immunorehabilitation should be considered. The major goal is to restore physiological parameters of immune system regarding regional normal ranges. Immunorehabilitation should be performed until complete recovery of a total of immune parameters and functions;
- ***step-by-step-immunological monitoring***;
- ***long-term and cyclic (multistep) courses of immunorehabilitation*** which number depends on the severity of immune abnormalities identified during immunological examination and may ranges from 2 or 3 to 5 or 6 or more. The duration of complex ambulatory-sanatorium immunorehabilitation should be at least 3 years;
- ***the terms of initiating immunorehabilitation*** (after verifying the diagnosis of immune disorder).

The development of key principles and methods of immunorehabilitation of

patients with immune dysfunctions resulted in beneficial clinical immunological effects and stable (12 months or more) clinical remission in 95% to 98% of patients.

In conclusion, I would like to highlight that the researches in the field of immunorehabilitation still have a long way to go, and future results will allow it to take a deserving place amongst other scientific disciplines [17, 19, 20].

The key results of our studies on the novel concept, immunorehabilitation of patients with immune system dysfunction, can be summarized as follows.

The aim of immunorehabilitation is to restore abnormal immune homeostasis, i.e., to normalize immunological parameters and to provide the recovery from acute disease or stable remission in chronic conditions.

Considering this, we should shape our understanding of the difference between immunocorrection and immunorehabilitation. The criterion of the efficacy of immunocorrection is the normalization of one or several immunological parameters. However, clinical effect is not guaranteed, moreover, the imbalance of immunoregulatory cells may re-occur. But when therapeutic factors recover immune system functions, the recurrences of chronic disease disappear or minimize, and the remission is significantly longer (i.e., stable), we are able to speak about immunorehabilitation of patients with immune dysfunction.

Verified diagnosis (main disease and comorbidities) is required for immunorehabilitation.

Immunorehabilition should be started early from the recognition of immune disorder (or, sometimes, probable immune deficiency) and verified clinical diagnosis regarding the signatures of main disease and its complications as well as comorbidities.

Individual approach is required.

Immunorehabilitation methods (differential, rational, complex) combine balneologic and pre-existed physical factors and pharmaceutical agents.

The procedures should be prescribed in a definite sequence and in adequate dosages. This is particularly important since consecutive procedures may enhance or attenuate each other. For example, we have demonstrated that in chronic bronchitis speleotherapy should precede radon baths by 2 or 3 hours while medical adaptative (preparative) and training procedures, i.e., exercises and massage, should be performed ahead of other procedures in the morning (from 9 a.m. till 11 a.m.). The number of immunorehabilitative procedures should not exceed patient's adaptation potentialities as well. These procedures should be started with small doses and performed according to the principles of hyposensitization thus making adaptation period easier. However, the

efficacy of immunorehabilitation depends on therapeutic doses and regimens, from the one hand, and their mechanisms of action and functional system of homeostasis, from the other hand.

Continuity should be absolute at every stage of immunorehabilitation, i.e.,

- institutional hospital of immunological department of the hospital;
- immunological sanatorium or rehabilitation center;
- outpatient department.

Each subsequent step of immunorehabilitation should be started considering the results of the previous step.

Rehabilitation of patients with immune dysfunction is one of the key issues in immunorehabilitology. I was lucky to be at its origin. Currently, immunorehabilitology may be regarded as follows:

Immunorehabilitology is a research area concerned with the recovery of immune system functional activity to physiologically normal levels under the effect of complex systemic therapeutic and preventive measures (both pharmacological and non-medical ones) to provide the recovery from acute diseases or stable clinical immunological remission with minimal (or even without) recurrences in chronic conditions.

While being closely associated with clinical immunology, in recent years immunorehabilitology has demonstrated its **individuality and independence**.

Immunorehabilitative methods are currently used in almost every medical and sanatorium-resort institution [17, 19].

The development of targeted pharmacological agents which affect certain components of immune system is of special interest in recent years. However, the development of novel original drug requires much time (5 to 12 years) and costs. Moreover, regulatory authorities impose very high requirements to the development, manufacturing, and introduction of novel pharmacological agents in terms of certification and licensing according to GLP (Good Laboratory Practice), GMP (Good Manifacturing Practice), and GCP (Good Clinical Practice) [23].

The introduction of personalized approach is another important aspect of the development of novel drugs (including immunomodulators) as well as immunorehabilitative methods. Personalized medicine is also called "4P medicine" where the 4Ps are Predictive, Preventive, Personalized, and Participatory. Published data suggest that in 2017, personalized medicines will comprise one-third of the global pharmaceutical market.

Intensive studies on the development of targeted immunomodulators

(including monoclonal antibodies) are underway worldwide. Certain progress was achieved in the development of targeted immunomodulators for various disorders. Thus, patients older than 12 years with severe IgE-dependent atopic asthma and other allergic disorders (atopic dermatitis, chronic urticaria) are treated with monoclonal antibodies against IgE (omalizumab), interleukin-5 (mepolizumab/Nucala), and other interleukins (IL-4, IL-9, IL-13, IL-33 etc.).

In addition, novel molecular diagnostic and therapeutic modalities for allergic disorders as well as recombinant allergic vaccines (which provide a basis of modern molecular allergology) were developed in recent years. Studies on the role of short peptides in various disorders and ageing performed by V.Kh. Khavinson et al. are very promising for the development of targeted immunomodulators. It was demonstrated that peptides Thymogen (EW), Vilog (ICE), and Crystagen (EDP) are effective for the recovery of thymus immune function both in experimental animals and in clinical practice (i.e., radiotherapy and chemotherapy for cancer, thymectomy, depression of regenerative processes etc.).

Immune system recognizes and destroys tumor cells. Abnormal regulation of the interaction between the signals from receptor co-inhibitors and co-stimulators (or immune "checkpoints" which modulate activation of T cells, key mediators of antitumor immunity) plays crucial role in tumor-associated immunosuppression. Monoclonal antibodies against negative immune regulators (e.g., CTLA-4 and PD-1/PD-L1) for anticancer treatment demonstrated considerable therapeutic success.

James P. Allison (University of Texas) developed innovative and highly effective method of cancer immunotherapy. Instead of targeting specific tumors, he focused his investigations on a specific protein, CTLA-4 (CD 152), which prevents the attack of immune system on tumor cells. He found that the blockage of this protein releases T cells which target and destroy tumor cells. Monoclonal antibodies against CTLA-4 (Ipilimumab) produced the best outcomes in patients with melanoma.

Monoclonal antibodies against PD-L1 and PD-L2 (Nivolumab and Pembrolizumab) are clinically effective as well. The investigations on molecular genetic aspects of the regulation of antitumor effects of immune-mediated targeted antitumor vaccines, tumor-specific clones of T cells and NK cells are very promising for the studies on cancer immunotherapy.

We should not rest on our laurels but highlight future perspectives. Currently, the most important questions are immunorehabilitation of patients who were exposed to various ecologically unfavorable factors, elderly persons, patients with AIDS, patients after severe injuries (including surgical ones) etc.

The studies on various mechanisms of immunorehabilitation and the development of its fundamental basis are important as well. The researches on the associations between immune parameters and other systems (nervous, endocrine, respiratory, circulatory etc.) are very promising. Therefore, the diagnosis of certain immunological abnormalities and specific immune defects without identification of potential etiology and pathogenic mechanisms of immune deficiency or without taking into account clinical functional and biochemical abnormalities as well as the diversity of disease variants and stages will scarcely help the patient. Complex study of a set of parameters and careful clinical immunological monitoring should be the basis for effective immunorehabilitation.

Despite current progress, this is just the beginning of the long thorny but important way that we should go together. Already today the names of many scientific, research, and medical institutions include the term "immunorehabilitation". Many scientists perform the studies on immunorehabilitation.

The aim of any researcher who will devote his/her professional life to immunorehabilitation is to make feasible contribution to this important scientific field required for population health. World Immunopathology Organization (WIPO) which headquarters is located in Moscow is the leading institution coordinating further studies on immunorehabilitation. Immunorehabilitation centers were organized under the aegis of WIPO in many Russian regions as well as in USA, Israel, Germany, Czech Republic, Austria, Bulgaria, Hungary, Australia, Thailand, Singapore, Poland, Netherlands, and Cyprus.

International Journal of Immunorehabilitation is published since 1994 in two languages (English and Russian). Many of my followers in various countries and regions worldwide joined in the development and studies on the issues, key methods, and principles of immunorehabilitation. Monographs, papers, and reviews are published, theses are defended, scientific congresses, conferences, and symposiums are held, i.e., immunorehabilitation as a science has a life fully.

Therefore, the path from the beginning of immunotherapy (the first use of diphtheria toxoid for diphtheria) to personalized targeted immunorehabilitation was traversed over slightly more than 100 years.

We are at the beginning of a new era, on the threshold of advances in clinical immunology which will promote the development of novel methods of targeted immunorehabilitation of patients with immune dysfunction [11, 13, 17, 19, 20].

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Omalizumab Therapy for Asthma and Urticaria

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B428R9022

Introduction

Omalizumab is a humanized monoclonal IgG_1 antibody directed to human immunoglobulin E (IgE). It has been approved world-wide for use in patients with moderate to severe allergic asthma (1). It is administered by subcutaneous injection and the dose varies according to the patient's IgE blood level and body weight. It is typically given monthly, consistent with its half-life as an IgG class antibody. The IgE level typically drops close to zero within 24 hrs., after a single dose, and blood levels typically rise toward the end of the dosing interval. Omalizumab is thought to be incapable of binding to cell surface IgE, does not cross-link IgE on the surface of basophils or mast cells, and therefore does not activate those cells. Recent observations, however, indicate that one effect of omalizumab is to facilitate dissociation of cell bound IgE (2) into the surrounding fluid (plasma or interstitial fluid), and this suggests that some interaction with cell-bound IgE might actually take place but without concomitant cell activation. A consequence of the prominent diminution of free IgE in the circulation is downregulation of the high affinity IgE receptor due to receptor internalization (3). Receptor downregulation takes a few days to 1-2 weeks in basophils, and 1-2 months in mast cells (4).

The Role of Omalizumab in The Therapy of Asthma

Since omalizumab removes IgE antibody from the circulation, the assumption was that it would ameliorate asthma that has a prominent allergic component and that has been borne out, in general. Nevertheless, its utility in practice is as an add-on therapy for patients whose asthma is difficult to control i.e. patients for whom long-acting sympathomimetics and high-dose inhaled corticosteroid, with or without leucotriene antagonists or parasympathetic inhibitors, are insufficient to control symptoms. The initial clinical studies consisted of four randomized, double-blind, placebo-controlled trials consisting of patients who were skin test positive to one or more perennial allergens maintained on a stable dose of an inhaled corticosteroid. Those receiving omalizumab had fewer exacerbations and required a lesser dose of the inhaled steroid and diminished

use of their rescue inhaler (5, 8). The effect was sustained for a year during an extension-phase study (9) and it was similarly effective and well-tolerated in children (10) as well as patients 50 years and older with severe persistent allergic asthma (11).

Allergen inhalation studies also support its effect in reversing many of characteristics of IgE-mediated allergic reactions. It reduces both the early and late phase reactions (12), decreases eosinophil numbers in sputum (13), and down regulates Fc receptors on basophils, mast cells, and dendritic cells (4, 14).

More recent studies demonstrate reduction of corticosteroid burden on patients with moderate or severe persistent allergic asthma (15) when added to baseline therapy. It may be particularly effective in those in whom disease-specific IgE levels account for a low proportion of total IgE (16). There appears to be a consistent positive effect on disease exacerbation rate, emergency room visits, steroid-dependence, and inflammatory markers. However, there is little effect on markers of pulmonary function such as FEV_1/FVC. Thus it affects the clinical course more than pulmonary function per say. The overall response rate of allergic asthmatics to omalizumab is 63% (16).

Omalizumab for Chronic Spontaneous (Idiopathic) Urticaria

Omalizumab is now employed with regularity for the treatment of patients with chronic spontaneous urticaria. It is "second-line" in the sense that it ought to be used once it is clear that patients have failed to have a satisfactory response to antihistamines based on a recent publication that argues in favor of this approach (17). While the most recent published guidelines suggest adding it after patients have failed antihistamines, (both H_1 and H_2 receptor blockers) and/or leucotriene antagonists (18, 19) it is listed as one choice among others.

An updated guideline which is being prepared will eliminate both H_2 antagonists and leucotriene antagonists, whose utility are questionable, and suggest using omalizumab when high-dose H_1 antagonists (at least 4 times the dose used to treat allergic rhinitis) fail and prior to trying cyclosporine which is the third consideration.

The rationale for employment of omalizumab in this fashion differs from its utility for asthma because chronic spontaneous urticaria is not "allergic" in the usual sense. There is no identifiable exogenous allergen. Yet it is clear, based on the response to omalizumab, that IgE itself has an important role in disease pathogenesis that is not yet understood. A number of possible effects that could ameliorate urticaria formation in CSU are listed in Table I.

The original idea is based on the observation that 35-40% of patients have a

circulating IgG antibody directed to the alpha subunit of the IgE receptor (20) while 5-10% have IgG anti IgE (21, 22).

In either instance, cross-linking to receptors (or two IgE's) at the cell surface can activate the cell (23) and activation of complement, liberating C5a (24) augments the release of histamine.

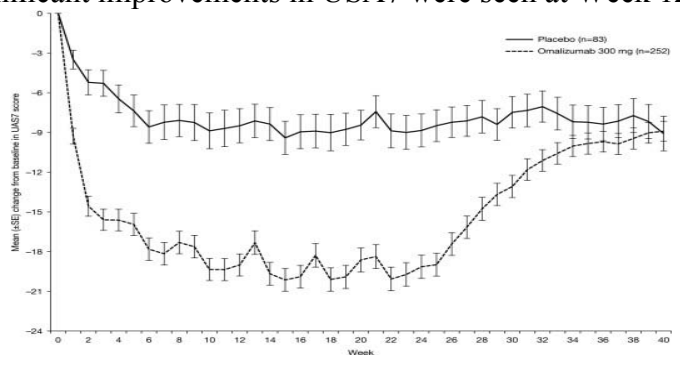
Since omalizumab binds IgE leading to down regulation of the IgE receptor, treatment actually removes the antigen. If the cell-surface density of unoccupied receptors falls sufficiently so that cross-linking two in proximity is not possible, the IgG antibody would not activate the cells. A proof-of-concept initial study of 12 patients, which was single-blind and placebo-controlled demonstrated improvement in 11 and 7 became asymptomatic (25).

All subjects were in the autoimmune associated subpopulation with positive antibody to the IgE receptor many of whom also had anti thyroid antibodies.

This was followed by a dose-escalation, double-blinded study with placebo-control which demonstrated significant improvement with doses of 150 mg or 300 mg monthly (26) as well as a study demonstrating similar efficacy in patients selected for a positive test for IgE anti thyroperioxidase (27). It also became clear that omalizumab is effective in patients lacking autoimmune associations (28) suggesting that the effect of treatment i.e. lowering plasma IgE and cellular IgE receptors, appears to "desensitize" cutaneous mast cells so that they become relatively unresponsive. This lead to three phase 3 studies of over 300 patients each which demonstrated remarkable efficacy at the 300 mg dose (Fig. 1) over a 6-month trial period (29, 31). The response rate of these studies varied for 65-70%, the placebo response was 25-30% (which is seen in all such studies), and when the treatment was stopped, symptoms recurred and progressively increased up to the placebo level. Omalizumab was then approved for treatment of CSU in the US, followed by Europe, Australia, Brazil, and then others. It is administered monthly; approval is for both the 150 mg and 300 mg dose although it is clear that the result with 300 mg is superior.

Thus in contrast to asthma, the dose given is independent of plasma IgE level or body weight.

Glacial: Significant improvements in USA7 were seen at Week 12 (secondary



Mechanistic considerations are summarized in Table I, however the ability of omalizumab to cause dissociation of IgE from the basophil (and presumably cutaneous mast cell) surface is of particular interest. It occurs within a day and may account for the very rapid response of some patients to treatment (32); for example, urticaria remits in some within 72 hrs. The peak response rate; however, when examined over time is 8-10 weeks i.e. after two injections which is more consistent with the timing of receptor down-regulation.

The less-dramatic effects on asthma may relate to aspects that are independent of IgE or mast cells or the effect of omalizumab on pulmonary mast cells may differ from that of cutaneous mast cells. Mast cells do have different physiologic and pathologic properties depending on their reaction.

For example, opiates activate skin mast cells but not pulmonary mast cells, and skin mast cells have C5a receptors, while pulmonary mast cells do not.

Basophils are recruited to the skin by chemotactic factors secreted from mast cells, endothelial cells, and perhaps other infiltrating cells so that patients are basopenic during periods of active urticaria (33). This reverses with omalizumab treatment (34) as well as spontaneous remission should that occur.

Blood basophils of patients are hyporesponsive to agonists acting through the IgE receptor (for example rabbit IgG anti human IgE) (22) which reverses as patients improve. The functional hyporesponsiveness (i.e. histamine secretion) seen in about 50% of patients appears to be due to increased intracellular phosphatases (35) which de-phosphorylate signal transduction molecules needed for cell secretion. It is not clear whether thus is an abnormality that is of pathogenic significance or is secondary to the urticarial process.

Omalizumab is approved for use in moderate to severe allergic asthma and for chronic spontaneous urticaria. It is clear that it is effective in many types of physical urticaria (36) including delayed pressure urticaria (37) which is refractory to many therapeutic modalities, as well as allergic rhinitis (38), chronic sinusitis with nasal polyps (39), bronchopulmonary aspergillosis (40), exercise-induced anaphylaxis (41), and possibly atopic dermatitis (42).

Each of these requires further clinical research to demonstrate efficacy in larger populations of patients, but it is clear that omalizumab has broad utility in allergic patient and those with immune-mediated disorders that are often seen in practices of Allergy/Clinical Immunology, Ear, Nose, and Throat, and Pulmonary Disease.

Table 1

Immunologic Associations Identified in Patients with CSU
1. IgG antibody to high affinity IgE receptor (IgG anti $Fc_eRI\alpha$) in 30-40%
2. IgG antibody to IgE 5-10%
3. Increased incidence of Hashimoto's Thyroiditis
4. IgG antibody to thyroid antigens (anti thyroglobular and anti peroxidase) – 25%
5. IgE antibodies to thyroperoxidase
6. Positive ANA-speckled pattern 30%
7. Expression to Th-2 initiating cytokines in skin biopsies including TSLP, IL25, and IL33[43]

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Clinical and Laboratory Biomarkers in Patients with Chronic Urticaria

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B428R9036

Introduction

Chronic spontaneous urticaria and angioedema (CSU) is one of the most frequent skin diseases, affecting up to 10% of the population, and in a considerable proportion of patients it interferes considerably with the normal life in areas such as sleep, work, education, or leisure activities. As for any other chronic medical condition, for the practicing clinician it is important to have biomarkers that can be used to establish the severity, predict the course, and design the most adequate treatment for the disease.

In this paper we will discuss the clinical features and complementary markers that may be useful for the management of patients with CSU. The following sections are included in this review: 1) Clinical markers. 2) Laboratory markers.

Clinical markers of CSU severity and time to spontaneous remission

The clinical features that have been studied as markers of severity and prognosis in patients with chronic urticaria (CU) are shown in Table 1.

Those include: age, gender, duration, presence of angioedema, comorbidity of Inducible urticaria, comorbidity of hypertension and metabolic syndrome, comorbidity of aspirin/NSAID hypersensitivity, lack of response to the treatment, and positivity of the autologous serum skin test (ASST).

Age

Improvement rates of CSU are significantly higher in patients less than 19 years old as compared to adults [1], although not all of the studies show the same effect of age [2].

Gender

Chronic urticaria occurs more frequently in women between 20 and

59 years old [3], and the quality of life is significantly more interfered in females especially in the domains itching/embarrassment and limits looks [4]. According to Gregoriou and coworkers female sex is associated to worse prognosis of CSU [5].

In children there are contradictory data, since some studies report higher rates of remission in girls [6], whereas in other investigations the prognosis in girls was unfavorable [7].

Disease duration

The duration of CU correlates with disease severity [8, 9]. In the study by Gregoriou and coworkers the duration of urticaria at the moment of the initial consultation was associated with worse prognosis [5], and this observation also holds true for cold urticaria [10] and for children [11], although other authors did not find a relation between duration of CU and remission [2]. Duration of CSU is likely to be longer in patients with high disease severity [12].

Association of wheals and angioedema

Most patients with CSU have concomitant wheals and angioedema, and the rate of urticaria and angioedema is 33 to 67%, whereas 29 to 65% show exclusively wheals, and 1 to 13% present angioedema without wheals [13].

A number of investigations have found that the presence of angioedema in patients with CSU is associated with longer disease duration and worse prognosis [5, 8, 9, 14-17].

Comorbidity of Inducible Urticaria

The combination of urticaria precipitated by physical factors, more frequently symptomatic dermographism and delayed pressure urticarial, occurs in 10 to 50% of patients with CSU [15]. The prognosis and duration of CU are worse in patients who present CSU associated to physical urticaria [17].

Comorbidity of Hypertension and Metabolic Syndrome

Chang and coworkers have observed that CSU is associated with an increased risk of hypertension [18]. Also, recently it was reported that hypertension is associated with longer duration of CSU [19].

Metabolic syndrome (MS), the combination of central obesity, dyslipidemia, hypertension, and hyperglycemia, is significantly more prevalent in patients with CSU than in healthy controls and is associated with lack of control of urticaria [20]. Patients with MS and CSU are older and show increased levels of circulating inflammatory markers.

Comorbidity of aspirin (ASA) and nonsteroidal anti-inflammatory drug (NSAID) hypersensitivity

Up to 40% of patients with CSU experience disease excerbations when they receive ASA and other COX-1 inhibitors. CSU associated with ASA/NSAID hypersensitivity has been designated ASA/NSAID-exacerbated cutaneous disease (AECD) in the current classification of hypersensitivity reactions to ASA and NSAIDs [21].

A recent study compared the clinical characteristics of patients with AECD with those of ASA/NSAID tolerant CSU patients. It was observed that in the first subset CU was more severe, showed a longer duration, was more often associated with atopy and angioedema [22], and this finding was confirmed in a study by Shin and coworkers [23].

Lack of response to the treatment

In children, the response to antihistamines correlates with higher remission rates and has a better prognosis [14]. Antihistamine-resistant CSU is associated to other clinical features of severity, including atopic asthma, rhinitis and rhinosinusitis, thyroid disease, and hypertension [24].

Additional studies have shown that antihistamine-resistant CSU is associated to increased complement C5a fraction, higher disease activity, longer duration, higher rates of positive autologous serum skin test (ASST) [25], and elevated D-dimer plasma levels [26].

Positive Autologous Serum Skin Test (ASST)

According to several studies a positive ASST is associated with longer duration of CSU [27-33]. A few studies could not confirm this association [2, 34, 35].

Laboratory markers of severity and prognosis

Laboratory alterations that can be utilized for the follow up of the clinical course of patients with CSU are summarized in Table 2, and include basophils and basophil activation, the measurement of several inflammatory markers in the serum, autoimmune phenomena, markers of activation of the extrinsic coagulation pathway, IgE and atopy, and serum vitamin D levels.

Basophils and basophil activation

When present, blood basopenia in CU patients correlates with the urticaria activity score [36, 37], is likely related to the recruitment of basophils to the skin [38], and inhibited by corticosteroids [39].

A reduced IgE-mediated histamine release is present in CSU [40-43]

whereas patients with a basophil responder phenotype to anti-IgE have longer disease duration, more exacerbations, and more severe pruritus [44], and the expression of CD203c, a marker of basophil activation, is increased and correlates with urticaria severity [45].

Inflammatory markers

A number of factors that reflect systemic inflammation are increased in the serum of patients with CSU, and are associated with the course of the disease. Those include C-reactive protein (CRP) [46-49], Interleukin-6 (IL-6) [50], Interleukin-18 (IL-18) [50,51], Complement C3 and C4 [52], Vascular endothelial growth factor [53], B-cell activating factor (BAFF) [54], matrix metalloproteinase-9 (MMP-9) [47, 55].

Autoimmunity

Several authors have observed that the positivity of the ASST is associated with longer duration and more severity of CU [5, 31-33, 38]. Also, the titers of anti-FcεRI or anti-IgE autoantibodies correlate with more severe CU [43].

On the other hand, it has been proposed that a positive ASST is a predictor of CU control [56].

Autoimmune responses directed to thyroid gland antigens are observed in 12 to 29% of patients with CSU [57], and patients with coexistent thyroid autoimmunity show a more severe and prolonged CU [5, 58].

Activation of the extrinsic coagulation pathway

An increase of plasmatic markers of thrombin generation and fibrinolysis has been observed in patients with severe CU [59-61]. Rabelo-Filardi and coworkers proposed that the increase of prothrombin fragments 1+2 and D-dimer correlate with CU severity [9], while it has been reported that the activation of coagulation/fibrinolysis parallels disease activity and decreases during remissions [60, 62].

IgE and atopy

A higher proportion of patients with increased serum IgE have moderate to severe CU when compared with CU patients who have normal IgE values [63].

About 54% of patients with CSU produce IgE antibodies directed to thyroid peroxidase [23, 64]. Interestingly, it has been reported that cold urticaria can be passively transferred in vitro with monomeric IgE from patients suffering the disease [65].

An increased prevalence of atopy is present in NSAID-induced urticaria [66, 67], and in ASA/NSAID-exacerbated cutaneous disease [22]. Additionally, the activity of CSU correlates with the positivity of skin prick tests to mite allergenic

extracts [68], whereas the atopic trait is linked to cholinergic urticaria [69].

Vitamin D

Low serum vitamin D levels are associated with increased severity and duration of CSU [70], and it has been proposed that vitamin D could be used as adjuvant therapy for CU [71].

Conclusions

- **In patients with CSU the duration of the disease correlates with its severity and prognosis.**
- **Prognostic biomarkers that have been postulated for the follow-up of CU include D-dimer levels, histamine release test, and markers of basophil activation.**

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Table 1. Clinical markers of CSU severity and time to spontaneous remission

➢ Age
➢ Gender
➢ Disease duration
➢ Presence of angioedema
➢ Combination of CSU and inducible urticaria
➢ Hypertension and metabolic syndrome
➢ Aspirin/NSAID hypersensitivity
➢ Lack of response to the treatment
➢ Positive autologous serum skin test (ASST)

Table 2. Laboratory markers of severity and prognosis in patients with chronic urticaria

Marker	Remarks
Basophils and basophil activation	Basopenia Abnormal basophil function Basophil activation
Inflammatory markers	C-reactive protein Interleukin-6 (IL-6) Interleukin-18 (IL-18) Complement C3 and C4 Vascular endothelial growth factor (VEGF) B-cell activating factor (BAFF) Matrix metalloproteinase-9 (MMP-9)

Autoimmunity	ASST Anti-thyroid autoantibodies
Activation of the extrinsic pathway of the coagulation system	D-dimer Prothrombin fragments 1+2
IgE and atopy	Serum IgE Positive prick skin tests to aeroallergens (mites)
Vitamin D	Serum vitamin D levels

Atopic Dermatitis in Children: Differential Approach to the Choice of Treatment Strategy

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B428R9038

Abstract

The study considers advantages of differential approach to the treatment of children (Ch) with different immunopathogenetic phenotypes (IPGF) of atopic dermatitis (AD). It has been found that patients with non-IgE-mediated type of AtD was not sensitization to IgE to house dust mite allergens (HDMA) and was observed a decline in macrophage-phagocytic component of immune system (MPCIS). It was shown, that the IgE-mediated phenotype includes 3 forms of AtD: allergic form; mixed form (in combination with allergic rhinitis, asthma); immunocompromised form (ICF). Allergic and mixed have a proven sensitivity to non-eliminated HDMA, which promoted allergization and provoked the development of symptoms in the course of AtD, and in Ch with ICF of AtD a decrease in parameters of MPCIS (such as phagocytic number, phagocytic index). On the basis of immune system disorders (ISD), has been developed a different complex of immunotherapy (CIT). The developed algorithm of examination and diagnosis of patients with AtD allows the choice of an adequate CIT in accordance with IPGF based on detected of immune system disorders (ISD). Implementation of individual treatment allowed to reduce the risk of developing severe and chronic AD and to improve the quality of life of patients.

Keywords: Atopic dermatitis, children, immunopathogenetic phenotypes, subcutaneous allergen specific immunotherapy, cytokines, comprehensive programs of immunotherapy

Introduction

Atopic dermatitis (AtD) is a chronic inflammatory allergic disease (ADs) of the skin with multifactorial pathogenesis. Its development proceeds against the background of various and interdependent genetical, ecological,

immunological, psychological, biochemical and other pathological processes, the most important of which is dysfunction of skin barrier. The skin barrier protective dysfunction causes the acceleration of secondary infection overlay and extraneous antigens penetration through damaged corneal layer.

The prevalence of adults' and children's AtD is steadily increasing all over the world and now reaches over 1-20% (approximately 20% for children and 1% -3% for adults). Two principal strategies for resolving of unsolved problems in management of ADs under consideration at present include application of allergic-specific immunotherapy (AIT) [1,2], which is aimed at long-term specific modulation of immune response towards immune tolerance to cause-significant allergens, and use of biological response modifiers for minimization of pathological immune reactions. The combined strategies [3-10] involving both approaches can ensure successful management of AD [11-13]. It is of great importance to carefully screen the patients with AtD [14-15] who should be placed on patient-specific combined therapy according to revealed immune and or/non-immune disorders and phenotype of the disease.

Immunopathogenetic phenotype (IPGP) determined in accordance with the results of complete medical assessment becomes a key factor for selection of optimal therapy for AtD, allows to provide for case-specific combined therapy regimens for this disease. In this connection, development of algorithm for assessment, diagnosis and treatment of children (Ch) with various AtD IPGP is a crucial task.

Background

The main purpose of the study was to argue the need of a differential approach to the treatment of Ch with different IPGF of AtD.

Methods

300 Ch aged 3-17 with moderate course of AtD in the exacerbation phase were examined. The control group was comprised of 30 healthy Ch of the same age. The patients underwent general clinical assessment, clinical assessment and immunoallergological assessment (IAA) aimed at detection of clinical and laboratory signs of allergen-induced inflammation. General clinical examination included clinical blood and urine analyses and screening for parasitic and viral infections. IAA consisted of: immunogram (cellular, humoral and phagocytic component of immune system), determination of levels of pro- and anti-inflammatory cytokines (by fluorescence immunoassay); skin testing,

challenge or elimination testing (if indicated), total and specific IgE blood testing. House dust mite allergens (HDMA) were used as a cause-significant allergen. Clinical assessment was aimed at collection of allergic anamnesis and evaluation of severity of clinical symptoms according to Scoring of Atopic Dermatitis (SCORAD) scale (Table 1).

Tabl. 1

DETECTION OF CLINICAL AND LABORATORY SIGNS OF ALLERGEN-INDUCED INFLAMMATION	
General Clinical Assessment	
Clinical analyses of blood and urine	
Screening on parasitic and viral infections	
Clinical Assessment	
Collection of allergic anamnesis	
Evaluation of severity of clinical symptoms according on a SCORAD scale	
Immuno-Allergological Assessment	
Immunogram	Evaluation of cellular, humoral and phagocytic components of immune system
Pro- and anti-inflammatory cytokines	IL-4, IL-13, INF-y
Allergological assessment	skin tests, provocation or an elimination tests (if indicated), the definition of general and specific of IgE (sensitization to the definition of cause-significant allergens)
Allergological assessment	skin tests, provocation or an elimination tests (if indicated), the definition of general and specific of IgE (sensitization to the definition of cause-significant allergens)

On the basis of the revealed disorders, several comprehensive programs of immunotherapy (CIT) were developed. For IgE-mediated forms of AtD were proposed CIT, including basic therapy (BT) and subcutaneous allergen-specific immunotherapy (SCIT) on an accelerated regimen. In cases when reduced functional activity of macrophage-phagocytic component of immune system (MPCIS) was detected, an immunomodulator (IM) was added. Selection of IM was based on its capacity to affect the cells of monocytic-macrophagal nature, increase of macrophages' cytotoxicity toward bacterial antigens and virus-infected cells, as well as correction of imbalance in cytokine profile Th1 and Th2 and intensifying the production of IFN-y, which eventually contributed to lower rate of infectious complications of AtD. For non-allergic form of AtD, the BT +IM were used (Fig. 1). BT included: elimination of cause-significant allergens, application of anti-inflammatory (topical and systemic) therapy (cetirizine-based medications in age-specific dosage variances, topical glucocorticosteroids, such as Advantan, Elocom, Triderm, Pimafucort, emollients – series Mustela, Avene, Dardia), correction of gastrointestinal dysfunction (enzymes: Kreon 10 000, Linex – according to age).

Fig. 1

INTEGRATED MULTIMODALITY IMMUNOTHERAPY PROGRAMS FOR VARIOUS PHENOTYPES OF ATOPIC DERMATITIS

Allergic/mixed form of Ig-E-mediated AtD	• Basic traditional therapy (BT) • Subcutaneous AIT (SCIT) on an accelerated regimen with HDMA
Combined form of Ig-E-mediated AtD	• BT • SCIT on an accelerated regimen with HDMA • Immunonodulator (IM), acting on the cells of MPCIS
Non-IgE-mediated phenotype of AtD	• BT • IM, acting on the cells of MPCIS

Results

The examination divided patients into two main EPGF-groups: the first group (1G) with IgE-mediated and the second group (2G) with non-IgE-mediated forms of AtD (Table 2).

Tabl. 2

CHARACTERISTIC OF IMMUNOPATHOGENETIC PHENOTYPES OF ATOPIC DERMATITIS AND REVEALED IMMUNE DISORDERS

Parameters	IgE-mediated phenotype of AtD (forms)			Non-IgE-mediated phenotype of AtD
	Allergic	Mixed***	ICF ****	
Sensitization to HDMA*	Present	Present	Present	Not present
Reduction in parameters of MPCIS** (phagocyte number, phagocyte index, NCT-test)	Within the expected range for age	Within the expected range for age	Statistically significant reduction	Statistically significant reduction

Note:
* HDMA - house dust mite antigens
** MPCIS - macrophage-phagocytic component of the immune system
*** Mixed form - the combination of AtD with allergic rhinitis and/or allergic bronchial asthma
**** ICF (immunocompromised) form - allergic AtD in combination with immune disorders in macrophage-phagocytic component of immune system

The patients with non-IgE-mediated type of AtD (2G) showed no IgE-sensitization to HDMA (Fig. 2) and observed a decline in MPCIS [16]: phagocytic index down to 44.2+2.4%, phagocytic number down to 2.7+0.2 microbial bodies (Fig. 3). The IgE-mediated phenotype includes 3 forms of AtD: allergic form; mixed form (in combination with allergic rhinitis, asthma);

immunocompromised form (ICF). Allergic and mixed have a proven sensitivity to non-eliminated HDMA, which promote allergization and provoke the development of symptoms in the course of AtD, and in Ch with ICF of AtD a decrease in parameters of MPCIS (such as phagocytic number, phagocytic index) was also found (Fig. 2 and Fig. 3).

Fig. 2

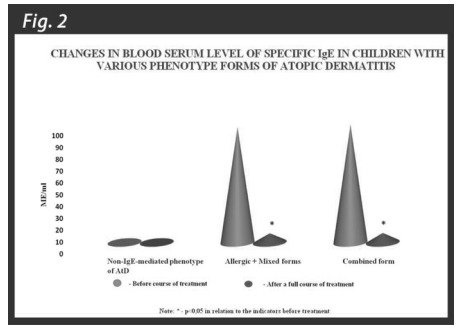
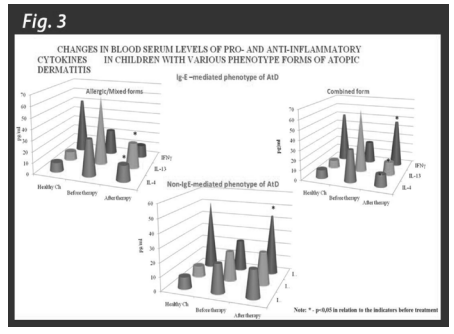
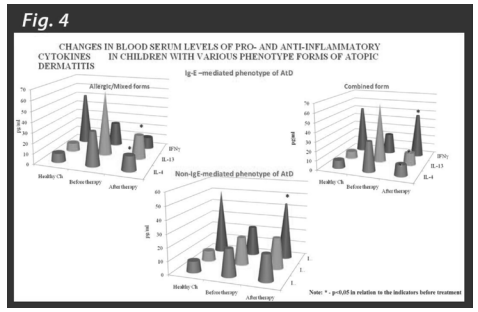


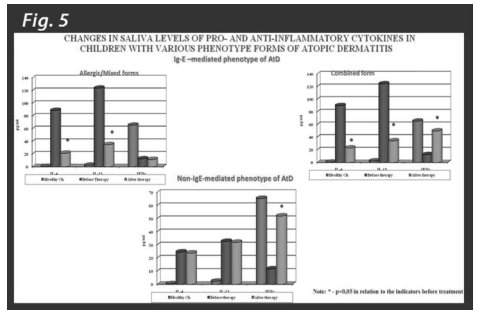
Fig. 3



Application of pathogenetically substantiated therapy contributed to intensification of IFN-y synthesis and decrease in both serom and saliva levels of anti-inflammatory cytokines IL-4 and IL-13 (Fig.4 and Fig.5), which stimulate secretion of IgE and participate in Th2-type immune reactions [17].

On the basis of immune system disorders (ISD), we developed a different complex of immunotherapy (CIT). For 1G it has been suggested to conduct a regimen of comprehensive IT, consisting of BT and SCIT in an accelerated scheme. CIT with IM allows to provide SCIT on an accelerated regimen and to increase effectiveness of treatment significantly. In 2G we have used BT and IM. Comparative study of changes in citykine status (levels of cytokines IL-4, IL-13 and INF-y in blood serum and saliva) in Ch with AtD has allowed to determining the advantages of including CIT in a multimodality therapy program. Application of CIT as a part of multimodality therapy helped to reduce the level of specific IgE, IL-4 from 55.2+4.2 pg/ml to 38.1+3.4 pg/ml ($p \leq 0.05$), to increase production of INF-y from 21.1+2.0 pg/ml to 44.6+3.5 pg/ml ($p \leq 0.05$). After a course of treatment the level of cytokines in biological fluids of patients from group which received both BT and CIT were approaching the same in healthy persons (Fig.4 and Fig.5).





Conclusion

The developed algorithm of examination and diagnosis of patients with AtD allows the choice of an adequate CIT in accordance with IPGF based on detected ISD. The above improves the treatment of Ch, increases the duration of remission, reduces the risk of the developing severe and chronic AD, it improves their quality of life [18,19], provides pharmacotherapy and medical services costs reduction, and it is more cost-effective [20-25].

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Chemically Modified Allergens - Allergoids in Specific Immunotherapy of Respiratory Allergy

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B428R9030

Abstract

The results from research on antigenicity and allergenicity of chemically modified preparations (allergoids) from grass pollens and house dust mites are presented in this review. The efficacy and immunological changes that occur during the course of immunotherapy with Bulgarian allergoids were assessed also.
The modification of allergens to allergoids has been proved by: Determination of final amino groups; Gel-filtration on Sephadex and Isoelectric focusing.
The allergenicity of the allergoids has been assessed by Skin Prick Tests. The antigenicity of the allergoids has been assessed by: Ouchterlony test – double diffusion in agar-gel.
53 patients with seasonal allergic rhinitis or bronchial asthma, sensitized to grass pollen allergen and 21 patients with bronchial asthma, sensitized to house dust mite are included for specific immunotherapy/hyposensitization with allergoids. Before the start of therapy after the first, second and third year of treatment the levels of total and allergen-specific IgE antibodies, as well as blocking IgG_4 antibodies are evaluated. A scoring system of subjective reporting of the condition of patients during therapy is used.
Comparing the number and size of positive skin reactions after skin prick tests with unmodified allergens and their corresponding allergoids show that allergoid possess weaker allergen activity compared to their native allergens.
Antigenicity of chemically modified allergens is affected minimally.
Studied Bulgarian allergoids have good therapeutic efficacy. The treatment with modified allergens causes strong immunological effects in allergic patients by forming high titers of allergen-specific IgG4 antibodies. At the same time in patients treated successfully with allergoids, there is a clear tendency to reduce the levels of total and allergen-specific IgE antibodies. All this is in favor of the broadly introducing of the allergoids in the clinical practice for

the purposes of the specific hyposensitization (immunotherapy) of the atopic respiratory allergic disease.

Keywords: allergen, chemically modified allergen, allergoid, specific immunotherapy

Introduction

The allergens possess two main properties:

- Allergenicity – the ability to stimulate formation of specific antibodies (mainly IgE), which sensitize human body.
- Antigenicity – connected with the formation of other kinds of antibodies, having no relation to the sensitization (mainly protective "blocking" antibodies).

Chemically modified allergens (allergoids) are allergens devoid of, or with reduced allergenicity, but with well preserved antigenicity [1,2].

Two very important effects are achieved with the introduction of the allergoids in the clinical practice: The risk of unpleasant side reactions in the course of treatment significantly decreases and more over the possibility for introducing in the patient the higher doses of allergoid, thus obtaining better clinical results [3].

Considering all this in recent years we have developed and implemented in practice two chemically modified allergens: one from grass pollens (*Dactylis glom., Festuca sp., Lolium per., Secale cer., Phleum prat. Arrhenaterum elatius*) and other - from house dust mite *D. pteronyssinus* (D.pt.).

In this review, we present the results of our research on antigenicity and allergenicity of chemically modified preparations. The efficacy and immunological changes that occur during the course of immunotherapy with Bulgarian allergoids were assessed also.

Methodology

The modification of allergens to allergoids has been proved by:

- Determination of final amino groups – $NH_{2;}$
- Gel-filtration on Sephadex;
- Isoelectric focusing [4].

The allergenicity of the allergoids has been assessed by:

- Skin Prick Tests (SPT).

The antigenicity of the allergoids has been assessed by:

- Ouchterlony test – double diffusion in agar-gel [5].

Patients included for specific immunotherapy/hyposensitization with allergoids:

53 patients with seasonal allergic rhinitis or bronchial asthma, sensitized to grass pollen allergen and 21 patients with bronchial asthma, sensitized to house dust mite are enrolled in the studies. 40 from the patients are female and 34 – male. The patients are from 17 to 61 years old.

The severity of the disease was estimated as follows: 6 patients with light, 27 – with moderate and 41 with severe form.

The course of subcutaneous specific immunotherapy (SCIT) is held year round. Before the start of therapy after the first, second and third year of treatment the levels of total and allergen-specific IgE antibodies, as well as blocking IgG_4 antibodies are evaluated.

A scoring system of subjective reporting of the condition of patients during therapy is used.

Results

We found that the concentration of free amino groups in chemically modified allergens is approximately 10 - 200 times less than that in the native allergens (Table 1).

These results confirm the thesis of Marsh [2] that an essential element of the mechanism in obtaining of allergoids is the interaction of amino groups of protein extracts with the aldehyde group of formaldehyde.

Table 1. Determination of concentration of free NH2-groups in the allergens and its respective allergoids compared with standard concentration of α-leucin

Allergens and Allergoids	Concentration of the samples in mg/ml	A340	LEU/mg
Grass pollen allergen	0.25	0.720	1.0×10^{-3}
Grass pollen allergoid	1.60	0.110	2.6×10^{-5}
D. pt. allergen	0.5	0.900	5.2×10^{-2}
D. pt. allergoid	1.5	0.011	7.8×10^{-3}

Gel filtration on Sephadex is a convenient method to assess the modification of various allergoids. By elution profiles of the native allergen and allergoid, presented in Fig. 1 A and B, we can assess that the impact of formaldehyde substantially alter the molecular mass in the modified products. In region of the elution profile, which respond to the high molecular weight of allergoid (at the elution volume 40-50 ml), occurs well defined peak, which in the native allergen is lacking.

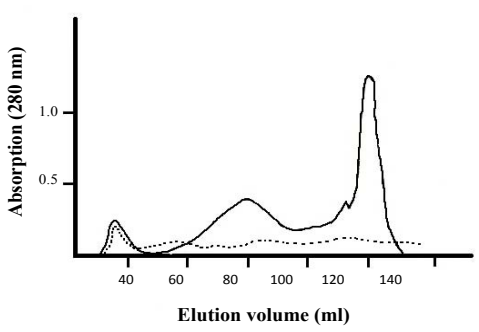

Fig. 2 A. Isoelectric focusing of D. pt. allergen (1,2,3) and its respective allergoids (5,6,7).

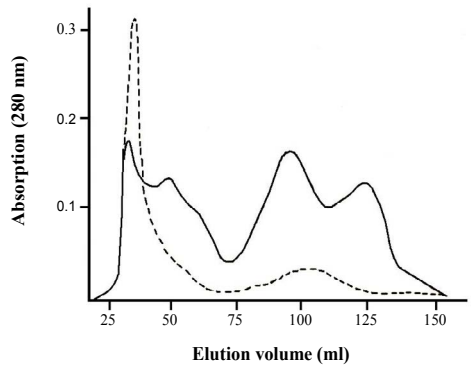

Fig 1 B. The elution profile of Grass Pollen allergen and its respective allergoids on Sepadex G-50

Fig. 1. Gel-filtration on Sephadex-G 75 and G-50

Comparative presentation of the elution profiles of the allergen and the corresponding allergoid illustrates the mechanism of modification with formaldehyde, which is creating a numerous intermolecular bond, leading to enlarging the molecules of the modified extract.

The simultaneous isoelectric focusing of the native allergens and the corresponding allergoids, modified with formaldehyde, revealed differences in isoelectric points (pI) of proteins in extracts (Fig 2. A and B).

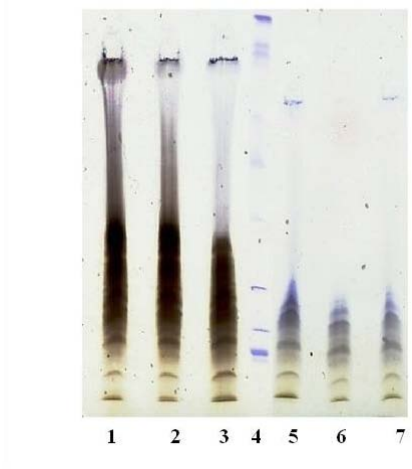

Fig. 2 A. Isoelectric focusing of D. pt. allergen (1,2,3) and its respective allergoids (5,6,7).

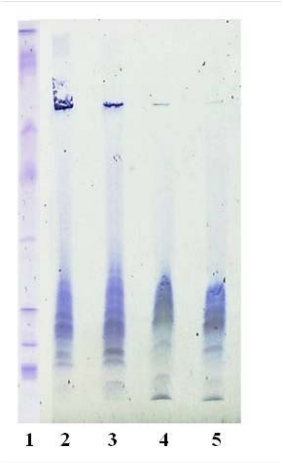

Fig. 2 B. Isoelectric focusing of Grass pollen allergen (2,3) and its respective allergoids (4,5).

Fig. 2. Isoelctric focusing

The data indicate that the protein profile of the unmodified allergens (Fig 2. A 1, 2, 3 and Figure 2. B. 1, 2) is characterized by a evenly distribution of the proteins throughout the pH gradient of the plate (pI of proteins are from 3.5 to 9.3).

The proteins of the chemically modified allergens focus near the anode in a relatively narrow range of pH (pI 3.5 to 5.5). The observed differences in the picture of the protein profile of allergoids most likely due to the reaction of formaldehyde with a positively charged amino acid residues of the proteins in the extract.

Comparing the number and size of positive skin reactions after SPT with unmodified allergens and their corresponding allergoids show that allergoid possess weaker allergen activity compared to their native allergens.

Evidence from studies with pollen allergoid has shown that the resulting allergenicity is reduced by 90 - 99 % against initial values. This is very clearly demonstrated by the fact that histamine release from sensitized basophils or mast cells drops a 1000-fold in the case of allergoids as compared to the original allergen. Substantially diminishes also allergoid ability to elicit response in skin tests on allergic patients, where they have been observed to be from 200 to 2000 times less allergenic (Table 2.)

Table 2. Allergy skin prick test /weal in mm/ with Grass pollen allergen and its respective allergoid in 76 allergic patients

Skin Prick Test (weal in mm)	Patients	Grass pollen allergen	Grass pollen allergoid		
		1000 PNU/ml	5000 PNU/ml	2500 PNU/ml	1000 PNU/ml
Mean diameter	76	7.21	4.58	3.89	< 2
Median diameter	76	7.0	5.0	4.0	2.0

Comparison between the size of the skin reaction induced by the allergen in diagnostic concentration and the reaction, elicited by allergoid in identical concentration, demonstrated that the chemically modified allergen from house dust mites had lost about 50% of a skin-sensitizing activity of the allergen.

The results from of SPT demonstrate that, even used in the highest concentration, allergoid (which is concentrated twice) - is not able to cause skin-allergic reaction, whose dimensions are comparable to those induced by the unmodified allergen (Table 3).

Table 3. Allergy skin prick test /weal in mm/ with House dust mite allergen and its respective allergoid in 64 allergic patients

Skin Prick Test (weal in mm)	Patients	Mite allergen	Mite allergoid	
		1000 BU	3000 BU	1000 BU
Mean diameter	64	6.27	4.59	3.3
Median diameter	64	6.0	4.0	4.0

A comparative analysis of the antigens of the allergens and their corresponding allergoids in double diffusion in agar-gel using a rabbit hyperimmune serum obtained after immunization of experimental animals with natural allergens is shown in Fig. 4 and 5.

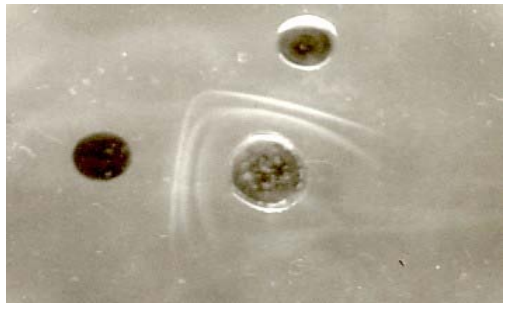

Fig. 4. Double diffusion in agar-gel of rabbit anti-D. pt. allergen serum /central well/ to D. pt. allergen /left well/ and to its respective allergoid /right well/.

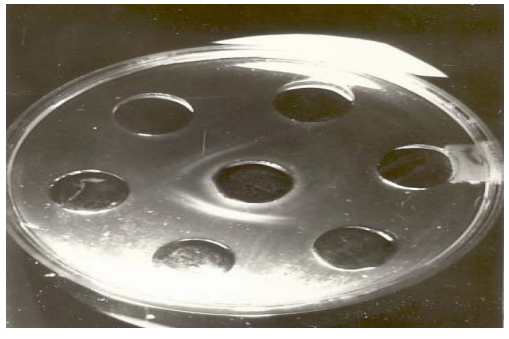

Fig. 5. Double diffusion in agar-gel of rabbit anti-Grass pollen allergen serum /central well/ to Grass pollen allergen /down well/ and to its respective allergoid /up well/.

The data indicate that antigenicity of chemically modified allergens is affected minimally. Thus the allergoids after regular application are capable to stimulate the immune system for production of IgG_4 "blocking" antibodies.

The slightly reduced antigenicity of modified preparation can be compensated by the application of larger amounts of allergoid during the course of specific immunotherapy without any danger of adverse allergic reactions.

After the third year of specific hyposensitization in 85% of treated patients is register strong or moderate effect from the therapy and if we add the number of patients with a little effect from SCIT, it can be concluded that 90.6% of patients obtain clinical benefits (Table 4.). No clinical effect from the SCIT with grass pollen allergoid has been observed only in 5 patients (9.4%).

Table 4. Clinical results from the specific hyposensitization, carried out with mixed Grass pollen allergoid

Term of treatment	Treated patients	Clinical results from specific hyposensitization							
		Strong effect		Moderate effect		Little effect		Without effect	
		number	%	number	%	number	%	number	%
1 year	53	20	37.5	20	37.5	6	11.3	7	13.2
2 years	53	22	41.5	22	41.5	3	5.6	6	11.3
3 years	53	36	68	9	17	3	5.6	5	9.4

Similar results are observed during assessment of clinical effect from immunotherapy with allergoid from house dust mites (Table 5.).

Table 5. Clinical results from the specific hyposensitization, carried out with D. pt. allergoid

Term of treatment	Treated patients	Clinical results from specific hyposensitization							
		Strong effect		Moderate effect		Little effect		Without effect	
		number	%	number	%	number	%	number	%
1 year	21	8	38	5	23	4	19	4	19
2 years	21	10	48	4	19	3	14	4	19
3 years	21	12	57	3	14	3	14	3	14

In comparison to the data before treatment after three year SCIT there is a statistically significant reduction in the level of total and specific IgE antibodies and a significant increase of «blocking» IgG_4 antibodies (Fig. 6.).

A correlation between the changed antibody levels and subjective evaluation of the condition has been observed also.

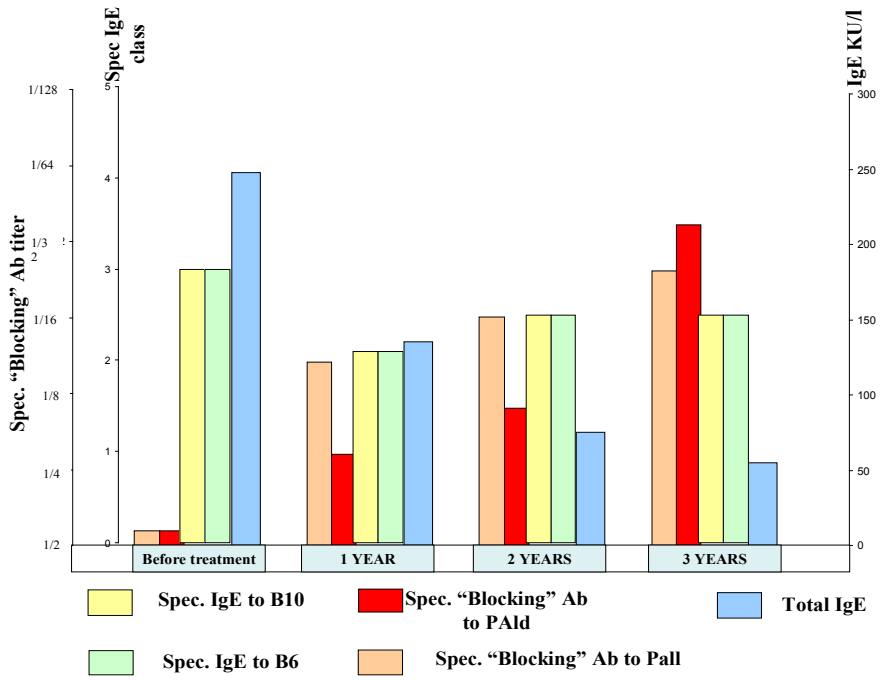

Fig. 6. The level of specific IgE and IgG4 (blocking antibodies) to grass pollen allergen and its respective allergoid in patients with respiratory allergy carried out 3 years specific immunotherapy with the latest.

The obtained data give evidence that studied Bulgarian allergoids have good therapeutic efficacy, which is due to the well-preserved immunogenicity of preparations. The treatment with modified allergens causes strong immunological effects in allergic patients by forming high titers of allergen-specific IgG_4 antibodies. At the same time in patients treated successfully with allergoids, there is a clear tendency to reduce the levels of total and allergen-specific IgE antibodies.

These encouraging results have even more value if one takes into mind the fact that in the course of therapy are registered single local allergic side effects and only in 4 patients (7.4%) was stopped increasing the dose of allergoid due to the strengthening of symptoms of primary allergic disease.

Conclusion

Having in mind the obtained results we are permitted to conclude that:

- The allergoids possess good therapeutic effectiveness;
- In the course of the treatment were observed in the patients single, weak local reactions only in 7-8% from them;

- It was possible to introduce in the patients twofold higher doses of allergoids in comparison with the allergens;
- Allergoid immunotherapy leads up to the formation of high titers of specific protective "blocking" IgG4 antibodies;
- After immunotherapy with allergoids decrease in the level of the total IgE and some of the specific IgE appears;

All this is in favor of the broadly introducing of the allergoids in the clinical practice for the purposes of the specific hyposensitization (immunotherapy) of the atopic respiratory allergic disease.

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Interleukin-8 and RANTES at Early Stages of Allergen-Specific Immunotherapy in Polyvalent Sensitized Patients

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B428C0011

Abstract

35 children with persistent allergic rhinitis caused by polyvalent sensitization were treated with allergen-specific immunotherapy. Serum level of IL-8 and RANTES has been detected on 0, 7, 30 and 90 days. Initial level of IL-8 was detectable in 34,3% patients and gradually decreased from Me 2,1[Q1-Q3 0-2, 9] to Me 0,0[Q1-Q3 0,0-0,0] pg/ml ($p<0,05$). Serum level of RANTES rised in 8-10 times if initially it was below10000 pg/ml but was down in 2-3 times if it was more than 10000 pg/ml.

Keywords: allergen-specific immunotherapy; children; IL-8; RANTES

Introduction

The allergen-specific immunotherapy (ASIT) is the only one method capable of change the natural history of atopic diseases. The efficiency of the ASIT is substantiated by the complex of modifications of the immune reactivity many of which are unclear till now [1].

Obvious disadvantage of the majority of ASIT mechanisms studies is estimation of only initial and ending values of the parameters for the characteristics of the changes without analysis of their intermediate processes regardless of long duration of the treatment and variable doses of an allergen at early stages of the treatment.

Information about dynamics of the immunological reactivity at early stages of the ASIT both contribute to understand the underlying mechanisms and also can help us to predict the efficiency of the treatment.

The aim of this study was to characterize the dynamics of two proinflammatory cytokines (IL-8 and RANTES) in children with persistent allergic rhinitis

(PAR) caused by polyvalent sensitization at early stages of the ASIT.

Methodology

30 children aged 7-13 suffering from PAR have been prospectively observed during ASIT with 3 allergens including house dust mite and two pollen ones.

Inclusion criteria: confirmed diagnosis of PAR; the duration of the exacerbations>50 days/year; the remission of the diseases (including pharmacological remission) at the beginning of the study; confirmed sensitization to house dust mite and outdoor inhaled allergens.

Exclusion criteria: nonallergic diseases of the upper respiratory tract; exacerbation of the diseases during treatment; ASIT in the past; exacerbation of the disease during ASIT.

The course of subcutaneous ASIT started in November-December and was carried out completely in 3 years [2].

The severity of the symptoms has been assessed before and after the treatment.

The efficiency of the treatment has been considered as "excellent" if the contact with the causative allergen didn't result in any symptoms; as "good" if symptoms were transient or mild; as "acceptable" in maintenance of symptoms but their severity was less than before the treatment; as "unacceptable" in the maintenance or worsening of the patient's condition.

The serum level of IL-8 and RANTES (ELISA, «Bender MedSystems», Austria) has been assessed on 0, 7, 30 and 90th day of ASIT. Serum samples were stored at -20 ºC during 3-4 months. The dynamics of serum cytokines level was evaluated retrospectively after the accomplishment of the course of ASIT. All patients have been divided in two groups depending on the efficiency of the treatment. The data of the patients with excellent/good and acceptable results were analyzed separately.

Results and discussion

ASIT is based on multiple cascade changes of immunological reactivity.

Now it's impossible to select the main mechanism allowing to recommend a single available biomarker for the prognosis and monitoring of ASIT [2].

The description of any initial and ending immunological parameters without characteristics of their intermediate dynamics regardless long duration of the treatment is obvious disadvantage of the majority of such studies. More over final effects of ASIT causing the efficiency of the treatment are induced by whole allergen dose administered for a long time. Along with that allergen doses at the beginning of ASIT are very low and potentially can induce unequal

effects influencing the final results of the treatment.

Clinically the result of the treatment has been diagnosed as excellent/good in 56,7% (17/30) and acceptable in 43,3% (13/30) children. Unacceptable result has been diagnosed in nobody. Earlier we reported about the possibility of different dynamics of some biomarkers in patients with excellent/good and acceptable efficiency of the ASIT [3].

But present study doesn't demonstrate any difference between these groups.

More over separated analysis of the dynamics of the studying parameters in depending on the efficiency of the treatment didn't show any available changes but any significant changes have been revealed if the whole group was studied.

Interleukin 8 (IL-8) is a promising marker for many clinical conditions and currently being applied by various subspecialties of medicine either for the purpose of rapid diagnosis or as a predictor of prognosis. Nevertheless, IL-8 level increased as a result of many inflammatory conditions, so careful interpretation of IL-8 level is required to make correlation with desired clinical condition's diagnosis or prognosis. In previous reports we showed the possibility of activation of inflammatory process during ASIT so a panel consisting of more then one biomarker including IL-8 will be promising diagnostic and prognostic approach for many clinical situations including ASIT.

A major property of IL-8 during the inflammatory process is chemotaxis of target cells to the site of inflammation including neutrophils, T cells and basophils. Neutrophil adhesion to and transmigration across the endothelium are regulated by IL-8 and once neutrophils arrive to the site of inflammation, IL-8 further stimulates those cells to carry out phagocytosis, thus increasing the efficiency of tissue repair. Studies have also shown that IL-8 also has other immunomodulatory effects including the ability to induce matrix metalloproteinase-9 expression, release of TNF-related apoptosis-inducing ligand and prime respiratory burst in neutrophils. At the same time an allergen exposure induces the release of higher levels of IL-8 and the cytokine enhances the survival of eosinophils [4].

In this study we have observed the cohort of 35 patients with PAR. Serum level of IL-8 and RANTES has been detected in 0, 7, 30 and 90 day (see Table 1, 2, Fig. 1, 2).

Initially detectable level of IL-8 has been shown only in the third of patients but at the same time it was markedly lower than in patients with acute or chronic inflammation [5]. We can explain this fact by the remission of the PAR and other inflammatory diseases requiring for the ASIT. Induction of immune tolerance during ASIT was characterized by the decrease of IL-8 serum concentration over the course of dose accumulation in ASIT. Gradual decrease of the concentration of IL-8 was accompanied with the reduction of the prevalence of detectable values from 34,3% (12/35) till 5,7% (2/35) (Table 1).

Table 1. The dynamics of serum level of IL-8 at early stages of ASIT (n=35)

Day/group	Parameter, Me [25%-75%]	
	IL-8, pg/ml	IL-8, detectable
	Me [Q1-Q3]	%
0 day	2,1 [0-2,9]	34,3
7 day	0* [0-2,4]	20,0
30 day	2,9** [0-5,0]	40,0
90 day	0,0 [0-0]***, ****	5,7*
	*p=0,032 – 0-7 days **p=0,045 – 7-30 days ***p=0,015 – 30-90 days ****p<0,001 – 0-90 days	*p =0,003 – 0-90 days

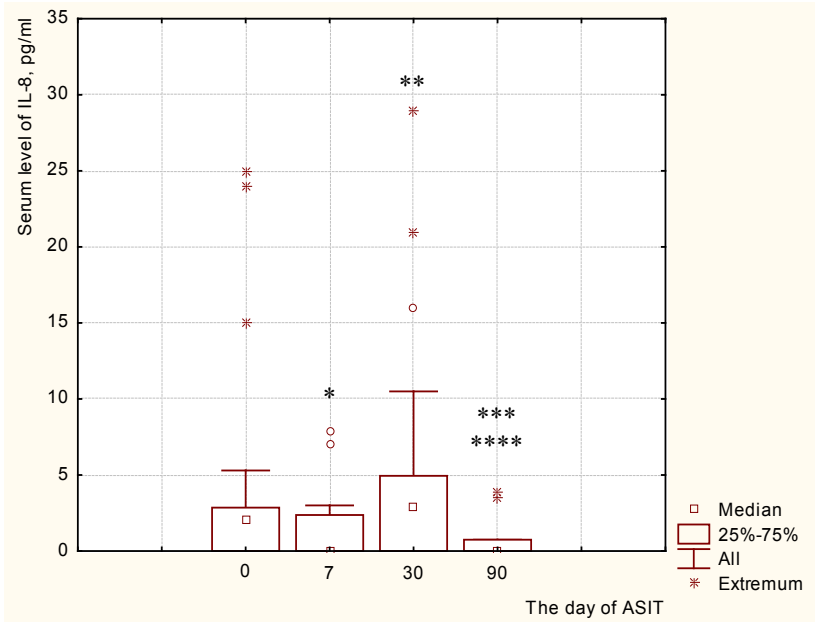

Fig. 1. The dynamics of serum level of IL-8 at early stages of ASIT (see comments in Table 1).

At the same time transient increasing of the IL-8 level occurred in 30 day but finally it was decreased to undetectable level in the most patients (94,3%).

This short-term increasing of the level of IL-8 may be caused by partially activated inflammation during the first month of the treatment. In the previous reports we demonstrated the possibility of subclinical enhancement of allergic inflammation at early stages of ASIT and short-term increase of eosinophils

count and eotaxin production. Despite these findings the treatment has been continued because any clinical symptoms were absent.

Another studied cytokine is RANTES. Expression of RANTES was initially considered to be T-cell specific both CD4+- and CD8+-cells. Findings of H.Oyamada revealed that mononuclear cells could produce RANTES but not eotaxin in response to a specific allergen in asthma patients. Any data about it is role in the mechanisms of ASIT are unknown. RANTES plays a key role in the airway inflammation in the late asthmatic response in which eosinophilia is typical. Indeed, RANTES production from PBMNC of asthma patients with eosinophilia was greater than that of patients without eosinophilia [6].

Table 2. The dynamics of serum level of RANTES at early stages of ASIT (n=35)

Day/ group	Parameter, Me [25% - 75%]		
	RANTES, pg/ml (n=35)	RANTES 1, pg/ml (n=28)	RANTES 2, pg/ml (n=7)
	Me [Q1-Q3] (all)	Me [Q1-Q3] (all)	Me [Q1-Q3] (all)
0 day	2834 [2235-5235]	2559 [1944-3541]	30450 [21255-50275]
7 day	3301 [1871-22100]	2850 [1871-12550]	33550 [26750-43100]
30 day	14100 [2955-21400]	10600 [2955-20150]*	25850 [16000-32150]
90 day	20850 [20950-32695]*	29850 [20950-32695]**, ***	14950 [8700-23569]*, **
	*p=0,041 0-90 days	*p=0,036 – 7-30 days **p=0,025 – 30-90 days ***p=0,012 – 0-90 days	*p=0,046 – 30-90 days **p=0,037 – 0-90 days

RANTES1 – based level <10000 pg/ml, RANTES2 – based level >10000 pg/ml

If based RANTES serum level has been less than 10000 pg/ml it gradually increased to 30th day and any more to 90th day (Table 1, Fig. 2). Total increasing may be estimated as 8-10-fold. It was noticed that on 90th day the concentration of RANTES in the group with low values raised to initial level in the second group. But if based RANTES serum level has exceeded than 10000 pg/ml it diminished in 2-3 times.

It causes the interest to RANTES role in the induction of immune tolerance in ASIT and study of it's dynamics during the treatment. Sanz C. etc. have studied initial and final of serum concentrations of RANTES and nothing changes have been determined [7].

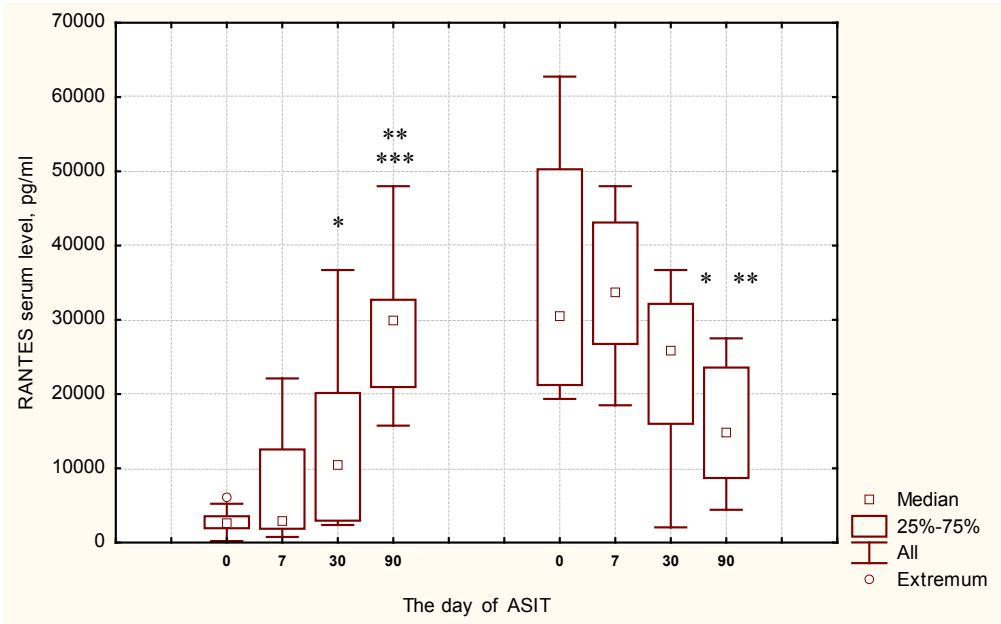

Fig. 2. The dynamics of RANTES at early studies of ASIT (see comments in Table 2).

In our study at first it has been seen that serum level of the RANTES raised only on 90th day but it masked controversial direction of the dynamics which has been demonstrated if separated analysis in depending on initial level of RANTES has been carried out.

Conclusion

Thus, IL-8 and RANTES are involved into mechanisms of ASIT at early stages of treatment. The peculiarities of the dynamics of their serum level at this time aren't associated with the insufficient effectiveness of the treatment but reflect the duration of ASIT. We suppose that primarily low concentration of IL-8 reflects clinical health of patients and it is short-term increasing at the first month of ASIT may be caused by possible temporal activation of allergic inflammation. It is gradual decreasing later to undetectable level is associated with obtaining of complete dose of an allergen and immune tolerance is established.

Controversial dynamics of serum level of RANTES is interesting for the future investigations. We supposed that T-cells involved into forming of immune tolerance during ASIT can up- or down-regulate RANTES reaching any middle level. Another cause may be the multiplicity of the origin of the cytokine leading to involvement into the process in various ways.

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Climate Change, Pollen Allergy and Asthma

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B428R9013

Introduction

It is now widely accepted that the earth's temperature is increasing, as confirmed by warming of the oceans, rising sea levels, glaciers melting, sea ice retreating in the Arctic and diminished snow cover in the Northern Hemisphere.

Moreover, changes are also occurring in the amount, intensity, frequency and type of precipitation as well as the increase of extreme weather events, like heat waves, droughts, floods and hurricanes; (1, 2). The recent Working Group I Report of the Intergovernmental Panel on Climate Change (IPCC) states "most of the observed increase in globally averaged temperatures since the mid-20th century is very likely due to the observed increase in anthropogenic greenhouse gas concentrations" (1).

Observational evidence indicates that recent regional changes in climate, particularly temperature increases, have already affected a diverse set of physical and biological systems in many parts of the world (1, 2).

A rapid rise has been observed in the number of hot days and severe meteorological events such as the 2003 heat wave where temperatures of 35°C and greater were reached resulting in around forty thousand excess deaths across Europe (3). Sea levels have also started to rise as an effect of a regression of the polar ice packs. Both events have led to water deprivation in certain areas, often associated with water degradation which potentially could result in population migration and the effects on health that result from mass population movement.

Migration studies provide useful information on the role of environmental factors, including climate changes, on the development of atopy and asthma (5-8). Migration involves exposure to a new set of pollutants and allergens

as well as changes in housing conditions, diet and accessibility to medical services, all of which are likely to affect migrants' health. Atopy and asthma are more prevalent in developed and industrialized countries as compared with undeveloped and less affluent countries, and the effect of migration is age and time-dependent: early age and longer time spent in the new environment increase the likelihood of developing allergenic symptoms such as asthma, rhinoconjunctivitis, or eczema. Migrants should, therefore, be aware of the potential for developing allergies and/or asthma. Strategies for primary prevention in high risk atopic individuals and secondary prevention guidelines should be developed both for populations in developing countries and for immigrants from such countries to atopy-prevalent developed countries.

Climate changes will influence the development of allergic respiratory diseases (5-19). Climate affects local and national food supplies, air and water quality, weather, economics and many other critical health determinants.

Climate change thus represents a massive threat to global health that could affect many disease factors in the 21st century.

There is also a link between climate changes and air pollution, and an individual's response to air pollution depends on the source and components of the pollution, as well as on climatic agents (9, 10). Some air pollution-related episodes of rhinitis and asthma exacerbation are due to climatic factors that favour the accumulation of air pollutants, such as ozone, at ground level.

Studies have demonstrated some effects of ozone over respiratory symptoms, acute decreases in lung function, increased airway responsiveness, airway injury and inflammation and systemic oxidative stress. Gent et al (11) examined the simultaneous effects of ozone and PM2.5 at levels below EPA standards on daily respiratory symptoms and rescue medication use among children with asthma. Daily respiratory symptoms and medication use were examined prospectively for children with physician-diagnosed asthma. Ozone level (but not PM2.5) was significantly associated with respiratory symptoms and rescue medication use among children using maintenance medication.

A 50-ppb increase in 1-hour ozone was associated with increased likelihood of wheeze (by 35%) and chest tightness (by 47%). The highest levels of ozone (1-hour or 8-hour averages) were associated with increased shortness of breath and rescue medication use. No significant exposure-dependent associations were observed for any outcome by any pollutant among children who did not use maintenance medication (a marker of asthma severity).

The key determinants of greenhouse gas emissions are energy production, transportation, agriculture, food production and waste management, and attempts at mitigating climate change will need to address each of these. However, while there is some uncertainty about predicting future meteorological trends, whatever interventions may be put in place to ameliorate climate change, it

is likely that the world will experience more hot days, fewer frost days, and more periods of heavy rain and consequent flooding (12). Paradoxically it is likely that there will be more periods of drought. A huge increase in CO2 concentrations during the last two decades has been experienced (figure 1).

However, it is important to consider that after CO2 emissions are reduced and atmospheric concentrations stabilize, surface air temperature continues to rise slowly for a century or more.

The effect of climate changes on allergic and respiratory diseases

A body of evidence suggests that major changes involving the atmosphere and the climate, including global warming induced by human activity, have an impact on the biosphere and human environment (3, 18, 43-45).

A synthesis of the health effects due to climate change is presented in figure 2.

Figure 2. Potential Health Effects of Climate Change

Climate events	Agriculture, forestry	Human health impact
Heavy precipitation events: frequency increases over most areas	Damage to crops; soil erosion, inability to cultivate land, water logging of soils; Adverse effects on quality of surface and groundwater; contamination of water supply	Deaths, injuries, infectious diseases, allergies and dermatitis from floods and landslides
Area affected by drought	Land degradation, lower yields/crop damage and failure; livestock deaths; land degradation; More widespread water stress	Increased risk of food and water shortage; increased risk of water- and food-borne diseases; cardiovascular disorders
Number of intense tropical cyclones	Damage to crops; wind throw of trees; Power outages cause disruption of public water supply	Increased risk of water- and food-borne diseases; asthma
Incidence of extreme high sea level	Salinization of irrigation and well water; Decreased freshwater availability due to saltwater intrusion	increase in stress-related disease; other allergic conditions

Studies on the effects of climate changes on respiratory allergy are still lacking and current knowledge is provided by epidemiological and experimental studies on the relationship between asthma and environmental factors, eg, meteorological variables, airborne allergens and air pollution. Climate change is correlated with allergens for several reasons:

(i) increase and faster plant growth;
(ii) increase in the amount of pollen produced by each plant;
(iii) increase in the amount of allergenic proteins contained in pollen,
(iv) increase in the start time of plant growth and therefore the start of pollen production and
(v) earlier and longer pollen seasons.

Climate changes affect allergenic plants and pollen distribution worldwide (13, 17-20).

There is also considerable evidence that subjects affected by asthma are at increased risk of developing obstructive airway exacerbations with exposure to gaseous and particulate components of air pollution (11).

Climate change coupled with air pollutant exposures may have potentially serious adverse consequences for human health in urban and polluted regions.

Data also suggest that air pollution can lead to the development of asthma (14, 15, 16).

It is not easy to evaluate the impact of climate changes and air pollution on the prevalence of asthma in general and on the timing of asthma exacerbations, but the global rise in asthma prevalence and severity indicates that air pollution and climate changes could be contributing.

Effect of climate change on pollen allergy

Pollen allergy is frequently used to study the interrelationship between air pollution and allergic respiratory diseases (rhinitis and asthma). Epidemiologic studies have demonstrated that urbanization, high levels of vehicle emissions and westernized lifestyle are correlated with an increase in the frequency of pollen-induced respiratory allergy in people who live in urban areas compared to those who live in rural areas (18).

Studies on plant responses to elevated CO2 concentrations indicate that plants exhibit enhanced photosynthesis and reproductive effects and produce more pollen (13, 17, 18, 19). An earlier start and peak of the pollen season is more pronounced in species that start flowering early in the year. Moreover, plants flower earlier in urban areas than in the corresponding rural areas with earlier pollination of about 2-4 days. Meteorological factors (temperature, wind speed, humidity, thunderstorms etc) along with their climatic regimes (warm or cold anomalies and dry or wet periods etc), can affect both biological and chemical components of this interaction. In addition, by inducing airway inflammation, air pollution overcomes the mucosal barrier, leading to the priming of allergen-induced responses.

Climate changes might induce negative effects on respiratory allergic diseases favouring the increased length and severity of the pollen season, the

higher occurrence of heavy precipitation events and the increasing frequency of urban air pollution episodes.

The main diseases of concern are asthma, rhino-sinusitis, COPD and respiratory tract infections, but the extent to which these are affected will vary according to the proportion of susceptible individuals in a given population.

Areas of greater poverty with limited access to medical care will suffer more as will those areas with less well developed medical services which are likely to include migrating populations and those with the greatest population growth.

With warming over the longer term, changing patterns of plant habitat and species density are likely, with gradual movement northward in the Northern Hemisphere, and further south in the Southern Hemisphere. The change in land use might also play a relevant role, especially for some important allergenic species, such as grasses. Since most data come from the analysis of distribution of airborne pollen, these findings are potentially biased by the occurrence of long and medium distance transport episodes of allergenic pollen (21, 22).

Climatic factors (temperature, wind speed, humidity, thunderstorms etc) can affect both components (biological and chemical) of this interaction (23-29). By attaching to the surface of pollen grains and of plant-derived particles of paucimicronic size, pollutants could modify not only the morphology of these antigen-carrying agents but also their allergenic potential. In addition, by inducing airway inflammation, which increases airway permeability, pollutants overcome the mucosal barrier and could be responsible for “priming” the allergen-induced responses of pollinosis in allergic and atopic individuals.

However, the relationship between air pollution, pollen exposure and respiratory allergy is based on an individual’s response to air pollution, which depends on the source and components of the pollution, as well as on climatic agents.

Interaction between climate change and urban air pollution

Some air pollution-related episodes of asthma exacerbations are due to climatic factors that favour the accumulation of air pollutants at ground level, and some cities are continuously affected by pollution caused by motor vehicles (19, 26, 27). Air pollution can interact with allergen-carrying paucimicronic particles derived from plants (28). The paucimicronic particles, pollen-originated or not, are able to reach peripheral airways with inhaled air, inducing asthma in sensitized subjects. Air pollution – in particular PM, DEP, ozone, nitrogen dioxide and sulfur dioxide – have been shown to have an inflammatory effect on the airways of susceptible subjects, causing increased permeability, easier penetration of allergens into the mucus membranes, and

easier interaction with cells of the immune system (30). There is also evidence that predisposed subjects have increased airway reactivity induced by air pollution and increased bronchial responsiveness to inhaled allergens.

Some pollutants seem to have an adjuvant immunologic effect on IgE synthesis in atopic subjects – in particular, DEPs, which can interact in atmosphere with pollens or paucimicronic particles (27).

It is also important to consider that in the Mediterranean area (Greece, Spain, Italy etc), in California and other areas, hundreds of thousands of hectares of woods are destroyed each year by fire. Moreover, fire produces millions of tons of CO2 which play a role in the greenhouse effect (24-26).

Thunderstorm-related allergic respiratory diseases and bronchial asthma in pollinosis subjects

Thunderstorms occurring during the pollen season have been observed to induce severe asthma attacks in pollinosis patients (29-40).

Associations between thunderstorms and asthma morbidity have been identified in multiple locations around the world (32-47). The most prominent hypotheses for thunderstorm-related asthma are linked with bioaerosols, and involve the role of rainwater in promoting the release of respirable particulate matter.

After hydratation and rupture by osmotic shock during the beginning of a thunderstorm, pollen grains release part of their cytoplasmic content into the atmosphere, including inhalable, allergen-carrying paucimicronic particles such as starch granules and other cytoplasmic components (28, 29).

In summary, the occurrence of these epidemics is closely linked to thunderstorms; the thunderstorm-related epidemics are limited to late spring and summer when there are high levels of airborne pollen grains; there is a close temporal association between the arrival of a thunderstorm, a major rise in concentration of pollen grains and the onset of asthma epidemics.

As a consequence, subjects affected by pollen allergy should be alert to the danger of being outdoors during a thunderstorm in the pollen season.

Changes in the profile of local allergens

We urgently need to monitor changes in vegetation and airborne allergens arising from climate change so that new allergen vaccines can be available for immunotherapy. Allergists should also be alert to changes in insect, mite, fish and animal populations that could give rise to new environmental allergen exposures, with the potential for new allergic sensitizations and a concomitant increase in allergic respiratory diseases, increased severity of asthma, and anaphylaxis.

Conclusions

Climate changes affect many physical and biological systems including the immunologic and respiratory systems that are critical to human health, and it is foreseeable that environmental risk factors will have a stronger effect in the coming decades (40-47). Climate changes interact with and affect air pollution and pollinosis, which in turn increases the frequency and severity of asthma, and affects the clinical expression of allergic disease. Climate change affects the timing, dispersion, quantity, and quality of aeroallergens and the distribution and severity of allergic disease. Climate change alters local weather patterns including minimum and maximum temperature, rain precipitation, and storms, all of which affect the burden of allergic disease. A combined approach comprises primary prevention by greenhouse gas mitigation to stabilize the climate, and secondary prevention by clinical intervention to minimize climate change–related increases in asthma and allergic disease (40). Climate changes in the future may depend on how rapidly and successfully global mitigation and adaptation strategies are deployed. The effect of human intervention and efforts to minimize changes in vegetation and aeroallergen exposure remains to be seen.

Reducing air pollution might contribute to lessening of the impact of climate change on pollen and thus directly on patients, while recognizing that ozone, the key pollutant associated with climate change, may be the major driver of pollutant/pollen interactions.

What can we do to decrease the effects of environmental factors affecting respiratory allergic diseases? Suggested measures are as follows: encouraging policies to promote access to non-polluting sources of energy; reducing the private traffic in towns and improving public transport; decreasing the use of fossil fuels and controlling vehicle emissions; planting non-allergenic trees in cities, and in this context the proposed implantation of new trees should be evaluated by allergy specialists in order to avoid high allergenic species.

Many measures to reduce greenhouse gas emissions may have positive benefits for health. These co-benefits will offset at least some of the costs of climate change mitigation and should be taken into account in international negotiations.

In conclusion, strategies to reduce climatic changes and air pollution are political in nature, but citizens, and in particular health professionals and societies, must raise their voices in the decision process to give strong support for clean air policies at both national and international levels.

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Preparing the Workforce for the Changing Practice Environment

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B428R9014

Abstract

The global burden of asthma continues to grow and constitutes a major healthcare challenge when preparing the next generation of providers for a re-designed healthcare system. According to the World Health Organization, it is estimated that approximately 300 million individuals suffer from asthma and the number is expected to increase by 100 million by the year 2025 (WHO, 2007). Strengthening the human resource capacity by improving training and establishing continuing education programs for healthcare providers at all level has been as a suggested plan of action to address this challenge. A “train the trainer” model has been utilized in certain fields with positive outcomes, but is less frequently employed in healthcare education (Sanders, M. Reynolds, J. Bagatelle, W, Trem, J. O Connor, E. & Katz, D, 2015). Faculty training using the “train the trainer” approach can be a key component in preparing the next generation of providers to deliver safe and effective asthma care.

Keywords: healthcare education, train the trainer model, asthma

Introduction

There is a strong association between symptom burden and healthcare use such as emergency room visits and hospitalization. (Fuhlbrigge, et al, 2002).

In light of the growing number of people living with asthma and the shortage of healthcare professionals specializing in the disease, it is critical that providers are well prepared and willing to work on the front line to improve asthma care and self-management. Research shows that increased preparation for providers with interest and experience in a topic can strongly impact the wellbeing of patients and families (Beacham, 2016).

Changing Practice Environment

The Institute of Medicine has outlined ten important rules of performance in a re-designed healthcare system to help improve care (Finkelman, A, 2012).

The first rule is that "care is based on a continuous healing relationship".

In order for providers to acknowledge the client's right to self-determination and demonstrate that they value the client's beliefs values and preferences, it is recommended that patient/provider communication should be "horizontal" rather than "top- down". Goals should be short so that they can be easily achieved (Bonezzi A, Brandl, C & DeAngelis, 2011). Examining the client's health belief system to better understand different world views and offer evidence based recommendations will help to reduce conflicts. A re-designed healthcare system includes safety as a systems priority. In asthma care, safety issues include poor asthma control because of frequent symptoms, greater use of rescue medications, functional impairment or worsening function continues to be a problem for many clients. A re-organization of tasks and personnel performing those tasks may require a fundamental re-thinking. Who is currently performing the task of educating the next generation of providers and do they have the appropriate skill level?

Methodology

The purpose of faculty development is to facilitate the development of faculty skills, foster an environment in which faculty feel empowered to continually work toward improved educational scholarship. In order to address the challenge and meet the growing educational needs in asthma care, a dedicated faculty training program using a "Train the Trainer" (TTT) model is proposed. This model is one in which content is gathered from experts to educate trainers in order to allow them to instruct target audiences. They can then disseminate the information to others in a timely fashion making it cost effective and sustainable. (Sanders, et al, 2015). A structured curriculum helps to regulate the how and when the information is delivered (Slutsky, P, Bryant, Stephens, 2001).

Train the Trainer model

A train the trainer educational model established by an organization gathers content from experts to educate trainers which then enables them to instruct target audiences. The advantage of this model is its ability to be replicated and information can be easily disseminated in a timely manner. (Sanders et al, 2015; Yong, et al, 2016; Greif, et al, 2015; Mayrhofer, 2016). The expert

trainer teaches the non-expert how to administer an intervention as well as trains others to do the same. It is different than traditional teaching models because it provides a cascade effect, thereby increasing the available pool of individuals with essential knowledge necessary to support learning.

Steps in the Process

The steps in the process to begin a TTT program include the following: Step 1: Obtain funding from a national foundation dedicated to supporting faculty development in asthma education Step 2: Launch a pilot program by a nurse specialist or a certified asthma educator Step 3: Begin interactive training program at a local academic center Step 4: Conduct lectures, small group discussions, workshops with asthma education experts and patient panels Step 5: complete mentored independent project benefitting education, research and patient care Step 6: Include mentorship by members of a multidisciplinary team Step 7: Attend a support or community group Step 8: Nominate scholars to advance educational scholarship and present new and innovative educational strategies to others. Share research in journals, webinars and disseminate sample curriculum on line with available resources.

Adult Learning Theory

According to adult learning theory (Knowles, M., Holton, E. Swanson, R., 2015), adult learners come to each learning event with a unique background of knowledge and experience. They are motivated to learn if they can share what they know and build on their prior experience because this will validate their expertise. Faculty are self-directed and want control over what and how they are learning. They are motivated to learn if they can make decisions not only about the content but the process in which they learn, therefore some independence is recommended.

When a train the trainer, session is in progress, it is important that participants are able to participate actively in the learning process, so that they can practice new skills or test their newly found knowledge prior to leaving the learning session.

VARK Learning Styles

A "train the trainer" model should approach teaching using a variety of strategies because it must fit the learner's style and preference. Learning preferences may include kinesthetic, visual, auditory or multimodal. Case studies/problem solving/simulation help learners anchor new skills and

knowledge. Learning can be enhanced when learners have an opportunity to reflect, review or personally relate to the material presented and discuss how to apply it.

Results

Employing a pyramid model such as "train the trainer" approach for faculty development can be used to enhance the depth of knowledge about evidence based guidelines for asthma and increase the level of confidence in developing course content, lectures and clinical mentorship in the undergraduate setting.

Forming an academic/practice partnership is an important bridge to build to improve care (Niederhauser, 2016).

A "train the trainer" model supports basic and ongoing collaboration of others to advance research and education. Additional goals for this model include the development of long term relationship between guest faculty, scholars and experts at nationally recognized asthma centers so that faculty can better prepare undergraduates with significantly more skills and knowledge about evidence based guidelines in asthma care.

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Climate Change, Extreme Meteorological Events (Thunderstorms During Pollen Seasons) and Asthma Attacks

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B428R9014

Keywords: Climate change and Thunderstorms, Climate change and pollen Allergy, Bronchial asthma, Severe asthma, Near fatal asthma, Thunderstorm-related asthma, Meteorological factors and asthma, Prevention of Thunderstorm-related asthma

There are observations that thunderstorms occurring during pollen seasons can induce severe asthma attacks in pollinosis patients (1).

According to current climate change scenarios, there will be an increase in intensity and frequency of heavy rainfall episodes, including thunderstorms, over the next few decades, which can be expected to be associated with an increase in the number and severity of asthma attacks both in adults and in children (2, 3).

Associations between thunderstorms and asthma attacks have been identified in multiple locations around the world (4). So, called "thunderstorm asthma" is characterized by asthma outbreaks possibly caused by the dispersion of more respirable allergenic particles derived from pollen and spores (1, 5, 6).

Thunderstorms have been linked to asthma epidemics, especially during the pollen seasons, and there are descriptions of asthma outbreaks associated with thunderstorms, which occurred in several cities, prevalently in Europe (Birmingham and London in the UK and Napoli in Italy) and Australia (Melbourne and Wagga Wagga) (1, 4, 7) (Table 1). The thunderstorm-asthma outbreaks are characterized, at the beginning of thunderstorms, by a rapid

increase of visits for asthma in general practitioner or hospital emergency departments. Subjects without asthma symptoms, but affected by seasonal rhinitis can experience an asthma attack. No unusual levels of air pollution were noted at the time of the epidemics, but there was a strong association with high atmospheric concentrations of pollen grains such as grasses or other allergenic plant species. However, subjects affected by pollen allergy should be informed about a possible risk of asthma attack at the beginning of a thunderstorm during pollen season.

On 21 November 2016 in Melbourne there was a dramatic event with 8 deaths and 8500 patients who needed medical treatments in Emergency departments of Melbourne Hospital for asthma attacks (7).

This in Melbourne has been the worst event of thunderstorm-asthma.

Table 1. Epidemics of thunderstorm-associated asthma outbreaks (1, 4, 7)

Year	Country	Observations
1983	UK	26 sudden cases of asthma attacks in relation to thunderstorms.
1992	Australia	Late spring thunderstorms in Melbourne can trigger epidemics of asthma attacks (five to 10-fold rise).
1997	UK	Asthma or other airways disease hospital visits. 640 cases who attended during a 30-h period on June 1994, nearly 10 times expected number.
1992-2000	Canada	18 970 hospital ED asthma visits among children 2-15 years of age. Summer thunderstorm activity was associated with an OR of 1.35 (95% CI 1.02–1.77) relative to summer periods with no activity.
1993-2004	USA	215 832 asthma ED visits; 24 350 of these visits occurred on days following thunderstorms. Significant association between daily counts of asthma ED visits and thunderstorm occurrence. Asthma visits were 3% higher on days following thunderstorms.
2000	Australia	Asthma visits during thunderstorms. History of hayfever and allergy to ryegrass are strong predictors for asthma exacerbation during thunderstorms in spring.
2001	Australia	The incidence of excess hospital attendances for asthma during late spring and summer was strongly linked to the occurrence of thunderstorm outflows
2002	UK	A case-control study of 26 patients presenting to Cambridge University Hospital with asthma after the thunderstorm Alternaria alternata sensitivity is a compelling predictor of epidemic asthma in patients with seasonal asthma and grass pollen allergy and is likely to be the important factor in thunderstorm-related asthma.
2004	Italy	Six cases of thunderstorm-related asthma because of pollen (Paretaria).
2010	Italy	20 cases of thunderstorm-related asthma because of pollen (olive tree).
2010	Australia	Epidemics of ‘thunderstorm asthma’ that occurred in Melbourne during spring 2010. The approach of spring, together with high winter rainfall in and around Melbourne that heralds another severe pollen season, raises the risk of allergic rhinitis and asthma in pollen-sensitive individuals.
2016	Australia	Epidemics of thunderstorm asthma in Melbourne with 8 deaths and 8500 in emergency department.

One of the first observations regarding thunderstorms and asthma outbreaks was provided by Packe and Ayres at the East Birmingham Hospital (Birmingham, UK) on July 6 and 7, 1983. These authors described a remarkable increase in the number of asthma emergency department admissions during the hours of a thunderstorm. In a 36-h period, 26 asthma cases were treated in the emergency department, compared with a daily average of two or three cases in the days preceding the outbreak.

Another asthma outbreak occurred in London, UK, coinciding with a heavy thunderstorm on June 24, 1994, when a large increase in the number of visits for asthma at the emergency departments of London and the southwest of England was observed. Several patients who were examined, who were not known to be asthmatics or were affected only by seasonal rhinitis, experienced an asthma attack. During a 30-h period from 6 p.m. on June 24, 1994, 640 patients with asthma or other airways disease (283 of whom were not known to be asthmatic and 403 were affected only by seasonal rhinitis) attended several emergency departments, nearly 10 times the expected number of 66 patients.

In total, 104 patients were admitted (including five to an intensive care unit) (574 patients attributable to the thunderstorm).

Other asthma outbreaks during thunderstorms have been described in Australia. In Melbourne, other than the dramatic outbreak of 21 november 2016, two large asthma outbreaks (rapid increase in hospital or general practitioner visits for asthma) coincided with thunderstorms. In WaggaWagga, 215 asthmatic subjects attended the local emergency department, 41 of whom required admission to hospital.

In south eastern Australia, Marks et al. (8) observed that the incidence of excess hospital attendances for asthma during late spring and summer was strongly linked to the occurrence of thunderstorm outflows and demonstrated that the arrival of a thunderstorm outflow was accompanied by a large increase in the concentration of ruptured pollen grains in ambient air.

Thunderstorm-related asthma was observed in Naples, Italy, on June 3 2004, when five adults and one child received treatment in emergency departments. One patient was admitted to an intensive care unit for a very severe bronchial obstruction and acute respiratory insufficiency following a sudden thunderstorm. All individuals were outdoors when the thunderstorm struck. In one severe case, a female sensitized only to Parietaria pollen allergen, soon began to show symptoms of intense dyspnoea, which gradually worsened.

She was taken to hospital where she was intubated and given high intravenous doses of corticosteroids. She was discharged a few days later. This patient had previously suffered from seasonal asthma but had been asthma-free for the past few years and did not need continuous therapy. None of the other five subjects took anti-allergic and/or anti-asthma drugs regularly.

All six patients were sensitized with allergic respiratory symptoms upon exposure to Parietaria pollen, but were not sensitized to grasses. Parietaria is an Urticacea that is widespread in the Naples area of Italy with a spring and summer pollen season that is, in part, coexistent with that of grasses.

During the thunderstorm, the concentration of airborne Parietaria pollen grains was particularly high, with a peak of 144 grains/m3 being recorded on June 3, 2004. Air pollution levels for both gaseous and particulate components based on the hourly concentrations of nitric dioxide, ozone and respirable particulate matter were not particularly high in Naples on June 3 and 4, 2004.

Subjects with sensitization to Parietaria who were indoors in Naples with the windows closed during the night between June 3 and 4, 2004, did not experience asthma attacks. No moulds or viruses were involved in the Naples epidemics.

Other outbreaks and/or case reports have been described in Barletta, Cartagena, Atlanta.

A similar phenomenon has been suggested for moulds after the observation of a possible key role of sensitization toAlternariaspecies in thunderstormrelated asthma.

Characteristics of described epidemics of thunderstorm-associated asthma
The occurrence of epidemics is closely linked to thunderstorm
The thunderstorm-related epidemics are limited to late spring and summer when there are high levels of airborne pollen grains
There is a close temporal association between the arrival of the thunderstorm, a major rise in the concentration of pollen grains and the onset of epidemics
Patients with pollen allergy, who stay indoors with windows closed during thunderstorm, are not involved
There are no high levels of gaseous and particulate components of air pollution
There is a major risk for patients who are not under antiasthma correct treatment

Although much remains to be discovered about the relationship between an increase in the number of asthma attacks and thunderstorms, reasonable evidence exists in favour of a causal link between them in patients suffering from pollen allergy. The most prominent hypotheses for thunderstorm-related asthma are linked with bioaerosols, and involve the role of rainwater in promoting the release of respirable particulate matter (1). Pollen grains can be carried by thunderstorm at ground level, where pollen rupture would be increased with release of allergenic biological aerosols of paucimicronic size, derived from the cytoplasm and which can penetrate deep into lower airways. In other words, there is evidence that under wet conditions or during thunderstorms, pollen grains may, after rupture by osmotic shock, release into the atmosphere part of their content, including respirable, allergen-carrying cytoplasmic starch granules (0.5 – 2.5 lm) or other paucimicronic components that can reach lower airways inducing asthma reactions in pollinosis patients.

These allergens can likely penetrate deeper into the lung, provoking more severe symptoms. It has been suggested that grass pollen starch granules are the most likely cause of associations between thunderstorms and asthma (8).

Suphioglu et al. (9) showed that ryegrass pollen grains contain a large amount of starch granules coated with allergens. After being ruptured in rainwater by osmotic shock, each grain can release 700 starch granules, which are small enough to penetrate the airways and trigger asthma attacks in previously sensitized subjects. Later Taylor et al. (10) hypothesized that the turbulent front of the advancing outflow releases more pollen from flowering grasses and grass pollen may release large amounts of paucimicronic allergenic particles, that is cytoplasmatic starch granules containing grass allergens (allergenbearing starch granules), after rupture by osmotic shock during thunderstorms.

Even though thunderstorms can induce severe asthma attacks or exacerbations, they are neither frequent nor responsible for a high amount of disease exacerbation. This constitutes a major concern nowadays as the possibility of thunderstorm-associated asthma outbreaks have become of dramatic actuality due to the “highly likely” increase in frequency of heavy precipitation events, including thunderstorms, projected by the climate change scenarios for the future decades (2, 3).

In summary, the occurrence of these epidemics is closely linked to thunderstorm and they are limited to late spring and summer when there are high levels of airborne pollen grains. There is a close temporal association between the arrival of the thunderstorm, a major rise in the concentration of pollen grains and the onset of epidemics. As a consequence, subjects affected by pollen allergy should be alert to the danger of being outdoors during a thunderstorm in the pollen season.

Authors declare they haven't conflict of interest.

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Probiotics and Allergy

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B428R9029

Abstract

The enormous number of controversial experimental and clinical data in the literature about the imminomodulation capacity of intestinal microbiom and its role in allergic diseases and the capacity of the *probiotics* for the primary prevention of allergies exist. The author presents contemporary information about this problem on the basis of the carried out by WAO in 2015 for the first time meta-analysis including 23 double-blind placebo-controlled studies and the resent data from Italian Society of Neonatology. The mechanisms of action of the gut microbiom as a immunomodulator which is similar of those of the *probiotics* in terms of prevention of the allergic diseases is discussed.

The protective role of intestinal microbiom/*probiotics* against sensitization of human organism is to suppress the Th2 immune response by increasing the number of CD25+CD4+ regulatory T lymphocytes which release IL-10, TGF-β, FoxP3 able to stimulate Th1 Ly to synthetized Inf-γ, IL-2, IL-12, TNF-α which suppress production of IgE antibodies. By this way intestinal mircobiom/*probiotics* may modulate the immunologic and the inflammatory system responses and thus to influence development of sensitization and allergy.

This mechanism of action closely coincides with the "hygienic hypothesis" in allergology. Concrete recommendations – strong and conditional about the use of *probiotics*, their efficacy and side effects when they are intended for primary prevention or treatment of different allergic conditions are presented.

Particular attention is given to *pregnant women at high risk for allergy in their children, breastfeeding women and infants at risk.* In conclusion, the author summarizes that are needed much more well planed and organized clinical trails, with specific design in order to allow scientifically based data about the real value of *probiotics* in clinical practice in terms of their effect on the development and prevention of allergic diseases. What is until now well known and recommended is that *probiotics* can be used in pregnant women, breastfeeding women and infants for prevention of eczema/atopic dermatitis.

There are not sufficient data showing the beneficial effect of *probiotics* in

the course or prevention of allergic rhinitis, bronchial asthma and any other allergies.

Keywords: Probiotics, atopic dermatitis, bronchial asthma, food allergy

The enormous number of controversial experimental and clinical data in the literature, many of them in a form of meta-analysis about the imminomodulation capacity of intestinal microbiom and its role in allergic diseases and the capacity of the probiotics for the primary prevention of allergies exist (1, 2, 3, 4, 5, 6, 7).The immunomodulation activity of the Intestinal microbiom attracts the interest of the researchers and clinicians to the different probiotics which contain living microorganisms that when administered to humans in adequate doses may confer a health benefit. They have been proposed to modulate immune response and have been advocated as a therapeutic and preventive interventions for allergic diseases.

The protective role of Intestinal microbiom against sensitization of human organism is: to stimulate CD25+CD4+ regulatory T lymphocytes which release IL-10, TGF-β, FoxP3 able to stimulate Th1 Ly to synthetized Inf-γ, IL-2, IL-12 TNF-α which suppress production of IgE antibodies and to suppress Th2 immune reactivity related with IL-4 and IL-13 release. By this way intestinal mircobiom may modulate immunologic and inflammatory system responses and thus, influences development of sensitization and allergy in terms of the quality and quantity of different microorganisms (fig. 1). This mechanism of action closely coincides with the "hygienic hypothesis" according to which the rise in the prevalence of allergic diseases could be caused by reduced exposure to micro-organisms, with consequent alteration in the balance of the immune response – the decrease of Th1 pattern of cytokine release suppressing the IgE synthesis and the predominant role of Th2 immune reactivity involved in IgE-mediated allergy.

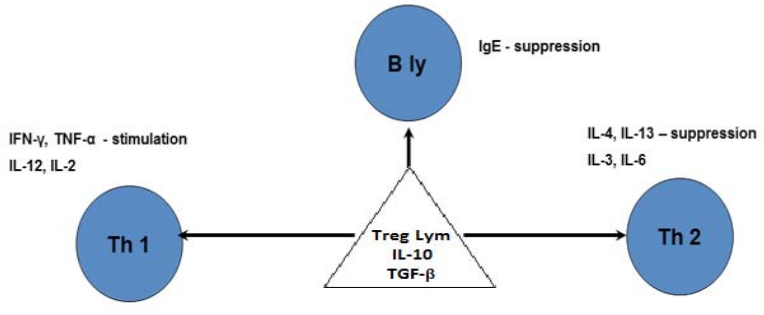

Fig. 1. Mechanism of action of microbiom/probiotics

All these above mentioned controversial issues in terms of the action of probiotics in prevention or treatment of allergic hypersensitivity stimulate the WAO via McMaster University in Canada to organize the only until now very large systematic review on randomized control trials of probiotics (21 published studies) realized by a team of 22 medical institutions from 12 countries (8) in order to assess their real role in this respect. Simultaneously with this study the Italian Society of Neonatology – a team of 12 leading pediatricians – carried out an investigation during several years on the use and clinical efficacy of probiotics in pediatric practice (9). On the basis of these double-blind, placebo-controlled clinical trials of oral probiotic supplementation the WAO presents a several recommendations concerning their efficacy and side effects when they are used for primary prevention or treatment of different allergic conditions. The recommendations are focused on three group of patients: *– pregnant women at high risk for allergy in their children – breastfeeding women – infants.* The recommendations are of two grades of strength – strong or conditional. *Strong recommendations* mean: for patients – most individuals would like the recommended course of action, and only small part of them would not; for clinicians – most individuals should receive the intervention; for policy makers – the recommendation can be adopted as a policy in most situation. *Conditional recommendations* mean: for patients – the majority of individuals would want the suggested course of action, but many would not; for clinicians – recognize that different choices will be appropriate for individual patients and to help them to make decision consistent with their values and preferences. For policy makers – policy-making will require substantial debate and involvement of different stakeholders.

Considering the results of these studies and all available data in this field *the WAO recommendations about pregnant women at high risk for allergy in their children* (*mainly in the last 3 months of pregnancy*) state that: there is high value on prevention of eczema in children when pregnant women use probiotics (*conditional recommendation*); there is relatively lower value on avoiding possible adverse effects of probiotics; there is lack of evidence that probiotics when are given to pregnant women prevent any other allergy – allergic rhinitis, asthma, food allergy. *The WAO recommendations about breastfeeding mothers are:* – to use probiotics in women who breastfeed infants at high risk of developing allergy because there is a net benefit resulting primarily from prevention of eczema (*conditional recommendation*); there is a very low certainty that there is any effect of probiotics use by breastfeeding mothers on the development of other allergies in their children; there is a lack of evidence that probiotics can prevent any other allergies when are given to breastfeeding women; the risk of any adverse effects is low. Follow-up in the included studies ranged from 4 to 36 months' infants used probiotics, *the WAO*

recommendations about healthy children are: to use probiotics in infants at a risk of developing allergies, because there is a net benefit resulting primarily from prevention of eczema atopic dermatitis (*conditional recommendation*); the studies failed to demonstrate a statistically significant effect of probiotics on development of allergic rhinitis or asthma in children. Development of food allergy was measured in 5 rendomized studies and no difference between probiotics and placebo arms was noted.

Conclusion

In conclusion we have to summarize that are needed much more well planed and organized clinical trails, with specific design in order to allow scientifically based data about the real value of probiotics in clinical practice in terms of their effect on the development and prevention of allergic diseases and to answer the following crucial questions: – Evaluation which of three discussed populations should receive probiotics? Whether there is large benefit with supplementation in one or combination of these populations? Which population is target? Evaluation whether any effect of probiotics depends on the species and the strains of microorganisms? Evaluation of the effects of different ways of administration of probiotics – as a milk or dairy supplement, stand-alone supplement? It is not clear when the administration of probiotics should be started and how long they should be used? Is the effect of natural probiotics in food different from that of supplementation?

What is until now well known and recommended is that probiotics can be used in pregnant women, breastfeeding women and infants for prevention of eczema/ atopic dermatitis. There are not sufficient data showing the beneficial effect of probiotics in the course or prevention of allergic rhinitis, bronchial asthma and any other allergies.

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Monitoring the Efficacy of Treatment in Children with Risk of Asthma

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B428R9049

Asthma is the most common chronic disease of childhood throughout the world including Georgia. The tendency of substantial increase of its prevalence and severe progression is being mentioned. Prevention of the disease, as well as effective diagnostic and treatment methods have great importance for managing this problem. The modern approaches in the prevention and treatment of asthma are delivered by GINA, "Global Strategy for Asthma Management and Prevention". The main recommendations of this initiative have already been using in different countries with consideration of national peculiarities.

Using leukotriene inhibitors during obstruction of respiratory system is one of the main recommendations of the project. The cysteinyl leukotrienes, LTC4, LTD4, and LTE4, play an integral role in pathophysiology of asthma [4, 7]. The unique mechanism of leukotriene receptor antagonists (LTRA) action results in a combination of both bronchodilator and anti-inflammatory effects [4. 9].

Considering the fact that optimal place of these drugs in asthma management is still under review, our work implies the monitoring of effectiveness of treatment with Montelukast, as one of the leukotrien receptor antagonist.

Keywords: Cysteinyl leukotrienes inhibitors, Corticosteroids, Bronchial Asthma

The aim

of the study is to evaluate the effect of Montelukast - leukotriene inhibitor in children population with risk of bronchial asthma.

Materials and Methods

The research was conducted at National Institute of Allergology, Asthma and Clinical Immunology, Georgian Academy of Sciences, Tskaltubo, Georgia.

104 patients (5-18 year, 43 girl, 61 boy), with risk of bronchial asthma were involved into the research. They were registered at the clinic for specification of diagnosis and treatment. Diagnosis of asthma in children is difficult because of the complex nature of the disorder in the young. Accurate diagnosis in primary care remains an important challenge. Considering it, the patients with the symptoms and signs described below (which are characteristic for asthma) were determined as a risk group:

- More than one of the following symptoms: wheeze, cough, difficulty breathing, chest tightness, particularly if these symptoms:
  - were frequent and recurrent.
  - were worse at night and in the early morning.
  - Occurred in response to, or are worse after, exercise or other triggers, such as exposure to pets, cold or damp air, or with emotions or laughter.
- Personal history of atopic disorder;
- Family history of atopic disorder;
- Widespread wheeze heard on auscultation.

Peak Expiratory Flow (PEF) and spirometry were used for evaluation of patients' condition. Peak Expiratory Flow (PEF) rate was estimated with apparatus, "Climent Clark".

For evaluation, the bronchoreactivity and bronchoconstriction, the following provocation tests were used:

- Inhalation with vapor of distilled water with "Thomax I" (average rate of nebulizer 4cc/min, particle size - 2to 5 Microns), duration – 3-4 min. PEF indicator was measured before and 5 and 10 min. after inhalation by peakflow-meter.
- 6 minutes physical exercise (running in place). PEF indicator was written 5 and 10 minutes after loading.

Computerized spirometry by apparatus "SPIROLAB 3" was conducted for verification of external respiratory changes and estimation level of bronchial obstruction. Besides Peak expiratory flow (PEF), the following parameters were studied and analyzed: forced expiratory volume in 1 sec (FEV1), forced volume vital capacity (FVC), FEV1/FVC ratio (FEV1 %) - Tiffeneau-Pinelli index.

2 groups of patients were determined based on above mentioned indicators: group I – 47 patients with external respiratory dysfunction and group II – 57 patients without changes. The scheme of treatment for these groups was the following: group 2 patients underwent symptomatic therapy during 1 week under observation. group 1 was divided into subgroups according to the treatment method: patients on inhaled corticosteroids (ICS) treatment (group 1a, n=24) and patients on ICS treatment added leukotriene inhibitor – montelukast (group 1b, n=23) (table 1).

For estimation, the effect of conducted treatment and accordingly condition of patient we used GINAs indicators [8]. Effect of treatment was evaluated based on the data of repeated spirometry conducted in 6 months. Recommendation of clinical practice "Management of asthma in general medical practice" was used for evaluation of asthma control [9].

Obtained results were statistically treated bythe student's *t*-distribution (SPSS 20.0). For minimal level of significance was taken $p<0,05$.

Results

Our results show that considering the functional condition of bronchial-pulmonar system 57 patients out of 104 had no any changes of external respiration, although 47 patients underwent these characteristic changes: decreased PEF with 20% and more was mentioned in 36 patients during physiological conditions, in 7 patients - during inhalation with vapor of distilled water and in 4 patients - brocho - constriction and subsequent PEF decrease was triggered by physical loading.

All 104 patients were undergone the spirometry measurement – as results of what all group 1 patients (n=47) with decreased PEF had different types of changes external dysfunction according to the results of spirometry (table 1, 2), although in other 57 patients no changes were described.

Table 1. Spirometry data in patients of group 1a treated by IGC scheme (n=24)

Parameters	Before treatment	after IGC treatment
PEF - Peak Expiratory Flow	73%	85%
FEV-1 - Forced ExpiratoryVolume1 sec	69%	80%
FVC - forced volume vital capacity	82%	84%
FEV1/FVC ratio (FEV1 %) - Tiffeneau-Pinelli index	61%	81%

Table 2. Spirometry data in patients of group 1b treated by IGC + leukotriene inhibitor (n=23)

Parameters	Before treatment	after IGC treatment + leukotriene inhibitor
PEF - Peak Expiratory Flow	73%	87%
FEV-1 - Forced Expiratory Volume1 sec	69%	84%
FVC - forced volume vital capacity	82%	89%
FEV1/FVC ratio (FEV1%) - Tiffeneau-Pinelli index	61%	82%

As our results show (table 2), PEF was obviously increased in the patients of group 1a (by 12%) ($p<0,001$), as well as in group 1b (14%) ($p<0,001$).

Concerning the FEV1 – it was detected elevation of the indicator in both groups, but the indicator was rather increased in the group treated by combined method (inhaled glucocorticoid+leukotriene inhibitor): FEV-1 was increased by 15% ($p<0,001$), in group 1a – by 11% ($p<0,005$). FVC - forced volume vital capacity – increase by 2% in group 1a, and by 7% - in group 1b.

After treatment in both groups FEV1/FVC ratio (FEV1%) - Tiffeneau-Pinelli index was dramatically increased (20% ($p<0,005$) and 21% ($p<0,05$)) and detected slight difference (1%) was not reliable ($P<0,1$).

Besides these spirometry parameters, duration of inter-exacerbation periods also was estimated during treatment period.

Table 3. Duration of inter-exacerbation periods in patients (n- number of patients)

Only inhaled glucocorticoid		inhaled glucocorticoid + leukotriene inhibitor	
1 week and more (controlled)	Less than 1 week (uncontrolled)	1 week and more (controlled)	Less than 1 week (uncontrolled)
n=23	n=1	n=23	n=0

Discussion

Bronchial asthma is a chronic inflammatory disease of the lower airways characterized by episodic breathlessness, wheezing, chest tightness and coughing. Numerous cell types, including eosinophils, T cells, mast cells, basophils, and neutrophils, play a role in triggering airway inflammation [1].

Leukotrienes (LTs) including cysteinyl leukotrienes (CysLTs) and LTB4

are the most potent inflammatory lipid mediators and play a central role in the pathophysiology of asthma and other inflammatory diseases. These biological molecules mediate a plethora of contractile and inflammatory responses through specific interaction with distinct G protein-coupled receptors (GPCRs) [1, 4, 6, 7]

Leukotrienes are derived from the metabolism of membrane phospholipids within alveolar macrophages, eosinophils, mast cells and neutrophils [2, 6].

Phospholipase A2, the cytosolic enzyme, cleaves arachidonic acid from the lipid bilayer, generating free arachidonic acid. The 5-lipoxygenase enzyme acts upon free arachidonic acid, generating leukotriene A4. Leukotriene A4 is then converted to different types of leukotrienes – C4, D4 and E4 – collectively known as the cysteinyl leukotrienes. There are two known receptors to which cysteinyl leukotrienes bind: CysLT-1, which binds to all three cysteinyl leukotrienes and is found on eosinophils, monocytes, airway smooth muscle cells, neutrophils, B cells, plasma cells, and tissue macrophages, and CysLT-2, which has higher affinity for leukotriene C4 and leukotriene D4, and is detected in many tissues of the body, including the cardiovascular system, adrenal glands, the nasal epithelium, and mucus glands [7].

Most of the knowledge of the pathophysiological role of LTs in asthma is currently limited to CysLT1 receptor -mediated effects, whereas the roles of the CysLT2 receptor and other emerging receptors are not well-known [7].

Nowadays inhaled corticosteroids (ICS) are the most effective controllers used in the treatment of asthma. These drugs can effectively suppress the characteristic inflammation in asthmatic airways, because directly reach the airway and intensively inhibit airway inflammation [3, 5, 6].

The molecular mechanisms whereby ICS suppress inflammation in asthma is based on the fundamental mechanisms of gene transcription as they can activate and suppress many genes relevant to understanding their action in asthma [5].

The most striking effect of glucocorticoids is to inhibit the expression of multiple inflammatory genes (cytokines, enzymes, receptors and adhesion molecules). This cannot be due to a direct interaction between glucocorticoid receptors and GRE, as these binding sites are absent from the promoter regions of most inflammatory genes. It is more likely to be due to a direct inhibitory interaction between activated glucocorticoid receptors and activated transcription factors, such as nuclear factor-kappa B and activator protein-1, which regulate the inflammatory gene expression. Glucocorticoid receptors interaction with negative GREs (glucocorticoid response elements) may suppress gene transcription and this may be important in mediating many side effects of corticosteroids [3].

Cysteinyl leukotrienes cause bronchoconstriction, mucus secretion,

increased vascular permeability and eosinophil migration to the airways, and also promote smooth muscle proliferation. Their synthesis and release appear not to be blocked by corticosteroid therapy [6]. At the same time, acting via the type 1 leukotriene (CysLT1) receptor, these proinflammatory mediators have numerous effects in the lungs, including decreased activity of respiratory cilia, increased mucus secretion, increased vaso-permeability, and promotion of eosinophil migration into airway mucosa. Blocking studies show that Cys-LTs are pivotal mediators in the pathophysiology of asthma [7]. Because of abovementioned leukotriene inhibitors are increasingly involved in treatment of asthma for declining the side effects of inhaled corticosteroids. In presented study all patients (who were administered leukotrene inhibitors) were treated with montelukast – one of the leukotriene inhibitors.

Montelukast is a selective CysLT1 receptor antagonist that reduces asthmatic inflammation and airway resistance and prevents bronchoconstriction [6].

Many studies have compared leukotriene inhibitors with other asthma treatments, including treatment with inhaled glucocorticoids. The results of these works are not similar [4, 6].

Elaboration of the results indicated that add-on therapy with CysLT1 receptor antagonists enables a reduction in the dose of inhaled glucocorticoids required to control asthma. According to our opinion, as LT pathway is relatively steroid-resistant [6], the combination of LTRAs and inhaled glucocorticoids led to increase therapeutic efficacy in subgroups of patients whose asthma is LT driven.

As for silent periods between exacerbations by using both treatment methods were similar - asthma was controlled.

Conclusions

The spirometry data were improved in both research groups after 6-month treatment, with prevalence of combined therapy - elaboration of the results showed that involvement of leukotrien inhibitor in the treatment led to decrease the dosage of glucocorticoids and in one case it was completely replaced by montelukast.

In conclusion, addition of leukotrienantagonist to inhaled glucocorticoids maintains control of risk of asthma and enables the dose of inhaled glucocorticoids to be reduced without compromising efficacy. So, our results indicate positive role of montelukast – leukotrien inhibitor in the treatment of such kind of disease.

On the basis of our results and considering current scientific studies in this field it is obvious that the potential effect of CysLT1 receptor antagonists or LT synthesis inhibitors in treatment of mind and severe forms of asthma requires further study.

Limitations

Age and sex distribution was not considered in the presented research.

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High Prevalence of Bronchial Asthma in Patients with Severe Plaque Psoriasis: a Retrospective Dermatological Clinic-Based Study

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B428C0010

Abstract

Psoriasis is a chronic inflammatory systemic disease. Evidence shows an association of psoriasis with Chronic Obstructive Pulmonary Disease, including bronchial asthma (BA). No studies have been conducted in Russian population of Psoriasis patients.

Methods

PsO pts with BA were identify in hospital Database reporting and coding by International Statistical Classification of Disease and Related Health Problems (ICD-10) between 2010 - 2015 years. This study included 889 psoriasis patients.

Results

145 out of 889 pts (16.3%) had BA. In PsA pts BA was found in the same number of cases as in PsO pts ($p<0.05$). BA was found in significantly more cases in old F. pts compare to young F. pts ($p<0.05$). BA was found in significantly more cases in old M. pts compare to young M. pts ($p<0.05$).

Conclusions

BA are common for hospital-treated cohort pts with severe plaque PsO. Old M. pts with severe plaque PsO significantly often suffer from BA compared to Young M. pts. Old F. pts with severe plaque PsO significantly often suffer from BA compared to Young F. pts.

Keywords: psoriasis, bronchial asthma, psoriatic arthritis, comorbidities

Background

Psoriasis is a chronic inflammatory skin disease affecting 2–3% of worldwide population.

Chronic plaque psoriasis, the most common form of psoriasis vulgaris, is characterized by sharply demarcated erythematous papules and plaques with scales and with various distribution, severity and course.

Psoriasis results from interaction between an individual's genetic susceptibility, specific environmental factors, and immune mechanisms.

Today there is increasing evidence to substantiate that psoriasis is not just a disease of the skin but a systemic inflammatory disease. The systemic inflammation in psoriasis generates elevation of C-reactive protein, homocysteine, and inflammatory cytokines such as TNF-a, IL-6, IL-17, IL-20, IL-22, and IL-23, which may contribute to the overall morbidity and mortality in these patients [1].

Numerous studies have evaluated the increased prevalence of comorbid diseases and risk factors in psoriatic patients, including obesity, metabolic syndrome, cardiovascular disease, psoriatic arthritis, autoimmune disease, psychiatric illness, liver disease, smoking, malignancy, chronic obstructive pulmonary disease, sleep apnea, and alcohol abuse. Insight into the overlapping pathogenesis of these comorbidities of psoriasis highlights the importance of immune-mediated mechanisms in these disease states [2, 3].

Comorbidities tend to increase with age.

Moderate to severe psoriasis (>10% of body surface area) is frequently associated with psoriatic arthritis and metabolic diseases, like abdominal obesity, diabetes, non-alcoholic fatty liver disease, dyslipidemia, metabolic syndrome, chronic kidney disease and Chronic Obstructive Pulmonary Disease [4].

Chronic Obstructive Pulmonary Disease (COPD) is a lung disease that includes chronic bronchitis, emphysema and asthma.

Asthma is a chronic disease involving the airways in the lungs. These airways, or bronchial tubes, allow air to come in and out of the lungs. Asthma is a common chronic disease worldwide and affects approximately 26 million persons in the United States. The pathophysiology of asthma is complex and involves airway inflammation, intermittent airflow obstruction, and bronchial hyperresponsiveness. In bronchial asthma, airway inflammation is characterized in most cases by an increased number of activated T-lymphocytes, particularly CD4 + Th2 cells, and sometimes eosinophils and mast cells. The most notable difference of chronic severe asthma compared with mild to moderate asthma is an increased number of neutrophils [5].

Previous studies have reported a positive correlation between psoriasis and chronic obstructive pulmonary disease (COPD); however, no studies have been conducted on Russian population of Psoriasis patients [6, 7].

Psoriasis, as well as other skin diseases, can affect the patient's self-esteem, interfering with all aspects of quality of life.

Psoriasis is a chronic inflammatory skin disease and the patients require long-term systemic basic anti-inflammatory and/or anti-cytokine targeted

therapies. According to modern concepts [2, 3], the choice of treatment for PsA and Ps depends not only on the clinical manifestations and disease activity, but also on the presence of a patient of a comorbid disease.

Comorbidity diseases influence on the PsO and the PsA results of treatment.

This results has practical value. So, at the PsA existence of an atherosclerosis, obesity, fat hepatosis indicates not achievement of remission or the minimum activity of a disease in 1 year of therapy by inhibitors of tumor necrosis factor-α.

It is shown that depression of an index of body weight at sick PsO improves the response to treatment by systemic drugs on PASI on average for 30% [1].

No studies have been conducted for a research of prevalence of comorbidity disease in patients with severe plaque psoriasis. Studying of prevalence of bronchial asthma at patients with a psoriasis is a new and urgent task.

Objectives

to evaluate the prevalence of BA comorbidity in a hospital-based cohort of patients (pts) with severe PsO.

Methods

All potential study subjects have a diagnosis of psoriasis confirmed by a dermatologist. The sources of recruitment are varied and include patients with a range of psoriasis types (primarily chronic plaque psoriasis) and severity.

Patients are mainly recruited from dermatology clinic in Moscow. The severity of skin symptoms was assessed by the Psoriasis Area and Severity Index (PASI).

889 pts (Male (M) -516/Female (F) -329) with moderate-to-severy plaque PsO, mean age 50.4±17,6 years, mean PASI 49.4±0.56, PsO duration 21,5±14,7 years were included. 302 out of 889 pts (33.9%) had PsA and 587 out of 889 pts (66.1%) had PsO alone. PsA pts were older then PsO pts – 55.3±13.7 and 50.4±17.6 ($p<0.001$). PsO pts with BA were identify in hospital Database reporting and coding by International Statistical Classification of Disease and Related Health Problems (ICD-10) between 2010 - 2015 years.

When was create the initial database used spreadsheet software MS Excel 2010. Statistical data processing performed using the software packages Statistica 10.

Statistical significance of the differences of the characteristic values in the two groups were determined using nonparametric Mann-Whitney test, and in 3 and more using the nonparametric criterion Kruskall Wallace.

To understand the relationships between variables was used the Spearman rank correlation coefficient. For comparison, the indicators used non-parametric Wilcoxon test. To describe quantitative and ordinal data were used mean and standard deviation (M ± S). All $p<0.05$ were considered to indicate statistical significance.

Results

We identified 889 patients with severe psoriasis. 145 out of 889 pts PsO (16.3%) had BA. In PsA pts BA coding as J45 was found in the same number of cases as in PsO pts – in 48 out of 302 pts (15.9%)/in 97 out of 587 pts (16.5%) accordingly ($p<0.05$). BA coding as J45 was found in significantly more cases in old F. pts compare to young F. pts - in 48 out of 261 pts (18.4%) and in 15 out of 113 pts (13.3%) accordingly ($p<0.05$). BA was found in significantly more cases in old M. pts compare to young M. pts - in 41 out of 212 pts (19.3%) and in 41 out of 305 pts (13.5%) accordingly ($p<0.05$).

Conclusion

BA are common for hospital-treated cohort pts with severe plaque PsO. Old M. pts with severe plaque PsO significantly often suffer from BA compared to Young M. pts. Old F. pts with severe plaque PsO significantly often suffer from BA compared to Young F. pts. Screening and accurate management of bronchial asthma and other Chronic Obstructive Pulmonary Disease are needed [8, 9]. Dermatologists caring for patients with psoriasis should be aware of this association, consult a general practitioner or pulmonologist, and advise the patients to stop smoking and reduce additional risk factors for asthma [5].

The main factor associated with asthma is smoking, followed by lung inflammation, which is responsible for small airways thickening and alveolar destruction. There is evidence that COPD seems to be a more complex disorder than only airways obstruction. The inflammation appears to be the link between COPD, BA and various other diseases such as metabolic syndrome and psoriasis [6,7,10].

Ungprasert P and Srivali N were conducted a systematic review and meta-analysis of case-control and cross-sectional studies that compared the risk of COPD in patients with psoriasis versus non-psoriasis participants. Autors have been analysed seven studies met our inclusion criteria and were included in the data analysis. The pooled odds ratio of COPD in patients with psoriasis versus control was 1.45 (95% CI, 1.21-1.73). The statistical heterogeneity was high with an I (2) of 91%. Therefore, our study provided evidence to support the increased risk of COPD among patients with psoriasis [9].

The presence of comorbidities has important implications in the approach to patients with psoriasis. The integral approach of psoriasis should include the identification of cardiovascular risk factors and metabolic diseases, the adaption of treatments to the existing comorbidities, as well as the evaluation of existing psychological/psychiatric disorders, in order to achieve a long-term control of the disease and improve the cumulative quality of life. Early and aggressive treatment of severe psoriasis, PsA and associated comorbidities may influence the well-being and probably the longevity of patients [11].

Our findings support the view of the high frequency of comorbid disease in PsO. The high incidence of co-morbidities such as cardiovascular disease, gastrointestinal damage, liver, affects the choice of therapy Ps and PsA and results of treatment with systemic drugs and genetically engineered biological agents.

Comorbid pathology contributes to a more severe course of the underlying disease and as a result the development of functional disorders, deterioration in the quality and life expectancy of patients with Ps and PsA.

Now in dermatologic and rheumatological clinics not enough attention is paid to questions of detection of comorbidities in PsO and PsA. For the decision of this problem will be promoted as development of multidisciplinary approach to maintaining such patients, and development at the national level of references on identification and prevention of comorbidities diseases in PsO and PsA patients.

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Grand-Parental Factors Associated with Grand-Children's Asthma Status in Early Childhood: Lifeways Cross-Generation Cohort Study, Republic of Ireland

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B428C0028

Abstract

Background

The transgenerational effect of risky exposures on childhood asthma is not well known. This analysis examined parental and grandparental factors associated with the index grand-child's developing asthma during their first 9 years of life.

Methods

Participants included 878 children from the Lifeways Cross Generational Cohort Study and their parents and grandparents (maternal grandmother (MGM), maternal grandfather (MGF), paternal grandmother (PGM), and paternal grandfather (PGM)). The outcome of interest was childhood asthma (ever vs. never having asthma) Grandparents' smoking status was the main predictor. Parents' smoking habit and history of asthma, mother's age, the index child's gender and region of residence were considered as secondary predictors. Analyses were performed separately for each child's grandparent, by grouping them in paternal and maternal lineages as well as by grandparental gender. Associations were estimated by using logistic regression.

Results

Amongst the 878 children, 20.5% (n=180) had ever suffered from asthma.

Compared with never smokers and after adjustment for all co-variables, MGM and MGF ever smokers showed a tendency to be inversely associated with

grandchildren with asthma, whilst PGM and PGF smokers showed a positive association tendency; however, none of the associations were statistically significant (MGM: Adjusted Odds Ratio (AOR) (95% CI) = 0.90 (0.54-1.49); MGF: AOR=0.97 (0.47-2.00; PGM: AOR=1.38 (0.67-2.85); PGF: AOR=1.65 (0.68-4.00)). No associations were found when combining each grandparent's smoking habit. Being a male child and both parents having a history of asthma were positively and statistically associated with offspring's asthma in each of the four lineages: MGM lineage (Boys: AOR=1.62 (1.14-2.31), mother's asthma: AOR=2.96 (1.67-5.26), father's asthma: AOR=2.28 (1.02-5.10)); MGF lineage (Boys: AOR=1.64 (1.15-2.33), mother's asthma: AOR=2.95 (1.66-5.24), father's asthma: AOR=2.30 (0.97-5.46)); PGM lineage (Boys: AOR=1.61 (1.13-2.31), mother's asthma: AOR=2.92 (1.63-5.23), father's asthma: AOR=2.46 (1.10-5.49)); PGF lineage (Boys: AOR=1.70 (1.18-2.47), mother's asthma: AOR=2.94 (1.62-5.33), father's asthma: AOR =2.58 (1.13-5.92)).

Conclusion

Children's asthma status was associated with that of their parents, but there was no significant evidence of grandparental impact of smoking on that outcome.

Keywords: Smoking, Asthma, Childhood Asthma, Parental Smoking

Introduction

Asthma has become an increasingly serious global public health concern. High prevalence of asthma, asthma-linked symptoms (e.g. wheeze), and other allergy-related disorders (e.g. Nasal allergy) among both adult and child populations have been reported worldwide [1-3]. Tobacco exposure during the pre-and post-natal period has been found to be an important risk factor for asthma onset and exacerbation in children [4, 5]. There has been limited research into the transgenerational transmission of smoking effects on childhood asthma and other smoking related illnesses (e.g. respiratory diseases).

Some studies have shown that maternal smoking habits can be associated with the offspring's early childhood asthma and that the smoking habit of the grandmother when pregnant with their grandchild's mother can contribute to an increased risk of asthma in the grandchild, suggesting a cross-generational transmission from the maternal line [6]. However the pathways through which risky exposures, especially those before and during the foetal period affect

the health status of future generations are not well understood. Moreover, few studies have examined the transgenerational transmission effect of smoking on the aetiology of childhood asthma across two generations [7].

Understanding the role of both grandmaternal and grandparental smoking on the onset of asthma in the grandchild might contribute to a better understanding of whether there are any potential biological or epigenetic cross generational mechanisms that may link these two public health concern, and therefore, inform health policies and guidelines.

The aim of the present study was to examine parental and grandparental factors associated with the index grand-children's experience of asthma during their first 9 years of life, in particular smoking status.

Methods

Data Source and study population

Participants included 878 live born, singleton children (index-children) from the primary 1092 Lifeways Cross Generational Cohort Study children.

In addition, the index-child's parents and grandparents (maternal grandmother (MGM), maternal grandfather (MGF), paternal grandmother (PGM), and paternal grandfather (PGF) were also included. Detailed information of the Lifeways Cross-Generation Study design, methods, questionnaire and tools, as well as the follow up, has been previously described in detail elsewhere [8, 9].

Briefly, the Lifeways Cross-Generation Study is a prospective longitudinal study established between October 2001 and January 2003 in the Republic of Ireland with an average follow up of 10 years for this analysis. It aimed to study the health status, diet and lifestyles patterns of index-children, their parents and grandparents as well as to examine potential cross-generations health-related links. Information about demographics, health conditions, lifestyles and behaviours, household and neighbourhood-linked socioeconomic factors for mother, father, grandparents and the index-child were collected via self-completion questionnaires and reporting forms, hospital records, and the general practitioners' (GP) health records. In addition, Lifeways participants' dietary patterns were obtained through the application of a validated food frequency questionnaire (FFQ). Measurement of anthropometric parameters and collection of biological samples at ten years of follow up was also obtained for a subsample of participants recruited to the study.

Some of the key factors related to the index-child's asthma status, in particular those related to grandparental smoking status and parents' history of

asthma had unknown values, therefore multivariable multiple imputation (MI) using the Fully Conditional Specification (FCS) approach [10] was performed.

100 datasets were imputed and the resulting pooled dataset was used in the present analysis.

Main outcomes

Childhood asthma was defined as ever or never on 10-year follow up. Ever asthma was any recorded episode or diagnosis of asthma during the first nine years of the child's life, based on the child's medical records, or if the mother reported that her/his offspring had been diagnosed with asthma; otherwise it was considered as never asthma.

Predictors

Grandparents' smoking status was considered as a main predictor. It was treated as ever smoked (reported to be smoker or a regularly past ex-smoker at study baseline) otherwise it was considered never.

Secondary predictors: Index-child sex and place of residence (east versus east region of the Republic of Ireland); mother's smoking status, ever (been a smoker during her life course up to the index-child's delivery) versus never; mother's age at the index-child's delivery, mother's medical history of asthma, mother's educational level (University studies versus up to secondary studies), father's smoking status, ever (reported to be a smoker or a regularly past ex-smoker at study baseline) and father's medical history of asthma.

Statistical analysis

For the pupose of the present study, the smoking status of each index-child's grandparent (MGM; MGF; PGM; PGF) was analysed separately and by combining it by parental lineage (maternal grandparents and paternal grandparents). Univariate and adjusted associations between childhood asthma and the main and secondary predictors were performed on the pool imputed dataset estimated by using logistic regression. All the analyses were performed at 95 CI% level. Stata software (version 13) was used for performing all the analyses.

Results

Overall population description (based on the non-imputed dataset)

For the overall population during the first nine years of life, 20.5% (n-180) of children had ever suffered from asthma compared with 79.5% (n=628) who had never suffered from childhood asthma, 48.41% were males and 66.17% lived in the eastern region of the Republic of Ireland. Regarding the child's parents, mothers' mean age was 30.8 (±5.8%) years old at the index-child's birth, 64.2% had ever smoked across the lifetime, 10.45% had a history of asthma and 51% had third-level education, whilst 45.8% and 11.9% of fathers had ever smoked and had history of asthma respectively.

Concerning grandparents, 45.9% of the MGM, 52.34% of MGF, 45.4% of PGM, and 52.87% of PGF were ever smokers grandparents.

Adjusted Association (based on the pooled imputed dataset) between grandparental smoking status and secondary predictors

Table 1 shows the adjusted associations between maternal grandparents' smoking and mother and father related factors in both MGM and MGF analysed models. Compared with never asthma sufferers, after adjustment for all co-variables, MGM and MGF ever smokers showed a tendency to be inversely associated with grandchildren's asthma; however, none of the associations were statistically significant. Being of male gender and having a mother with a history of asthma were associated (showing similar magnitude) with having suffered with asthma during the first years of life in both MGM and MGF models. Father asthma association with offspring asthma was clearly observed in the MGM smoking model and borderline significance in MGF model. When combining maternal grandparents' smoking status, similar associations as previously described were found.

Table 1. Adjusted association between maternal grandparents' smoking and grandchild ever asthma during the first nine years of life

	MGM Smoking Model	**MGF Smoking Model**	**Maternal grandparents Smoking Model**
	Ever asthma (versus Never)	**Ever asthma (versus Never)**	**Ever asthma (versus Never)**
Exposure (Adjusted model)	**Adjusted OR (Low-Up 95%CI)**	**Adjusted OR (Low-Up 95%CI)**	**Adjusted OR (Low-Up 95%CI)**

MGM ever smoked (vs never)[1]	0.90(0.54-1.49)	---	---
MGF ever smoked (vs never)[2]	---	0.97(0.47-2.00)	
Maternal grandparents(any) ever smoked (vs never)[3]	--	--	1.02(0.57-1.84)
Male-child (vs female-child)	1.62(1.14-2.31)	1.64(1.15-2.33)	1.63(1.15-2.31)
East Region (vs West)	1.18(0.80-1.74)	1.17(0.79-1.74)	1.17(0.79-1.74)
Mother's age(years)	1.01(0.98-1.04)	1.01(0.98-1.05)	1.01(0.98-1.04)
Mother ever smoked (vs never)	0.99(0.67-1.46)	1.01(0.69-1.48)	1.00(0.68-1.47
Mother ever asthma (vs never)	2.96(1.67-5.26)	2.95(1.66-5.24)	2.93(1.66-5.20)
Mother's third-level education (vs up to 2nd-level)	0.89(0.61-1.30)	0.89(0.61-1.31)	0.89(0.61-1.30)
Father ever smoked (vs never)	1.09(0.73-1.64)	1.09(0.72-1.63)	1.08(0.72-1.62)
Father ever asthma (vs never)	2.28(1.02-5.10)	2.30(0.97-5.46)	2.32(1.00-5.41)

Pro > F= 0.001; 2. Pro > F: 0.004; 3. Pro > F= 0.002

Table 2 shows the adjusted associations between paternal grandparents' smoking and mother and father related factors in both PGM and PGF analysed models. PGM and PGF smokers showed a positive tendency to be associated with their grandchildren's asthma status; however it was not statistically significant. Similar to the MGM and MGF smoking models (Table 1), being a male child and having parents with a history of asthma was positively and statistically significantly associated with experienced asthma during the first nine years of life (Table 2). Again, the magnitude of the associations between parents' history of asthma and their offspring's asthma status were similar in the three grandparental models (Table 2).

Table 2. Adjusted association between paternal grandparents' smoking and grandchild ever asthma during the first nine years of life

	PGM Smoking Model	**PGF Smoking Model**	**Paternal grandparents Smoking Model**
	Ever asthma (versus Never)	**Ever asthma (versus Never)**	**Ever asthma (versus Never)**
Exposure (Adjusted model)	**Adjusted OR (Low-Up 95%CI)**	**Adjusted OR (Low-Up 95%CI)**	**Adjusted OR (Low-Up 95%CI)**
PGM ever smoked (vs never)[1]	1.38(0.67-2.85)	---	---
PGF ever smoked (vs never)[2]	---	1.65(0.68-4.00)	

Paternal grandparents(any) ever smoked (vs never)[3]	--	--	1.79(0.81-3.94)
Male-child (vs female-child)	1.61(1.13-2.31)	1.70(1.18-2.47)	1.66(1.16-2.38)
East Region (vs West)	1.12(0.74-1.70)	1.12(0.74-1.69)	1.12(0.74-1.69)
Mother's age(years)	1.01(0.98-1.05)	1.01(0.97-1.04)	1.01(0.98-1.04
Mother ever smoked (vs never)	1.00(0.68-1.48)	0.93(0.62-1.39)	0.96(0.65-1.42)
Mother ever asthma (vs never)	2.92(1.63-5.23)	2.94(1.62-1.39)	2.92(1.62-5.28)
Mother's third-level education (vs up to 2nd-level)	0.93(0.62-1.39)	0.87(0.59-1.28)	0.90(0.61-1.32)
Father ever smoked (vs never)	1.03(0.67-1.58)	1.11(0.72-1.71)	1.06(0.70-1.61)
Father ever asthma (vs never)	2.46(1.10-5.49)	2.58(1.13-5.92)	2.58(1.13-5.88)

Pro > F= 0.002; 2: Pro > F: 0.003 1. 3. Pro > F: 0.001

Discussion

In the present study we aimed to examine the relationship between grandparental smoking status and key mother and father-related factors and the index-child developing asthma during the first years of life. There was no association found between grandparental and parental smoking and the index-child developing asthma. Existing literature has reported that prenatal and postnatal maternal and paternal smoking induces the onset of asthma and asthma related symptoms such as wheeze in their offspring, particularly in early childhood [4, 11]. Regarding grandparental smoking, our results showed no association between the smoking status of the four grandparents and their grandchildren developing asthma when the parental asthma status was taken into account.

From the few studies that have examined the effect of grandparental smoking on asthma in their third generation-members, it has been reported that maternal grandmother's smoking status during the index-children's maternal foetal period is related to a higher asthma risk in their grandchildren [6]. Other studies in the same field, showed no evidence that grandparental smoking from the maternal line influenced their grandchildren developing asthma; however they did find some evidence for the paternal line [7].

It is important to highlight that the relatives' smoking status considered here was ever smoked during the life course up to the first trimester of the

foetal period of the studied children for grandparents and father; and until delivery for the mother's smoking. Although the specific time when the index-child's relatives had started to smoke or for how long they had smoked, was not available, we used ever versus never smoking in order to capture the long-term onset smoking habit as it is hardly likely that grandparents and parents who were not smokers started to smoke later in their life or during their grand-child's foetal period. Similar to other studies [12, 13], we found that the parental history of asthma is strongly associated with their offspring onset of asthma during the first years of life, the maternal associations being higher in magnitude, if compared with the paternal line.

Conclusion

Children's asthma status was associated with that of their parents, but there was no significant evidence of grandparental impact of smoking on that outcome.

Conflict of interest: The authors declare no conflict of interest.
Funding: Health Research Board of Ireland

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Use of Mini Nutritional Assessment Tool for Screening Nutritional Pathological States and Associated Factors in a Chronic Respiratory Disease Hospitalized Cohort

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B428C0057

Abstract

Malnutrition is often related to patients with severe chronic respiratory disease and involves an unfavorable prognosis. Detecting the risk of malnutrition in hospitalized patients with respiratory disease and intervening on it could improve the prognosis and quality of life.

Materials and methods
This study was conducted in June 2016. We evaluated nutritional state and associated factors in 56 patients. Of these, 26 were diagnosed of chronic respiratory disease. We used Mini Nutritional Assessment validated test (MNA) to evaluate nutritional state with 3 possible outcomes (malnutrition, normonutrition, and risk of malnutrition).

Results
Regarding analytical parameters, the average value of vitamin D level was 8 ng/ml. The prevalence of malnourished patients was 38.5%, while 61.5% of patients were at risk of malnutrition. The percentage of patients normally nourished was 0%.

Conclusions
Our respiratory patients are at high risk of malnutrition and require close monitoring and appropriate nutritional assessment and treatment. MNA is an easy and useful tool to evaluated nutritional state.

Keywords: Mini nutritional assessment, malnutrition, chronic respiratory disease

Introduction

Traditionally, weight loss has been associated with advanced stages of different respiratory diseases (chronic obstructive pulmonary disease-COPD, pulmonary fibrosis, lung cancer).

Malnutrition is often related to patients with severe COPD. In addition, "pulmonary cachexia syndrome" has been described as loss of fat-free body mass causing muscle wasting and affects 25-40% of these patients.

It is associated with unfavourable prognosis and accelerated worsening in functional status. [1]

Studies in hospitalized patients between 1995 and 2011 show a prevalence of malnutrition ranging between 16.6% and 47.3% [2]. In the hospitalized elderly, nutritional risk is estimated at close to 36.6% and the estimated prevalence of malnutrition is 20.6%. It is complex to find data on nutritional preventive measures and the most prevalent deficits in cohorts of elderly patients with respiratory disease. [3] A decline in mortality and costs of morbidity treatments has been observed in patients with a good nutritional status. Therefore, it is fundamental to know the nutritional status of our patients in order to improve their prognosis [2]. By defining the prevalence of nutritional disorders in our population, we can estimate their importance in our chronic patients, optimize resources for their intervention and enhance the training of professionals involved in their management [4]. To achieve this, a useful tool is Mini Nutritional Assessment validated test (MNA). This is a simple, quick and non-invasive test recommended for early malnutrition risk detection that consists of 18 items referring to dietary intake, anthropometric measurements, associated comorbidity and lifestyle. MNA correlates highly with clinical assessment and objective indicators of nutritional status (energy intake, vitamin status, albumin level and body mass index) [3] (Tables 1 and 2).

Tables 1 and 2. Mini Nutritional Assessment MNA

First complete screening section. If the result is 11 or less, continue with the assessment to achieve a Malnutrition Indicator Score.

Table 1. Screening

SCREENING	
A.- Has food intake declined over the past 3 months due to loss of appetite, digestive problems, chewing or swallowing difficulties? 0 = severe decrease in food intake 1 = moderate decrease in food intake 2 = no decrease in food intake	D.- Has suffered psychological stress or acute disease in the past 3 months? 0 = yes 2 = no
B.- Weight loss during the last 3 months 0 = weight loss greater than 3kg (6.6lbs) 1 = does not know 2 = weight loss between 1 and 3kg (2.2 and 6.6 lbs) 3 = no weight loss	E.- Neuropsychological problems 0 = severe dementia or depression 1 = mild dementia 2 = no psychological problems
C.- Mobility 0 = bed or chair bound 1 = able to get out of bed/chair but does not go out 2 = goes out	F.- Body Mass Index (BMI) = weight in kg/(height in m)^2 0 = BMI less than 19 1 = BMI 19 to less than 21 2 = BMI 21 to less than 23 3 = BMI 23 or greater
Screening score (subtotal max. 14 points) 12-14 points: Normal nutritional status 8-11 points: At risk of malnutrition 0-7 points: Malnourished	

Table 2. Assessment

ASSESSMENT	
G.- Lives independently (not in nursing home or hospital) 1 = yes 0 = no	M.- How much fluid (water, juice, coffee, tea, milk...) is consumed per day? 0.0 = less than 3 cups 0.5 = 3 to 5 cups 1.0 = more than 5 cups
H.- Takes more than 3 prescription drugs per day 0 = yes 1 = no	N.- Mode of feeding 0 = unable to eat without assistance 1 = self-fed with some difficulty 2 = self-fed without any problem
I.- Pressure sores or skin ulcers 0 = yes 1 = no	O.- Self view of nutritional status 0 = views self as being malnourished 1 = is uncertain of nutritional state 2 = views self as having no nutritional problem
J.- How many full meals does the patient eat daily? 0 = 1 meal 1 = 2 meals 2 = 3 meals	P.- In comparison with other people of the same age, how does the patient consider his/her health status? 0.0 = not as good 0.5 = does not know 1.0 = as good 2.0 = better

K.- Selected consumption markers for protein intake • At least one serving of dairy products (milk, cheese, yoghurt) per day • Two or more servings of legumes or eggs per week • Meat, fish or poultry every day 0.0 = if 0 or 1 yes 0.5 = if 2 yes 1.0 = if 3 yes	Q.- Mid-arm circumference (MAC) in cm 0.0 = MAC less than 21 0.5 = MAC 21 to 22 1.0 = MAC greater than 22
L.- Consumes two or more servings of fruit or vegetables per day? 0 = no 1 = yes	R.- Calf circumference (CC) in cm 0 = CC less than 31 1 = CC 31 or greater
Malnutrition Indicator Score: Normal nutritional status: 24 to 30 points At risk of malnutrition: 17 to 23.5 points Malnourished: Less tan 17 points	

Objective

Learn the nutritional characteristics of a cohort of elderly patients diagnosed with chronic respiratory disease admitted to a long stay unit.

Material and methods

Prevalence study of nutritional disorders and associated factors in an institutionalized cohort of elderly multi-pathological patients diagnosed with chronic respiratory disease.

Included are those patients diagnosed with respiratory failure of at least one year of evolution who have presented at least grade 2 dyspnoeas per MRC (Medical Research Council), FEV1 <65% or oxygen saturation registration less than 90%.

For nutritional assessment, the Mini Nutritional Assessment validated test was used, with three possible outcomes (malnutrition, normonutrition, and risk of malnutrition). Nutritional deficits analytical screening was performed and an associated risk factors questionnaire was used. The latter took into account the following variables: dysphagia, feeding tube, diabetes mellitus, oral nutritional supplements, dementia, source of infection and surgery during current admission.

Data collection was performed by the physicians responsible for all patients admitted to our unit meeting the criteria indicated above.

R statistical processing software in the University of Cadiz version for data processing and free office suite LibreOffice 5.0.1 for drafting and presentation of data were used.

Results

The number of patients with chronic respiratory disease was 26 of a total of 56 patients admitted to the long stay unit. 53.8% of them were men and 46.2% were women. The mean age was set at 79.15 years (minimum 55, maximum 93), and the mean stay at 32.54 days (min. 2 and max. 72).

As to the analytical parameters, the mean level of haemoglobin was 11.0 g/dL (min 8, max 13. SD-standard deviation 2.28); total cholesterol 171.0 mg/dL (min 92, max 315. SD 59.7); triglycerides 163 mg/dL (min 75, max 299. SD 65.5); serum iron 47.50 mcg/dL (min 12, max 96. SD 21.7); albumin 3.80 g/dL (min 3, max 4. SD 0.5); vitamin B12 394.50 pg/mL (min 136, max 637. SD 172.3); folic acid 6 ng/mL (min 3, max 11. SD 4.3) and vitamin D 8 ng/mL (min 4, max 12. SD 7.07).

Of the total study population, 4 patients were in palliative treatment; the rest remained in active treatment and rehabilitation.

Nutritional assessment using the MNA questionnaire revealed 38.5% of patients were malnourished, while 61.5% of patients were at risk of malnutrition.

The percentage of patients normally nourished was 0%.

As associated factors, 53.8% of patients had dysphagia or swallowing disorder; 7% carried a nasogastric feeding tube and 46.2% received treatment with oral nutritional supplements; 30.8% of patients were diabetic and the same percentage exhibited the presence of dementia; 76.9% had an infectious focus at the time of the study; and 23.1% had undergone surgery during the current admission.

Conclusions

- In our setting, the use of MNA is a feasible and fast tool to detect nutritional disorders.

- All patients had vitamin D deficiency. This finding should be monitored in respiratory patients to provide proper supplementation, especially if they receive medication associated with calcium metabolism disorders such as corticosteroids.

- In our cohort of respiratory patients, the prevalence of malnourished patients is 38.5%. In a hospital setting any number of patients suffering malnutrition is unacceptable; therefore, we must understand the causes of it, since it can be attributed to advanced disease stage and patient age.

- The risk of malnutrition is 61.5% and the percentage of normally nourished patients is 0%. Again, this could be explained by the degree of complexity of patients admitted to a long stay unit.

- Still, we must accept that our respiratory patients are at high risk of malnutrition and require close monitoring and appropriate nutritional assessment and treatment.
- MNA test should be integrated in our daily work in order to detect possible nutritional disorders that may adversely affect the prognosis of patients with COPD.
- While the limited number of patients studied does not allow the results to be extrapolated to the population level, it is a wakeup call concerning the aspects studied.

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The Prognosis for the Development of Chronic Lung Disease in Very Immature Infants

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B428C0036

Abstract

The present paper focuses on the analysis of the predictive value of perinatal risk factors and genetic factors particularly the major histocompatibility complex in diagnostics of the development of chronic lung disease (CLD) - bronchopulmonary dysplasia (BPD) in very immature infants. Based on the Wald diagnostic test, maternal and other perinatal risk factors, clinical and laboratory findings, X-ray and immunologic characteristics were used to construct a diagnostic table of prognostic risk factors for developing CLD.

Keywords: chronic pulmonary disease, bronchopulmonary dysplasia, very immature infants, A. Wald diagnostic test, prognostic risk factors

Introduction

In recent years, there has been an increase in the incidence of preterm births with a tendency towards decreasing gestational age at birth all over the world including Russia [1-3]. Bronchopulmonary dysplasia (BPD) is a common chronic lung disease (CLD) of prematurity occurring in newborns of less than 32 weeks gestation [4-6]. The development of BPD is associated with adverse long-term outcomes regarding both the functions of respiratory organs, visual organs and neurologic development as well as increasing mortality rate in the pediatric group under discussion [7]. To predict possible negative sequels and to identify incipient problems is the ultimate goal of providing neonatal and outpatient care for premature infants at risk of BPD [8-10].

Optimization of instituting adequate ventilation, prenatal prevention of

respiratory distress-syndrome, postnatal surfactant prevention and therapy as well as a decrease in oxygen exposure contributed to the reduction of the development of "old" BPD in most cases. However, this did not result in a significant reduction in "new" BPD incidence [11]. Insufficient awareness by pediatricians of CLD, late diagnostics and as a result, inadequate management of infants with BPD give both medical and social importance to the issue [11].

Most researchers consider BPD to be a multiorgan and multifactor disease with genetic impact [11-14]. In view of this, considering risk factors, immunologic aspects of the development of BPD in order to predict this pathology occurrence is of great theoretical and practical importance [12, 15, 16, 17].

Purpose

to evaluate the prognostic value of perinatal and genetic risk factors particularly the major histocompatibility complex in diagnostics of the development of CLD in very immature infants.

Patients and methods

The study group comprised infants born below 32 weeks gestation with CLD (n=111), the comparison group – infants without BPD (n=97). The study appears to be retro – and perspective one.

The inclusion criteria in the study group consisted of: gestation less than 32 weeks, age more than 1 month, BPD, parental consent. *The exclusion criteria* – gestation over 32 weeks, age less than 1 month, oxygen independence at 28 days of age, absence of clinical signs of BPD, parental disagreement. Diagnoses of BPD and its forms was established in accordance with the 2008 working classification of the main clinical forms of bronchopulmonary diseases in children [18]. Clinical criteria applied consisted of: respiratory therapy, oxygen dependence at 28 days of age and over to support blood oxygenation level – $SaO_2 \geq 90\%$, respiratory insufficiency (RI), bronchoobstructive syndrome, on X-ray: interstitial edema alternating with areas of increased transparency of lung tissue, ribbon-like seals, fibrosis [18]. Diagnosis of the classical BPD form in prematurely born infants was established with the presence of RDS history, hard artificial ventilation instituted within more than 3 days, chest X-ray findings of lung hyperinflation, fibrosis and bulls. Diagnosis of the new BPD form in prematurity was made with the absence of hard artificial ventilation parameters, the administration of surfactant preparations, and X-rays showing homogeneous opacity of the lung tissue ("nebula") without its swelling with small or large seals, areas of increased transparency. *Evaluation of BPD severity* was made in terms of anamnesis, clinical and X-ray criteria of the disease severity taking into account oxygen dependence at 36 weeks post-menstrual age or during hospital discharge (if it was earlier) [18, 19].

We applied conventional investigation methods, besides we studied the distribution of Class I HLA-region genes (HLA - A, B loci) and Class II (DR loci) of the study group infants and their mothers using the Terasaki compliment-dependent microlymphocytotoxic test and the PCR method with sequence-specific primers (PSR-SSR, «Protrans», Germany). The blood samples of healthy subjects (118 samples of HLA antigens) living in the Republic of Bashkortostan were used as controls.

Statistical processing of data obtained was performed with the Windows 7 program by using the "STATISTICA 6,0" (StatSoft) licensed program with the help of parametric and non-parametric statistics depending on the nature of the distribution value (the Kolmogorov-Smirnov criterion with constructing a histogramm). For determining diagnostic value parameters of BPD risks we used the non-parametric procedure by A. Wald. The thresholds were determined. Their crossing by summing up diagnostic coefficients (DC) allowed to make a prognosis for the development of BPD in the premature infant with respiratory pathology. The value of the threshold sum of the DCs was (+13) and (–13). The sign appeared to be informative with DC≥2,0 and J≥0,25. The standard error was no more than 5% ($p<0,05$).

Results

Comparative analysis of perinatal anamnesis of the study patients showed that the development of severe respiratory disorders in premature infants with the consequent development of BPD occurred under the impact of diverse adverse maternal factors (Table 1). In our studies, the absence of antenatal steroid prophylaxis was the most significant risk factor for the development of CLD (Table 1).

Table 1. Maternal risk factors for the development of BPD in very immature infants

	Statistical index
	RR [95% CI]
CLD	1,7 [1,2–2,0]*
Acute respiratory tract infection (ARTI) during pregnancy	1,9 [1,5–2,1]*
Cytomegalovirus (CMV)	1,6 [1,1–1,8]*
Chronic fetoplacental insufficiency (CFPI)	1,4 [1,1–1,9]*
Hydramnios	1,7 [1,3–2,0]*
Threatened abortion	1,6 [1,2–2,0]*

Aggravated obstetric history (AOH)	1,8 [1,3–2,1]*
Colpitis	1,4 [1,1–1,8]*
Absence of antenatal steroid prophylaxis	2,6 [1,1–9,9]*

Note. RR – relative risk; CI – confidential interval
** – RR – statistical significant*

Susceptibility to a number of diseases is genetically determined and is often related to the HLA-complex [20]. It is established that mothers of infants with BPD have an increased incidence of HLA-specificities of A10 (21,1 versus 8,5% in controls, RR=2,5; p=0,035) and A28 (15,2 versus 2,5%, respectively, RR =6,2; p=0,002). These genes of HLA-region A locus appear to be additional maternal risk factors of the development of BPD in their babies. The positive moderate relationship between certain alleles of the major histocompatibility complex of the mother and the severity of BPD course (HLA A28, r_s=+0,37; B40, r_s=+0,47, p<0,05) has been established.

Neonatal risk factors of the development of BPD. In the study group, 93,9% of premature infants' Apgar score was significantly lower at birth: mean 3 [2; 4] and 4 [3; 5], respectively; (p=0,003) and in the dynamics at 5 min after birth: mean 5 [4; 6] and 6 [5; 7], respectively (p=0,001). In the study group, ventilation was provided for 92% of infants (193 patients) and in the comparison group - for only 56,7% (55 patients) (χ^2=33,1; p= 0,0005). Respiratory support for premature infants is presented in Table 2. The mean duration of mechanical ventilation administration to the study patients significantly exceeded that of controls. Among infants with BPD, those with the need for 10-20 day ventilation (41,4%) and over 20 days (20,7%) predominated, while in the comparison group - 82,5% of premature infants were ventilated up to 6 days (p<0,05).

Only 13 study patients (12,6%) required repeated intubation. The duration of the oxygen administration to BPD infants exceeded 30 days and was as much as 3,5 times longer than in the comparison group (Table 2).

Table 2. Respiratory support to infants with the development of BPD and without the disease, Me [25; 75]

Signs	Groups		P-value
	Study	Comparison	
Mechanical ventilation (MV), days	12 [7; 19]	1 [0; 5]	<0,001
Oxygen tent, days	0 [0; 4]	0 [0; 0]	<0,001
NCPAP, days	3 [0; 7]	0 [0; 2]	<0,001

Mask, days	15 [10; 20]	4 [1; 5]	<0,001
Oxygen dependence, days	35 [30; 42]	7 [5; 11]	<0,001

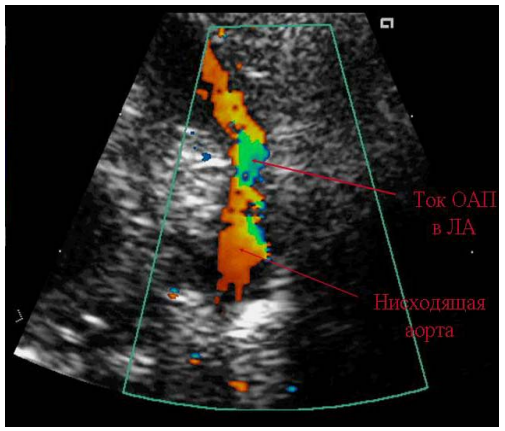
Note. Statistical significance of differences between groups was assessed by the Mann-Whitney U Test

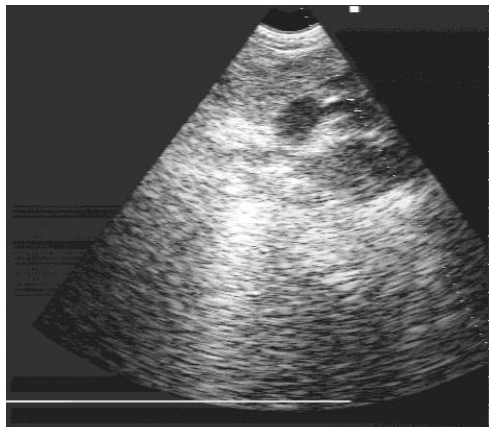
The incidence of endotracheal prophylactic administration of Curosurf - a natural surfactanct - to both group patients did not differ and was 61,2% (79 infants) in the study group and 55,6% (50 infants) in the comparison group (χ^2=0,3; p=0,47). A number of recent studies have documented the absence of advantages of the prophylactic surfactant therapy for extremely preterm newborns (with ELBW) provided that the full course of antenatal steroids for respiratory distress syndrome (RDS) prophylaxis and the use of NCPAP as a starting point of respiratory therapy are available. However, the authors of the 2013 European consensus guidelines on the management of infants with RDS concentrate on the fact that these outcomes cannot be extrapolated on the whole population of extremely preterm infants without taking into account the specific conditions of healthcare institutions [21]. In our studies, the development of classical BPD in preterm infants inversely correlated with the administration of Curosurf (rs=-0,378; p=0,0001).

The study group infants had marked health disorders: intoxication (63,3 vs 31,0%; χ^2=18,3; p<0,001), thermolability (50,5 vs 31,0%; χ^2=8,2; p=0,004), acrocyanosis (55,8 vs 29,9%; χ^2=14,4; p=0,001), edematous, hemorrhagic syndromes (respectively, 22,5 vs 4,1%; χ^2=19,8; p<0,001; 13,5 vs 5,2%; χ^2=4,38; p<0,04), respiratory disorders with bronchoobstructive syndrome (59,8% vs 16,2%; χ^2=71,7; p<0,001). These hospitalized infants had a 4-fold increase in apnea occurrence (40,6 vs 10,3%; χ^2=25,12; p<0,001). The majority of infants with BPD had Type II and Type III respiratory insufficiency (RI).

Infants with cyanosis of the skin during more than a month were at high risk for developing BPD: RR=3,0 [2, 4 – 3, 6].

Somatic pathology of prematurity in the study group was presented by diagnoses of intrauterine infection (27,9 vs 12,4%, χ^2=6,72; p=0,01), neonatal pneumonia (78,2 vs 48,4%, χ^2=17,7; p<0,001), sepsis (22,5 vs 10,3%, χ^2=4,68; p=0,031) and functioning patent ductus arteriosus (PDA) (18,4 vs 5,5%, χ^2=4,68; p=0,031) (Fig.), type 2-3 retinopathy (10,9 vs 1,1%; χ^2=4,04; p=0,045), hypotrophy (40,0 vs 13,8%; χ^2=22,2; p<0,001). Severe intraventricular hemorrhage (IVH) more than 80% (p<0,05) prevailed in the study group.





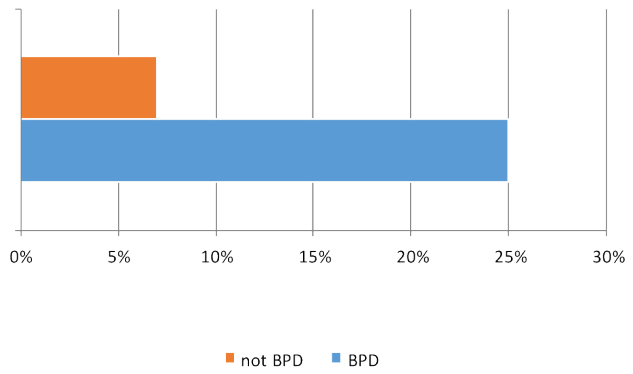


Fig. Functioning PDA in very immature infants*
*Note – * – significance of differences – p< 0,05*

Based on the statistical analysis, it has been shown that a great number of diverse neonatal factors have a significant impact on the development of BPD (Table 3).

Table 3. Neonatal risk factors for developing BPD in very immature infants

The study factor	**Statistic index**
	RR [95% CI]
Sepsis	1,4 [1,1-1,8]*
Intrauterine infection	1,5 [1,1-1,8]*
Neonatal pneumonia	2,1 [1,4-3,1]*
Congenital pneumonia	1,3 [0,9-1,7]
Type III IVH	1,8 [1,1-2,8]*
Functioning PDA	1,6 [1,1-1,9]*
Severe hypoxia at birth (Apgar 0 – 3б)	1,4 [1,1-1,9]*
Total cynosis of the skin	3,0 [2,4-3,6]*
Type II-III RI	2,2 [1,8-2,6]*
MV more than 6 days	2,6 [2,0-3,4]*
The infant's sex (male)	1,7 [1,3-2,3]*
Postnatal hypotrophy	2,0 [1,5-2,4]*

Most perinatal risk factors for BPD had been previously determined [9, 11, 15, 22, 23] and were confirmed by our studies on small premature infants. One of the most pressing issues of modern clinical medicine is identifying disease markers, that is, the most common signs for certain nosological forms. This fact is of utmost importance for determining the role of genetic mechanisms in the pathogenesis, individual prognosis for the development, course and outcomes of BPD [24].

The frequency of occurrence of a certain antigen in CLD patients was studied comparing the frequency of the carrier of the same antigen in "the healthy" group of the particular population. In the group of premature infants with BPD, there was a significant increase in the frequency of occurrence of HLA-specificities of A28 (allele frequency - 0,0678 vs 0,0127 in controls, OR–5,65; p<0,05), B22 (allele frequency - 0,0508 vs 0,0169 in controls, OR–3,1; p<0,05). Minimal and significant among OR values were B18 (allele frequency - 0,0169 vs 0,0762 in controls, OR–0,209; p=0,02), B16 (allele frequency - 0,0694 vs 0,0805 in controls, OR–0,197; p=0,02), DR11 (allele frequency - 0,0423 vs 0,1179 in controls; OR–0,33; p=0,02). Despite the fact that OR index for B21, DR9 and DR14 was more than 2, the detailed statistical processing with determining χ^2 and two-tailed Fisher's test showed the differences to be insignificant.

A marked correlation between ventilation duration and the presence of HLA A2 antigen in the infant (r_s=+0,722, p=0,043), need for high-frequency ventilation of the lungs and the given antigen (r_s=+0,722, p=0,04), as well as with HLA B27 (r_s=+0,8, p= 0,017) and negative relationship between A1 antigen r_{s+}-0,723, p=0,04) was established. The duration of repeated ventilation correlated with HLADR17 (+0,716, p=0,0001) in the premature infant. The correlation between the development of the classical BPD form and the presence of B15 antigen in the premature infant was determined (r_s=+0,388, p=0,003).

The major histocompatibility complex is the most polymorphous genetic structure in the genome. The fact of BPD association with certain HLA-antigens may be considered for forming risk groups with an early beginning of preventive measures to reduce the pathology severity and improve the diseases outcomes as well as for the probabilistic prediction for the disease severity.

Genes of DR loci of Class II HLA-region have limited expression. They are presented only on B-lymphocytes, macrophages, activated T-lymphocytes and help immunocompetent cells interact in the development of an immune response. Since HLA-DR genes present immune response genes, the data obtained should be taken into account while taking both preventive and therapeutic measures for premature infants with BPD. However, one should keep in mind that the population studied was not homogenous. Currently, there is no evidence of absolute association suggesting that every infant with an HLA-antigen related to the disease in his phenotype is potentially sick.

The data obtained in the study of maternal and other perinatal risk factors, clinical and laboratory findings and immunogenetic characteristics have been used to construct the diagnostic table of prognostic factors according to A. Wald. Based on the Wald's test, DC of current factors are summed up while studying the patient. If the score is more than 13, the probability of the patient to be in the risk group for CLD is 95%, if it is more than 17 - 99%. If the score is 13 and less the probability of the patient to be in the patient group without CLD is 95%, if it is 17 and less - 99%.

According to Table 4, among maternal risk factors the highest DC is typical of acute respiratory infections during pregnancy and hydramnion.

Table 4. Diagnostic table for predicting BPD in very immature infants

Signs	Possible options	DC	J
1	2	3	4
Maternal risk factors			
Inflammatory genital diseases	yes	+4	0,27
Family history: spontaneous abortion	yes	+7	0,52
Hydramnion	yes	**+6**	**0,58**
ARTI in the 1-st trimester	yes	**+8**	**0,62**
ARTI in the 2nd-3ed trimester	yes	**+9**	**0,38**
Neonatal findings			
Ventilation duration for more than 6 days	yes	+2	0,86
Ventilation duration for more than 10 days	yes	**+16**	**3,24**
Pneumothorax	yes	**+11**	**0,69**
The infant's Apgar score of 2 and less at 1 min after birth	yes	+4	0,3
The infant's Apgar score of 5 and more at 1 min after birth	*yes*	*-4*	*0,28*
RI 1 ст. (based on SaO_2)	yes	-8	2,51
RI 2 ст. (based on SaO_2)	yes	**+7**	**1,51**
Bronchoobstructive syndrome	no	-4	1,15
	yes	**+9**	**2,31**
Cyanosis	no	-4	1,23
	yes	**+9**	**2,59**
Apnea	no	-2	0,39
	yes	+6	0,74
Edema syndrome	yes	**+8**	**0,83**
Male	yes	+2	0,28
Female	yes	-3	0,34
Pneumonia	no	-4	0,66
Functioning PDA	yes	**+13**	**1,21**
Postnatal hypotrophy	no	-3	0,48
	yes	+5	0,65
Type III IVH	yes	**+11**	**0,7**

Note: DC – diagnostic coefficient ≥ 2, J – criterion of the sign's information value ≥ 0,25

The high information value of DC is typical of such indices as ventilation for more than 10 days, bronchoobstructive, edema syndrome predominant in the clinical picture complicated by pneumothorax, cyanosis of the skin.

As for comorbid conditions, the highest prognostic risk factor for BPD is determined for functioning PDA and severe IVH. Among X-ray findings, the identification of interstitial changes, uneven pneumatization of the lungs and detection of bulls and cysts are of importance in predicting the development of BPD (Table 5). Among laboratory criteria, the most informative signs of risks for BPD were anemia, thrombocyropenia, leukopenia and increased IgA.

This is common in intrauterine antigen stimulation.

Although the risk for the development of BPD in premature infants largely depends on predisposing factors, it should be noted that this pathology develops not in all preterm infants at risk. Predisposition to chronic lung disease is determined by the infant's HLA-phenotype. It has been shown that infants with HLA A28, B22 are at the highest risk for the development of BDP, and infants with B16, B18 and DR11 antigens are at the lowest risk.

Table 5. Diagnostic table of predicting BPD in preterm infants based on X-ray, laboratory data and HLA-phenotype

Signs	Possible options	DC	J
1	2	3	4
X-ray criteria			

Hyperinflation signs	yes	+3	0,66
	no	-5	0,92
Interstitial changes	no	-9	3,12
	yes	**+8**	**2,83**
Increased transparency of lung tissue	yes	**+7**	**1,09**
	no	-1	0,31
The unevenness of pneumatization	yes	**+7**	**1,49**
	no	-3	0,7
Bulls, cysts	yes	**+10**	**0,6**
Laboratory criteria			
Thrombocytopenia (thrombocytes $50*10^9$)	yes	**+8**	**0,94**
Leukopenia less (leucocytes less than $5*10^9$)	yes	**+5**	**0,45**
Anemia at birth less (Hb less than 100 g/l)	yes	**+4**	**1,02**
Hypoproteinemia at birth (total protein less than 35 g/l)	yes	+3	0,86
Azotemia (urea more than 8 mmol/l)	yes	+4	0,38
Hyperimmunoglobullinemia IgA (more than 0,13 g/l)	yes	**+8**	**0,26**
HLA - specificity			
A1	yes	**-5**	**0,52**
A2	yes	**-6**	**1,9**
A3	yes	**-4**	**0,35**
A11	yes	**-7**	**0,59**
A19	yes	**-6**	**0,48**
A28	yes	**+3**	**0,26**
B5	yes	**-6**	**0,53**
B8	yes	**-4**	**0,27**
B13	yes	**-5**	**0,31**
B16	yes	**-10**	**0,92**
B18	yes	**-10**	**0,85**
B22	yes	**+5**	**0,25**
DR1	yes	**-5**	**0,56**
DR4	yes	**-5**	**0,43**
DR11	yes	**-8**	**0,95**
DR15	yes	**-6**	**0,65**

Note. DC – diagnostic coefficient ≥ 2; J – criterion of the sign's information value ≥ 0,25

Thus based on the present study, we could identify the most important perinatal and immunogenetic risk factors for the development of BPD in small premature infants. Results obtained confirm the current concept that the given pathology is of multifactor and polygenic nature.

The diagnostic tables of predictors of the development of CLD in preterm infants including both clinical and history data, laboratory and X-ray findings as well as HLA-typing results have been constructed for general practitioners.

Hopefully, these tables will be suitable for early identification of patients at high risk of BPD and timely carrying out preventive measures against severe complications.

Conclusions

1. Maternal risk factors for the development of BPD in premature infants include chronic bronchopulmonary diseases, abortion history, threatened abortions and acute respiratory infections during the current pregnancy, inflammatory genital diseases. Significant perinatal risk factors for the development of CLD in prematurely born infants include: nonmodified endogenic factors – genetic predisposition, male gender; modified endogenic factors – chronic fetoplacental insufficiency, hydramnion, low Apgar's score at birth, functioning PDA or/and exogenic factors – the absence of antenatal hormonal and postnatal surfactant prophylaxis of RDS, ventilation for more than 6 days, congenital and postnatal infections, severe IVH, postnatal hypotrophy.

2. The system of human leucocyte antigens is of great importance in the development of bronchopulmonary dysplasia. The presence of HLA A28, B22 in the infant, and HLA A28, A10 in the mother is a genetic marker for bronchopulmonary dysplasia. The presence of HLA B16, B18, DRB1*11 in the infant is a resistant factor for its development. The relationship between HLA B15 antigens in the infant and the development of typical bronchopulmonary dysplasia has been established. The relationship between HLA A28 and B40 in the mother and severity of disease has also been established.

3. Complex evaluation of perinatal and immunogenetic factors in premature infants can be used to predict chronic pulmonary diseases and to confirm indications as well as to take preventive and early therapeutic measures.

4. The proposed modified Wald table is helpful for predicting the development of BPD in the presence of perinatal risk factors and/or identification of HLA A28 and B22 alleles, B15 in the infant and A28, A10 and B40 in the mother.

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Evaluation of the Global Susceptibility Trends of *Pseudomona Aeruginosa* in Patients with Acute Exacerbations of Chronic Obstructive Pulmonary Disease

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B428C0072

Summary

Pseudomona aeruginosa is recognized as a relevant pathogen in respiratory infections and Chronic Pulmonary Obstructive Disease (COPD) patients, and has become an important cause of gram-negative infection. It is the most common pathogen isolated from patients who have been hospitalized longer than one week, and it is a frequent cause of nosocomial infections.

Objectives

To analyse susceptibility of *Pseudomona aeruginosa* isolated obtained from respiratory samples to antibacterial agents (aminoglicosides, carbapenems, betalactams and quinolones) for treatment of infected COPD patients, and to determine the epidemiological characteristics of the isolates involved.

Material and Method

A total of 100 Gram-negative isolates from COPD patients diagnosed of respiratory infections were included for 8 years. The samples were collected from Primary Care and hospitalized patients (sputum, bronchial aspirates, bronchoalveolar lavage and blood cultures). Only one isolate per species per patient was accepted. Isolates were identified to the species. Susceptibility to antibacterial agents: aminoglicosides (Amikacin), carbapenems (Imipemen), and betalactams (Cefepime, Ceftazidime, Piperacilin-Tazobactam) and

quinolones (Ciprofloxacin and Levofloxacin) was determined by broth microdilution (Walk-Away Systems, Beckman) and breakpoints following the method recommendations of the Clinical and Laboratory Standars Institute (CLSI) and EUCAST guidelines.

Results

Amikacin and levofloxacin again showed a significant increase in susceptibility (from 87.4 to 92.7% and from 70.7 to 76.4%, respectively, $p<0.05$); The increase in susceptibility of ciprofloxacin approached statistical significance (71.2% to 76.0%, $p=0.057$).

Conclusions

Susceptibility of *P. aeruginosa* to most agents remained unchanged.

Globally, quinolones showed significant differences, demonstrating increasing susceptibility. The susceptibility of Piperacillin-tazobactam also increased (but was not significant). The increase shown in antibacterial susceptibility to ciprofloxacin and especially for levofloxacin, strength the role of quinolones in the treatment of respiratory infections caused by *P. aeruginosa*. Amikacin continues to be an effective alternative.

Keywords: Antimicrobial Susceptibility, Pseudomona aeruginosa, respiratory infection, Chronic Obstructive Pulmonary Disease

Introduction

Pseudomona aeruginosa is beginning to be recognized as a relevant pathogen in Chronic Obstrutive Pulmonary Disease (COPD), associated with an intense airway inflammation, damage and poor prognosis for those with the disease. The prevalence of *P. aeruginosa* infection in acute exacerbations of COPD is estimated to be 4% but increases to as much as 13% among patients with advanced airway obstruction. P. aeruginosa has been somewhat refractory to antimicrobial therapy, with almost no drugs inhibiting > 90% of isolates.

This bacteria has become an important cause of gram-negative infection, especially in patients with compromised host defense mechanisms. It is the most common pathogen isolated from patients who have been hospitalized longer than 1 week, and it is a frequent cause of respiratory infections.

Objectives

To analyze the change of susceptibility of *P. aeruginosa* isolates against to four antimicrobial agents from patient attended in our Health Area to implement protocols for preventing and treating of patients diagnosed of respiratory tract infection.

Material and methods

For this study, a total of 100 Gram-negative isolates from COPD patients diagnosed of Respiratory Infections (RI) were included for 8 years.

The samples were collected from a Health Area (Primary Care and Hospital).

The origins of the samples were: sputum, bronchial aspirates, bronchial lavages and blood cultures. Only one isolate per species per patient was accepted into the study. They were cultivated following standard procedures from January 2008 to December 2015.

Isolates were identified to the species. Susceptibility (MICs) was determined using the CLSI broth microdilution method (Walk-Away Systems, Beckman) and the breakpoints were considered according to the Clinical Laboratory Standard Institute guideless (CLSI) and EUCAST guidelines. The antimicrobial agents analyzed were: aminoglicosides (Amikacin), carbapenems (Imipemen), betalactams (Cefepime, Ceftazidime, Piperacilin-Tazobactam) and quinolones (Ciprofloxacin and Levofloxacin).

Results

The main changes observed in our study are represented in the Figure 1: Increases in susceptibility were larger and statistically significant for all studied agents except piperacillin-tazobactam. Amikacin and levofloxacin showed a significant increase in susceptibility (from 87.4 to 92.7% and from 70.7 to 76.4%, respectively, $p<0.05$). The increase in the susceptibility of ciprofloxacin approached statistical significance (71.2% to 76.0%, $p=0.057$).

The increases for the other agents that showed significant trends in the overall analysis were not statistically significant in the sensitivity analysis.

The figure 2 represents the main changes happened in susceptibility for 4 antibacterial agents.

Figure 1. Evolution susceptibility trends for P. aeruginosa 2008-2015

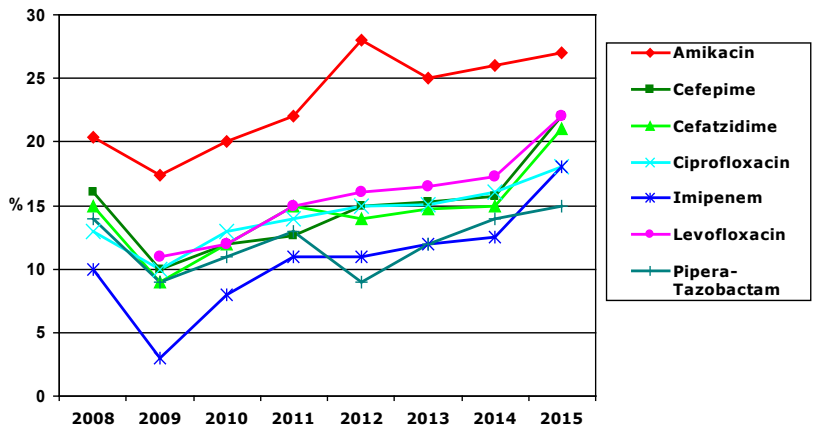
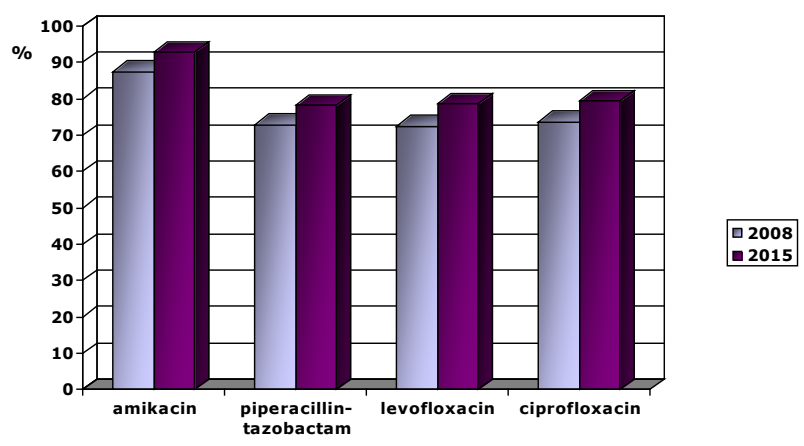


Figure 2. P.aeruginosa: increasing trend in susceptibility (2008 to 2015)



Conclusions

Our results are similar others european studies in which ciprofloxacin and levofloxacin showed a statistically significant increasing trend in susceptibility ($p<0.05$) and piperacillin-tazobactam, but the trend was not statistically significant ($p=0.096$). The increasing susceptibility rates reported here may support those published findings, although other undetermined factors probably also contributed to these trends.

Globally, quinolones showed significant differences, demonstrating increasing susceptibility. The susceptibility of piperacillin-tazobactam also increased (but was not significant). The increase shown in antibacterial susceptibility to ciprofloxacin and especially for levofloxacin, strength the role of quinolones in the treatment of respiratory infections caused by *Pseudomona aeruginosa*. Amikacin continues to be an effective alternative.

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Hormonal Relationship in the Ontogenesis of the First Three Years of Life at Recurrent Respiratory Diseases

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B428C0061

Frequent respiratory diseases – a marker of violation of adaptation of the child to factors of external and internal environment which is intimately bound to features of ontogenetic development and genetic determinancy. [1, 6].

All range of the state of health, disease, and also state between health and a disease is bound to system of adaptation reactions. According to the theory of the common adaptation syndrome of Hans Selye, [6], reaction to a stress proceeds stagely and is characterized by a particular complex of changes in neuroendocrinal system, affecting the level of nonspecific resistance of an organism, its anti-inflammatory potential and metabolism [4, 5]. H. Selye, having formulated the provision on the common adaptation syndrome, suggested to allocate three stages: alarms, resistance and exhaustions.

Developing these representations, it was offered in a resistance stage on character of endocrine indexes to allocate catabolic and anabolic phases (in parallel with the inductive and productive phase of the immune answer), phases of increase in glucocorticoids and STH respectively. Inadequate dominance of catabolic or anabolic agents in a hormonal profile (disharmonious reaction) defines manifestations of violation of adaptation and respectively the nature of pathological process as private manifestation of a condition of a disadaptation.

Not numerous literary data allow to assume that dysfunction of immune system with change of a cytokine profile can be the cornerstone of dishormonal violations. This circumstance is urgent for consideration of formation of frequent respiratory incidence at children of an early age when formation of adaptation potential depends on degree of a maturity of nervous and endocrine systems. [1,4.] The purpose of the real research was studying features of a hormonal regulation at children of the first three years of life which often have respiratory diseases.

Keywords: Hormonal relations, often ill children, sporadically ill children, immunity, cortisol, somatotropic hormone,insulin

Materials and research techniques

85 often ill children (OIC) and 68 sporadic the ill children (SIC) aged up to three years who were divided into three age groups are examined: 6 months-12 months., 12 months-24 months., 24-36 months. Fig 1.

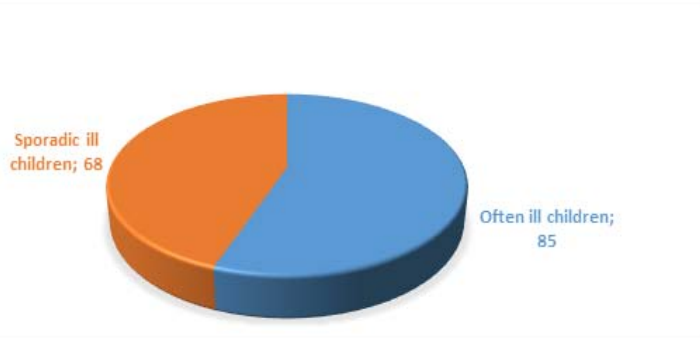

Fig. 1. Distribution of examinees by groups

Determination of content of insulin (I) of hormone of body height (STH) and Cortisol (Co) to blood of children was carried out by method of an enzyme immunoassay with use of reference sets. Assessment of a significance of exogenetic and internal causes on the frequency of incidence of children was carried out by questioning of parents.

Statistical processing of results

In work the studied sizes were presented in the form of selective mean value and a standard error of average size. Reliability of distinctions of average sizes of independent selections was estimated by means of a parametrical Student criterion at the normal distribution law and nonparametric criterion of Mann-Whitney at difference of an index from normal ($p< 0,05$). For the characteristic of interrelation between indexes used correlation analysis. Binding force was estimated according to Cheddok's table. All statistical procedures were carried out with use of a package of the Statistica 6,0 application programs (StatSoft, USA).

Results of researches

At assessment of a significance of the factors influencing formation of frequent respiratory incidence it was established that from psychosocial

factors in both groups on incidence frequency the greatest impact is exerted by material living conditions, but with a larger significance at OIC (30% and 19% respectively, $p<0,05$), then social diseases in a family (21% and 18%) (tab. 1).

Table 1. The maintenance of STG, insulin and hydrocortisone at OIC and SIC of the first three years of life.

Age of children	OIC	SIC	OIC	SIC	OIC	SIC	
	Insulin micromole unit/ ml.		somatotropic hormone nmole/l		cortisol nmole/l		
1-6 months		17,8±2,3	18.2±3,1*	9,4±0,32	5,1±0,21*	820±71,2	400±32,3*
6-12 months		21,8±2,24	16,8±1,26*	8,7±0,4	3,6±0,22*	816±31,8	500±42,1*
12-36 months		5,8±0,12	4.2±0,31	7,8±0,22	5,1±0,31	640±78,8	800±55,2*

*Note: *changes, reliable in comparison with SIC are designated*

To a lesser extent, the inexact family composition (14% and 11%), attention degree to the child (11% and 9%) affect. Influence of medicobiological factors on the frequency of incidence of children of an early age also differs. At OIC the frequency of occurrence of perinatal lesion of a CNS (55% and 37%, $p<0,01$), the adverse course of pregnancy (32% and 24%, $p<0,05$), pathology of childbirth (19% and 15%) in comparison with SIC is highest. Frequency of such factors as nonrational feeding, low weight at the birth is less often noted in both groups, a prematurity. Results of a research of the hormonal status of children showed that at the age of the first half of the year of life children of both groups had almost identical whereas the maintenance of STH and the Hydrocortisone at OIC considerably exceeded that at SIC content of insulin. Data are presented in the table No. 1. In an early ontogenesis insulin is one of backbone hormones of cell-like metabolism as carbohydrates serve as the main substratum of a cell-like anabolism. Apparently, deduction of datum level of this hormone is in essence significant for preservation of activity of an organism. Tension of systems of adaptation at OIC of this age group occurs generally due to change of other links of a hormonal regulation on what specifies higher content of STH and Co in OIC blood in comparison with OIC, and also high degree of correlation of change of these hormones ($R=0,72$).

Data are presented in the table No. 2. In the second half of the year of life at OIC the tendency to increase in content of insulin in blood whereas this index remained same with SIC, as well as in the first six months is noted. Also, the maintenance of STH and Co in blood both OIC, and SIC changes. At OIC the

level of hormone of body height remains same high, and at SIC decreases in comparison with younger age group. The maintenance of Co is also higher, than at SIC. Studying of correlative communications showed that OIC of this age group exists the strong connection between change of STH and insulin (R=92), and also between Co and STH (R=64). Data are presented in the table No. 1. The received results suggest that the age of the second half of the year of life is critical on formation of the interhormonal communications forming the adaptation potential of the child in the next years. Apparently, at this age at OIC the pathological form of the interhormonal relations is put that in a consequence affects the frequency and duration of incidence and significantly affects indexes of body height of children. It is known that OIC have deviations in body weight, weak muscle system more often. Aged from a year up to three years adaptation reactions already gain "adult" character from children and the main hormone, in charge of response of an organism to a stress is Co.

Results of our research showed that the orientation of change of maintenance of Co in OIC and SIC blood is opposite. At the OIC level of this hormone becomes lower, than in children of younger age group that allows to assume a possibility of exhaustion of a hormonal link of adaptation. At SIC, on the contrary, higher content of Co is established that it is probably bound to the adequate answer of hypophysial and adrenal system to the sharp period of a disease. Data are presented in the table No. 2.

Table 2. Correlative dependence of STH, insulin and hydrocortisone of children of the first three years of life.

Correlative index	STH/insulin		Co/STH		Co/insulin	
Age	OIC	SIC	OIC	SIC	OIC	SIC
1-6 months	r=0,74	r=0,38	r=0,63	r=0,27	r=0,54	r=-0,41
6-12 months	r=0,42	r=0,34	r=0,42	r=0,42	r=0,31	r=0,34
12-36 months	r=0,38	r=0,26	r=0,34	r=0,23	r=0.41	r=-0,36

Thus, results of researches allow to believe that in the first half a year of life insulin is a backbone factor both at OIC and at SIC which maintaining provides formation of adaptive reactions due to change in other links of a hormonal regulation. But at OIC tension of processes of an anabolism is noted on what specifies high content in STH blood. In process of maturing of a hormonal regulation change of the functional relationship between links of endocrine system is noted. But in the conditions of a stress at OIC after 6 months insulin

keeps a role of the leading regulator of cell-like metabolism, STH decreases very slightly. It is apparent that processes of body height at OIC are supported due to high tension of a hypophysial link of a regulation that perhaps defines repeatability of recurrent respiratory diseases and poor effectiveness of treatment-and-reabilitation actions. Rigider correlative communications between the STH level and insulin (=0,74), a hydrocortisone and STH (=0,63) are found in children with the perinatal damages of the central nervous system (PDCNS) since the period of a neonatality. The imbalance of hypophysial and adrenal system, and also hormone of insulin is noted at OIC since the period of a neonatality and remains during the whole first year of life and is especially expressed in 6 months. The most profound and essential changes are noted at the children born from mothers with endocrinopathies.

Changes in the hormonal status leads to violation of processes of adaptation, both in the first months of life, and during the subsequent age periods. These changes can be considered as one of predictors of development in children of recurrent respiratory diseases. The special attention is deserved in this plan by the children born from mothers with primary endocrinopathies and children with manifestations of PDCNS

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Antibiotics Administration Policy and Antibacterial Susceptibility in Isolates from Critical Patients with Respiratory Infections in the Intensive Care Unit

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B428C0058

Introduction

The prescription of antimicrobials in critical patients is one of the therapeutic interventions most frequently used in Intensive Care Units due to the own pathologies of the patient and the risk of intrahospitalary infections.

The proper use of these drugs influences the clinical evolution of patients, bacterial resistance and health costs.

Methods

Study: Observational, descriptive, longitudinal and retrospective. Period: January 2014 to December 2016. Sources: Databases (CNDD 2016) form the computer program of the Emergency Admission Service and the one from Dispensing Registry of the Pharmacy Service. Location: 6 beds average a year. Hospitalizations/year: 373 (2014) + 404 (2015) + 117 (2016) Stays: 1328 (2014) + 1229 (2015) + 1354 (2016). Origin of the samples: bronchial aspirates, sputum and blood culture. Antimicrobial consumption: in Daily Dose Defined (DDD c-d)/100 days of stay. Antibiotics tested: Imipenem (IMP), Oxacillin (OXA), Penicillin (PEN), Piperacillin-Tazobactam (P/Z), Aztreonam, Amoxicillin-clavulanic (AMC), Vancomycin (VAN), Daptomycin (DAPT), Gentamicin; Levofloxacin (LEVO).

Results

1. The most commonly used antimicrobials (approximately 80%) of DDD, were: levofloxacin, imipenem, daptomycin, linezolid and piperacillin-tazobactam. On the other hand, we observed a gradual decrease in the consumption of cefotaxime and amoxicillin-clavulanic.
2. Average consumption of antimicrobials in 3 years was 196.27 DDD/100

c-d and the distribution for years was: 219.70 (2014), 157.35 (2015); 211.78 (2016).

3. Positive blood cultures (n: 92): Distribution by gender and species: 13 (12%) *P.aeruginosa* and *E.coli* (2 BLEAS), 10 ScoN (11%) (9 R oxacillin), 11 (12%) *E.faecalis,* 9 (10.12%), *K.pneumoniae* (1 BLEA), 6 (6%) *Proteus* spp (1 BLEA) and *S. aureus* (1 SAMR), 4 (3.6%) *S.marcenscens*, 3 (2.7%) *X.maltophilia*, 10 (11%) Other Enterobacteria.
4. Penicillin, oxacillin and amoxicillin-clavulanic acid were the antimicrobials of the beta-lactam group, in contrast to which our isolates had a higher percentage of resistance, which led us to search for antimicrobial therapeutic alternatives (Imipenem).
5. Resistances found in the group of beta-lactamase inhibitors (amoxicillin-clavulanic), makes it necessary to orientate the prescription to other broad-spectrum antibiotics (piperacillin-tazobactam) that presented a lower resistancerate.

Conclusions

It is necessary to know the resistance profile of our work areas so that the choice of antimicrobial is the most successful possible. Prescription-indication studies are required to determine the actual susceptibility of antimicrobials and to modify prescription patterns. Regardless of the intrinsic resistance of the microorganisms studied, the high consumption of certain antimicrobials could be conditioning the selection of resistant strains (levofloxacin) and thus justifying the described resistance.

Keywords: Antimicrobial Susceptibility, Intensive Care Unit, respiratory infections, Intrinsic resistance

Summary

The prescription of antimicrobials in critical patients is one of the therapeutic interventions most frequently used in Intensive Care Units due to the own pathologies of the patient and the risk of intrahospitalary infections. The proper use of these drugs influences the clinical evolution of patients, bacterial resistance and health costs.

Objectives

In order to carry out an adequate management of the main anti-infective drugs used in the treatment of respiratory infection (Intensive Care Unit), we carried out the following study.

Material and Method

a. Clinics parameters	
• **Study**	Observational, descriptive, longitudinal and retrospective.
- Period	January 2014 to December 2016.
- Sources	Databases from the computer program of the Emergency Admission Service and the one from dispensing registry of the Pharmacy Service.
• **Location**	6 beds average a year.
• **Income/year**	373 (2014) + 404 (2015) + 117 (2016).
• **Bed-stays/year**	1328 (2014) + 1229 (2015) + 1354 (2016).

b. Microbiological parameters:

For this study, a total of 100 Gram-negative isolates from patients diagnosed of Respiratory Infections (RI) were included for 3 years. The samples were collected from patients admitted in Intensive Unit Care. The origins of the samples were: sputum, bronchial aspirates, bronchial lavage, and blood cultures. Only one isolate per species per patient was accepted into the study.

They were cultivated following standard procedures from January 2008 to December 2015.

Susceptibility (minimal inhibitory concentration) was determined using the CLSI broth microdilution method (Walk-Away Systems, Beckman) and the breakpoints were considered according to the Clinical Laboratory Standard Institute guidelines (CLSI) and EUCAST guidelines. The antimicrobial agents analyzed were: ciprofloxacin and levofloxacin, piperacillin-tazobactam, amikacin and levofloxacin.

c. Pharmacy parameters:

The use of antibiotics in hospitalized patients was obtained through the Hospital Pharmacy Service, using as technical unit of measure the Defined Daily Dose (DDDs c-d) per 100 days of stay: (DDD c-d)/100 days of stay.

Antibiotics tested:
• Imipenem (IMP)
• Oxacillin (OXA)
• Penicillin (PEN)
• Piperacillin-Tazobactam (P/Z)
• Aztreonam (AZT)
• Amoxicillin-clavulanic (AMC)
• Vancomycin (VAN)
• Daptomycin (DAPT)
• Gentamicin (GM)
• Levofloxacin (LEV)

Results

1. Positive blood cultures (n: 92): Distribution by gender and species: 13 (12%) *P.aeruginosa* and *E.coli* (2 BLEAS), 10 Negative Coagulase Staphylococco ScoN (11%) (9 R oxacillin), 11 (12%) *E.faecalis*, 9 (10.12%) *K.pneumoniae* (1 BLEA), 6 (6%), *Proteus spp* (1 BLEA) and *S.aureus* (1 SAMR), 4 (3.6%) *S.marcenscens*, 3 (2.7%) *X.maltophilia*, 10 (11%) Other Enterobacerias.
2. Penicillin, oxacillin and amoxicillin-clavulanic acid were the antimicrobials of the beta-lactam group, in contrast to which our isolates had a higher percentage of resistance, which led us to search for antimicrobial therapeutic alternatives (Imipenem). (Table 1)
3. Resistances found in the group of beta-lactamase inhibitors (amoxicillin-clavulanic), makes necessary to orientate the prescription to other broad-spectrum antibiotics (piperacillin-tazobactam) that presents a lower resistance rate.
4. Average consumption of antimicrobials in 3 years was 196.27 DDD/100c-d and the distribution for years was: 219.70 (2014), 157.35 (2015); 211.78 (2016). (Table 2)
5. The most commonly used antimicrobials, approximately 80% of DDD, were levofloxacin, imipenem, daptomycin, linezolid and piperacillin-tazobactam. On the other hand, we observed a gradual decrease in the consumption of cefotaxime and amoxicillin-clavulanic acid (Table 2).

Table 1. Antibacterial Resistance. (years 2014-2016)

Antibiotics	Percentage (%)
IMP	23,43
OXA	58,82
PEN	71,42
P/T	18,33
AZT	27,9
AMC	47,16
VAN	3,57
DAPT	0,03
GM	22,22
LEV	33,33
LIZ	14,28
CFT	37,25

Table 2. Antimicrobial consumption. (years 2014-2016)

Antibiotics	DDD c-d/100 bed-stays
IMP y cilastatin	17,14
OXA	2,63
P/T	15,17
AZT	0,64
AUG	11,85
VAN	6,19
DAP	16,12
CTX	10,21
LIZ	16,12
GM/ TOB	1,18/4,47
LEV	22,37

Conclusions

Antibiotics administration policy to critical patients should be based on the fulfilment of a set of rules about its use. The strategies proposed in recent years to optimise effectiveness and minimise adverse effects should be applied cautiously. Constant evaluation should be practiced in the obtained results and adapted to meet the needs of each Intensive Care Unit.

It is compulsory to evaluate the resistance profile of our work areas so the choice of the antimicrobial agent is the most successful one. Regardless of the intrinsic resistance of the microorganisms studied, high consumption of certain antimicrobials could condition the selection of resistant strains (levofloxacin) and thus justifying the described resistance.

Prescription-indication studies are required to determine the actual susceptibility of antimicrobials. It is necessary to carry out long-term antimicrobial prescription-resistance studies that allow us to confirm our results.

Knowledge of these patterns of consumption will be useful to develop a consensus in antimicrobial policy as well as serve as a basis for developing a correct management of antimicrobials in our daily clinical practice.

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Prevalence of Extended-Spectrum Beta-lactamase Producing Microorganisms and Respiratory Infections

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B428C0073

Introduction

Respiratory tract infections caused by multidrug-resistant pathogens (beta-lactamase production microorganisms) are becoming a major concern for treatment constituting an additional burden for individual patients and for the Health Care System.

Objective

To determine the prevalence and etiology of resistant microorganisms responsible for lower respiratory tract infections in hospitalized patients.

Methods

A total of 100 bacterial isolates from patients diagnosed with respiratory infections were studied for three years in a Health Area (Emergency Unit, Pneumology Department and Intensive Care Service). The origin of the samples was: sputum, bronchial aspirate, pleural fluid, bronchoalveolar lavage and blood cultures. The study of antibacterial susceptibility (Walk away, Beckman) was determined using the CLSI broth microdilution method (Walk-Away Systems, Beckman) and the breakpoints were considered according to the Clinical Laboratory Standard Institute guidelines (CLSI) and EUCAST guidelines 2011. It was confirmed by diffusion method (disk and E-test), resistant isolates detected. Epidemiological and clinical data of patients were analyzed: age, gender, hospitalization in the previous three months, diabetes, Chronic Pulmonary Obstructive Disease (COPD), asthma, interstitial lung disease and treatment with antibiotics in the last month.

Results

Klebsiella pneumoniae was the most predominant bacterial species

(32%), followed by *Pseudomonas aeruginosa* (12%), *Serratia marcencens*, *Enterobacter spp* and *Escherichia coli*. The prevalence of resistant bacterial isolates ESBL producers was 31%, being *Pseudomonas aeruginosa* the most predominant (44.2%), followed by *Klebsiella pneumoniae* (31.6%). No strains of Acinetobacter baumannii were isolated. Of the different clinical variables studied stand out from the point of view of respiratory infection, history of COPD and other variables the hospitalization and treatment history prior to admission. 5. Have a higher risk of infection by multi-drug resistant pathogens: patients with COPD, those who have had a hospitalization in the last 3 months and those who have taken at least one course of antibiotics.

Conclusions

We results are very similar to European studies, however we should not forget to make focus on the management of these patients, and the need for the implementation of strategies for infection control to prevent the spread of these strains.

Keywords: ESBLs microorganisms, lower respiratory Infection

Introduction

The prevalence of extended-spectrum beta-lactamase (ESBL) producing gram-negative bacilli has increased in recent years. Respiratory tract infections caused by multidrug-resistant pathogens (beta-lactamase production) are becoming a major concern for treatment constituting an additional burden for individual patients and for the Health Care System.

ESBLs have been identified in several members of the enterobacteria family, as well as in *Pseudomonas aeruginosa.* During the years 1980 and 1990 *Klebsiella spp* was as responsible for the production of these enzymes, giving rise to an increase in the production of ESBL by *Escherichia coli*, mainly from the enzyme CTX-M.

The distribution of each microorganism among all Gram-negative bacilli-ESBLs is similar to that of other publications, with *Escherichia coli* being the most common microorganism by difference, followed by a lower prevalence of *Klebsiella pneumoniae* and *Klebsiellla oxytoca*. *Escherichia coli-ESBL* has been more frequently associated with urinary tract infections and bacteriemias.

In our case, more than a third of the positive cultures for this microorganism were made in clinical samples of blood. On the other hand, *K. pneumoniae-ESBL* has been implicated as an important cause of bacteriemias and respiratory tract infections. In our investigation, it was identified in greater proportion in urocultures, followed by blood cultures and respiratory samples.

Objectives

We have proposed to describe the trend of bacterial resistance to antibiotics in the main pathogens that cause respiratory infections, highlighting some essential aspects in each bacterial group.

Matherial and Method

A total of 100 bacterial isolates from patients diagnosed with respiratory infections were studied for 3 years in our Health Area (Emergency Unit, Neumology Service and Intensive Care Service). The origin of the samples was: sputum, bronchial aspirate, pleural fluid, bronchoalveolar lavage and blood cultures. The study of antibacterial susceptibility (Walk away, Beckman) was determined using the CLSI broth microdilution method (Walk-Away Systems, Beckman) and the breakpoints were considered according to the Clinical Laboratory Standard Institute guideline (CLSI) and EUCAST guidelines 2011. It was confirmed by diffusion method (disk and E-test), resistant isolates detected. Epidemiological and clinical data of patients were analyzed: age, gender, hospitalization in the previous three months, diabetes, Chronic Pulmonary Obstructive Disease (COPD), asthma, interstitial lung disease and treatment with antibiotics in the last month.

Results

1. *Klebsiella pneumoniae* was the most predominant bacterial species (32%), followed by *Pseudomonas aeruginosa* (12%), *Serratia marcencens*, *Enterobacter spp* and *Escherichia coli*.
2. The prevalence of resistant bacterial isolates ESBL producers was 31%, being *Pseudomonas aeruginosa* the most predominant (44.2%), followed by *Klebsiella pneumoniae* (31.6%). No strains of *Acinetobacter baumannii* were isolated.
3. Of the different clinical variables studied stand out from the point of view of respiratory infection, history of COPD and other variables the hospitalization and treatment history prior to admission.
4. Have a higher risk of infection by multi-drug resistant pathogens: patients with COPD, those who have had a hospitalization in the last 3 months and those who have taken at least one course of antibiotics.

Conclusions

Our results are very similar to some european studies, however we should not forget to make focus on the management of these patients, and the need

for the implementation of strategies for infection control to prevent the spread of these strains. The use of antibiotics in humans is often excessive and inappropriate. In the United States, despite evidence and guidelines for clinical practice, 71% of acute bronchitis in adults were treated with antibiotics between 1996 and 2010, with a significant increase over the years. Prescription of antibiotics is a complex process in which physicians have different degrees of training, motivation, workload and knowledge.

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Skin Immunologic Tests and Latent Tuberculosis Infection

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B428C0023

Latent TB infection (LTI) - asimptomatic nontransmissiv tuberculosis infection with hidden persistence of Mycobacteria in the host organism [1].

The phenomenon of persistence of bacteria causes an increased interest around the world [2, 3, 4, 5]. The problem of differentiation of latent and active TB is very important, but difficult and not yet completely solvable [6].

Penetration of Mycobacteria in the body can lead to the following:

1. under the influence of protective forces of the organism Mycobacterium all perish;
2. as a result of the multiplication of Mycobacterium tuberculosis develops;
3. a small number of bacilli to survive but remains in the body in the “asleep” so called “dormant” - latent TB infection;
4. Mycobacteria present in “asleep” state, once again begin to multiply,the disease developes [7].

According to the World Health Organization (WHO), one third of all humanity has LTI 5-20% of those infected there is a risk of development of active tuberculosis (TB) during their lifetime and in the majority of cases TB develops in 2-5 Byears of infection [8]. The gap between infection and the development of tuberculosis is unique for contagious disease [9]. Thus, the LTI is a tank of the future TB. Infection control (measures to reduce the incidence, early diagnosis and treatment) is the most important strategy to combat TB.

The decrease in the number of people infected with M. tuberculosis and preventing new cases of disease is achieved by preventive therapy of persons with LTI [10, 11, 12]. Therefore for infection control should be more clearly delineate the range of persons with LTI in need of preventive therapy.

The main method of determination LTI are tuberculin tests revealing delayed type hypersensitivity.

For carrying out the reaction using PPD (purified protein derivative).

However, the PPD contains more than 200 antigens derived from M. bovis and M. humanus, a part of these antigens locate in other non-tubercular

mikobacteria. For this reason, a positive test can be registered not only in case of MBT infection, but also in, nontuberculous mycobacteria infection as well as a certain period after BCG vaccination [13, 14, 15]. Decording of M. tuberculosis genome [16] permit to use separate specific MBT proteins for diagnosis of tuberculosis. In the genome of the MBT around 4000 proteins coded and genes profile expressed at different stages of infection can vary [17, 18, 19, 20, 21]. The two most widely used for diagnostic purposes antigens ESAT-6 (Early Secreted Antigenic Target 6) and CFP-10 (Culture Filtrate Protein 10) encoded in the zone of RD1 M. tuberculosis genome and, they express when reproduction of Mycobacteria and absent in M. bovis BCG and most non-tuberculosis Mycobacteria. They are related to the virulence of M. tuberculosis [22, 23, 24, 25, 26]. These antigens have been used to create diaskintest (tuberculous recombinant allergen) that represents a complex of recombinant protein CFP-10 and ESAT-6 produced genetically modified culture of Escherichia Coli BL-21 (DE3)/p CFP-ESAT and destined for intradermal test with the purpose of revealing delayed-type hypersensitivity [27, 28, 29].

The purpose of the study

With the aim of finding objective indicators (other than tuberculin test and history data)to identify LTI and to compare the diagnostic possibilities of tuberculin intradermal tests (TST) and samples with allergen tuberculous recombinant-diaskintest (DST) to identify activation of LTI were studied the results of these two skin immunological tests carried out in parallel at the 100 children and teenagers.

Materials and methods

Among 100 study was 61 males and 39 females aged from 2 to 17 years of age, the average age of 8.7±0.4 years old. All have been carefully assembled histories, clinical and x-ray examination, explored the peripheral blood (complete blood count) and sputum examination on the presence of AFB.

Detection of cellular immune response carried out using diaskintest based on an evaluation of delayed-type hypersensitivity. We used the intradermal injection of diaskintest at a dose of 2mkg in 0.1 ml, containing ESAT6-CFP-10 (Lecco, Russia) present in virulent strains of Mycobacterium tuberculosis.

The reaction were evaluated visually after 72 h and measured the size of induration in millimetres. The result was considered negative in the absence of infiltration, doubtful if hyperemia without infiltration, positive if there is infiltration (papules) of any size, hyperergic-when the diameter of infiltration 15 mm and more, formation vesicle and necrosis and (or) the presence of lymphangitis, lymphadenitis. For the evaluation of delayed-type hypersensitivity also conducted intradermal tuberculin test (routine method)

Statistic calculating of data performed using computer programs Microsoft Excel for Windows, Statistica.

Results and discussion

During the research it was found that 99.0±1.0% of examined persons may be considered infected. Only one TST reaction was doubtful (4 mm diameter papule formation). Hyperergic reaction to tuberculin intradermal injection has evolved from 19.0±3.9% surveyed, papule with the formation of vesicles from 5.0±2.2%. At the same time, positive reaction to the DST was detected only in 25.0±1.0% of the surveyed hyperergic reaction in 16.0±3.7%. In 75.0±4.3% surveyed reaction to DST was negative.

Table 1. Results of skin immune tests (TST and DST)

The severity of reaction	TST n =100	DST n =100
Positive	99.0±1.0%	25.0±1.0%
Hyperergic	19.0±3.9%	16.0±3.7%
With the formation of vesicles	5.0±2.2%	3.0±1.7%
Doubtful	1.0±1.0%	0.0%
Negative	0.0%	75.0±4.3%

As a result, a comprehensive clinical-radiological and laboratory study among surveyed revealed 3 patients with tuberculosis. In 17 years old patients, TST-doubtful (papule with diameter of 4 mm) DST-hperergic reaction (papule with a diameter of 17 mm), complaining of weakness, loss of appetite, productive cough, radiographically changes were identified, allowing diagnosis “infiltrative tuberculosis in the phase of destruction”, in the sputum AFB+. In 14 years old patient DST positive with the formation of papule (diameter of 10 mm) and a negative TST tuberculouse mesenteric adenitis was identified. And finally, 16 years old patient, with hsyperergic DST (papule-18 mm with vezikule) and hyperergic TST (17 mm papule) identified tuberculosis of cervical lymph nodes. Significantly less positive reaction to the DST (25.0±4.3%) than the TST (99.0±1.0%) have surveyed possible because, as shown above, the DST identifies the intensification of latent TB infection as antigens presented in DST expressed when reproduction of Mycobacteria and associated with virulence of the MBT. Match results of TST and DST was determined largely by the hyperergic reactions (9 people). Interest results of TST (papule with a diameter of 4 mm) and with a diameter of 14 mm papules formation on DST from patient with infiltrative pulmonary tuberculosis in the

phase of destruction. Thus, we have got several contradictory data necessitate further investigation with a view to a comparative analysis of the effectiveness of two skin immunologic tests in detecting latent TB infection and its activation is necessary.

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Remodeling of Phenotype $Cd16^{+}Cd11b^{+}$ Neutrophilic Granulocytes in Acute Epstein-Barr Viral Infection

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B428R9048

Abstract

Neutrophilic granulocytes (NG) – the very important cells of the innate and adaptive immune system, involving in the incredibly quick implementation of antiviral defense and protection. Comparative analysis of the level of intensity of the expression of molecules CD16 and CD11b on the membrane NG revealed three distinct subpopulations in the population of $CD16^{+}CD11b^{+}NG$: $CD16^{bright}CD11b^{dim}$, $CD16^{dim}CD11b^{dim}$, $CD16^{bright}CD11b^{dim}$ in healthy subjects and three distinct subpopulations – $CD16^{+}CD11b^{+}NG$: $CD16^{bright}CD11b^{bright}$, $CD16^{bright}CD11b^{dim}$, $CD16^{bright}CD11b^{dim}$ in patients with severe acute Epstein-Barr viral (EBV) infection. It was found, that in healthy individuals prevails $CD16^{bright}CD11b^{dim}NG$ subpopulation, in patients with severe acute Epstein-Barr viral infection subpopulation $CD16^{bright}CD11b^{brigh}NG$ dominates.

We propose, that the high levels of expression of CD16 and CD11b was necessary for realization of antiviral activities of NG in defense against EBV infection. Remodeling of the NG phenotype $CD16^{+}CD11b^{+}N$ into subpopulation $CD16^{bright}CD11b^{bright}NG$ was appeared in severe acute EBV infection in comparison with healthy individuals who didn't have subpopulation $CD16^{bright}CD11b^{bright}NG$. Subpopulation $CD16^{bright}CD11b^{bright}NG$, detecting in severe acute EBV infection, had features of a high cytotoxiety ($CD16^{bright}$) and suppressive influences ($CD11b^{bright}$). Further studies are needed to determine the functional roles of the subpopulation $CD16^{bright}CD11b^{bright}NG$ in severe acute EBV infection as well as to study potency of the target immunomodulating influences for positive transformation this phenotype NG with the purpose of prevention of an emergence of bacterial complications.

Keywords: neutrophilic granulocytes, subpopulation, acute viral infections

Background

Neutrophils granulocytes (NG) are the key effector cells of the innate and adaptive immune system, realizing different antimicrobial mechanisms. NG possess the arsenal of defensive strategies, such as: reactive oxygen species, phagosytosis, realizing of antimicrobial granule contents, formation of neutrophil extracellular traps (NETs). NG can very quickly realize those main defensive functions. From the other side NG realize different regulatory influences on T and B cells, nature killers, dendritic cells and macrophages.

Over last 2 deade, many authors have demonstrated and documented that neutrophilic granulocytes have the very important role in the antiviral defense [1, 2, 3, 4]. More recently there have been several reports indicating exist of several phenotypes of NG with distinct functions in peripheral blood. It was shown that NG exhibit a high degree of plasticity and functional heterogeneity.

There are few NG populations and subpopulations with different features.

The membrane of NGs express many different molecules – receptors of the receptors' that realize functions of those cells. The levels of the receptors' expressions are depending from the physiological or pathological conditions and the scenarios of the immune response. There are the great spectrum of NG membrane receptors: histocompatibility complex, adhesion structures, pattern recognition receptors (PRR), receptors for cytokines, immunoglobulins, complement components, hormones, neuropeptides, histamine, kinases, membrane molecules to other cells etc. [1, 5, 6, 7, 8]. Differentiation of NG into different functional phenotypes has been described Pillay and c-workers [5, 6] in experimental studies with LPS in healthy adults and in patients with bacterial sepsis and trauma. They have shown three different subsets of NGs: mature ($CD16^{high}CD62L^{high}$), immature ($CD16^{low}CD62L^{high}$) and suppressive ($CD16^{high}CD62L^{low}$). Recently B.Cortjens and co-workers [12] demonstrated occurrence of distinct and unique NG' subset responses during severe viral and secondary bacterial infection in infants. They have characterized four heterogeneous NG' subsets in the blood of infants with severe viral respiratory infection: mature, immature, progenitor and suppressive. The progenitor subset NG had $CD16^{low}CD62L^{low}$ phenotype. The suppressive NG' subsets were found only in infants with viral and bacterial co-infections.

NGs constitutively equipped with two receptors: CD11b (Mac-1 receptor or a component complement receptor CR3b) and CD16 (FcγRIII, binds IgG with low affinity). In the physiological state in healthy individuals the expression of these receptors on membrane NG are low. During the inclusion, NG into immune response, after contact with bacterial or viral antigen, there is additional translocation of intracellular reserve pools of CD16 and CD11b to the surface membrane of NGs, which results in a multiple increase of number of highly

equipped activated NG. It is well known that membrane receptors CD16 and CD11b play an important role in the implementation of the phagocytosis, in formation of neutrophils extracellular network (NET), antibody dependent cellular cytotoxicity (ADCC) at infections processes of the different nature.

CD11b is activating receptor and can triggers NG for realization of phagocytic function. In absence of CD11b on membrane of NG, phagocytic function NG was destroyed. [7, 9, 10, 11]. At the same time the levels of the simultaneous expression of the receptors CD16 and CD11b on the surface membrane of NG in normal and pathological conditions has been little studied.

Materials and methods

We had studied 28 patients both sexes aged from 18 to 28 years old with the severe acute viral tonsillitis, having clinical symptoms of the early stages of the disease. Control group consist 25 healthy volunteers both sexes aged from 18 to 28 years old. The PCR and the serological diagnostic methods were used for detection of the acute Epstein Barr infection (EBV) (AEBVI).

The simultaneously expression of molecules CD16, CD11b on surface membrane of NG of peripheral blood was investigated by flow cytometry on CYTOMICS FC 500 (Beckman Coulter, USA), using the panel of monoclonal antibodies: CD16-ECD, CD11b-PC5 and appropriate isotype controls.

It was estimated the percentage of number of $CD16^{+}CD11b^{+}$NG and it was made the differentiation of subpopulations, using the value of the middle fluorescence intensity (MFI), that according the density of expressed surface molecules CD16, CD11b on membrane of NG.

Results

The diagnose of severe acute EBV infection was supported in 100% of cases using the PCR method and the serological diagnostic methods. IgM VCA and IgG EA were detected in 100% of cases. IgG EBNA have 21,4% of patients, IgM VCA+IgG EA – 78,6% (early first infection), IgM VCA+IgG EA+IgG EBNA – 21,4% of patients (late first infection). The replication of EBV was found using PCR method in the blood in 87,7%, in the saliva and the urine in 14,3% of cases "Fig.1". The obtained data had demonstrated that the level of $CD16^{+}CD11b^{+}$NG was decreased in patients with severe acute viral infection in 1,24 times in comparison with healthy volunteers ($p<0,05$).

Comparative analysis of MFI CD16 and MFI CD11b at population $CD16^{+}CD11b^{+}$NG

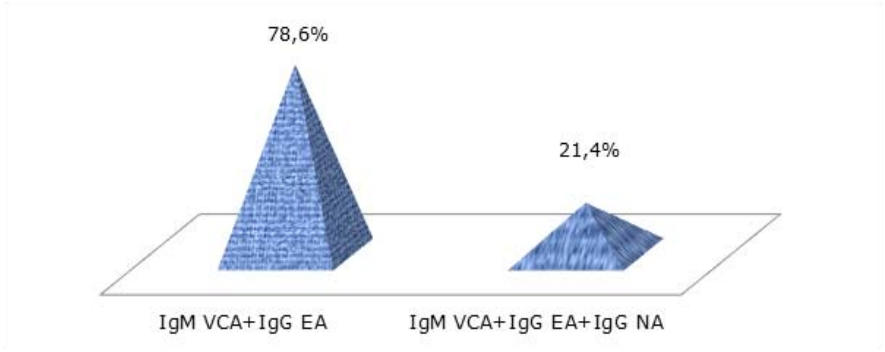

Figure 1. Serological profile in severe acute Epstein-Barr viral infection

was revealed significant differences in expression levels of studied receptors in acute viral infection compared to control. It is found that at AEBVI, compared to healthy controls, in subpopulation $CD16^{+}CD11b^{+}$NG MFI CD16 increased to 2,5-fold ($p<0,05$) and the MFI CD11b significantly increased to 1,5-fold ($p<0,05$). Estimation of the received data of relative content of $CD16^{+}CD11b^{+}$NG in healthy individuals and AEBVI, taking into account of expression level of CD16 and CD11b, gives phenotypic picture, showing different intensity equipping by CD16 and CD11b receptors. This may be an illustration of various switching mechanisms of NG in the immune response at infectious processes "Fig.2".

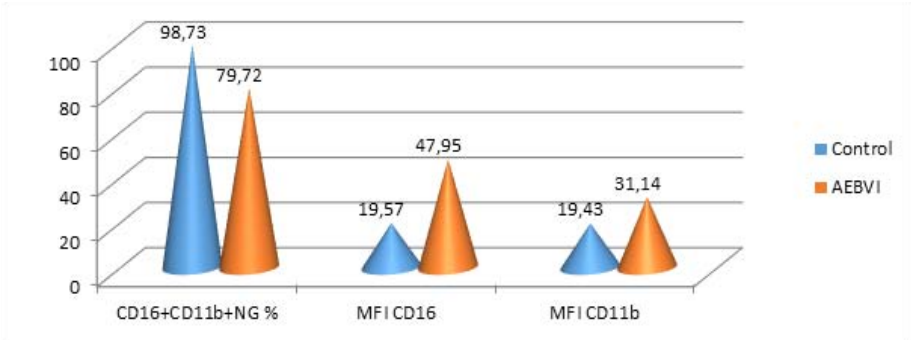

Figure 2. $CD16^{+}CD11b^{+}$NG and high Mean Fluorescence Intensity (MFI) in severe acute Epstein-Barr virus infection (AEBVI)

A more detailed analysis of the level of the expression of the studied receptors $CD16^{+}CD11b^{+}$NG have identified four subpopulations that were differed in density of the expression of CD16 and CD11b receptors in healthy volunteers and patients with EBV infections. The subpopulation of high-density expression of both receptors ($CD16^{bright}CD11b^{bright}$), the subpopulation of high-density

expression of CD16 and low CD11b ($CD16^{bright}CD11b^{dim}$), a subpopulation with a low density expression of both receptors ($CD16^{dim}CD11b^{dim}$); and the subpopulation with a low density expression of CD16 and high density expression of CD11b ($CD16^{dim}CD11b^{bright}$) were identified "Fig.3".

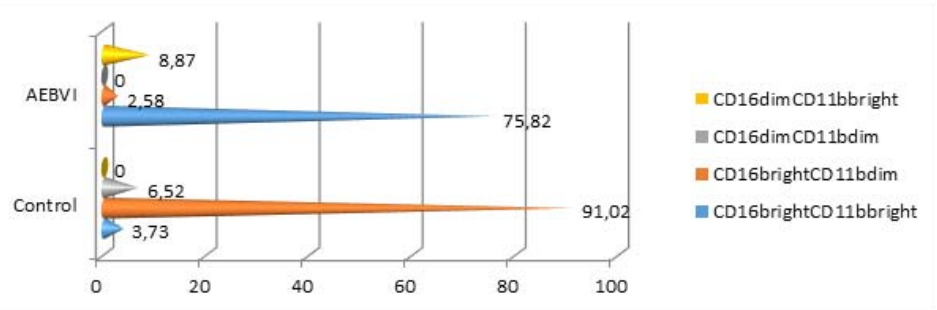

Figure 3. Profiles $CD16^{+}$ $CD11b^{+}$NG in severe acute Epstein-Barr viral infection (AEBVI)

At the same time it was shown the different levels of the expression of the surface membrane receptors CD16 and CD11b of NG in healthy volunteers and in patients with severe acute EBV infection. Thus, the subpopulation $CD16^{bright}CD11b^{dim}$NG was dominated in healthy individuals, consisting 91,02±5,23% of $CD16^{+}CD11b^{+}$NG, at the same time in group with acute viral infection the level of $CD16^{bright}CD11b^{dim}$NG was lower and consisted only 2,58±0,80% ($p<0,001$). From the other side the subpopulation $CD16^{bright}CD11b^{bright}$NG was dominated, consisting 75,82±2,28 of $CD16^{+}CD11b^{+}$NG in patients with the acute EBV infection. In healthy volunteers the subpopulation $CD16^{bright}CD11b^{bright}$NG was completely absent.

We had demonstrated, that $CD16^{+}CD11b^{+}$NG can change their phenotype in early stage of the acute viral infection - the acute EBV tonsillitis, in which subpopulation $CD16^{bright}CD11b^{bright}$NG appeared and dominated.

While the predominance of subpopulations $CD16^{bright}CD11b^{bright}$НГ in severe acute EBV infection of lymphoid ring of the nasopharynx in patients in a state of moderate severity or serious conditions demonstrates emergence of a well-equipped NG with high cytotoxic antiviral potential activity. Revealed enhanced expression of CD16 on NG when a viral infection may be due to the greater functional importance of the cytotoxic NG, expressing FcγRIII (CD16) for the implementation of ADCC associated with CD11b-dependent increase of adhesion and increased degranulation [10, 11].

Conclusion

We have demonstrated that the subpopulation/subset $CD16^{bright}CD11b^{dim}$NG was dominated in healthy individuals. The NGs with phenotype $CD16^{bright}CD11b^{bright}$ were absent in healthy volunteers, but were appeared and dominated in acute EBV infection. Pillay J. and co-workers in 2012 year [6] revealed the existence of a novel subset of mature hypersegmented human neutrophils with immunosuppressive activity $CD11c^{bright}CD62L^{dim}CD16^{bright}CD11b^{bright}$.

This subpopulation was able to suppress T cell proliferation through the release ROS and required expression of CD11b. Later Cortjens B. and co-workers in 2017 year had shown that in severe respiratory viral infection in infants the expression of activation marker CD11b was elevated in suppressive NG in comparison with mature NG. Those suppressive subsets NGs had also the highest expression of CD63 molecules on their surface membrane that had indicated the active degranulation of NGs. Those authors demonstrated that suppressive subset NGs had appeared in viral and bacterial co-infections in neonates.

We proposed that from the one hand the appearance $CD16^{bright}CD11b^{bright}$ NG with features of a high cytotoxiety (the high levels of the expression of CD16) and suppressive influences (high levels of the expression of CD11b molecules) are necessary for realization of antiviral activities of NGs in their fight against EBV infection. Those subpopulations of NGs should have a high antiviral activity. On the other hand its suppressive properties (high levels of the expression of CD11b) maybe in the future lead to different complications in the form of secondary bacterial infections.

Thereby, remodeling of the NG phenotype and the appearance of new NG subpopulation $CD16^{bright}CD11b^{bright}$NG with features of a high cytotoxiety and suppressive influences were identified in severe acute EBV infection. Further studies are needed to determine the functional roles of the subpopulation $CD16^{bright}CD11b^{bright}$NG in EBV and other herpes-viral infections as well as to study potency of the target immunomodulating influences for the positive transformation this phenotype NG with the purpose of a prevention of an emergence of bacterial complications.

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Clinical and Immunological Features of Hemorrhagic Fever with Renal Syndrome

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B428R9044

In Russia, hemorrhagic fever with renal syndrome (HFRS) ranks first among zoonotic viral infections and feral herd human infections.

The sources of infection are mainly wild rodents, the carriers of Hantaan, Puumala, Seoul, and Dobrava viruses. Clinical manifestations of human disease include intoxication, pain, and hemorrhagic syndromes. The disease is characterized by cyclic course with early, oliguric, polyuric, and convalescence periods [1].

Several data suggest that hantaviruses, causative agents of HFRS, significantly impact the nature of immune response to this disorder. The most typical changes are the leading role of CD8+ cells in virus eradication [2] and the effect of CD4+ type 1 helper T cells (Th1) [3] and regulatory T cells [4] on disease severity. Immunological abnormalities may result from the virus itself or renal disorder as it was demonstrated for other viral infections [5].

Less data on the role of innate lymphocytes (i.e., NK cells and NKT) are available; however, modern scientific literature comprises some evidence on NK cells cytokine regulation in HFRS [6].

Considering this, the aim of our study was to reveal the association between blood levels of NK cells and NKT and other immune cells in HFRS depending on disease severity.

Materials and methods

Peripheral blood was collected from 54 patients with HFRS admitted to Samara Hospital of the Samara State Medical University (Russia) between October 2013 and December 2016. Clinical diagnosis of HFRS was verified by serum detection of IgM and IgG antibodies against HTNV nucleocapsid protein (NP). Disease severity was assessed using clinical and laboratory diagnostic criteria of HFRS in Russia as follows: (i) renal failure without classic oliguric stage; (ii) symptoms of uremia, hemorrhage (skin and mucous membranes), and renal failure with classic oliguric stage; (iii) severe uremia, effusion, hemorrhage (skin and mucous membranes), and renal failure with

oliguria (urine output 50 to 500 ml/day) for ≤ 5 days or anuria (urine output<50 ml/day) for ≤ 2 days. 16 healthy volunteers matched on age and gender were controls.

Fresh PBMCs were isolated from whole blood by density gradient centrifugation using standard procedures. For surface-expressed antigens, PBMCs (approximately 2×10^6 cells/ml) were incubated with antibodies for 30 min at 4°C in the dark. For intracellular staining, cells were permeabilized using BD FACS-Perm2 (BD Biosciences) according to the manufacturer's instructions. After an additional wash, PBMCs were analyzed with four-color fluorescent-antibody staining.

Statistical analysis was performed using SPSS Statistic 21,0. For parameter comparisons between subject groups, a Mann-Whitney U test was used. The data in dot plots represent the median, minimum, and maximum. Spearman's test was used for correlations. *P* values of less than 0.05 were considered significant.

Results

Blood levels of various lymphocyte phenotypes in patients with HFRS as compared with controls are represented in Table 1.

Table 1. Immunological parameters in the blood of HFRS patients compared with controls

Immune cells	Phenotype	Median [minimum; maximum] (%)		p
		Patients with HFRS (n=40)	Healthy controls (n=16)	
Tlymphocytes	CD3+	68.0 [44.4; 86.0]	75 [62; 87]	0.008*
Thelper cells	CD3+CD4+	36.1 [5.5; 58.7]	41 [14; 57]	0.038*
Cytotoxic T cells (CTL)	CD3+CD8+	31.8 [10.4; 78.0]	28 [16; 71]	0.173
NKT cells	CD3+CD56+	5.4 [2.5; 8.1]	3.4 [2.3; 5]	0.041*
NK cells	CD16+CD56+	16.6 [11.0; 33.8]	12.9 [9.5; 28]	0.123
B lymphocytes	CD19+	12.6 [5.0; 25.0]	10.5 [2.5; 16]	0.159
Activated T cells	CD3+CD25+	4.2 [2.1; 9.6]	7.4 [2.6; 7.8]	0.108
RegulatoryT cells	CD3+CD4+FoxP3+	10.7 [4.0; 27.0]	3.0 [2.3; 8.1]	<0.001*
	CD3+CD8+FoxP3+	12.5 [3.5; 26.1]	0.4 [0.1; 4.4]	<0.001*
Cells expressing activated lectin receptor	CD16+CD56+ NKG2D+	48.6 [23.4; 71.6]	12.6 [9.6; 27]	<0.001*

*Note: n, the number of patients in each group; p, the probability of the difference between the data at baseline and after 1 year; * – the significance of the difference by Mann-Whitney test (p<0.05).*

As shown in Table 1, immunological changes in HFRS predominantly affect various types of T cells. The number of T cells reduces mainly through the decrease of T helper cells. It might be associated with significant increase of regulatory T cells since these lymphocytes negatively correlate with T helpers (p<0.05). NKT cell count increases moderately while the number of cells expressing activated lectin receptor NKG2D rises significantly. The latter includes cytotoxic cells (NK) and, to a lesser extent, CTL.

We attempted to identify cell populations and subpopulations associated with HFRS severity and revealed differences between moderate and severe disease course in three lymphocyte types, i.e., Bcells, NKcells, and NKG2D+ cells (see Fig. 1).

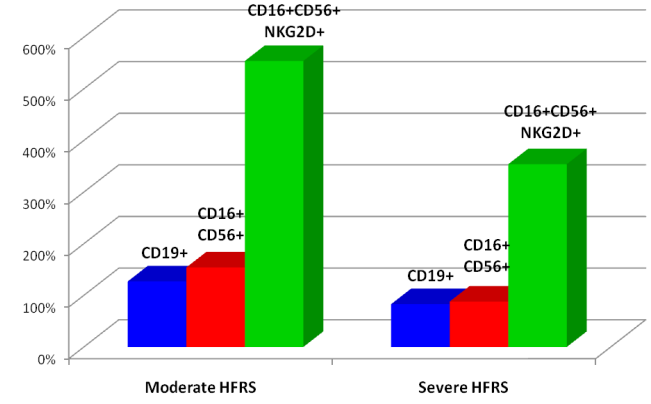

Fig. 1. The ratio of informative lymphocyte phenotypes in moderate and severe HFRS

In severe HFRS, B cell count decreases by 1.5 times, i.e., statistically significantly but not too much. The number of NK cells as well as the number of cells expressing NKG2D decrease by 1.7 times in severe HFRS.

The results of the analysis of correlations are represented in Table 2. As shown in Table 2, only regulatory T cells, NK cells, and cells expressing NKG2D demonstrate significant differences in correlations in the different severity of HFRS.

In moderate HFRS, regulatory T cells did not generate any correlations

while NK cells correlated with B cells. Cells expressing NKG2D demonstrated positive correlations with the number of cytotoxic T cells, NK T cells, and NK cells and negative correlations with the number of T helper cells and activated T cells.

In severe HFRS, negative correlations imply that the targets of immunosuppressive effect of regulatory T cells are predominantly T helper cells and NK T cells. NK cells were not involved into these correlations while NKG2D expression demonstrated positive correlation with cytotoxic T cells only.

Table 2. Correlation coefficients between lymphocyte phenotypes in HFRS

Lymphocyte phenotype	CD3+CD4+	CD3+CD8+	CD3+CD56+	CD16+CD56+	CD19+	CD3+CD25+	CD3+CD4+ FoxP3+	CD3+CD8+ FoxP3+	NKG2D+
Moderate HFRS									
CD3+	0.278	0.478	0.298	0.087	-0.073	-0.407	-0.218	-0.142	0.280
CD3+CD4+		-0.526	-0.197	-0.136	0.125	0.227	-0.304	0.071	-0.686
CD3+CD8+			0.229	0.142	-0.186	-0.651	0.032	-0.045	0.768
CD3+CD56+				0.680	0.076	-0.027	-0.087	-0.253	0.323
CD16+CD56+					0.378	-0.002	-0.359	-0.165	0.389
CD19+						0.076	-0.280	0.047	-0.006
CD3+CD25+							0.088	0.144	-0.542
CD3+CD4+FoxP3+								0.082	0.005
CD3+CD8+FoxP3+									-0.111
Severe HFRS									
CD3+	0.256	0.496	0.298	0.129	-0.011	-0.408	-0.248	-0.148	0.337
CD3+CD4+		-0.525	-0.181	-0.065	0.237	0.240	-0.377	0.152	-0.682
CD3+CD8+			0.192	0.099	-0.239	-0.644	0.058	-0.092	0.776
CD3+CD56+				0.685	0.054	0.015	-0.033	-0.376	0.295
CD16+CD56+					0.337	0.028	-0.302	-0.271	0.342
CD19+						0.092	-0.259	-0.029	-0.080
CD3+CD25+							0.091	0.182	-0.537
CD3+CD4+FoxP3+								0.154	0.061
CD3+CD8+FoxP3+									-0.180

Note: significant negative correlations are represented in blue, significant positive correlations are represented in dark blue.

Conclusion

Ourstudy shown that immune mechanisms of hemorrhagic fever with renal syndrome are mediated by T cells as well as innate immune cells, i.e., NK cells and NK T cells. Severe HFRS develops under functional predomination of regulatory T cells, cytotoxic T cells plays the key role while B cells and innate immune cells are less important.

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Summary - Hemorrhagic fever with renal syndrome (HFRS) is a feral herd disease caused by hantaviruses which manifestations include hemorrhages and

renal disorder. HFRS may be severe. Disease severity is generally tailored to immune mechanisms. The role of innate lymphocytes, i.e., NK cells and NKT, still remains elusive. Our study demonstrated that T helper cell blood count decreases in HFRS while the number of NKT and regulatory T cells increases. Severe HFRS develops under functional predomination of regulatory T cells, cytotoxic T cells plays the key role while B cells and innate immune cells are less important.

The Incoordination of Immunoregulatory Processes as a Springboard of Formation of the Immune-Mediated Diseases

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B428C0053

Secondary immunodeficiency is formed as a result of damage of the functioning of the immune system, causing inflammation as the primary response to any impact. It is then transformed either to chronic infectious process or results to the progression of autoinflamation [1, 2]. One of the fundamental principles of efficient performance of the immune system of its homeostatic role is the coordination of activation processes and suppression due to intraimmune regulation. Regulation mechanisms of the immune response are induced simultaneously with the starting of the effector reactions and imbalance of these processes may underlie the impaired immune response and development of different clinical variants of immune pathologies [3, 4].

The literature has accumulated extensive evidence of the role of various factors responsible for the direction and the immune response strength.

However, the responsible mechanisms for coordination of activation and suppression remain unclear [5, 6, 7, 8]. The question of the formation of different phenotypes of immune deficiency and identification of the key changes of immune status in chronic autoimmune or infectious inflammation on the background of disregulatory processes is being still discussed.

The aim of the study was to identify disregulatory formation mechanisms of autoimmune and infectious phenotypes of immune deficiency.

Materials and methods. 50 patients were examined in the dynamics of progression of HIV infection and 89 patients with multiple sclerosis (MS) at activation stage and full clinical remission. 40 healthy blood donors consisted the control group. Bioscience has investigated the subpopulation structure and the expression of activation (CD25, HLADR) receptors, the intracellular content of signal and effector factors (Foxp3, IFN-γ, IL-4) lymphocytes in the peripheral blood by the method of flow-laser cytometry using the Cytomics FC 500 cytometer (BeckmanCoulter) and monoclonal antibodies production Beckman Coulter. The results are presented as percent positive cells (Mean ±

SD). The assessment of proliferative capacity of T- cells was carried out using RBTL with radiometric count based on β-the counter BETA-2. The results were expressed in impulse per minute (imp/min) and the stimulation index (SI) was calculated, that is ratio the inclusion of mark in the presence and in the absence of mitogen PHA. The determination of the relative affinity of IgG antibodies to HIV antigens was carried out on the basis of the methodology R. W. Luxton, E. J. Thompson [9] with the calculation of the reduction factor affinity (KRaf). The content of cytokines in blood serum (IFN-γ, IL-4, TNF-α) was determined by immune-enzyme analysis method using test systems manufactured by "Vector-Best" (Russia). The data obtained were interpreted on the basis of the pathogenetic principle of the immune system assessment [10]. Analyzing the characteristics of immunogenesis, the stages of recognition, activation, proliferation, regulation and implementation effect were characterized. Statistical data processing was performed using software package Statistica 7.0.

Results. It has been revealed that people infected with HIV in the terminal stage of secondary acquired immunodeficiency at decrease as the total number of CD4+-lymphocytes and the number of naive CD4+CD45RA+T-cells providing the first detection of antigen the number of peripheral CD4+Foxp3+T-lymphocytes was increased. The indicator reflecting the proportion of Treg in the total pool of CD4+T cells, which is much higher than in the control group is mostly evident. In that case, proliferative ability of T-lymphocytes has been significantly suppressed, which SI RBTL has confirmed, although the expression of markers of early (CD3+CD25+) and late activation (CD3+HLADR+) having exceeded the reference values.

Hyperimmunoglobulinemia is revealed in the humoral link at significantly reduced ability to bind specific antigen which is confirmed by the value of the coefficient of affinity decrease of anti-HIV IgG antibodies. In addition, hypercytokinemia of opposed cytokines IL-4 and IFN-γ is recorded. Thus, in the conditions of the formed secondary immunodeficiency mediated by HIV infection substantially the coherence of processes of activation and suppression, involving immunoregulatory mechanisms associated with increased negative regulation are impaired (tab. 1).

In patients with MS during the clinical manifestation in circulation the number of T lymphocytes expressing the markers of early and late activation in the amplification of proliferative properties is increased which is verified by the importance of SI RBTL to T-cell mitogen. CD4+subpopulation is characterized by an increase of CD4+CD45RA+- naive cells in the decline both total CD4+CD25+Foxp3+regulatory lymphocytes and their portion of the total population of CD4+T cells. The increase of the number of mature B- cells and production of IgM is revealed in the humoral link. Cytokine spectrum blood serum changes are manifested by the increase of the content of IFN-γ and as a consequence by the changes in the ratio of immunoregulatory cytokines IFN-γ/IL-4 in the direction of cell-mediated processes. Thus, the obtained data indicate the dominance of the activation processes of the immune response that are supported by the weakening of the T-cell negative immune regulation as well (tab. 1).

Comparing the data obtained it is possible to summarize different clinical manifestation of immune-mediated pathology to be associated with different mechanisms of intramodal regulation disorders. Inhibition of recognition processes and proliferation in the amplification of negative regulation leads to the formation of immune pathology with a prevalence of infectious immune insufficiency. In activating the recognition, enhancing the proliferative properties of the lymphocytes and reducing immunoregulatory T-suppression processes intraimmune disregulation contribute to the development of the autoimmune phenotype of immune-mediated pathology.

Table 1. Parameters of cellular and humoral factors of the immune response in the AIDS HIV infection stage and during exacerbation of multiple sclerosis

Indices	AIDS	MS	Control
CD3+, %	51.20±3.77*	67.36±1.10	68.88±0.38
CD3+, 10^9/l	0.87±0.10*	1.91±0.06*	1.22±0.03
CD3+CD25+, %	3.01±0.50*	2.77±0.24*	2.15±0.17
CD3+HLA DR+, %	10.38±1.33*	9.49±0.51*	8.04±0.14
RBTL, SI	9.60±3.30*	72.12±1.97*	63.60±0.87
CD4+, %	6.60±0.85*	43.15±1.18	41.92±0.35
CD4+, 10^9/l	0.13±0.02*	1.23±0.05*	0.74±0.02
CD4+CD45RA+, %	1.4±0.7*	40.9±2.55*	29.2±6.1
CD4CD45RA, 10^9/l	0.03±0.02*	1.17±0.06*	0.4±0.1
CD4+CD25+Foxp3+, %	2.7±0.3*	0.42±0.02*	1.3± 0.3
CD4+CD25+Foxp3+, 10^9/l	0.046±0.005*	0.014±0.002*	0.02±0.01
CD4+CD25+Foxp3/CD4+, %	40.9±1.8*	0.97±0.03*	3.25±1.6
CD8+, %	42.20±3.45*	22.28±1.02	21.88±0.33
CD8+, 10^9/l	0.62±0.11*	0.63±0.03*	0.39±0.01
CD20+, %	6.80±1.06	7.36±0.46	6.20±0.24
CD20+, 10^9/l	0.09±0.02	0.21±0.01*	0.11±0.01
IgA g/l	2.74±0.20*	1.79±0.09	1.4±0.3
IgM g/l	1.95±0.19*	1.57±0.04*	1.1±0.1
IgG g/l	14.21±0.50*	10.82±0.25	10.3± 1.3
KRaf., %	67.9±6.9	-	-

TNF-α, pg/ml	20.96±5.85*	9.48±0.96*	1.14±0.16
IL-4, pg/ml	69.60±21.75*	0.85±0.36*	1.9±0.2
IFN-γ, pg/ml	53.49±23.03*	38.37±16.91*	6.2±3.3
$K_{IFN-\gamma/IL-4}$	0.8±0.5*	45.14±8.72*	3.3±1.5

*Note – * – p<0, 05 – statistical significance of differences of indicators in comparison with the control.*

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On Some Immunomodulating Properties of Microcloning Cultures of Stevia (*Stevia Rebaudiana, Bertoni*)

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B428C0039

Abstract

The 5% decoction of grows up in an open ground stevia leaves (SG) and received by a microcloning method one' (SC) influence on the ability of practically healthy person's (PHP) and mouse (M) blood leukocytes to migrate from a glass capillary. It has been shown that the SG' 5% decoctions just as SC' one modulates spontaneous migration of blood leucocytes from a glass capillary of both - human and mouse. But, there are some differences in this modulation, that dependent on the conditions of stevia cultivation. The SC leaves' decoction mainly suppresses spontaneous migration of mammalian leukocytes, just as SG' - stimulates it.

Keywords: Stevia Rebaudiana, Bertoni, 5% decoction of native and cloning grass' lives, immunomodulation

Stevia (*Stevia rebaudiana*, Bertoni) is a perennial shrub that is indigenous to Paraguay and Brazil. The leaf and its extracts have been used as a natural sweetener [4]. The leaf extract of stevia possesses many phytochemicals, which include austroinullin, β-carotene, dulcoside, nilacin, rebaudi oxides, riboflavin, steviol, stevioside, and tiamin with known antimicrobial properties against many pathogens [5]. Stevia is also well known in traditional medicine for its use in treatment of many diseases like diabetes, high blood pressure, and weight loss [6]. Stevia synthesizes sweet glycosides. In any countries this plant serves in some to one of sugar substitutes. However, FDA (Food – and Drug Administration) – the main department of the USA supervising safety of food and drugs, carries it to «to products with uncertain safety» [10]. Besides, it was shown that stevia sugars prime the uptake of antibiotics in *Staphylococcus aureus* and *Escherichia coli* [8]. Whole leaf extract acts

against the various morphological forms of Borrelia burgdorferi *in vitro* was shown [12] The investigation of anti-inflammatory and immunomodulatory activities of stevioside and its metabolite steviol suggested that stevioside attenuates synthesis of inflammatory mediators in LPS-stimulated THP-1 cells by interfering with the IKKβ and NF-κB signaling pathway, and stevioside - induced TNF-α secretion is partially mediated through TLR4 [2].

On another words, stevia possesses immunomodulating activity. However we have not found data about immunomodulatory or anti-inflammatory activity of extracts from stevia microclones' leaves.

Research objective

To study character of the 5% decoction from grows up in an open ground stevia leaves (SG) and received by a microcloning method one' (SC) influence on the ability of practically healthy person's (PHP) and mouse (M) blood leukocytes to migrate from a glass capillary.

Materials and methods of investigation

The blood leucocytes from the 40 practically healthy persons' (PHP) aged 29.3 ± 0.9 years was examined. Blood was taken from PHP finger with the help of vacutainers and used in experiments. Blood of 30 BALB/c mice was taken from the tail vein into the heparinized glass capillary.

Stevia lives for the study were obtained at State Institute of biology and medicinal plants of the Academy of Science of Turkmenistan, in the form of dried leaves, packaged in paper bags of 50 grams. Stevia' leaves, grown up in a ground, have been collected at vegetation height in 2015.

Cultivation of stevia was spent with use of traditional biotechnological method on the culture medium by Murasige - Skuga without addition of growth regulators [1]. Sprouts cultivated in test tubes at +24ºC, 70% of humidity and light exposure 6000 lux. Leaves of cloned stevia collected when runaway reached 20 sm at length and it is good vegetated. Leaves have been dried up indoors in the same conditions as a SG and then packaged in paper bags of 50.0 grams.

The 5% stevia lives' decoction (infusum ex 10:200) was prepared in accordance with requirements of the Pharmacopoeia (1991) [11].

The decoctions were prepared just before the experiment. Migration activity of leucocytes examined according to [9]. The results express as leucocytes' migration index (LMI). The obtained data were mathematically processed.

Results

It has been established that at a whale the blood leucocytes' migration capacity in presence of stevia decoction not significantly dependents on the mammalian kind **(Fig. 1)**. In both cases decoction of SG stimulates leucocytes' migration (human LMI at middle is 112,6±21,3 and mouse leukocytes' –128,5±11,7 respectively; P>.05), decoction of SC – depressed it (LMI at middle is 79,3±12,3 and 93,1±23,6 respectively; P>.05).

But this effect is realized in different number of cases and it depends on a kind of a mammal **(Fig. 2)**. So, it has been shown that the 5% SG leaves decoction stimulates human leucocytes' migration in 65%, depresses in 5% and not changes in 30% of cases. In the same time the 5% decoction of SC stimulates human leucocytes' migration in 5%, depresses in 95% and not changes in 0% of cases. The mouse leucocytes react to stevia decoction' presence just as leukocytes of the practically healthy person **(Fig. 3)**.

Thus, the SG' 5% decoctions just as SC' one modulates spontaneous migration of blood leucocytes from a glass capillary of both – human and mouse. But, there are some differences in this modulation, that dependent on the conditions of stevia cultivation. The SC leaves' decoction mainly suppresses spontaneous migration of mammalian leukocytes, just as SG' – stimulates it.

Medicinal plants remain an important source of new medicines. However, in the past decade, research into natural products in the pharmaceutical industry has declined. However, recent technological advances have led to a renewed interest in natural products in medicines discovery [7]. Use of plants in the medicine will promote increase in the market for medicinal plants in the World and it will undoubtedly depend on the production and processing of quality plant material [3]. In this aspect using of not only traditionally grown up medicinal plants, but also their cloning seems just perspective.

DRAWINGS AND DIAGRAMMES TO ARTICLE

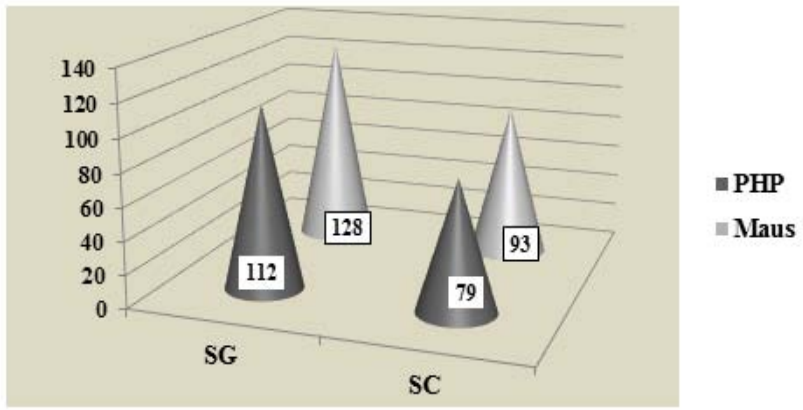

Fig. 1 The LMI meaning of human (PHP) and mouse (M) in dependence of stevia decoction' kind (SG – stevia ground, SC – cloning stevia).

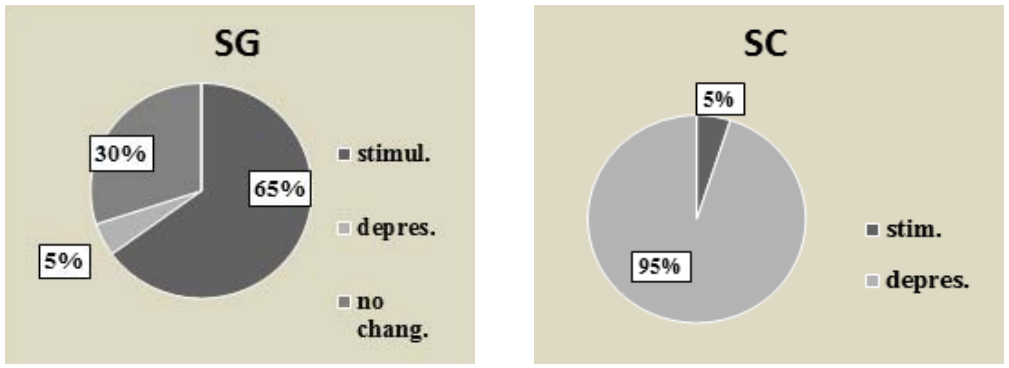

Fig. 2 Modulations of human leucocytes migration in decoctions of stevia ground – SG and stevia cloning – SC presence.

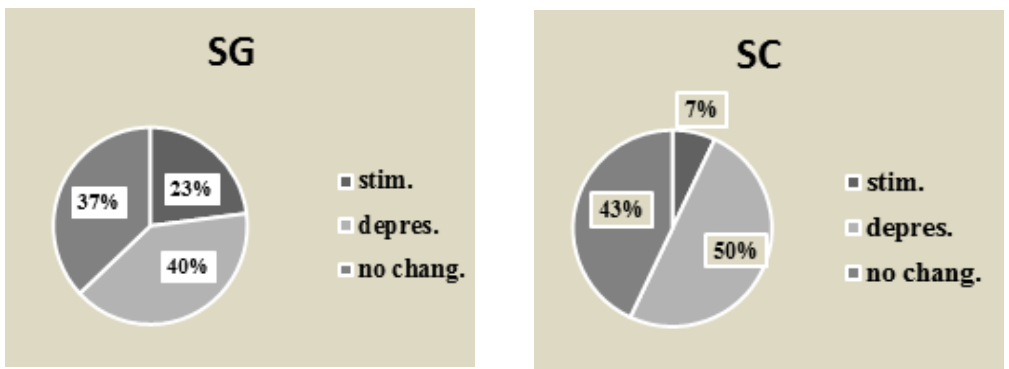

Fig. 3 Modulations of mouse leucocytes migration in decoctions of stevia ground – SG and stevia cloning – SC presence.

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The Nature of Immunological Changes in Plasmodium Falciparum Infected Children

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B428R9051

The article presents the research results of cellular and humoral immunity in children infected with *P. falciparum* malaria, conducted during the course of the disease and depending on the severity of the pathological process.

The variety and variability of the pathogen antigens explain the complexity and diversity of the *P. falciparum* malaria immunopathogenesis.

The manifestation period of malaria clinical symptoms detected depression of T-lymphocytes and CD4 +, thereby decreasing immunoreactivity index, and inhibition of specific antibodies synthesis with high values of circulating immune complexшs (CICs). Varying levels of cytokines changes in the dynamics of the infectious process were revealed depending on the severity of the disease, enabling to predict the course of *P.falciparum* malaria in children.

The data indicates the feasibility of immune disorders corrections in the early stages of the disease.

Keywords: malaria, cellular and humoral immunity, cytokines, children

Introduction

Pathogenic mechanisms of malaria infection are associated with the massive destruction of plasmodium infected red blood cells and the cascade development of immunological reactions [1-3]. A severe course of infection and systemic organ lesions are more frequently observed in *P. falciparum* malaria. Defects of immunoregulatory mechanisms of patient response may lead to the development of disease recurrence and a parasitical asymptomatic carrier state [3-6]. Many aspects of the pathogenesis of malaria still remain poorly understood, in particular the features of the development of specific immunity in children and associated with them the progress of malaria infection and disease outcomes. In the immune cells the most antimalarial activity has been shown by macrophages, T cells and a number of cytokines secreted by

them [7-9]. It is known that cell-mediated immunity only works in cooperation with the humoral immunity and with the participation of the complement system. There are very little information regarding these issues and activity of phagocytes in children affected with malaria in scientific journals, and the results of individual fragmentary studies are highly controversial and apply only to adult patients. The aim of this study was to investigate the mechanisms of immunological response in children with *P. falciparum* malaria.

Materials and Methods

We examined 124 patients with *P. falciparum* malaria at the age of 6 months to 14 years that were hospitalized in Clinical Infectious Diseases Hospital in Dushanbe, as well as at the regional hospital of Khatlon region of Tajikistan.

In most cases they were school – age children (56%). The analysis of morbidity revealed seasonality: the peak incidence was recorded in July-August and early autumn (September-October). 39 patients were diagnosed with mild forms of the disease, 60 – moderate form and 25 – severe form.

According to patient examination the mild and moderate forms of the disease were dominating (34 and 47.6% respectively), mainly due to their early admission. Clinical manifestations of malaria were depended on the age of children: in very young children early symptoms of intoxication prevailed, dyspepsia, diarrhea, fever – had an intermittent character and there was no typical malarial paroxysm. Malaria symptoms in children of older age groups did not differ much from the adults.

Verification of the diagnosis was based on clinical, epidemiological data and the results of microscopic examination of blood smear. In all patients, along with the standard, clinical and laboratory tests the number of indicators of the immune status were performed that included: the T-immunity (CD4+, CD8+ ,CD20+), the content of serum immunoglobulins of three main classes - A, M, G, the level of circulating immune complexes (CIC), C3 complement, as well as the concentration of key serum cytokines (TNF-α, IL - 1β , IL – 6, IFN-γ, IL – 2, IL – 4) that have been studied in the dynamics of infectious process (at the peak of the disease, early and late periods of recovery) and depending on the severity of the disease.

Results and discussion

The study of the immune response dynamics in *P. falciparum* malaria revealed disturbances of immunoregulatory mechanisms in the different forms of the disease and their severity depend on the severity of the disease.

The study of cellular and humoral protective factors revealed that in patients

with mild form of tropical malaria indicators of cellular immunity at all stages of the disease did not differ from those of the control group (54,6 ± 8,0%, 56,7 ± 8, 5 and 59,1 ± 10,0%, respectively, in the peak of disease, during the early and late recovery) ($p > 0,05$), but the immunoregulatory index (IRI) in the peak of disease was reduced (2.0).m Low production of early antibodies (IgM - 1,05 ± 0,9 versus 1,8 ± 0,7 g/l in control) in the early stages of the disease contributed to the reduction of the index of antibody activity (IAA).

High levels of IgG (13,6 ± 0,7 11.2 ± against 0.35 g/l in control, $P < 0.01$) nd CIC (2,4 ± 0,13 vs. 0.84 ± 0.04 g/l in the control, $p < 0.001$) decreased gradually toward recovery, but also late stage of recovery in the absence of parasites in the peripheral blood are still significantly higher than the control level (0,98 ± 0,04 g/l).

In the peak of the mild form of *P. falciparum* malaria in the composition of CIC immunoglobulin G was dominated, which, apparently, was associated with a more active IgG binding of the antigenic determinants of *P. falciparum*.

This was also reflected in the significant increase in the level of the absolute content of B cells in the period of the disease (30,7 ± 6,0 vs. 13,9 ± 3,5%, $p < 0,05$), which is obviously connected with the expressed neo - antigenic stimulation of B cell part of the immune system. The values of C3 blood serum in all periods of the disease remained normal. The dynamics of cellular and humoral immunity in patients with mild form of *P. falciparum* malaria showed a weak immune restructuring of the organism due to brief irritation of the immune system by antigens of the parasite. Obviously, it is linked to more frequent occurrence of relapses and repeated cases of the disease after suffering from mild form of *P. falciparum* malaria (77.3%).

Changes of the immune status in the moderate form of *P. falciparum* malaria were significantly different from those of the mild form. The most pronounced changes were observed at the peak of malaria infection and early recovery: the absolute number of T-lymphocytes were significantly reduced (43,1 ± 6,4 and 49,4 ± 7,0%, respectively, versus 66,7 ± 4,7% in control, $p < 0.001$) and CD4 + (18,6 ± 5,0 and 28.1 ± 6.3 g/l vs. 46.3 ± 5.0% in control, $p < 0.01$).

In these stages of the disease the activation of humoral immunity was observed, that was expressed in a significant increase in IgM concentrations of (1,8 ± 0,3 and 1,6 ± 0,58 g/l, against 1,02 ± 0,07 g/l in the control, $p < 0.01$) and the IAA index (2.4). In the peak of moderate form in the composition of the CIC large-sized complexes containing IgM was dominated. Active immune restructuring determines a favorable outcome of the disease, that is evidenced by the absence of cases of relapses and the formation of asymptomatic carriage with moderate course of illness.

Severe form of *P. falciparum* malaria was characterized by almost the same quantitative and qualitative changes of cellular immunity, as well as in

cases of moderate form, but there was a significant reduction of IRI during the crisis period (1.96 versus 2.77 in the control, p <0.01). Indicators of humoral immunity did not differ from those of healthy children, indicating the low production of antibodies M and G class with this form of disease severity.

In the peak of the disease a significant increase of CIC was observed (2,43 ± 0,2 vs. 0,84 ± 0,04 g/l, p <0.001) and, as in the moderate form immune complexes of large dimensions prevailed in their composition containing IgM. The obvious depression cell humoral protective factors and of high CIC values requires immunotherapy.

As a result, studies have found a significant increase of pro-inflammatory cytokines in almost all periods of the disease with a tendency of reduced rates in the period of late recovery, but not reaching the control values (p <0,05).

An exception is the content of IFN-γ: detected at a low level (p <0,05) in the peak of disease, following an increase in value in the period of recovery, which is a major factor, activating macrophages and promotes more effective destruction of intracellular pathogens. The findings suggest a substantial suppression of specific antiparasitic immunity in *P. falciparum* malaria. The level of cytokines correlated with the severity of the disease: the highest rates found in severe *P. falciparum* malaria, perhaps due to excessive activity of monocytes/macrophages, responsible for the production of pro-inflammatory components of regulation, as well as the release of reactive radicals. The involvement of the monocyte-macrophage cells, followed by the active elaboration of the whole complex of biologically active substances, contributing to cellular and circulatory disorders can be traced with moderate and severe *P. falciparum* malaria, it is probably one of the pathological links of severe anemia, brain damage and non-specific inflammation. In considering the role of cytokines in the development of these or other disorders one have to take into account the diversity of their spectrum of biological activity [1, 3, 6, 7]. It is known that IFN-γ, naturally called immune interferon, also has the ability to suppress the proliferation of erythrocyte germ cells [3, 8].

One particular interest is the study of pro-inflammatory cytokine antagonists – IL-4 produced by Th2-cells. The concentration of this cytokine was significantly greater than control values in all periods of the disease, the highest content was during the peak of *P. falciparum* malaria (p <0.01), suggesting an imbalance of immunoregulatory mechanisms of Th2 type.

The predominance of Th2-way immune response determines the suppression of cell-mediated immunity in the early stages of the disease. In addition, the imbalance of cell-cell interactions and the reduction of immunomodulatory properties indicate on failure of stimulation of own adequate immune response as a reaction to malarial infection caused by *P. falciparum*. Although replicative malaria pathogen activity must be an inductor of interferon production, it does

not happen due to lack of effective immunological response to various stimuli, including proinflammatory cytokines, pathogen itself and its products of metabolism. This may leads to severe course with the development of serious complications, and the concentration of these cytokines may be a predictor of it. It should be noted that imbalance of immune mechanisms is short and in the recovery period the dominance of Th1- type immune response which is induced by IFN-γ production is observed, as well as lower levels of pro-inflammatory cytokines that exacerbate cardiovascular and autoimmune processes. Increasing concentrations of IL-4 has compensatory rather than active counter - regulatory character to proinflammatory cytokines that implies more stabilizing function in the inflammatory response. Together, these identified features demonstrate the complexity and diversity of processes of immune cytokines in *P. falciparum* malaria.

P. falciparum malaria is characterized by depression of T cell immunity, the extent of which is depended on the severity of malaria infection.

It is important to note the low levels of T-lymphocytes in moderate and severe forms of the disease, a significant reduction of T helpers, relatively intact level of T suppressors and suppression of humoral immunity.

These changes are most pronounced in the moderate form of the disease, moreover, they are accompanied by a substantial increase in the functional activity of cells having killer cytotoxic activity. These changes can be considered as a regular and adequate, since they provide sanogenesis and complete recovery in *P. falciparum* malaria, in case of timely and effective chemotherapy, as well as due to the genetically determined immune reaction adaptation of organism aimed at binding and elimination of the pathogen and its antigens. The imbalance of cytokine profile in *P. falciparum* malaria is an important pathogenetic factor in the development of severe and recurrent forms of the disease, since the formation of a impaired immune response to parasitic antigens contributes to adverse outcomes.

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Impact of Il10, Ifn Gamma and Tnf Alfa in Evolution of Patients Affected by Chronic Delta Viral Infection

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B428R9054

Abstract

Hepatic disorder caused by the hepatitis D virus (HDV) is not always diagnosed and the efficacy of its actual therapy is rather low in our country.

The contribution of cellular immune responses to liver damage and elimination of the virus in hepatitis D infection has not yet been clarified due to insufficient data on this topic. And the identification of some immunological factors with possible impact on the evolution of HDV and determinants for the choice of antiviral therapy for patients with HDV are actual at the moment.

Aim of the study was to evaluate immunological parameters: IL10, TNF-alfa, IFN- gamma (ELISA) in relation by the activity of ALT and AST, BEA score, the degree of fibrosis (assessed by Fibroscan or Fibromax) in patients with chronic liver diseases induced by HDV. The study involved 170 patients with chronic Delta viral infection. The participants were divided into two groups: *1*. ARNHDV+/ADNHBV – (96 patients); *2*. ARNHDV+/ADNHBV- (74 patients). Conclusion: the study results suggest that Delta virus would have more immunopathogenic implications that should be taken into consideration in the study of patients with chronic Delta viral infection, titration of values of IL10, TNF-alfa, IFN gamma would also be appropriate in the choice of an adequate patient for antiviral therapy.

Bakground

The incidence of hepatic disorders caused by chronic Delta viral infection has been increasing in the Republic of Moldova, thus every 6-th HbsAg-positive subject is diagnosed to be antiHDV positive. It should be mentioned that in our country the screening of HDV early stage is not available, so patients with detected HDV have an advanced degree of fibrosis. The persistence of virus B and D depends of the host immune response an virological characteristic.

The host response to hepatitis viruses involves various components of the immune system, including T-lymphocyte immune-regulatory cytokines.

Cytokines are a group of protein molecules involved in various biological processes including growth, differentiation, cell survival, hematopoiesis, immunological functions, inflammation, apoptosis, necrosis and fibrosis.

The control of cytokine production is highly complex, while the effects of cytokines are widespread throughout multiple regulatory molecule networks.

The liver is a major organ in the production of cytokines. They play an important role in liver growth and regeneration, as well as in inflammatory processes including viral liver disease, in liver fibrosis and cirrhosis. Changes in various cytokine activities have been reported during HBV, HCV and rarely in HDV infections, while an imbalance of pro-inflammatory and anti-inflammatory cytokine production influences their immunopathogenesis. At the moment there are few therapy options to treat HDV (only PegInterferon/with or without analogous antiHDV nucleosides) and their efficacy is reduced. So, the correct identification of the group of patients with HDV for the administration of an adequate therapy is an actual problem that has to be solved.

Aim of the study

to evaluate the impact of the level of IL10, TNF alfa, IFN gamma in evolution of liver diseases induced by hepatic virus delta and their correlation with hepatic fibrosis and BEA score.

Objectives of research

1. To analyse the relative importance of the main demografical factors in chronic HDV infection and their correlation with severity of diseases.
2. To evaluate the clinical-biochimical features from patients affected by chronic HDV infection.
3. To determine the level of hepatic fibrosis (by Fibroscan/Fibromax) in patients with hepatic disorders due to chronic HDV.
4. To measere levels of interleukins: IL-10, TNF alfa and IFN gamma in patients affected by chronic HDV infection and their correlation of BEA score.
5. To correlate serum level of IL 10, TNF alfa and IFN gamma with biochimical markers of liver disease and with grade of hepatic fibrosis.
6. To evaluate the interrelations between level of IL 10, TNF alfa and IFN gamma with virusological response by antiviral therapy.

Material and methods

One hundred and seventy patients with chronic HDV were included in the study, all of them were born in the Republic of Moldova. All patients were subjected to: thorough history taking and clinical examination, liver functional

test, serological studies (HbsAg, antiHBcorAg, HbeAg, antiHBeAg, antiHDV Ag, antiHDV IgM and IgG, antiHCV by ELISA), ADN HBV and ARN HDV (quantitative) were revealed by the PCR method. 69 patients was investigated for immunological status: imunoglobulins A, M, G, E by ELISA; autoantibodies – ANA, antiLKM3, antiDNA – by ELISA or imunofluorescence methods, and interleukins: evel of IFN- gamma, TNF-alfa, IL2, IL10 were assessed by ELISA technique. Workup also included for all patients: abdominal ultrasound, superior endoscopy, and hepatic fibrosis was detected by Fibroscan in 57% of cases and by Fibromax – in 33%. All the patients were scored BEA using http://hepatitis-delta.org/physicians-and-scientists/calculators/. The SPSS 12 program was applied for the statistical analysis. In finally, all patients were divided into two groups: group I was formed of 96 patients with ARNHDV+/ADNHBV-; group II was formed of 74 patients with ARN HDV+/ADNHBV+.

Results

evaluation of patients' demographic data allowed determining the profile of a patient with HDV in our country: female predominance (54%), young age (39.5 ± 5.45 years), interfamilial route of HDV acquisition (31.8% of cases come from HDV/HBV positive families), health workers constitute about 26.7% of patients, and 28% of patients underwent splenectomy.

Table 1. Clinical date for studies group

Parameter	Groupe 1 (n=96)	Groupe 2 (n=74)	p
Age	42±2.8	33.5±6.1	P<0.05
Male:female	41:55	39:35	P<0.05
Splenectomy	19%	31%	P<0.01
Past antiviral therapy: Pegasis	22%	17%	P<0.05
Nucleotide/nucleoside analogous	9%	2.1%	P<0.01

The serologic study identified two groups of patients: one group with ARNHDV+/ADNHBV- (75%) and the other – with ARNHDV+/ADNHBV – (24.7%). Antiviral therapy with Peginterferon (within 48 weeks) was administered to 33% of patients, 1/3 of which discontinued the treatment within the first year due to adverse effects. The negativity of ARN HDV after the therapy occured in 28% of patients. The assessment of biological analysis of the studied patients revealed the increase of ALT 98.78 ± 26.7 mmol/l (86.32%), gGTP level 63.7± 13.6 U/l (69.5%), bilirubin indices 38.9 ± 11.7 mmol/l (38.4%), decrease of albumin 26.4 ± 3.79 g/l (41.3%), decrease of cholinesterase 3876.8 ± 982.7 U/l (43.87%), increase of INR 1.7± 0.67(43.6%),

significant thrombocytopenia <50.000 cel3 (61.5%). A 23% increase of alfa-fetoproteins was noted in the patients at the onset of study and in the period of the follow up their increase was 35.7%.

The study of cytokine level revealed the increase of TNF-alfa in patients with ARNHDV+/ADN HBV+ vs those of group I ($p<0.05$). Our study showed that TNF-alpha level increases in patients with chronic liver disease induced VHD and VHB reactivation. The level of these cytokines was also increased in patients with ARN HDV+/ADN HBV - after the treatment without virusologic response vs ARNHDV+/ADNHBV - not treated patients ($p<0.036$).

The increase of IL10 indices was revealed in ARNHDV+/ADNHBV- patients after the antiviral therapy vs the patients from group II ($p<0.048$).

The reduced value of IL 10 was noted in patients with increased ALT activity vs those with the ALT value within normal limits ($p <0.05$). The reduced value of IL 10 was identified in the patients from group II with the advanced degree of fibrosis F3-F4 ($p<0.05$). So it can be used to evaluate disease activity.

The relevant decrease was noted in the IFN-gamma value in the patients with ARNHDV+/ADNHBV - versus those of group II ($p<0.05$), also was indentified that patients after antiviral therapy with PegIFN (who achieved virusological response) had lower IFN-γ concentrations (88.2±15.3 pg/ml) than non-responder patients (162.8±19.3). Monitoring IFN-γ levels can aid clinicians to further identify high risk patients who may fail PegIFN therapy and allow for the adoption of appropriate strategies for more personalized medicine.

Table 2. Biochimical and immunological parameters in studies group

Parameters	Group 1 (n=96)	Groupe 2 (n=74)	p
ALT (U/L)	98.5±6.9	103.6±10.4	P <0.05
AST (U/L)	87.9±9.15	97.5±8.35	
Platelet count (x109/l)	97.5±11.6	71.3±13.7	P<0.05
IL10 (pg/ml)	14.9±3.2	10.5±2.7	P<0.01
TNF alfa (pg/ml)	0.81±1.21	3.31±0.87	P<0.001
IFN gamma (pg/ml)	81.4±6.7	120.6±13.2	P<0.01

The assessment of patients'dynamics revealed a progressive evolution in the hepatic process in 67 % of patients (biochemical decompensation and vascular decompensation). The assessment of fibrosis (by Fibscan) for all patients (n=170) revealed the prevalence of F4 in 44.3%, F3 - 27.3%, F2 – 27.4%; the Fibromax assessment revealed F4 in 31.7%, F3 – 27.6%, F2 – 32.3%, F1-18.4%.

Table 3. Evaluation of fibrosis in studies group

Parameters by Fibroscan	Group 1 (n=96)	Groupe 2 (n=74)	p
F1 (<7 kPa)	11.43%	25.6%	P<0.01
F2 (7.1-9.0 kPa)	16.6%	28.3%	P<0.05
F3 (9.1-12.0 kPa)	42.7%	33.7%	P<0.01
F4 (>12.1 kPa)	27.1%	11.7%	P<0.01

The study of fibrosis degree (Fibroscan) in patients in dynamics (on average within 7 years) revealed F4 – 53%, F3 – 31%, F2 – 16%. The assessment of BEA score pointed the presence of class A in 15%, class B – 39.8%, class C – 45.2%, but after the repeated assessment of those patients within the average period of 5 years the BEA data were : class A – 6%, class B – 47%, class C – 47%.

Discussion

In our country the incidence of HDV is high as we are experiencing a deficit of HDV screening at the level of primary care. In the majority of cases patients are diagnosed HDV only when they are examined by a specialist and this fact delays the early diagnosis of the disease. Our study shows that patients with HDV are of young age, good for work and constitute an important reservoir of intrafamilial infection. The patients were diagnosed HDV when they already had progressive hepatic disorder with biochemical decompensation and advanced degree of fibrosis. The BEA score revealed the prevalence of class C, that is the group of patients who are not applicable for antiviral therapy and can be put on the waiting list for liver transplantation only.

HDV has direct action on the hepatocytes and indirect (immunogenic) action, by the recent report that HDV viremia, despite high variability, has no correlation with biochemical activity or staging and grading of live [5].

Tabel 4. Authors and imunological studies in liver diseases by delta infection

Year	Autors	Studies
1990	Karrayanis et.al	Immunization of woodchucks with recombinant hepatitis delta antigen does not protect against hepatitis delta virus infection [8, 9]
1997	Nissini et.al.	MHC-II-restricted epitopes of the HDAg [12]
1998	Accapezzato et.al.	Extracellular processing of HDAg [1]
2004	Huang et.al	MHC-I-restricted epitopes [8]

2006	Aslan et.al	Perforin-positive CD4 T cells in HDV [2]
2009	Grabovski et.al	Strong HBV-specific T cell responses in HDV patients [4, 5]

The contribution of cellular immune responses to liver damage and elimination of the virus in hepatitis D infection has not yet been clarified due to insufficient data on this topic.

Over 100 different inflammatory cytokines have been identified, which regulate the balance between humoral and cell-mediated immunity.

Inflammatory cytokines participate in the defense against viral replications and modulating the host immune function. Th1 cytokines (e.g., interferon-γ and IL-2) are key mediators for host antiviral immunity, while Th2 cytokines (e.g., IL-4 and IL-10) may attenuate these inflammation responses.

Dysregulation of T-helper (Th1/Th2) cytokine production may play an important role in immunopathogenesis of chronic hepatitis D. In HDV patients, interferon-γ (IFN- γ) and IL-12 levels drop as a result of the enhanced IL-10 production, which serves as a possible down-regulator of IFN-γ. IL-10 is an important immunoregulatory cytokine and its main biological functions seem to be the limitation and termination of inflammatory responses and the regulation of differentiation and proliferation of T cells, B cells, natural killer cells, antigen-presenting cells, mast cells and granulocytes. Moreover, IL-10 promotes the development of a type 2 cytokine pattern by inhibiting IFN-γ production. [17]

Our study revealed some aspects concerning clinical and immunological evolution of patients with Delta infection, namely the relevant increase of IL 10 in patients with ARNHDV+/ARNHDV- after antiviral therapy (with PegInterferon) who obtained an effective virusologic response, on the other hand, IL 10 was detected in reduced values in the patients with advanced fibrosis and in the patients with increased ALT acrivity. These results suggest that IL 10, being an anti-inflammatory cytokine, plays a part in the evolution of chronic Delta virus infection. IL-10 has been reported to be one of the potential factors in establishing immune suppression and viral persistence.

The possibility of titration of these cytokines before the antiviral therapy would influence the adequate choice of patients. TNF-alfa was increased both in patients with ARNHDV+/ADNHBV+, and those with ARNHDV+/ADNHBV- who did not respond to antiviral therapy with Interferon, so TNF-alfa, being a pro-inflammatory cytokine, is of definite importance for patients with active double infection and for those who did not obtain any virusologic response after the therapy with Interferon.

Conclusion

The obtained results revealed an alarming situation concerning the intrafamilial transmission of HDV in our country on the background of the deficit of screening of this pathology at the level of primary care. It should be noted that Delta virus would have more immunopathogenic implications that should be taken into consideration on examination of patients with HDV and titration of IL10, TNF-alfa indices would be timely in the choice of adequate patients for antiviral therapy.

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The Study of Indicators of an Autoimmune Disease in the Dominance of Yeasts and Rare Species Escherichia in Conditions of Intestinal Dysbiosis

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B428C0026

Abstract

The paper presents the results of a microbiological and immunological examination of children with intestinal microbiocenosis disorder using vegetation of yeast fungi of the genus Candida and rare forms of bacteria of the genus Escherichia. The biological properties of yeast fungi have been studied.

The presence of autoimmune antibodies to the tissues of the human body has been revealed. In the blood of the first group examined were most often found APC (26.7, 10 and 0%, respectively). In patients with yeast and Escherichia dysbacteriosis of the intestine, the level of antimitochondrial antibodies was 25-35 IU/ml, which exceeds the reference indices (0-25 IU/ml). Also among the representatives of the first group were antibodies to smooth muscles in a titer of 1: 80 among 9 children (30%) in the absence of corresponding antibodies in the second and third group of subjects. The indices of interleukins – 17A, 23, 35, which participate in the development of autoimmune pathology are established.

Keywords: autoimmune pathology, autoantibodies, dysbacteriosis, interleukins

Introduction

In violation of microecological balance of the intestine, immunopathological reactions develop [1]. The problem of intestinal dysbiosis and allergy is still present [2]. There is also an increase in autoimmune pathology [3]. The incidence of mycosis is increasing annually due to the spread of immunodeficiency states [4]. Risks of development of clinically expressed candidiasis are associated with age and are maximal in the period of newborn, young children, pregnant

and elderly. The pathogenesis of candidiasis of the digestive system is determined by the ratio of microbial pathogenicity factors and the decrease of microorganism resistance. Fungal invasion is promoted by active proteinases, phospholipase, hyaluronidase, hemolytic factor, due to which fungi penetrate through the glycoprotein layer of the intestinal mucosa [5].

Nonspecific resistance of the gastrointestinal tract is represented mainly by the immune system associated with the intestine [6]. Intraepithelial lymphocytes that prevent the penetration of fungi of the genus Candida through lamina propria and aggregation in Peyer's patches, B-lymphocytes-producers of secretory IgA, IgM, blocking the ability of fungi to adhere, constitute the cellular part of this system [7]. Obligatnye representatives of normal intestinal microbiota (lactobacillus, bifidobacteria, E. coli) also play a protective role, reduce the ability of yeast fungi to inactivate lysozyme and form biofilms [8].

An imbalance in the cytokine status develops, immunoregulation is impaired [9]. It has been established [10] that the pro-inflammatory cytokines of the IL-17 family play a role in the development of chronic autoimmune processes; IL-23 stimulates the development of Th17 and the production of IL-17A. IL-35 is anti-inflammatory, suppressing the differentiation of Th17 and determining the balance in the development of inflammatory reactions [11].

Purpose of work

The aim of the work was to estimate the number of autoantibodies in the blood serum of patients with intestinal dysbacteriosis, with predominance of yeast fungi and rare species of Escherichia. Objectives of the study: to study the presence and level of autoantibodies and cytokines: interleukins-17A, 23, 35.

Materials and methods

Under supervision were 90 people aged 2 to 3 years. All patients were divided into three groups: 30 people with intestinal dysbacteriosis syndrome and dominance of yeast fungi of the genus Candida and rare species of Escherichia: E. coli inactive, E. blattae, E. hermannii, E. fergusonii, E. vulneris (first group), 30 children with intestinal dysbiosis 2 degrees (second group), 30 - without pathology (third group). Exercises were taken in accordance with the rules of aseptic and antiseptic in sterile containers, the amount of yeast fungi was determined when sowing on Saburo's nutrient media, the Escherichia was cultivated on Endo media. [12] Identification was carried out using biochemical sets of Candida-Test 21 (Lachema, Czech Republic), (BioMerieux, France).

To determine the level of autoantibodies, indirect immunofluorescence and ELISA were used. Antibodies in the serum to thyroglobulin (ATG), to mitochondria (AMA), to smooth muscle (ASM), antineutrophil cytoplasmic antibodies (ATCA), antiparietal antibodies (APC) were investigated. IL-17A, 23.35 serum levels were determined in ELISA using commercial test systems manufactured by Bender MedSystems (Austria) [13], Uscscn Life Science Ins.

Wuhan (China). The results were systematized in the program "STATISTICA 10".

Results

Analyzing the results of Fig. 1, it is necessary to note in the first group of the examined the more pronounced vegetation of yeast fungi of the genus Candida: C. albicans (40, 16.7 and 6.7%), C. glabrata (26.7, 13.3 and 0%), C. kefir (33.3, 20 and 10%).

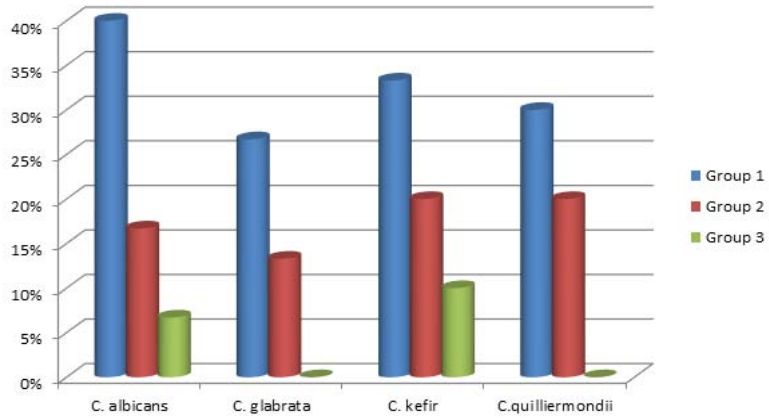

Fig. 1. Frequency of yeast fungi of the genus Candida

Table 1. The frequency of detection of autoantibodies in the serum of patients

Group study	ATG	AMA	ASM	APC
1	4 (13,3%)	35 IU/ml	9 (30%)	26,7%
2	3 (10%)	28 IU/ml	2 (6,7%)	10%
3	1 (3,3%)	0-25 IU/ml	0	0%

By stating the figures in Tab. 1, we can draw conclusions: among the surveyed the first group of APC (26.7, 10 and 0%, respectively) were more often found. In patients with yeast and Escherichia dysbacteriosis of the intestine, the level of antimitochondrial antibodies was 25-35 IU/ml, which exceeds the reference indices (0-25 IU/ml). Also among the representatives of

the first group were antibodies to smooth muscles in a titer of 1:80 in 9 children (30%) in the absence of corresponding antibodies in the second and third group of subjects.

In the blood of patients of the first group, interleukins - 17A, 23.35 ($p<0.05$) were detected more often (Tab. 2.).

Table 2. The content of interleukins in the blood serum

Group study	Number of subjects(n)	IL-17A	IL-23	IL-35
1	30	4,8±0,8*	48±5,1	51±4,02
2	30	2,3±0,2	31±3,2*	42±3,8
3	30	0,6±0,15*	26±2,8	31±2,9

** value p<0.05*

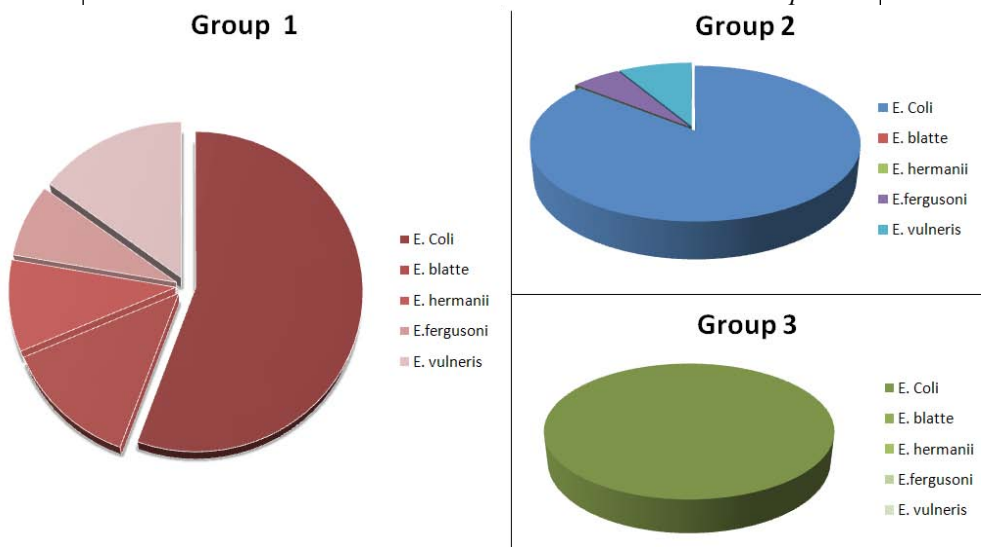

Fig. 2. Frequency of vegetation of Escherichia in the material under study

Rare species of bacteria of the genus Escherichia are more often detected in patients with grade 4 dysbacteriosis (Figure 2). Also, microorganisms isolated from patients of the first group had more frequent hemolytic activity (Tab. 3).

Table 3. Hemolytic activity of strains of bacteria of the genus Escherichia

Group study	E. coli	E. blatte	E. hermanii	E.fergusoni	E. vulneris
№ 1	6 (20%)	0	0	4 (40%)	2 (20%)
№ 2	2 (6,7%)	0	0	0	0
№ 3	0	0	0	0	0

Conclusions

Therefore, in patients with microecological disorders of the intestine with dominance of yeast fungi of the genus Candida and rare species of Escherichia: E. coli inactive, E. blattae, E. hermannii, E. fergusonii, E. vulneris more often determined autoantibodies to mitochondria and smooth muscles, interleukins - 17A, 23.35 ($p<0.05$). It is necessary to take into account the development of autoimmune processes in patients with dysbacteriosis of the fourth degree of intestines in children in the development of methods of prevention and therapy.

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Mortality, Lifetime and Predictors of Survival in Rheumatoid Arthritis Patients

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B428C0064

Abstract

The conducted study included 590 rheumatoid arthritis (RA) patients – 492 women and 108 men of 44.4±12.7 (mean±SD) years old (Me=44,2 years, 2625 visits) and the analysis of the autopsy data of 171 RA patients dead in Minsk clinical hospitals for the period from 1981 up to 2005. Overall death rates and partial mortality rates from musculoskeletal system and connective tissue diseases in adult population of Minsk for the 5-year period (2001-2005 years) were evaluated. The partial mortality rate from RA in Minsk for the 5-year period was 0.027% (CI95 0.019-0.039) and the average annual lethality rate from RA – 0.60% (CI95 0.13-2.65%). The average lifetime in RA patients dead in 2001-2005 years was 66.0±12.8 years in the general patient group.

The average lifetime in RA patients for the 25-year period increased by more than 10 years in the general patient group – from 55.6 to 66.0 years.

The average lifetime in RA patients with glucocorticosteroid dependence was lower than without it: 60.7±12.0 years vs 63.3±10.7 years. The average lifetime in RA patients was 59.8±12.4 years and 62.9±10.9 years in those with secondary amyloidosis and without it, correspondingly. The median of survival in the general group of RA patients was 69.1 years. The probability of surviving up to 50 years was about 95%, up to 60 years – 85% and up to 70 years – about 50%. The survival time in RA patients was significantly lower ($p<0.01$) in case of the following clinical conditions: RA onset at the age under 36 years, rapid RA progression during the first three years of the disease, Sjögren's syndrome, rheumatoid vasculitis and secondary amyloidosis.

Keywords: rheumatoid arthritis, RA, lifetime, mortality, predictors of survival

Introduction

The rheumatoid arthritis is considered to be a central, key problem of the modern rheumatology as any advances in understanding etiology, pathogenesis and treatment of the disease have the great influence not only on the development of rheumatology but on the medicine in general [1, 2].

In accordance with the published literature data the average lifetime in rheumatoid arthritis (RA) patients is 3-4 years shorter than life expectancy in women and 5-7 years shorter than life expectancy in men and it has considerable fluctuations which depend on the population studied [3, 4].

Up to date the mortality level, lifetime and predictors of survival in rheumatoid arthritis patients have not been studied in the Republic of Belarus.

Objectives

To estimate the partial mortality and the lethality in rheumatoid arthritis patients for the 5-year period as well as to estimate the rheumatoid arthritis patients lifetime and its trend for the 25-year period and to reveal the predictors of survival in rheumatoid arthritis patients.

Methodology

Overall death rates in adults in Minsk for the period from 2001 up to 2005 year, partial mortality rates from musculoskeletal system and connective tissue diseases (MSCTD) as well as the partial mortality rate from RA in adults of typical Minsk outpatient clinic № 12 service area for the 5-year period from 2001 up to 2005 year were determined.

Hospital mortality pattern for the principal rheumatic diseases in adults in Minsk for the 5-year period (2001-2005) was determined through the analysis of the postmortem examination reports of autopsies performed in Minsk City Clinical Pathologoanatomic Bureau for the mentioned period.

To estimate the RA patient lifetime and its trend for the 25-year period (1981-2005) and 5-year period (2001-2005) we analyzed the autopsy data of 171 RA patients on the basis of the postmortem examination report database of Minsk City Clinical Pathologoanatomic Bureau for the period from 1981 up to 2005.

The survival rates in rheumatoid arthritis patients (RA) were analyzed by Kaplan-Meier product-limit method. The study included 590 RA patients – 492 women and 108 men of 16.7 to 76.7 years old (2625 visits): M=44.4

(SD=12.7), Me=44.2 RA with disease duration up to 1 year at the time point of the first visit was diagnosed in 302 patients (233 women and 69 men).

The following scheme of human ontogenesis age periodization for people older than 16 years was employed (A.S. Leontyuk, 2000): the juvenile age – 17-21 (men – ♂), 16-20 (women – ♀); I period of the mature age – 22-35 (♂), 21-35 (♀); II period of the mature age – 36-60 (♂), 36-55 (♀); the elderly age – 61-74 (♂), 56-74 (♀) and the old age – 75-90 (♂ and ♀).

The predictors of survival in RA patients were determined by means of the thorough analysis of the postmortem examination reports for the 5-year period from 2001 up to 2005 year (n=37).

For the statistical analysis performance Statistica 6 software package by StatSoft Russia was implemented. The differences between qualitative parameters frequency in independent groups were estimated by Fisher exact test [5]. For the comparison of the alternative parameters failed to comply with the normal distribution Mann-Whitney U-test was used.

Results

Overall death rates in Minsk (the Republic of Belarus) and in the service area of Minsk typical outpatient clinic № 12 and partial mortality rates from musculoskeletal system and connective tissue diseases as well as from rheumatoid arthritis in adult population of Minsk outpatient clinic № 12 service area for the 5-year period (2001-2005 years) are presented in Tables 1 and 2 below.

Table 1. Overall death rates in adults in Minsk for the 5-year period from 2001 up to 2005

Parameter	2001	2002	2003	2004	2005	Mean
Minsk population, n[1]	1705900	1719500	1733900	1753600	1773300	1737240
Number of overall deaths in Minsk, n	15970	16844	16278	16362	16903	16471
Overall death rates in Minsk, %	9.4	9.8	9.5	9.3	9.5	9.5

1 – average resident population size in Minsk = (the population size in Minsk at the beginning of the year + the population size in Minsk at the end of the year)/2.

Table 2. Overall and partial death rates in adults of Minsk outpatient clinic № 12 service area for the period from 2001 up to 2005 year

Parameter	2001	2002	2003	2004	2005	Mean
Adult population of the outpatient clinic № 12 service area, n	53018	52215	50502	50486	50500	51344
Number of overall deaths in the service area, n	650	660	654	675	668	661
Overall death rates in the service area, ‰	12.3	12.6	13.0	13.4	13.1	12.9
Number of deaths from MSCTD in the service area, n	1	3	2	4	1	2.2
Mortality from MSCTD in the service area, ‰	0.019	0.057	0.039	0.079	0.020	0.043
Number of deaths from RA in the service area, n	1	2	1	2	1	1.4
Mortality from RA in the service area, ‰	0.019	0.038	0.020	0.040	0.020	0.027

The average annual mortality rates in 2001-2005 were 9.5‰ (CI95 9.3-9.7‰) in the general population of Minsk and 11.7‰ (confidence interval of 95% (CI95) was 11.6-11.9‰) in adults. The average annual mortality rate in adults of Minsk outpatient clinic № 12 service area was 12.9‰ (CI95 11.9-13.9‰) ($p>0.1$, non-significant). There were 11 patients dead from MSCTD for the mentioned 5-year period (2.2 per year) among them. The partial mortality from MSCTD in adults of Minsk outpatient clinic № 12 service area was 0.043‰ (CI95 0.032-0.058‰). The mortality from MSCTD made minor contribution to the overall death rate for the mentioned period - 0.33% (CI95 0.10-1.14%).

There were 7 rheumatoid arthritis patients among 11 patients dead from MSCTD. The partial mortality rate from RA was 0.027‰ (CI95 0.019-0.039‰) and the average annual lethality rate from RA for the 5-year period (i.e. average annual mortality rate among RA patients) – 0.60% (CI95 0.13-2.65%).

Hospital mortality pattern for the principal rheumatic diseases in adults in Minsk for the period 2001-2005 years is presented in Table 3 below.

Table 3. Hospital mortality pattern for the principal rheumatic diseases in adults in Minsk for the period 2001-2005 years

Parameter	2001	2002	2003	2004	2005	Total
Circulatory system diseases (CSD) [1] (chapters IX and XVII of the International Classification of Diseases 10 (ICD-10)						
Acute rheumatic fever	0	0	0	0	1	1
Chronic rheumatic heart diseases	34	33	41	33	28	169
Primary infective endocarditis	6	4	15	10	10	45
Secondary infective endocarditis	6	3	3	7	11	30
Primary cardiomyopathies	6	6	5	7	3	27
Circulatory system malformation (Q20-Q28)	1	1	3	1	1	7
CSD total number	53	47	67	58	54	279
Connective tissue diseases (CTD) [2] (chapter XIII of ICD-10)						
RA (primary disease)	3	1	1	7	15	29
RA (concomitant disease)	2	1	2	2	3	8
Ankylosing spondylitis (AS)	0	2	3	1	0	6
Systemic lupus erythematosus	2	0	0	5	1	8
Systemic sclerosis (primary)	0	1	1	0	1	3
Primary dermato(poly-)myositis	1	0	0	0	0	1
Primary systemic vasculitis (pSV)	4	2	2	5	2	15
Total number (CTD + RA + AS + pSV[2])	12	8	8	20	22	70

1 – CSD or circulatory system diseases supervised by rheumatologists.
*2 – CTD – connective tissue diseases, RA – rheumatoid arthritis, AS – ankylosing spondylitis, pSV – primary systemic vasculit*is.

For the period 2001-2005 years there were 21708 in-patient deaths registered in all Minsk clinical hospitals, 17313 among them – in adults. The number of autopsies performed for this 5-year period was 13283 (2657 per year) or 74.1% of in-patient deaths in adults in Minsk. This number of postmortem examinations is considerably higher than in Moscow, Russia (<35%) and incomparably higher than in Europe (<5%). According to some investigators opinion the autopsy frequency exceeding 35-40% of deaths is the minimal sufficient level for the objective estimation of hospital mortality and regional lethality [6].

Hospital mortality in adults in Minsk for the 5-year period was approximately 25% of the corresponding overall death rate. The expert judgment of the cause of death was implemented in every sixth adult dead in Minsk. These numbers

make possible to extrapolate our study results to the overall death rate and lethality in adults in Minsk.

The average annual number of autopsies in RA patients was 7.4 (37/5) or approximately 10% of the total annual number of autopsies in rheumatic diseases. On the basis of the estimation given above the predictable average annual hospital mortality in RA patients can vary from 9 to 10 patients (9.6 per year) or come to 0.28% (9.6/3463) of general hospital mortality. The average annual mortality in adults in Minsk for the mentioned period was 16291 cases.

As the hospital mortality was 21.3% (3463/16291) of overall death rate the predictable annual mortality from RA in adults in Minsk can be approximately 45 cases or 0.032‰ (CI95 0.024-0.043‰). This level closely approaches to the results of our estimation of the mortality from RA performed on the basis of database of Minsk outpatient clinic № 12 service area – 0.027‰ (CI95 0.019-0.039‰).

The average lifetime in RA patients dead in 2001-2005 years was 66.0±12.8 years (mean±SD) in the general patient group, 66.7±13.1 in women and 62.3±11.3 years in men with median 69.0, 71.0 and 66.5 years, correspondingly (Table 4).

Table 4. Average lifetime in RA patients for the 25-year

Period	Women, ♀			Men, ♂			♀+♂		
	Mean	SD	Median	Mean	SD	Median	Mean	SD	Median
1981-1985	56.6	9.3	57.0	52.7	20.6	61.0	55.6	12.9	58.0
1986-1990	62.9	9.3	63.0	56.0	13.7	62.0	61.2	10.7	62.1
1991-1995	62.0	9.5	62.0	56.5	9.0	58.0	60.9	9.6	62.0
1996-2000	63.7	10.2	63.5	65.1	7.7	63.5	64.0	9.7	63.5
2001-2005	66.7	13.1	71.0	62.3	11.3	66.5	66.0	12.8	69.0

As it appears from the table above the average lifetime in RA patients for the 25-year period increased by more than 10 years in the general patient group – from 55.6 to 66.0 years ($p_{2\text{-}t}$=0.0036 – two-way statistical significance of difference by Fisher's exact test). Such lifetime increase was typical both for women (from 56.6 to 66.7 years) and men (from 52.7 to 62.3 years). 2/3 RA patients (116/171) died at the age of 50-70 years. On average, the lifetime difference between women and men was approximately 4 years in favour of women.

The dependence on glucocorticosteroids was revealed in 53.2% (91/171) RA patients with equal frequency for both sexes: 54.1% (73/135) in women and 50.0% (18/36) in men, correspondingly ($p_{2\text{-}t}$=0.3309). The average lifetime in RA patients with glucocorticosteroid dependence was lower than without it:

60.7±12.0 years and 63.3±10.7 years, correspondingly ($p_{2\text{-}t}$<0.1).

According to the postmortem autopsy data, secondary amyloidosis was revealed in 31.6% (54/171) of RA patients with higher frequency in women than in men: 34.1% (46/135) and 22.2% (8/36), correspondingly ($p_{2\text{-}t}$=0.0851).

The average lifetime in RA patients was 59.8±12.4 years and 62.9±10.9 in those with secondary amyloidosis and without it, correspondingly.

The proportion of complete (non-censored) cases for the survival analysis was 27.3% (161/590, 138 women and 23 men). Testing for differences in survival between RA patients of both sexes did not reveal significant differences (Gehan generalised Wil-coxon test, p=0.6972), as a result we considered the problem in the general group of RA patients.

The median of survival in the general group of RA patients was 69.1 years (68.7 in men and 69.2 years in women), the probability of surviving up to 50 years was about 95%, up to 60 years – 85% and up to 70 years – about 50%.

The survival time in RA patients was significantly lower (p<0.01) in case of the following clinical conditions: RA onset at the age under 36 years, rapid RA progression during the first three years of the disease, Sjögren's syndrome, rheumatoid vasculitis and secondary amyloidosis (Table 5).

Table 5. The survival time in alternative subgroups of RA patients

Clinical condition	Median, years	Interquartile range (25-75%), years	p<0.01 (Mann-Whitney U-test)
RA onset at the age under 36 years	49.6	38.0-57.7	*
RA onset at the age after 36 years	65.2	60.3-71.2	
Rheumatoid vasculitis presence	57.2	52.5-60.3	*
Rheumatoid vasculitis absence	63.7	57.3-70.0	
Sjögren's syndrome presence	59.7	56.9-64.8	*
Sjögren's syndrome absence	63.9	56.7-70.4	
Systemic amyloidosis presence	58.2	39.4-62.0	*
Systemic amyloidosis absence	64.3	58.8-70.0	
Renalamyloidosis presence	56.1	45.5-61.3	*
Renalamyloidosis absence	64.1	58.6-69.6	
Chronic kidney disease presence	56.0	39.4-62.6	*
Chronic kidney disease absence	64.3	58.6-70.3	
Glucocorticosteroid dependence presence	59.6	54.9-66.2	*
Glucocorticosteroid dependence absence	66.9	61.9-71.9	

** – significant difference*

The survival in RA patients was also significantly reduced in case of chronic kidney disease (CKD) regardless of the age of RA onset. The median of survival in patients with RA and CKD was significantly lower than in RA patients without CKD ($p<0.0001$). Glucocorticosteroid dependence had the same influence on the median of survival in RA patients (7-year decrease of the median of survival was revealed in case of glucocorticosteroid dependence, $p<0.0001$). We noted that rheumatoid nodules presence and serologic status of the disease did not influence on the survival of RA patients ($p>0.1$).

Conclusions

1. The average annual mortality rates in adults were 11.7‰ (CI95 11.6-11.9‰) in Minsk and 12.9‰ (11.9-13.9‰) in the service area of Minsk outpatient clinic № 12. The mortality from MSCTD made minor contribution to the overall death rate for the studied 5-year period - 0.33% (CI95 0.10-1.14%). The partial mortality rate from RA was 0.027‰ (CI95 0.019-0.039‰) and the corresponding lethality– 0.60% (CI95 0.13-2.65%).
2. The average lifetime in RA patients dead in 2001-2005 years was 66.0±12.8 years in the general patient group (66,7±13,1 years in women and 62.3±11,3 years in men). The average lifetime in RA patients for the 25-year period increased by more than 10 years in the general patient group – from 55.6 to 66.0 years. On average, the lifetime difference between women and men was approximately 4 years in favour of women.
3. The average lifetime in RA patients with glucocorticosteroid dependence was lower than without it (60.7±12.0 years vs 63.3±10.7 years), that was similar to the average lifetime in RA patients with secondary amyloidosis and without it (59.8±12.4 years vs 62.9±10.9 years).
4. The median of survival in the general group of RA patients was 69.1 years (68.7 in men and 69.2 years in women). The probability of surviving up to 50 years was about 95%, up to 60 years – 85% and up to 70 years – about 50%.
5. The survival time in RA patients was significantly lower ($p<0.01$) in case of the following clinical conditions: RA onset at the age under 36 years, rapid RA progression during the first three years of the disease, Sjögren's syndrome, rheumatoid vasculitis and secondary amyloidosis. The median of survival in patients with RA and chronic kidney disease was significantly lower than in RA patients without it ($p<0.0001$).

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Dynamics of Morbidity and Mortality from Breast Cancer in Women of Childbearing Age and 50 Years and Older in North Ossetia-Alania in 1990-2014

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B428C0013

Abstract

Breast cancer (BC) is an actual problem of medicine and public health of all countries. The article presents the dynamics of incidence, mortality, survival status of cancer care. Marked by high morbidity, mortality, neglect and low survival of patients 5 years or more. The necessity of screening of the female population in the risk group for breast cancer.

Keywords: Breast cancer, morbidity, mortality, survival

Introduction

Breast cancer (BC) is the most common tumor in women worldwide [2, 4, 6, 7]. In Russia in 2014 was 65088 cases of breast cancer. The “rough” prevalence rate was 82,99 and standardized 48,85 per 100 thousand female population [1].

The detection of early (I-II) stages of the disease has made 68,1% and III and IV of 30.9%. 1% stage of breast cancer is not established [5]. Given that for stage II includes patients with regional metastases (PN+) and without (PN0), it can be assumed that the number of truly early-stage breast cancer does not exceed 20% [3]. The number of deaths from breast cancer in 2014 amounted to 22445 women. “Crude” death rate – a 29.08 and standardized – 15,30 per 100 thousand women [1].

The purpose of the study

Retrospective analysis of morbidity, mortality and survival in patients with breast cancer (BC) fertile age 50 years and older in the Republic.

Materials and methods

The data of the state statistical reporting forms of the Republican oncologic dispensary (ROD): No. 7 “Information about diseases malignant neoplasms”; No. 35 “Data on patients with malignant neoplasms”; № 5 (table C51) “the Distribution of deaths by sex, age groups and causes of death”; Data Cancer registry; RN table 2 “population by age and sex,” according to the state statistics service of the Republic.

Results

In 25 years KIND of given 5919 women with breast cancer of which 1460 (24.7%) of child-bearing (15-49 years of age) (Fig. 1). “Rough” age-specific incidence (Fig. 2).

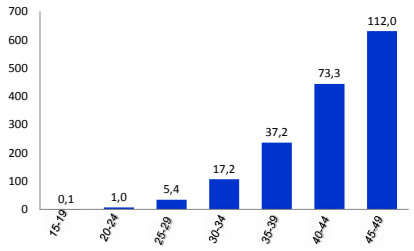

Fig.2 "Rough" age-specific incidence rates of child-bearing age (1990 - 2014)

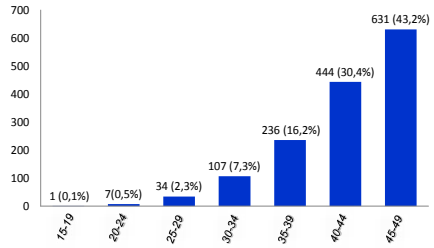

Fig.1 Absolute number and percentage of patients in fertile age (1990-2014).

From Fig. 2 shows that the “crude” incidence of child-bearing age increases with age and averaged 36,01 on 100 thousand women. population. Dynamics of incidence of breast cancer in fertile age by years is shown in Fig. 3.

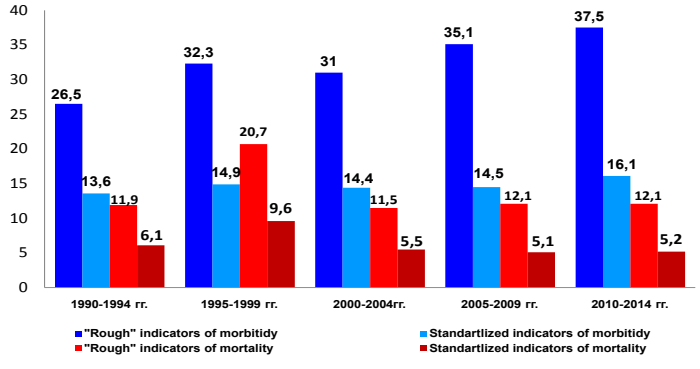

Fig.3 Dynamics of indicators of morbidity and mortality of child-bearing age in 1990-2014.

From Fig. 3 shows that the rate of incidence in absolute numbers in 2014 compared to 1990 has increased 1.65 times. Average “gross” the incidence rate increased 1.4 times, and standardized (world standard) 1.2 times.

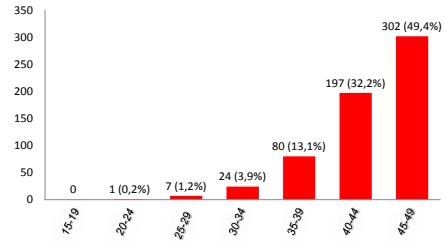

Fig.4 the Absolute number and percentage of deaths in child-bearing age (1990-2014).

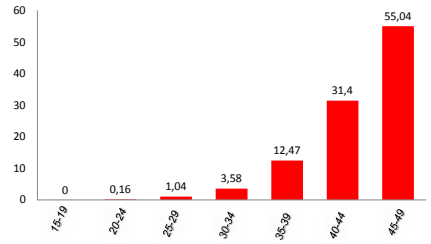

Fig.5 "Rough" age-specific mortality rates in fertile age (1990 - 2014)

Of 1460 patients with breast cancer of reproductive age during the observation died 611 (41,0%) (Fig. 4). “Rough” age-specific mortality in women of reproductive age (Fig. 5).

Dynamics of mortality of breast cancer in fertile age by years is shown in Fig. 3. The mortality rate in absolute numbers in 2014 compared to 1990 increased by 1.2 times. Average “gross” indicator increased marginally (1.01%), and standardized (world standard) fell by 1.17 times.

Life expectancy of 5 years or more of child-bearing age from the moment of diagnosis amounted to 52.6 per cent.

5919 from 4459 patients with breast cancer (75.3 per cent) were aged 50 years and older (Fig. 7). «Crude» incidence rates at age 50 years and older (Fig. 8).

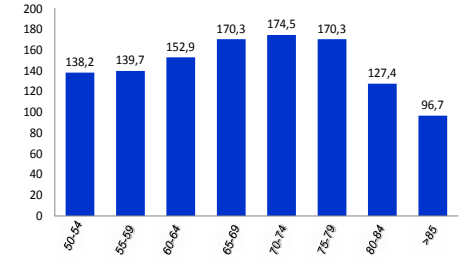

Fig.7 "Rough" age-specific incidence at age 50 years and older (1990-2014).

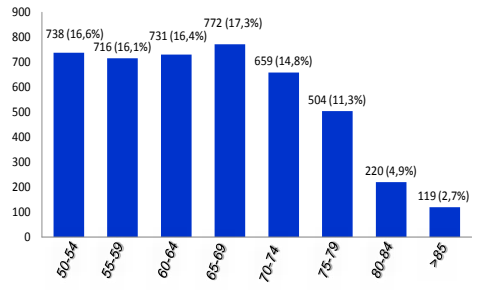

Fig.6 the Absolute number and the percentage of patients aged 50 years and older (1990-2014).

The peak incidence occurs in the age group 65-74 years – 171,78 on average. Dynamics of incidence of breast cancer at age 50 years and older by years (Fig. 9).

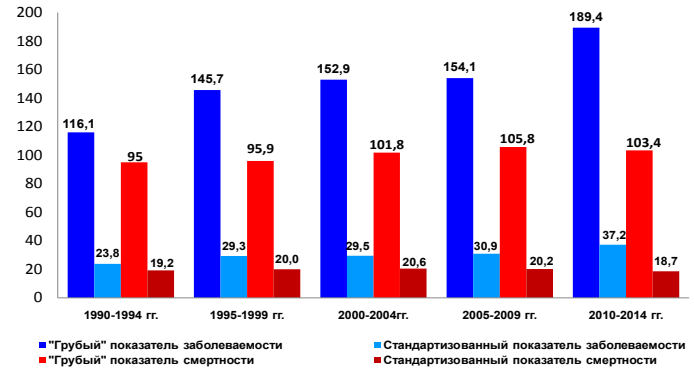

Fig.8 Dynamics in morbidity and mortality of 50 years and older in 1990-2014.

The absolute number of patients in 2014, compared to 1990 has increased 1.9 times. Average "gross", the incidence rate has increased 1.63 times, and standardized (world standard) in 1.57 times.

4459 registered patients of age 50 years and older died 2936 (65,8%) (Fig. 9). "Crude" mortality rates at age 50 years and older is shown in Fig. 10.

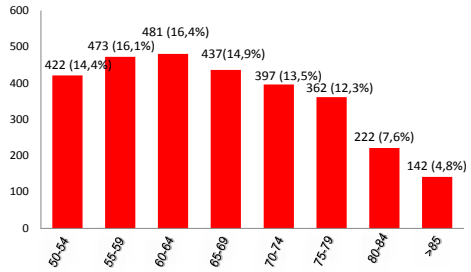

Fig.9 Absolute number and percentage of deaths aged 50 years and older (1990-2014).

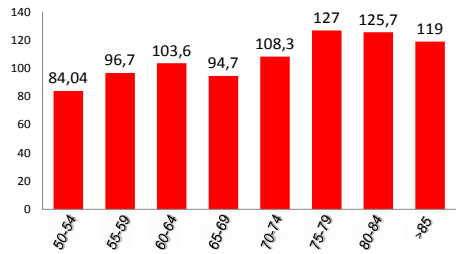

Fig.10 "Gross" age-specific mortality rates at age 50 years and older (1990-2014).

The peak of mortality in the age group 75-84 years – 123,9 on average.

Dynamics of mortality rates at age 50 years and older by years is shown in Fig. 8.

The absolute number of deaths in 2014, compared to 1990, increased in 1,1 times. The average "crude" mortality rate rose slightly (1.08 times), and standardized (world) have not changed.

Life expectancy at age 50 years and older 5 years or more since diagnosis was 51.8%.

Conclusions

The incidence of breast cancer in women of childbearing age is increasing, mortality is not reduced. The incidence in women 50 years and older is high and continues to grow. The peak incidence occurs in the age group 65-74 years.

The death rate is slowly, but growing. The peak of mortality in the 75-84 years age. The survival of childbearing age was 52.6%, at the age of 50 years and older is 51.8 per cent. The state of cancer care in breast cancer in the Republic is unsatisfactory.

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Prognostic Significance of CD4 Lymphocyte Deficiency in Previously Untreated Hodgkin's Lymphoma

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B428C0060

Abstract

Pre-treatment lymphopenia (PL) is included to the IPS score for advanced Hodgkin's lymphoma (HL) as one of negative outcome predictors.

In our earlier analysis on 838 patients with HL I-IV stages, the adverse prognostic value of PL was shown also for HL stages I-II. CD4 deficiency is known to accompany the impaired immunity in classical HL and was recently shown to play negative role in other malignancies. To explore the influence of pre-treatment CD4 lymphopenia on the efficacy of first-line therapy in patients with HL I-IV stages, we collected from the database the patients whose medical records contained results of flow cytometry of peripheral lymphocytes performed before treatment start. Total 162 patients were eligible for this retrospective study. Absolute lymphocyte count was decreased (≤1000 μL) in 53 (33%) out of 162 patients, with predominance in women (p=0.029) and unfavorable morphology (p=0,014); frequency of PL increased with stage (p=0.007) and IPS (p=0.000). Moderate CD4 deficiency (400-210 μL) was found in 36 (22%) of 162 patients; 10 (62%) out of 16 men and in 2 (10%) out of 20 women had normal lymphocyte count. Deep CD4 lymphopenia (≤200 μL) was found in 24 (15%) out of 162 patients; it was associated with age ≥45 (p=0.031), advanced stage (p=0.03) and IPS score ≥4 (p=0.000).

At a median follow up of 60 months, all patients with CD4 count ≤400 μL had lower progression-free survival (PFS) and overall survival (OS) compared with those without CD4 lymphopenia. In stages I-II favorable (n=13) progression occurred only in a patient with low CD4 count; OS was 100%.

In stages I-II unfavorable (n=29), six patients with CD4 deficiency had PFS 50% vs. 95% in the rest, p=0.007; OS was 30% vs. 100%, p=0.001. In stages

III-IV (n=120) patients with low CD4 count (n=53) had 5-year PFS 64% vs. 87%, p=0.006; OS was 70% vs. 95%, p=0.004. Subset analysis in 94 patients with stages III-IV and IPS 0-3 supported negative impact of CD4 lymphopenia (PFS 69% vs. 88%, p=0.054; OS 76% vs. 97%, p=0.058). This study shows that pretreatment lymphopenia is an independent prognostic factor for patients with HL I-II stages as well as for relatively favorable patients with stages III-IV patients having IPS 0-3.

Keywords: Hodgkin's disease, prognosis, immunology, lymphopenia, CD4 lymphocytes

Introduction

Pre-treatment lymphopenia (PL) is associated with negative prognosis in different malignancies, including lymphomas. Lymphocyte count <600 μL or <8% is accounted for as one of the seven risk factors in the International Prognostic Score (IPS) which was developed for patients with advanced Hodgkin lymphoma [1]. According to our previous analysis most strong adverse features belonged to a combination of absolute and relative lymphopenia [2].

Less is known about the influence of PL on the treatment outcome in patients with early stages of Hodgkin's lymphoma (HL). In the Medical Radiological Research Center (Obninsk, Russia) the negative prognostic value of PL for long term PFS and OS was shown on a prospective cohort of 838 patients with HL I-IV stages treated in 1998-2010. PL appeared to be an independent risk factor for patients with early stages HL and for those patients with stages III-IV who had IPS 0-3 [3]. Since 2010, the PL was added to the prognostic index which was used in MRRC as the basis for risk-adapted therapy in all stages of HL patients [4].

CD4 deficiency is known to accompany the impaired immunity in classical HL [5] and was recently shown to play negative role in various solid tumors and lymphomas [6-9]. As part of reactive microenvironment, CD4 lymphocytes play a key role in tumor-response regulation through the effector-cells activation and involvement in cell reactions. However, in the course of progression the tumor acquires a number of features that allow it to avoid immunological surveillance. In HL the cells of reactive environment play an important role in tumor-cell proliferation and outcome. Despite a huge amount of activated CD4 lymphocytes closely surrounding Reed-Sternberg cells and the full number of HLA-II-type molecules, as well as costimulatory molecules and adhesive molecules on Hodgkin and Reed-Sternberg cells, full cytotoxic immune response with participation of CD8 cells does not occur and tumor cells escape from being killed [5]. It remains unclear what subsets of HL

patients suffer from baseline CD4 lymphopenia.

To explore this question, the prognostic value of CD4 lymphocyte counts for long term PFS and OS was investigated on a retrospective cohort of 162 HL patients treated in MRRC (Obninsk) during 2003–2015 with a median 60 months of follow up.

Methodology

In a database of MRRC (n=838 patients) we performed a search of HL patients who had flow cytometry of peripheral blood lymphocytes used at initial examination. Total 162 HD patients (95 female and 67 male) were eligible for this study, 53 of them had low lymphocyte counts at presentation. All patients were referred to the Department of Radiotherapy and Chemotherapy for Hemoblastoses of MRRC between 2003-2015. Median age was 28 years (16-59 yr). All diagnoses were made using histological and immune-histochemical investigations according to WHO classification. Pretreatment examination included thoracic and abdominal computed tomography; ultrasound examination of all peripheral lymph nodes, abdomen, retroperitoneal and small pelvis areas; bone-marrow biopsy. Additional investigations were performed when indicated. Stages of disease were defined by Ann Arbor classification.

The patients were divided into 3 groups according to risk factors: Group 1(n=13) included patients with HL I-II stages without risk factors. Group 2 (n=29) comprised patients with HL I-II stages having risk factors (mediastinal bulk, isolated extranodal involvement, involvement of 3 or more lymphatic areas). Group 3 (n=120) included patients with Stages III-IV. Patients received risk-adapted combined modality therapy described elsewhere [4] The median follow-up for all patients was 60 months.

Immunophenotyping of peripheral blood lymphocytes was performed by flow cytofluorometers FACScan and FACS Calibur (Becton Dickinson).

Monoclonal antibodies to CD3, CD4 labeled with different fluorochromes were used to identify cells (Becton Dickinson Immunocytometry Systems – BDIS, USA).

The ч2 test was used to test the hypothesis that proportions were equal among 2 or more groups of patients. Five-year overall survival and relapse-free survival were analyzed with regard of the initial level of CD4+T subpopulation.

Kaplan-Meier method was applied to assess survival, the data were analyzed with statistical package SPSS 20 for Windows.

Results

Table 1 describes the baseline characteristics of the patients and the incidence of lymphopenia in different subgroups. In univariate analysis the frequency of absolute lymphocyte counts ≤1000 μL was higher in female, unfavorable morphology (nodular sclerosis Grade II plus lymphoid depletion), advanced HL and IPS ≥4. Although the incidence of moderate CD4 lymphopenia (400-210 μL) was found to vary in different subgroups, it was observed in all subgroups, and altogether in 36 (22%) of 162 patients. Moreover, among patients with lowered CD4 counts, 10 (62%) out of 16 men and in 2 (10%) out of 20 women had normal lymphocyte count, i.e. could not be purposely chosen for immunophenotyping.

Table 1
Frequency of pretreatment lymphopenia in Hodgkin's lymphoma

Characteristics	No (%)	Lymphocyte ≤1000 μL N (%)	*p* value[a]	CD4 Ly ≤200 μL N (%)	*p* value[a]	CD4 Ly ≤400 μL N (%)	*p* value[a]
Gender							
Male	67 (41)	16 (24)	**0.029**	7 (10)	0.276	16 (24)	0.815
Female	95 (59)	37 (39)		17 (18)		20 (21)	
Age							
≥ 45	17 (10)	8 (47)	0.093	6 (35)	**0.031**	3 (18)	0.864
< 45	145 (90)	45 (31)		18 (12)		33 (23)	
Morphology							
LP	5 (3)	3 (60)	0.112	0	0.091	2 (40)	0.404
NS Gr I	72 (44)	17 (24)		6 (8)		13 (18)	
NS Gr II	34 (21)	15 (44)		6 (18)		11 (32)	
MC	43 (27)	14 (32)		9 (21)		8 (19)	
LD	8 (5)	4 (50)		3 (38)		2 (25)	
NS Gr I + MC	115	31 (27)	**0.014**	15 (13)	0.297	21 (18)	0.136
NS Gr II + LD	42	19 (45)		9 (21)		13 (31)	
Stages							
I-II F	13 (8)	0	**0.007**	0	**0.030**	1 (8)	0.281
I-II U	29 (18)	5 (17)		1 (3)		5 (17)	
III-IV	120 (74)	48 (40)		23 (19)		30 (25)	
IPS score							
0-2	115 (71)	26 (23)	**0.000**	7 (6)	**0.000**	22 (19)	0.291
3	21 (13)	10 (48)		3 (14)		7 (33)	
≥4	26 (16)	17 (65)		14 (54)		7 (27)	

Bold characters designate a parameter with a significantly different distribution in the subgroups tested, defined by a p < 0.05. [a] The ч2 test was used to test the hypothesis that proportions were equal among 2 or more groups of patients. Ly=lymphocyte. LP=lymphocyte predominance. NS=nodular sclerosis. MC=mixed cellularity. LD=lymphocyte depletion. F=favorable. U=unfavorable.

The deep CD4 lymphopenia (≤200 μL) was found in 24(15%) out of 162 patients; it was associated with age ≥45 (p=0.031), advanced stage (p=0.03) and IPS score ≥4 (p=0.000). Fig 1. shows that overall survival (OS) and progression-free survival (PFS) for all 162 patients correlated with baseline CD4 counts. At a median follow up of 60 months, all patients with CD4 count ≤400 μL had lower progression-free survival (PFS) and lower overall survival (OS) compared with those without CD4 lymphopenia. In stages I-II favorable (n=13) progression occurred only in a patient with low CD4 count; OS was 100%. In stages I-II unfavorable (n=29), six patients with CD4 deficiency had PFS 50% vs. 95% in the rest, p=0.007; OS was 30% vs. 100%, p=0.001.

Among 120 patients with stages III-IV, those with low CD4 count (n=53) had 5-year PFS 64% (95% CI, 48-80) compared with 87% (95% CI, 79-96) in patients without CD4 deficiency, p=0.006. Overall survival in advanced HL with low CD4 count was 70% (95% CI, 53-88) compared with 95% (95% CI, 94-100), p=0.004. Subset analysis in 94 patients with stages III-IV plus IPS 0-3 supported negative impact of CD4 lymphopenia. PFS in 'low CD4' patients were 69% (95% CI, 51-87) vs. 88% (95% CI, 80-97), p=0.054; OS was 76% (95% CI, 54-99) vs. 97% (95% CI, 92-100), p=0.058.

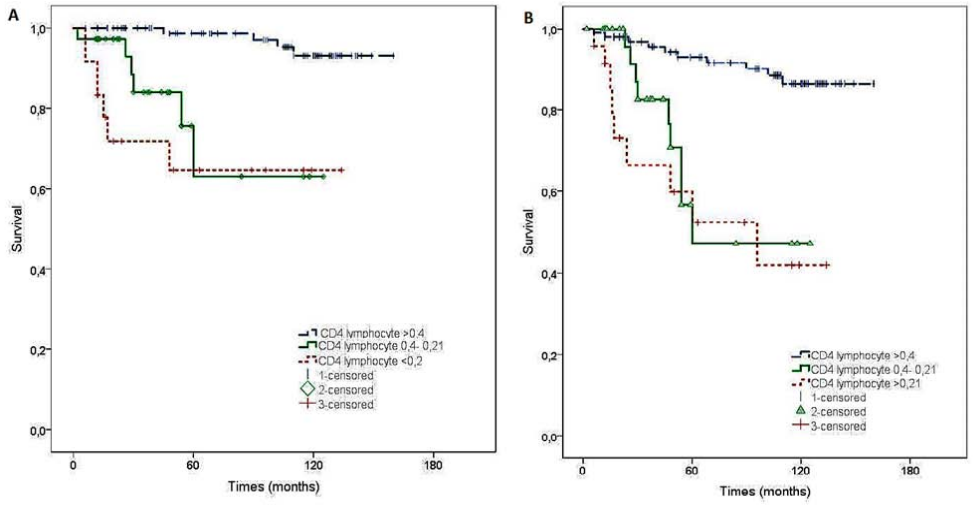

Fig. 1. Overall (A) and progression-free (B) survival according to baseline CD4 lymphocyte counts in 162 patients with Hodgkin's lymphoma I-IV stages.

Discussion

According to our earlier analysis, absolute lymphopenia (≤1000 μL) in previously untreated HL is relatively rare event with overall incidence 14% (116 of 838 patients) [3]. Decreased lymphocyte counts were found in 1.4% of patients with favourable stages I-II, in 7,5% of patients with unfavorable I-II stages and in 18,6% patients with stages III-IV. To our knowledge, in the course of different clinical trial no stratification is provided to account for possible adverse effect of pre-treatment lymphopenia.

A number of studies showed unfavorable prognostic significance of pretreatment CD4 deficience and our results support these data. This was also a risk factor for infections and hematological complications in the course of chemotherapy in patients with solid tumors [6, 8]. However, as yet there have been only a few reports on prognostic significance of CD4 lymphocyte deficiency in patients with malignanat lymphomas [7, 9].

We have studied prognostic significance of decreased CD4 lymphocyte counts in the group of 162 initial HL patients. An important conclusion from our study was the fact that, unlike IPS, a low CD4-cell count as a prognostic sign for overall and progression-free survival may be as well applied to patients with early-stage disease.

It should be noted that in our study the incidence rate of CD4 deficiency in patients without pre-treatment lymphopenia was 9%. This fact possibly

suggests under-treatment in this category of patients. Perhaps awareness of clinical importance of the CD4 deficiency would encourage the introduction of some changes in the treatment strategy for this patient category.

Conclusions

We may conclude that assessment of CD4 lymphocytes is an easily available method to evaluate prognosis in HL patients. We suggest that decreased CD4+ cell count should be considered when planning therapy for initial HL as an additional sign of unfavorable prognosis along with IPS factors and it can be used for early-stage HL as well. Presence of lymphopenia requires innovative therapeutic approaches that would improve the outcome in this patient group.

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Immunophysiology of Cancer. An Overview of New Generation of Visualizing and Cytotoxic Agents

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B428R9015

Abstract

Today, immunophysiology of cancer is a rapidly progressing field of science and antibody derivatives approved for anticancer therapy is big business.

The current trend is design of multifunctional agents that have toxic and/or visualizing modules, as well as targeting agents for addressed delivery.

The mini-review is focused on recent results of engineering constructs for cancer diagnostics and therapy developed in our laboratory.

Keywords: HER2, single chain antibody, DARPin, bispecificity, targeted delivery, barnase–barstar

Introduction

The progress achieved in studying the molecular basis of carcinogenesis has revealed subtle biochemical differences between tumor and normal cells and, thus, provided opportunities for developing therapies and diagnostics based on these differences. The targeted therapy concept involves the development of drugs specifically interacting with target molecules that are expressed in tumor cells but are not present in normal tissues.

The use of modular constructs based on immunoglobulin superfamily proteins for targeted drug delivery and diagnosis is the current trend in molecular medicine referred to as theranostics [1–4]. Design and evaluation of new high-affinity protein compounds that can selectively and efficiently destroy human cancer cells are a priority research area in biomedicine.

Molecules used as a targeted moduls

Monoclonal antibodies and their derivatives are widely used in clinical application for selective destruction of human tumors. Scaffold proteins, having the same affinity and specificity, surpass their corresponding monoclonal antibodies in physical and chemical properties. They possess such desired properties as a small size, which enables efficient tissue penetration, rapid

folding, high chemical, proteolytic and thermal stability and they do not tend to aggregate [4]. These features give scaffold proteins advantages over antibodies being used as binding moieties in multifunctional compounds for the diagnosis and treatment of human diseases.

The human epidermal growth factor receptor 2 (HER2 or ERBB2) is overexpressed in 20-30% of breast and ovary tumors [7, 8]. High level of HER2 expression usually correlates with aggressive tumor phenotype and enhanced metastasis [9]. Because HER2 is expressed at relatively low levels in normal epithelial cells, it makes this receptor an attractive target in cancer therapy [1].

The fragment of the antibody 4D5 scFv is used as a targeting module that is a single polypeptide chain in which the variable domains of light and heavy immunoglobulin chains are connected by short flexible linkers, and the constant domains are lacking. The 4D5 scFv fragment, as the targeting module, attracts attention because it is also capable of effectively recognizing HER2, but, unlike full length antibodies, it does not provide interaction with the receptors of immune system cells and complement system proteins [1].

As an alternative targeting module, the ankyrin repeat protein, DARPin_9-29, with high affinity for transmembrane receptor HER2 have been used for design of oncotheranostic agents. To facilitate an anticancer effect evaluation of constructed agents a fluorescent adenocarcinoma cell line SKOV-kat was designed (Fig. 1) [5]. It allows visualization of xenografted tumors in alive animals.

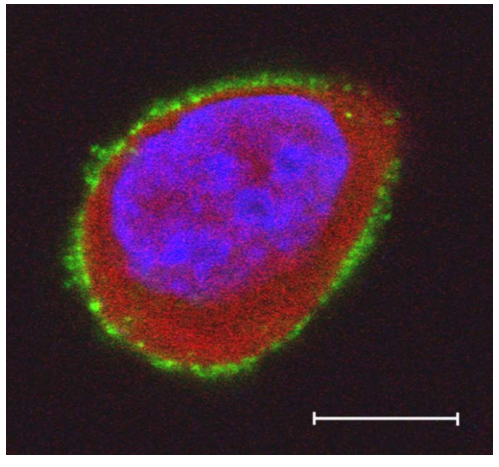

Fig. 1. Visualization of SKOV-kat cells. Fluorescence of SKOV-kat cells expressing protein Katushka (red) visualized by confocal microscopy; nuclei are stained with Hoechst 33258 (blue). Bar 10 μm.

Multifunctional anti-cancer recombinant proteins

Standard procedures for design of targeted imaging and therapeutic compounds are based on an attachment of recognizing molecules to visualizing agents or drugs. In frame of this approach the fully genetically encoded anti-receptor immunotoxins [5, 6], immunoRNase [7-10], and antibody-photosesitizers [11, 12] were constructed. A fluorescent proteins, Killer Red, miniSOG and a ribonuclease barnase were used as toxic principles.

They were fused to the single-chain scFv-fragment of anti-HER2/neu antibody 4D5 and/or DARPin_9-29 that recognizes the extracellular domain of cancer marker HER2. The all bifunctional fusion proteins demonstrated specific cytotoxic effect on HER2-positive human carcinoma cells. The highest effect was observed with pseudomonas exotoxin A fusion (Table 1).

Table 1. Citotoxicity of immunotoxin 4D5 scFv-ETA PE40, free ETA PE40, and free 4D5 scFv for cell lines with different levels of the HER2 expression. IC_{50} values are given with 95% confidence interval

Cell line	Citotoxicity, IC_{50}, nM					
	40 min			72 h		
	4D5 scFv-PE40	PE40	4D5scFv	4D5 scFv- PE40	PE40	4D5 scFv
CHO	>1000	>1000	>1000	8.7 (5.6–13.6)	2.9 (1.8–4.6)	>100
SKOV-kat	22 (5.7–85.3)	>1000	>1000	0.008 (0.006–0.013)	4.9 (1.3–18.4)	>100
SKOV-3	-	-	-	0.017 (0.011–0.025)	6.6 (3.1–14.0)	>100

Design of multimodule nanostructures for imaging and/or therapy

Nowadays nanobiotechnologies open up new possibilities for diagnostics and treatment of oncological, cardiovascular, autoimmune, and other diseases.

Rapid development of nanotechnology has stimulated considerable interest in their applications in life sciences. In particular, several unique features of nanoparticles appeared very attractive for their implementation as diagnostic and therapeutic complexes. An important attribute of the nanoparticles is the ability to bind various molecules ensuring biological activity of the particles; among these molecules are toxic and/or visualizing modules, as well as targeting agents for addressed delivery. In recent biomedical studies, much attention has been paid to the search for new methods of noninvasive imaging of the internal structure of biological objects. Instruments with a high spatial resolution have

been designed, and, consequently, optical methods for investigation are gaining widespread use [13-16].

A novel strategy, "Protein-assisted NanoAssembler", for design of heterostructures based on the ribonuclease barnase and its inhibitor, barstar, was suggested [17-19]. The barnase and barstar are small, stable, very soluble, resistant to proteases proteins. The complex between them is extremely tight with a $K_d \sim 10^{-14}$ M. The N- and C-terminal parts of both proteins are localized outside of the barnase barstar interface and are therefore accessible for fusion with targeting, visualizing or toxic compounds. The suggested strategy is applicable to virtually any proteins that can be functionally attached to the barstar and barnase molecules. It seems particularly well suited to the production of heterooligomeric constructs because the extremely specific barnase barstar interaction eliminates reliably the mispairing problems.

The important advantage of barnase·barstar over the majority of other dimerization modules is that their interaction ratio is precisely 1:1, and neither of the partners is aggregation prone.

A particular attention as new and unique therapeutic agents attract nanoparticles (NPs) that make it possible to solve old but still actual problems by principally new means and ways. A number of nanoparticle-based medications are already approved for therapeutic purposes. Important advantage of NPs is their developed surface, which can be decorated with biocompatible functional moieties, and thus form a versatile docking station. NP can serve as a nano-vehicle to host biologically significant modules, such as therapeutic, targeting and stealth modules for targeted delivery, diagnosis that guides and monitor effects of the NP-assisted therapy of pathology lesions. These properties provide foundations for significant emerging areas in applied biomedical science including (personalised) nanomedicine and theranostics.

In order to apply nanoparticles for imaging and/or therapy one needs to consider three key aspects: design of bright and photostable luminescent nanomaterials conspicuous on the background of the excitation light back-scattering and cell autofluorescence; amiable surface modification to enable facile interfacing with biomolecules, and modular engineering of the biomolecular complexes with targeting vectors (antibody, mini-antibodies or peptides) firmly attached to the NP for target delivery to specific cellular or tissue sites.

To develop a modular engineering concept (Fig. 2) we study self-assembly of polystyrene micro-and nanoparticles with two functionalities − magnetic and fluorescent – using proteinaceous "molecular glues", most notably, the barnase-barstar system (BBS). The obtained assemblies were tested for their resistance to high concentrations of chaotropic agents (urea and GdmHCl) as well as high temperature and low pH conditions causing denaturation of most proteins. In the majority of cases, the structures exhibit unusual stability and maintain apparently unaltered morphologies upon exposure to these conditions

for extended periods of time. Comparison of the BBS-system with other proteinaceous self-assembly systems (streptavidin_biotin, antibody_antigen, and protein A_immunoglobulin), showed that whereas their resistance to destruction is relatively comparable, the capacity to assemble under harsh conditions differs substantially.

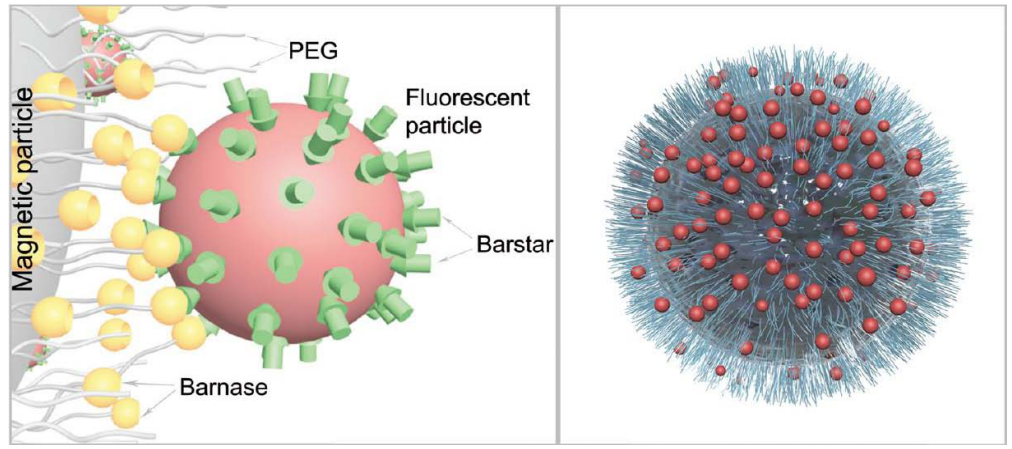

Fig. 2. The concept of multipoint contacts between the components of the colloidal assembly. Left: The multiple BBS pairs at the particle interface. Right: A general schematic view of an assembled structure.
PEG, polyethylene glycol [19].

The ability of the BBS-glued assemblies to retain their integrity in extreme conditions makes them attractive for a number of applications, taking into account the feasibility of utilization of modules of various natures as participants of self-assembly. The designed nanoparticle assembly approach may prove particularly advantageous for such applications where the remarkable durability of the assemblies becomes a feature of high value.

Examples embrace a broad spectrum including sensing of ecological pollutants in complex media, photonics and theragnostic approaches in medicine, also making use of multifunctionality offered by the assemblies [19].

Furthermore, the unexpectedly high "tensile strength" of the proteinaceous molecular glues described in this work sets one thinking of potential applicability of these self-assembled structures instead of, or alongside with, covalently linked entities. If for creation of *a fortiori* very durable and "reliable" structures at the nano-and microscale one would definitely choose chemical reactions as a means to build such structures, now, armed with the knowledge of exceptional stability of protein-assisted assemblies, one has access to a great variety of specific (naturally occurring or engineered) "molecular glues" that can be used for the same purposes and with similar efficiency. Moreover, utilization of specific non-covalent interactions adds to the flexibility of the

designed assembly systems and imparts higher controllability over the whole process of assembly than in the case of chaotic chemical reactions.

This universal platform was used for design of multifunctional agents for theranostics applications with important types of the nanoparticles, including well established quantum dots (QDs), luminescent nanodiamonds (LNDs), colloidal gold, magnetic NPs, and luminescent upconversion NPs. It provides a straight-forward technology to design a multifunctional nanoheterostructures "when the whole is greater than the sum of the parts" [20, 21].

Acknowledgments

This research was supported by RSF grants (no. 14-24-00106) in the part of construction of the immunotoxins and nanobioconjugates, and (no. 16–14–10321) in the part of cell studies.

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Analysis of Expression of Cancer-Testicular Antigens on the Tumor Cell Cultures of Bladder Cancer

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B428R9037

Abstract

Non-specific methods of immunotherapy have been successfully used for more than 40 years for activation of immune system in case of treatment of urothelial carcinoma. Development of method of specific immunotherapy in case of bladder cancer (BC) is a current problem. Cancer-testis antigens (CTA) are the most promising target in the context of creation of antitumor vaccines because they are distinguished by marked immunogenicity, they are detected in different types of tumors and have limited expression patterns in healthy tissues of grown-up organism. Development of personified dendritic cell antitumor vaccines against BC is related not only to selection of optimal antigens for activation of dendritic cells, but also to determination of optimal parameters of cultivation of cells for provision of maximum CTA expression by tumor cells. The work presents data in relation to research of peculiarities of CTA expression by tumor cultures of BC in case of long-term cultivation.

Keywords: Immunotherapy, bladder cancer, anti-tumor vaccine, cell culture, cancer-testis antigens, urothelial carcinoma cells

Introduction

Studies of recent years convincingly proved the leading role of immune system in antitumor protection of organism and became a starting point for development of different methods of immunotherapy of malignant growths [1]. Autologous dendritic cell vaccines activated with tumor-associated antigens [2-3] are considered the most efficient for formation of specific antitumor immune response [2-3]. Cancer-testis antigens (CTA) for activation of dendritic cells are successfully used for treatment of advanced stages of melanoma and kidney cancer [4, 5]. In healthy organism, only tissues of male

gonad and placenta express CTA, however, the same antigens are frequently present in different tumors. More than 100 specimens of this group of antigens were studied, about 30 of which were encoded in X-chromosome, including such highly immunogenic families as MAGE-A, GAGE, BAGE and NY-ESO-1. Expression frequency of CTA varies greatly depending on the tumor type. For melanoma, ovarian cancer, lung cancer and BC high expression of CTA is typical, while for colorectal cancer and prostate cancer the same is low [6]. Regarding the level of mutational load, urothelial carcinoma holds the third position among all malignant growths being surpassed in this regard only by melanoma and lung cancer, which creates particular opportunities for use of immunotherapy in case of bladder cancer (BC) [7-11]. Several studies showed high levels of CTA expression by BC cells [12]. CTA of MAGE group were registered at the rate of 43%, while NY-ESO-1 – from 35% [6] to 45.1% of studied tumor specimens [13]. Other works demonstrated presence of MAGE-A3 positive tumors in the amount of 46,5% [14] to 58,8%.

Facts pointing at correlation between disease prognosis and expression are interesting. Specifically, correlation between MAGE-A4 and MAGE-A9 expression and low relapse-free survival rate/survival rate without progression was established [15]. In this regard, research of CTA expression with urothelial carcinoma cells (UCC) in the process of cultivation of tumor material is considered important for creation of PDCAV against BC.

Background

Objective of the research was to study the level of expression of CTA (MAGE, NY-ESO-1, GAGE, BAGE) by UCC at different transits.

Materials and methods

In the work, tumor specimens from 54 patients with muscular invasive (MIF) and muscular non-invasive forms (MNF) of BC, separated immediately after performance of surgical intervention, were used. Written voluntary informed consents were obtained from all patients. Fragments of tumor tissue with a size of not less than 0,3 cm^3 obtained during the surgery were immediately placed into sterile containers with nutritive medium (NM) DMEM/F12 (Biolot, Russia) and delivered to laboratory. For disaggregation of cells, automatic mechanic method (with the use of Medimashin Dako, Denmark) was used. Obtained cell suspension was sequentially flowed through sterile nylon filters with pore diameter of 100 and 70 μm (DAKO, Denmark). Viability was evaluated with the

use of automatic meter Countess (Invitrogen, USA) by counting cells, colored with trypan blue. Cells were suspended in full nutritive medium (FNM) of the following composition: DMEM/F12 (Biolot, Russia) with addition of 20% Fetal Bovine Serum (Biolot, Russia) and growth factors: insulin, transferrin, selenium (Invitrogen, USA). Cultivation of UCC was performed in CO2-incubator «Heracel» (Termo Electron LTD GmbH, Germany). Passaging was performed with the use of mixture of equal volumes of Versen (Biolot, Russia) and Trypsin (Biolot, Russia) solutions [15]. Detailed information about each patient and characteristic of their tumor was obtained from medical record.

Immunophenotypic analysis of CTA expression was performed by means of flow laser cytometer BD FACS Canto II (BD Biosciense, USA). Blocking of Fc-receptors for prevention of non-specific coloring was performed by means of Human BD Fc Block (BD Biosciense, USA), diluted in Stain Buffer (BD Biosciense, USA). Incubation was performed in BD Perm/Wash solution.

Cells were washed twice in BD Perm/Wash bufer, pelleted by means of centrifugation for 10 min at 1000 rpm. Then $1x10^6$ of cells were incubated with specific antibodies (20 mcl/1 specimen): MAGE, GAGE3, BAGE, NY-ESO-1 (Santa Cruz Biotechnology, USA). Mathematical processing of data was performed with the use of package of statistical software SPSS 23.0 for Windows (SPSS, Chicago, IL, USA).

Results

As a result of the research, 10 consistently transferred UCC were obtained which were examined for presence of CTA expression at early (before 10) and later (over 30) transits [16-18]. Out of 10 analyzed specimens – 6 were represented with MIF BC and 4 – MNF BC. Pathohistological characterization of tumor specimens is presented in Table 1.

Table 1. Pathohistological characterization of specimens of BC tumor culture and CTA expression depending on the duration of cultivation

N	Age	Stage/ Grade	CTA expression at early transits (<10)				CTA expression at late transits (>30)			
			NY -ESO	GAGE	MAGE	BAGE	NY -ESO	GAGE	MAGE	BAGE
1.	61	T1/ GIII	+	+	+	+	+	+	+	+
2.	68	T2/ GIII	+	+	+	+	+	+	+	+
3.	71	T2/ GIII	+	-	+	-	+	-	-	-
4.	69	T3a/ GIII	-	+	+	-	-	+	-	-
5.	45	T1/GI	-	-	+	+	-	-	+	-
6.	74	T2/GII	+	+	+	-	-	+	-	-
7.	73	T2/ GIII	+	-	+	-	-	-	+	-
8.	56	T1/ GIII	-	-	-	-	-	-	-	-
9.	66	T1/GI	-	-	-	-	-	-	-	-
10.	70	T2/ GIII	-	-	-	-	-	-	-	-

Results of comparative studies showed that in UCC cultures at early transits CTA expression was detected frequently. Particularly, MAGE-70%; BAGE–30%; GAGE – 40%; NY-ESO-1 – 50%. In 70% of tumor cultures, expression of at least one of analyzed CTA and in 20% - of all researched CTA was registered.

In the process of cultivation decrease of the number of CTA, expressed by cell lines was noted (Fig. 1). It was established that CTA expression in specimens was nonhomogeneous. In case of prolonged cultivation of UCC (over 30 transits) percentage composition of cells, expressing CTA conclusively decreased -28.2±4.6%, up to total disappearance ($p \leq 0,05$).

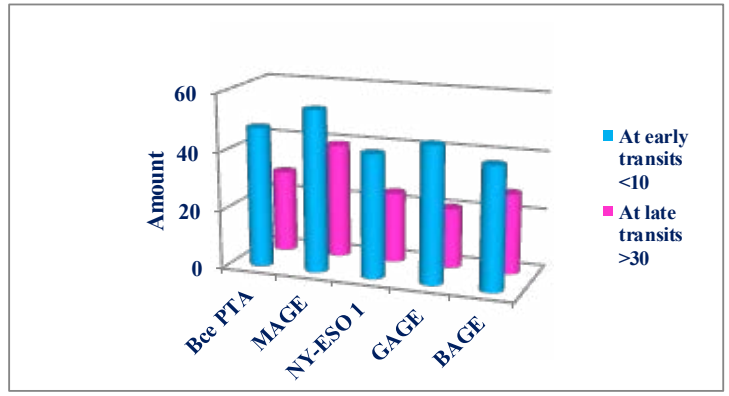

Fig. 1. Amount of CTA-positive cells in cultures of tumor cells of BC patients.

In the course of cytofluorometry research of specimens of tumor UCC of patient T. with MNF of high grade malignancy tumor (T1N0M0/GIII) at 5th (Fig. 2) and 30th (Fig. 3) transits, it was established, that CTA expression of MAGE group at 5th transit amounted to 78,6% as compared to 39,8 % if MAGE-positive cells, detected at the 30th transit.

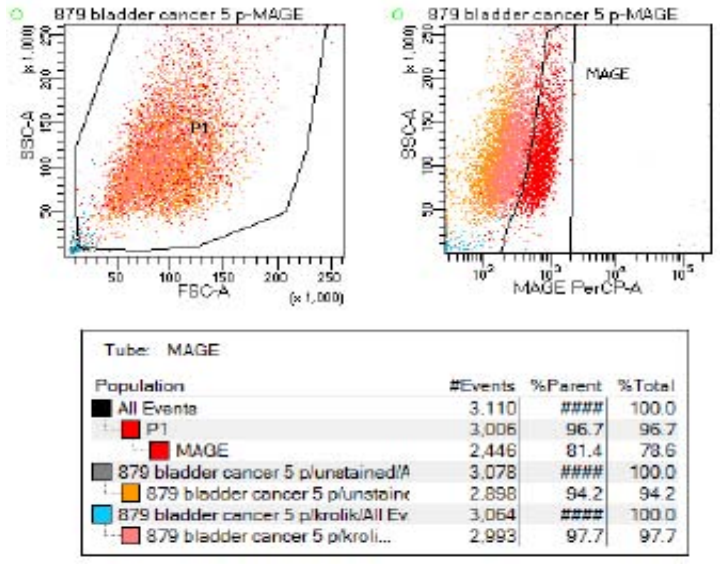

Fig. 2. Results of CTA expression of MADE group with tumor cells of urothelial carcinoma of patient T., 61 y.o. with MNF of high grade malignancy tumor (T1N0M0/GIII) at 5th transit

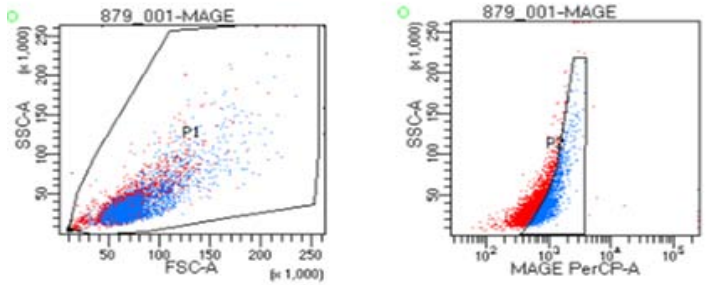

Fig. 3. Results of CTA expression of MADE group with tumor cells of urothelial carcinoma of patient T., 61 y.o. with MNF of high grade malignancy tumor (T1N0M0/GIII) at 30th transit

Total amount of UCC expressing CTA in one specimen at early transits amounted at an average to 48%, while CTA expression for UCC which underwent over 30 transits was significantly lower: 28,2% ($p<0,05$). MAGE expression amounted at an average to 55,4% at early transits and 39,3% at late transits. Portion of NY-ESO-1 expression positive cells in primary cell cultures amounted at an average to 42,3%, at late transits this value was significantly lower - 24,1% ($p<0,05$). Cells expressing GAGE and BAGE were detected in 46,8% and 41,9% at early stages of cultivation. In cultures, which underwent 30 transits and more this value amounted to 20,5% (11,9-33,9) and 27,5% ($p<0,05$).

Conclusions

In the process of cultivation of BC tumor cells for creation of antitumor vaccines, it is necessary to evaluate the level of CTA expression. UCC with high frequency of CTA expression may be used for preparation of personified autologous dendritic cell antitumor vaccines.

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Chromosome Aberrations and the Expression of Tumor-Associated Antigens by Tumor Cultures of Bladder Cancer with Long-Term Cultivation

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B428R9003

Abstract

High rate of mutational load in case of bladder cancer (BC), surpassed in this regard only by melanoma and lung cancer, determines tumor sensitivity to immunotherapeutic treatment methods and makes it one of the most promising targets for immunotherapy. Inhibitors of check-points such as CTLA-4, PD-1 and PD-L1 and antitumor vaccines have already proved their activity in case of these diseases. Supposedly, the very significant amount of mutations in the tumor, having high level of expression of tumor-associated antigens for their presentation in immune system, is the important factor of efficiency of immunodrugs. Development of personified dendritic cell antitumor vaccines against BC poses an urgent problem, which covers many aspects, necessary for its standardization. Particularly, in case of cultivation of tumor cells under *in vitro* conditions their transformation goes at higher pace in comparison with *in vivo* tumor development, which must be considered when selecting cell lines for research. The work presents details related to research of cytogenetic peculiarities of BC tumor cultures in case of long-term cultivation and detection of correlation between cytogenetic profile and expression of tumor-specific cancer-testis antigens.

Keywords: Immunotherapy, bladder cancer, anti-tumor vaccine, karyotype, cytogenetic profile, cancer-testis antigens

Introduction

Every year more than 430 thousand of new cases of bladder cancer (BC) are diagnosed, and in Russia, incidence rate of the disease tends to grow steadily [1-3]. In 95% of cases, BC is represented with transitional cell urothelial carcinoma.

In about 75% of cases, muscular non-invasive form of cancer is detected, in other 25% of cases BC is primarily represented with highly aggressive, muscular invasive form [4-6]. Clinical and morphological heterogeneity of tumor is caused by genetic diversity. Existence of two independent molecular ways of development of muscular invasive and muscular non-invasive forms of cancer is assumed [7-9]. The most noticeable are activating mutations in proto-oncogenes: H-RAS, FGFR3 and PIK3CA, typical for papillary tumors and inactivating mutations with participation of tumor suppressors: TP53, PTEN and RB1 in non-papillary tumors. According to data of several researchers [10], regarding the level of mutation load urothelial carcinoma holds the third position among all malignant growths being surpassed in this regard only by melanoma and lung cancer, defines perspectives of use of vaccine therapy for such disease.

Background

The purpose of this research was to study cytogenetic peculiarities of BC tumor cultures in case of prolonged cultivation and to reveal possible correlation of cytogenetic profile with expression of tumor-specific cancer-testis antigens (CTA).

Materials and methods

In the work, tumor specimens from 54 patients with muscular invasive (MIF) and muscular non-invasive forms (MNF) of BC, separated immediately after performance of surgical intervention, were used. Written voluntary informed consents were obtained from all patients. Fragments of tumor tissue with a size of not less than 0,3 cm^3 obtained during the surgery were immediately placed into sterile containers with nutritive medium (NM) DMEM/F12 (Biolot, Russia) and delivered to laboratory. For disaggregation of cells, automatic mechanic method (with the use of Medimashin Dako, Denmark) was used. Obtained cell suspension was sequentially flowed through sterile nylon filters with pore diameter of 100 and 70 μm (DAKO, Denmark); viability was evaluated with the

use of automatic meter Countess (Invitrogen, USA) by counting cells, colored with trypan blue; cells were suspended in full nutritive medium (FNM) of the following composition: DMEM/F12 (Biolot, Russia) with addition of 20% Fetal Bovine Serum (Biolot, Russia) and growth factors: insulin, transferrin, selenium (Invitrogen, USA). Cultivation of UCC was performed in CO2-incubator «Heracel» (Termo Electron LTD GmbH, Germany).

Passaging was performed with the use of mixture of equal volumes of Versen (Biolot, Russia) and Trypsin (Biolot, Russia) solutions [11]. As a result, 10 consistently transferred tumor cultures were obtained which were examined at early (before 10) and later (over 30) transits [12-13]. 24 hours before collection of metaphase chromosomes, colchicine in final concentration of 0.04 μg/ml was added to cultural flasks. Then after 24 hours, flask with monolayer of cells was placed for 1 minute into a cuvette with ice, following which by means of intensive shaking of the flask metaphase cells were separated from the surface and obtained suspension was poured into test tubes for centrifugation.

After pelleting of cells for 10 minutes at 1000 rpm hypotonic solution (0.075 M KCl) was added to the residue, with subsequent resuspension and exposure for 35-45 min at t=37°C. Then cells were pelleted for 10 min at 1000 rpm, supernatant was removed and along with careful stirring chilled fixative – methanol – acetic acid in the ratio of 3:1 was added drop by drop to the residue.

The last procedure was repeated three times. Afterwards cells were pipetted on chilled moist glasses and were placed for 24 h into thermostat at t=56ºC.

Then preparations were treated with trypsin solution with subsequent Giemsa staining. Preparations were analyzed with consideration for international classification of chromosomes. As a clone, 2-3 cells with identical damages were reviewed, and in case of monosomy (in case of absence of the same chromosome), there must be not less than three of these cells. Number of analyzed cells in the culture depended on the quantity of identified clones and lied within the range from 20 to 100. Structural and numeric changes of chromosomes were defined with consideration for modern nomenclature [14].

CTA expression was evaluated by means of flow cytometry method at FACS Canto II device with the use of antibodies to CTA: MAGE (FL-309), GAGE3 (N10), BAGE (R-15), NY-ESO-1 (E978) (Santa Cruz Biotechnology, USA).

Mathematical processing of data was performed with the use of package of statistical software SPSS 23.0 for Windows (SPSS, Chicago, IL, USA).

Results

Among damages, previously described in the guide on cytogenetics [15], in bladder tumors different changes in 9 chromosome, especially in 21p locus were detected, monosomy of chromosomes 1, 3, 6, 8, 13, 14 and 17, loss of Y chromosome were discovered. In FISH studies with the use of centromeric probes, specific mutations of 9 chromosome were detected in 44% of cases.

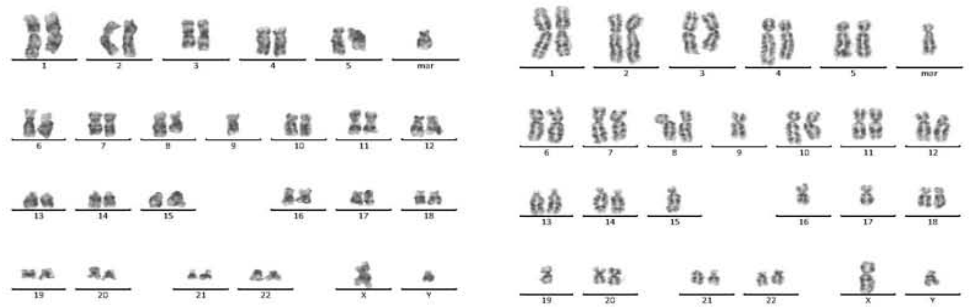
According to other data [16], this value reaches 60%. According to present-day views [17], such chromosomal irregularities correspond to the earliest stages of development of urothelial tumor and are not related to its progression.

Exactly in locus 21p of 9 chromosome there is a cluster of suppressor-genes of tumor growth p14 (ARF), p15 and p16 (MTS1, CDKN2), which encodes the protein, inhibiting cyclin-dependent kinase 4. Other big group comprises changes of 17 chromosome, in which suppressor-gene of tumor growth p53 is encoded. According to literature data [16], these changes amount to about 42% and correlate both to the degree of malignancy, and the stage of neoplastic process. 17p deletions are rarely encountered in superficial papillary tumors, but are frequently detected in case of muscular invasive cancer. According to some data, this mutation is related to dismal prognosis of disease state, early progression and metastasizing of tumor. In addition, mutations of the short arm of 13 chromosome where RB gene is located are typical for muscular invasive tumors. According to literature data [18], it is detected in 27% of cases.

In our studies it was established, that all researched tumor cultures had cytogenetic changes typical for BC. Mostly the following changes were identified: deletion of 9 chromosome (66,7%), absence of Y-chromosome (50%) and monosomy of 13 and 17 chromosomes (33,3%). In rare cases changes in chromosomes 1, 3, 7 and trisomy of 7 chromosome were detected.

Comparative studies showed that in case of prolonged passaging, in a part of cultures significant increase of the number of genetic changes in a form of division of previously homogeneous population into subclones differing in ploidy (up to 56 chromosomes) and quantity of changed chromosomes is observed, which complies with data of other researchers [16]. It can be assumed, that transformation of tumor cells under *in vitro* conditions goes at higher pace in comparison with *in vivo* tumor development. For example, in Fig. 1 (a) cytogenetic analysis of the culture of cells of urothelial poorly differentiated carcinoma of high malignant potential (T1N0M0 hight grade) of patient T. (61 y.o.), examined at 9th transit, is shown. Changes in karyotype affect 9 pair of chromosomes, in which deletion of the short arm in locus 21p occurs (del (9) (p21). In Fig. 1(b) cytogenetic profile of tumor culture of the same patient at

the 34th transit is shown. Increase of the number of chromosomal mutations and monosomy of 15, 16, 17, 19 chromosomes are noted.



(a) (b)

Fig. 1. Cytogenetic profile of the culture of cells of urothelial carcinoma (T1N0M0 hight grade) at 9th transit (a) with karyotype: 46, XY, -9,+mar and 34-the transit (b) with karyotype: 42,XY, -9, -15, -16,-17,-19,+mar.

Maximum number of chromosomal changes was detected at late transits.

In Fig. 2, karyotype of cells of urothelial poorly differentiated carcinoma of high malignant potential (T2N0M0 hight grade) of patient P. (68 y.o.) at 36th transit is shown: 53-55, X, -Y, t (1;7), + del (1) (q22) x2, + del (1) (q21), + del (1) (p22), + del (2) (p13), + del (3) (p), 4, + 5, del (7) (q11.2), t (7;12) (p;q), + t (9;12), + del (11) (q), 12q^{+}, + t (12;17), t (13;17) (q;p) ,15p^{+}, + 16, + der (16), -17, -18, + 19, -21, -22.

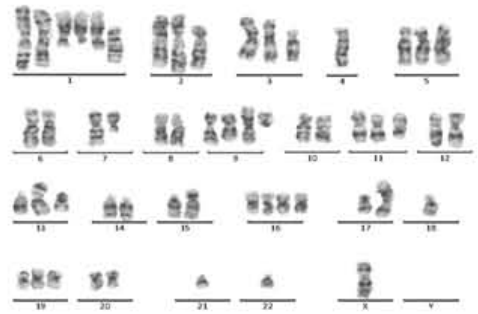


Fig. 2. Cytogenetic profile of the culture of cells of urothelial carcinoma (T2N0M0 hight grade) at 36th transit with karyotype: 53-55, X,-Y, t(1;7),+del(1)(q22)x2,+del(1)(q21),+del(1)(p22),+del(2)(p13),+del(3)(p),4,+5,del(7)(q11.2),t(7;12)(p;q),+t(9;12),+del(11)(q),12q+,+t(12;17),t(13;17)(q;p),15p+,+16,+der(16),-17,-18,+19,-21,-22.

In Fig. 3 karyotype of the culture of cells of urothelial poorly differentiated carcinoma of high malignant potential (T2N0M0 hight grade) of patient P. (71 y.o.) at 38th transit is demonstrated: 53, X-Y,i(1q),+del(1)(p22),del(1)(q12), del(1)q11, del(1)(q23), del(3)(p11),+der(6), del(7)(q12),+t(9;12),-10,del(11)(p13),der(11),-13,-15,+16,-17, 18,+20,-21.

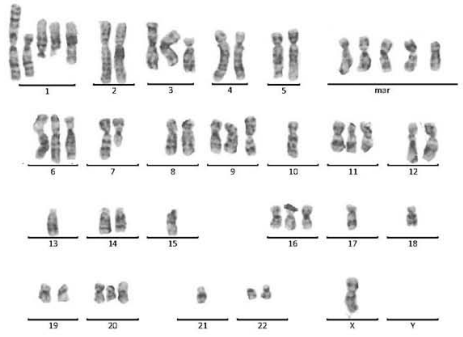


Fig. 3. Cytogenetic profile of the culture of cells of urothelial carcinoma (T2N0M0 hight grade) at 38th transit with karyotype: 53,X-Y,i(1q),+del(1)(p22),del(1)(q12), del(1)q11, del(1)(q23), del(3)(p11), +der(6),del(7)(q12),+t(9;12),-10,del(11)(p13),der(11),-13,-15,+16,-17, 18,+20,-21.

After comparison of the results with data, obtained by flow cytometry method, certain correlation of these changes to decrease of CTA expression (GAGE, BAGE, MAGE и NY-ESO-1) ($p<0,05$) was established.

Particular cultures in the course of multiple transits preserved both cytogenetic profile and consistent CTA expression, which makes them promising for further use in immunotherapy in case of BC.

Conclusions

As can be seen from the above, in the process of cultivation of BC cells it was established, that for creation of personified autologous dendritic cell antitumor vaccines it is necessary to use autologous tumor cells at early transits (not later than tenth). Use of allogenic cell cultures characterized by consistency of cytogenetic changes and stable expression of tumor-associated antigens is promising for creation of allogenic antitumor vaccines and their use for scientific and research purposes in case of BC.

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Comparative Characteristics of the Level of Expression of Tumor-Associated Antigens in Various Forms of Invasion of Bladder Cancer

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B428R9035

Abstract

Bladder cancer (BC) in patients is characterized by different extent of invasion and malignancy. Inhomogeneity of immunological and biological markers, moderate level of evidence of their clinical and diagnostic significance requires performance of further studies for research of new and discovery of optimal combinations of existing immunobiological BC markers and establishment of possible differences. The most highly immunogenic antigens are cancer-testis antigens (CTA), expression of which in case of different forms of BC is poorly explored. The work contains comparative data from research of CTA (MAGE, BAGE, NY-ESO-1, GAGE) expression in case of invasive and non-invasive forms of BC. Difference of CTA expression profile depending on the form of BC invasion is demonstrated, which opens opportunities for their use for creation of anti-tumor vaccines in case of BC.

Keywords: immunotherapy, bladder cancer, urothelial carcinoma, anti-tumor vaccine, cell culture, cancer-testis antigens, various forms of invasion

Introduction

Bladder cancer (BC) ranks second in terms of occurrence rate among malignant growths of urinary tracts and ninth among all malignant growths [1, 2]. Every year more than 400 thousand of new cases of BC are registered in the world [3, 4], and in Russia, incidence rate of the disease also tends to grow steadily. During the period from 2004 to 2014 this value increased by 14,13% in men and by 27,51% in women. In total from 8,76 to 10,2 cases per

100 thousand of people respectively. According to the report on the status of oncological assistance to the Russian population for 2015 during the decennial period from 2005 to 2015 incidence rate of BC within the territory of Russia increased from 46,0 to 68,3 per 100 thousand. 15438 new cases of BC were registered in 2015 without consideration for mortality during the first year from the moment of establishing of diagnosis, which amounted to 16,5% [5]. Incidence rate of urothelial cancer significantly varies between different geographic regions (from 1.8 to 27.1 per 100 thousand men and from 0,5 to 4,1 per 100 thousand women), coming out on top in countries with predominance of European population [6]. The most common form of tumor is transitional cell urothelial carcinoma, which is about 95% of cases of this disease.

In about 75-80% of cases, muscular non-invasive BC is detected, in remaining 20-25% of cases highly aggressive muscular invasive form of the disease is diagnosed [7]. If prognosis for muscular invasive form of cancer is always unfavorable, muscular non-invasive BC in this regard is a non-homogenous group of tumors with 80% risk of relapse of non-invasive form and 20% risk of progression [8]. In studies of recent years, significant attention was given to evaluation of influence of expression of tumor-associated antigens on disease prognosis. Particularly this relates to cancer-testis antigens (CTA).

High immunogenicity and unique profile of expression in human body makes CTA a promising candidate for creation of antitumor vaccines and possible progression marker in case of variety of malignant growths including BC [9]. In a number of studies, high levels of CTA expression by BC cells were demonstrated. It was established that CTA of MAGE group were detected in 43% of tumors, NY-ESO-1 in 35% [10]. Other studies reported 46,5% or 58,8% of MAGE-A3 positive tumors [11, 12], and reported discovery of NY-ESO-1 in 45.1% of examined tumor specimens. Data about correlation between the degree of tumor invasion and CTA expression are controversial [10, 14], consequently, they require additional studies.

Background

Objective of the research is to perform comparative study of CTA expression (MAGE, NY-ESO-1, GAGE, BAGE) by urothelial carcinoma cells (UCC) in case of different forms of BC invasion.

Materials and methods

In the work, tumor specimens from 54 patients aged from 37 to 82 with

muscular invasive and muscular non-invasive forms of BC, separated immediately in the course of performance of surgical intervention, were used.

Written voluntary informed consents were obtained from all patients.

Fragments of tumor tissue with a size of not less than 0,3 cm^3 obtained during the surgery were immediately placed into sterile containers with nutritive medium (NM) DMEM/F12 (Biolot, Russia) and delivered to laboratory. For disaggregation of cells, automatic mechanic method (with the use of Medimashin Dako, Denmark) was used. Tumors specimens were mechanically disaggregated and underwent cryopreservation for further studies. For disaggregation of cells, automatic mechanic method (with the use of Medimashin Dako, Denmark) was used [15, 17]. Obtained cell suspension was sequentially flowed through sterile nylon filters with pore diameter of 100 and 70 μm (DAKO, Denmark). Viability was evaluated with the use of automatic meter Countess (Invitrogen, USA) by counting cells, colored with trypan blue. Detailed information about each patient and characteristic of tumor was obtained from the medical record. Specimens containing at least 5x10*6 of cells were frozen in cryopreservation media containing 90% of bovine fetal serum (Biolot, Russia) and 10% of dimethyl sulfoxide (DMSO).

Cryotubes were placed into programmable freezer Mr. Frosty™ Freezing Container (Thermo Scientific ™, USA) with freezing rate of 1°C per minute, optimal for maintaining of cell viability, and were stored at the temperature of -80°C until further studies. CTA expression (MAGE, BAGE, NY-ESO-1, GAGE) was evaluated by means of flow cytometry method at FACS Canto II device (BD Biosciense, USA). Blocking of Fc-receptors for prevention of non-specific coloring was performed by means of Human BD Fc Block (BD Biosciense, USA), diluted in Stain Buffer (BD Biosciense, USA).

Incubation was performed in BD Perm/Wash solution. Cells were washed twice in BD Perm/Wash bufer, pelleted by means of centrifugation for 10 min at 1000 rpm. Then $1x10^6$ of cells were incubated with specific antibodies (20 mcl/1 specimen): MAGE, GAGE3, BAGE, NY-ESO-1 (Santa Cruz Biotechnology, USA). Mathematical processing of data was performed with the use of package of statistical software SPSS 23.0 for Windows (SPSS, Chicago, IL, USA).

Values with confidence interval of not less than 95% where $p \leq 0.05$ were considered statistically significant.

Results

In total, 24 tumor specimens were examined in the work, out of which 18 (75%) were represented with muscular invasive form of BC and 6 (25%) were represented with muscular non-invasive form.

In the course of examination of tumor specimens, taken from patients with muscular invasive BC, it was established, that NY-ESO-1 expression was in 7 (38,9%); MAGE in 15 (83,3%); GAGE in 8 (44,4%) and BAGE – in 9 (50%) of cases. Two tumor specimens of this form expressed all 4 studied CTA (11,1%), while five tumors expressed 3 out of 4 studied antigens (27,8%).

Expression of at least one of studied CTA was detected in 88.9% of cases (16 specimens). Overall, each tumor specimen of muscular invasive BC had on its surface from one to four represented CTA. Research of tumor specimens of muscular non-invasive BC showed that NY-ESO-1, MAGE and BAGE expression was registered in individual specimens (one antigen in each tumor), which amounted to 16.7% of cases for each CTA. GAGE was detected in 2 tumor specimens (33.3%). In two specimens, CTA expression was absent, and in two specimens expression of two studied CTA at once was detected (33.3%).

In 66,7% of cases one of 4 studied antigens was represented (Table 1).

Table 1. Comparative characteristics of CTA expression level in case of different forms of BC invasion

BC invasion forms	Tumor associated BC antigens									
	NY-ESO-1		MAGE		GAGE		BAGE		Total	
	n	%	n	%	n	%	n	%	n	%
Muscular non-invasive Ta-T1	7	38,9	15	83,3	8	44,4	9	50	16	88,9
Muscular invasive T2-T4	1	16,7	1	16,7	2	33,3	1	16,7	4	66,7

In the course of examination of CTA expression level both as a whole and for each antigen individually, reliably significant increased CTA expression in case of muscular invasive BC in comparison to non-invasive tumor form was ascertained ($p<0.05$). CTA expression by tumor cells of urothelial carcinoma of patient L, 61 y.o. with muscular invasive tumor form of high degree of malignancy is shown in fig. 1 (T2N0M0/GIII).

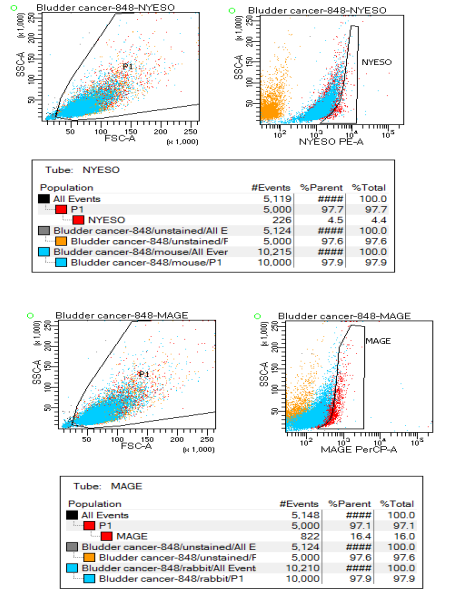

Fig. 1. CTA expression (MAGE, BAGE, NY-ESO-1, GAGE) by tumor cells of urothelial carcinoma of patient L., 61 years old with muscular invasive tumor form of high degree of malignancy (T2N0M0/GIII).

Conclusions

As can be seen from the above, preliminary analysis of CTA expression profile (MAGE, BAGE, NY-ESO-1, GAGE) demonstrated that it is significantly higher in case of muscular invasive forms than in case of muscular non-invasive BC tumors. This opens possibilities and prospect for use of CTA for specific immunotherapy and development on their basis of multicomponent multiantigen dendritic cell anti-tumor vaccines against BC.

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The Effect of the Exogenous Glucosamine Hydrochloride and Chondroitin Sulfate on Biochemical Indices by the Experimental Inflammation of the Parodentium Tissues

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B428R9006

Abstract

In the article the evaluation issues of the effect of the exogenous glucosamine hydrochloride and chondroitin sulfate on biochemical indices of the blood serum as well as of the parodentium tissues by the experimental inflammation are examined. The experimental model of gingivitis has been formed in 40 white rats of the Wistar line presenting the control and the main groups; additionally, the parodentium of 20 intact animals has been studied.

It has been revealed that the usage of glucosamine hydrochloride and chondroitin sulfate by the experimental inflammation of the parodentium tissues leads to the normalization of the main inflammation indices including the general proteolytic activity and the activity of the acid phosphatase as well which indicates the anti-inflammatory and the parodentium protective effects.

Keywords: biochemical indices, inflammation, experiment, parodentium

Introduction

In spite of the high achievements of modern medicine the inflammatory-dystrophic lesions of the parodentium tissues take the leading part among the dental disorders and represent a considerable medical and social problem [5, 9]. It is known that the stroma of the parodentium complex is represented by the different types of connective tissue that are subject to the constant destruction in conditions of the inflammation process which causes the loss of dentogingival and dentoalveolar attachment as well as the loss of bone tissue

and the loss of tooth as the consequence [15, 16]. The medicamental treatment as a part of the complex therapy for inflammatory-dystrophic disorders of the parodentium must be aimed at different levels of the pathological process [8, 10].

The most important and effective for the treatment of this pathology is the prescription of remedies that not only possess the manifested anti-inflammatory effect but also intensify the regeneration processes in the dental ligamentous apparatus [13, 14]. Being the natural structure component of biomembranes the glucosamine takes the leading part in the protective function of the gum epithelium provided by the glucosamineglycanes which are a part of the gluing substance between the cells of the laminated pavement epithelium that maintain the mechanical strength of the cell wall and prevent from the penetration of microorganisms and their toxins into the underlying tissues [1, 2].

The usage of glucosamine provides the decrease in the mechanical tissue deformations as well as the optimization of their reciprocal position and blood supply [3, 6]. The second area is the stimulation of anabolic and regenerative processes in the connective and other types of tissues. It is known that the intake of the glucosamine stimulates the biosynthetical processes in the connective tissue which leads to the shortening of its recovery period after the lesion by means of the physical or chemical factors [4, 7]. The third area of the protective effect of glucosamine is the inhibition of activity of the lysosomal enzymes that are destroying the connective tissue and the other types of organic tissues as well [11]. It is especially evident in the presence of inflammatory and dystrophic processes in the connective tissue. There are some data that indicate the considerable role of the hyaluronic acid in the regulation of the capillary-connective structures and also in the fact that glucosamineglycanes provide the protection of the parodentium tissues against the bacterial and toxic agents [12].

The purpose of the study is to evaluate the effect of the exogenous glucosamine hydrochloride and chondroitin sulfate on biochemical indices of blood serum as well as of the parodentium tissues by the experimental inflammation.

Materials and methods of the study

The experimental studies have been performed on 60 white rats of the Wistar line with the mass about 220-250 g that have been kept on the standard food and water ration in accordance with the hygienic standards. The experiments have been carried out in accordance with the International principles of the

European convention about "The Protection of Vertebrate Animals Used in Experiments and for Other Scientific Purposes" (Strasbourg, 1986), «The General Ethical Principles for Experiments on Animals» (Russia, 2011) as well as in accordance with the principles of the proper laboratory praxis (National Standard "The Principles of the Proper Laboratory Praxis", state standard R 53434-2009) and the positive conclusion of the Ethical Committee N. 32 of 12.02.2014. The study has been performed within the confines of the State Task of the Ministry of the Public Health Care of the Russian Federation for the "Stavropol State Medical University" of the Ministry of the Public Health Care of the Russian Federation for implementation of scientific researches and innovations within the theme "The Parodentium Tissues in Regeneration and Immunomodulation" of 14.01.2014 № 302/09 together with the Russian Research Institute of Sheep and Goat Breeding and the Stavropol State Agricultural University (Stavropol).

The animals have been divided into 3 groups: 1st group – the intact animals (20 animals); 2nd group – rats suffering from the experimental gingivitis (20 animals); 3rd – rats suffering from the experimental gingivitis that have been orally given the water solution of glucosamine hydrochloride and chondroitin sodium sulfate (Theraflex®, Sagmel, Inc., USA) in the dose of 30 mg/kg 3 times a day (20 animals); (the experimental period made up 90 days: 60 days – the modeling of gingivitis; 30 *cymoк* – the treatment period).

The model of the experimental gingivitis has been formed in two phases: within the first phase – by means of creation of dysbacteriosis in the oral cavity (intramuscular injections of the lincomycin hydrochloride in dose of 30 mg/kg two times a day within 5 days) and further local lesions of gum and tissues of the vestibule of mouth by means of the application of the apitoxin suspension (in dose of 1 mg/kg two times a day within 5 days). The applications have been performed on two areas of the vestibule of mouth between the under lip and the lower incisors and between the upper and lower molar teeth and the right cheek.

The treatment has started on the next day after the pathology reproduction had been completed. The general proteolytic activity in the blood serum and the homogenates of the parodentium tissue has been revealed in accordance with the method of B.F. Erlanger (on the fission of the N-a-benzoyl-D, L-arginine paranitroanilide). The determination of the activity of the alkaline and the acid phosphatases in the blood serum has been performed in accordance with the method of Bodanskiy (1992) that is based on the ability of enzymes to chip the non-organic phosphate off from the b-glycerophosphate. For determination of the activity of the alkaline phosphatase the alkaline solution of the

b-glycerophosphate has been used while for the acid phosphatase the acid solution of the b-glycerophosphate has been used. The statistical processing of the received study materials has been carried out by means of the single-factor analysis of variance and the Newman's multiple comparison in the PrimerofBiostatistics 4.03 program for Windows. The differences of $p<0,05$ have been considered to be correct.

Results of the study

As the results of the experimental morphological study has revealed the development of the clinical pattern of gingivitis has been accompanied by the changes in the biochemical indices in the blood serum and the gum tissue of rats. The study of the general proteolytic activity (GPA) in the blood serum of rats suffering from the experimental parodentium inflammation has revealed the increase of its indices in comparison with the data received on the intact animals. As far as the level increase of the general proteolytic activity in the blood serum indirectly indicates the presence of the inflammatory process in organism, the highest indices have been registered on the 5^{th}-10^{th} day of experiment.

Under the influence of the exogenous glucosamine hydrochloride and chondroitin sulfate in the main group the decrease of GPA up to the indices of the intact rats on the 20^{th} day of experiment has been revealed. In addition since the 10^{th} day of observations, the level of the total activity in the main group has been correctly lower than the same index of the control group (table 1).

Table 1. The general proteolytic activity (millimole/t*s) in the blood serum of rats within the interactive experimental gingivitis without treatment and by the usage of the exogenous glucosamine hydrochloride and chondroitin sulfate (M±m)

Subject of the study	Study period, days			
	5	10	15	20
Intact rats n=20	2,05±0,04			
Control group (rats suffering from experimental gingivitis), n=20	5,02±0,08	4,09±0,06*	2,95±0,13*	2,44±0,15
Main group (rats suffering from experimental gingivitis +Theraflex®), n=20	4,15±0,07	3,04±0,03**	2,19±0,12**	2,03±0,09*

*Note: *the indices are correct in comparison with the indices of the intact animals, p<0,05; ** the indices are correct in comparison with the indices of the animals from the control group (gingivitis), p<0,05*

Therefore, the usage of the exogenous glucosamine hydrochloride and chondroitin sulfate has provided the level decrease of the universal inflammatory index notably the general proteolytic activity which testifies to the specified positive influence of the exogenous glucosamine hydrochloride and chondroitin sulfate on the systemic homeostasis.

The study of the general proteolytic activity in the homogenates of the parodentium tissue has also enabled the determination of changes similar to the ones observed in the blood serum (table 2).

Table 2. The general proteolytic activity (millimole/t*s) in the homogenates of the parodentium tissue of rats within the interactive experimental gingivitis without treatment and by the usage of the exogenous glucosamine hydrochloride and chondroitin sulfate (M±m)

Subject of the study	Study period, days			
	5	10	15	20
Intact rats n=20	2,05±0,04			
Control group (rats suffering from experimental gingivitis), n=20	5,98±0,37*	5,46±0,23*	5,19±0,45	4,36±0,21*
Main group (rats suffering from experimental gingivitis +Theraflex®), n=20	6,09±0,43**	4,93±0,45**	4,09±0,22*	3,92±0,55*

*Note: *the indices are correct in comparison with the indices of the intact animals, p<0,05; ** the indices are correct in comparison with the indices of the animals from the control group (gingivitis), p<0,05*

In the group of animals suffering from the experimental gingivitis without treatment the increased general proteolytic activity during the entire experimental period has been detected, but it's maximal increase has been observed on the 5^{th}-10^{th} day.

Under the influence of the exogenous glucosamine hydrochloride and chondroitin sulfate the general proteolytic activity in the homogenates of the parodentium tissues has decreased up to the normal level on the 20^{th} day of experiment. From the 15^{th} day and till the end of the experiment the GPA indices had the lower value in comparison with the animals of the control group. Thus the usage of the exogenous glucosamine hydrochloride and chondroitin sulfate by the experimental inflammation of the parodentium tissues has the anti-inflammatory and the parodentium protective effect and normalizes the proteolytic process. By the experimental gingivitis in addition to the intensification of proteolytic processes the activization of the lysosomal

enzymes of the acid phosphatase has been detected both in the blood serum and in the homogenates of the parodentium tissue (table 3) which indicated the damage and destruction of the cell membranes of parodentium.

Table 3. The activity of the acid phosphatase in the blood serum (mckat/l) and the homogenates of the parodentium tissue (mckat/g) of rats within the interactive experimental gingivitis without treatment and by the usage of the exogenous glucosamine hydrochloride and chondroitin sulfate (M±m**)**

Subject of the study	Study period, days			
	5	10	15	20
Blood serum				
Intact rats n=20	1,22±0,06			
Control group (rats suffering from experimental gingivitis), n=20	1,94±0,07*	1,58±0,09*	1,44±0,06*	1,37±0,09
Main group (rats suffering from experimental gingivitis +Theraflex®), n=20	1,42±0,04**	1,35±0,03**	1,27±0,08*	1,19±0,05
Homogenates of the parodentium tissues				
Intact rats n=20	3,55±0,27			
Control group (rats suffering from experimental gingivitis), n=20	7,09±0,22*	6,12±0,23*	4,92±0,43*	3,25±0,44*
Main group (rats suffering from experimental gingivitis +Theraflex®), n=20	5,77±0,25**	4,97±0,46**	3,22±0,45*	3,23±0,73*

*Note: *the indices are correct in comparison with the indices of the intact animals, p<0,05; ** the indices are correct in comparison with the indices of the animals from the control group (gingivitis), p<0,05*

The normalization of the activity of the acid phosphatase in the blood serum and the homogenates of the parodentium tissues in animals of the control group has taken place only on the 20th day of the experiment. In the main group of animals the normalization of this index has been apparent in the blood serum only on the 10th day while in the homogenates of the parodentium tissues – only on the 15th day. Because the acid phosphatase is one of the inflammation indices the decrease of its activity under the influence of the exogenous glucosamine hydrochloride and chondroitin sulfate testifies to the positive effect of these substances on the inflammatory process and the shortening of the recovery period for rats suffering from the experimental gingivitis.

Conclusion

Thus the usage of the exogenous glucosamine hydrochloride and chondroitin sulfate by the experimental inflammation of the parodentium tissues positively influences the biochemical indices of blood serum as well as those of the parodentium tissues: it leads to the normalization of such inflammation indices like the general proteolytic activity and the activity of the acid phosphatase which indicates the anti-inflammatory and the parodentium protective effects of the used remedy.

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Translational Methods for Solving the Tasks of the Project to Develop Bioengineering System and the Neuronet Processes of Diagnostic

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B428C0044

Abstract

In the present study is considered the complex scientific approaches to solving the problem translation of fundamental research into the practice of medical diagnostics. For this purpose, has been formed the research team, consisting of groups possessing knowledge in different areas.

For the purposes realization of the project has been developed a biotechnical system, including technical device for input an electrophysiological information in mode on-line.

The paper presents a comparative analysis of classification of the degree of an activity of the autonomic nervous system by means of an artificial neural network trained by algorithm of back-propagation of error and by means of artificial neural network trained by combining algorithm of back-propagation of error and variant of method stochastic training Cauchy.

Performed medical trials have shown high clinical efficiency of aggregated neural network algorithm classification of the degree activity of ANS. The presented objects in form of autoregressive clouds (ARC) at using of given algorithm in the 100% cases were recognized correctly. The errors of classification amounted 0%.

Keywords: pulse sensor, biotechnical system, neurocomputing, neural network classification algorithm, the algorithm back-propagation, stochastic training algorithm Cauchy, combination of training methods

Introduction

Artificial neural networks (ANN) are used for decision a wide range of

problems in the mathematical modeling of poorly structured and poorly formalized processes in various technical systems. It should be noted also that these systems belong to ones of the most effective and, therefore, are highly by the demanded in biomedical research. The mathematical description of the processes assessment of the status objective of the leading physiological systems of the human organism is associated with the processing of a large array of numeric data received via an input device the electrophysiological information.

However, for the sake of truth it should be emphasized that as precursors of a given area of neuronet investigations were scientific works associated with probabilistic methods analysis of electrophysiological information by means of biotechnical system differential diagnosis of the change of human functional state. [3, 4, 5].

In medical practice neuronet technologies were used for analyzing signals of photoplethysmographic pulse in patients with vascular disease of the lower extremities [1].

In the problems of classification required a new sample attributed to a certain group. The ANN let you create rules of decision-making in the learning process. In the problems of this sort, as a rule, is necessary to have the training set of data that were previously classified by the experts in the studied question.

The approaches for ANN training based on the use of the algorithm the back-propagation of error were described in the work [2].

Actual is also carrying out investigations connected with evaluation of the effectiveness of different methods of training artificial neural networks to solving the problem of classification of functional states of humans.

To achieve this goal, it is necessary to solve the following tasks:

- elaborate a biotechnical system for entering and processing of electrophysiological data in on-line mode;
- develop the model ANN for solving the problem of classification;
- create a training algorithm based on the algorithm of back-propagation of error;
- form a training algorithm based on the combination of back-propagation of error with stochastic Cauchy training;
- work out a several computer applications for the evaluation of the adequacy of the developed models;
- compare the results of study on the basis of clinical criteria of effectiveness algorithms for recognizing the state of activity ANS in terms of sensitivity and specificity.

For the decision of formulated tasks were used the methodology of the system analysis, neurocybernetics, control theory and the theory of modeling.

Material and research methods

General strategy to achieve the objectives was the organization of the cluster translational medicine operating on contractual basis.

The solution of the tasks of the project within the established cluster carried out translational research team of biophysicists, engineers, designers, system programmers and doctors.

Implementation of the objective functions in it is provided by crowdsourcing ideology as with the involvement of the financial component of the project, and with the recruitment of the necessary competences and intangible assets of its subdivisions to carry out the project. It is imperative to attract employees with strategic thinking skills.

The infrastructure of the cluster is shown in Figure 1 and includes 5 modules:

1. Creative module generating innovative ideas with the decomposition of the project objectives and functions;
2. System Programming Module;
3. Manufacturing module for the implementation of biotechnical systems of diagnostics and treatment;
4. Technology transfer module;
5. Clinical research module.

The cluster Coordinator provided overall project management.

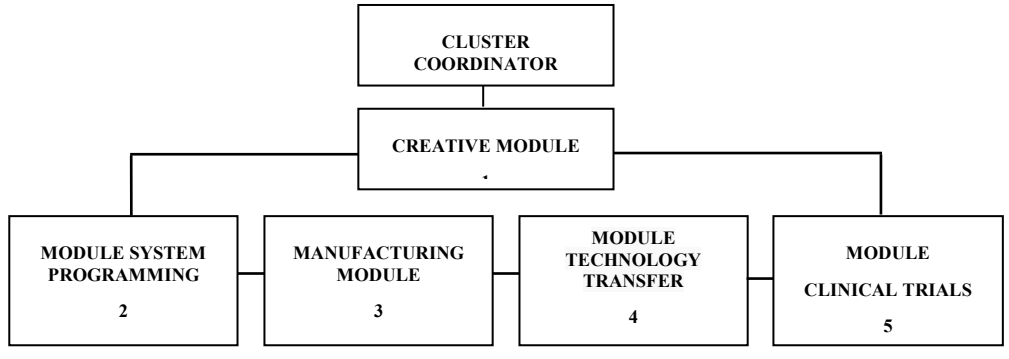

Fig. 1. The structure of the cluster for translational medicine project

Functionally, each module is belonged to some departments of higher educational institutions of Belgorod, Voronezh, as well as enterprises in the city of Belgorod and Kursk. Computer modeling of artificial neural network trained by combining gradient and stochastic methods was carried out in the programming environment Lazarus.

On the basis of system analysis was designed biotechnical device for entering electrophysiological information in mode on-line and presented on figure 2.

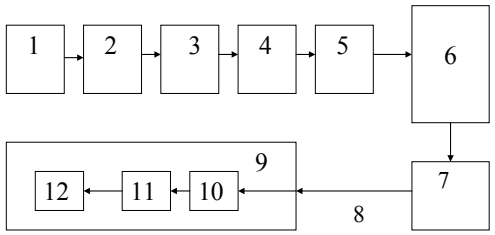

Fig. 2. The structure of the biotechnical system

Into device was integrated a pulse sensor (1-5), a microprocessor with ASCII signal converter (6), converter USB Universal Serial Bus and USB bus (7), USB bus (8), personal computer (9).

For the personal computer has been developed program-determinant of a USB device (10), the program converter of protocol USB bus in the COM-port protocol (11) and neural network application program (12).

The classification of the degree activity of ANS is carried out by means of two-layer type of network trained by algorithm of back-propagation of error and by means of artificial neural network trained by combining algorithm of back-propagation of error and variant of method stochastic training Cauchy (Fig.3).

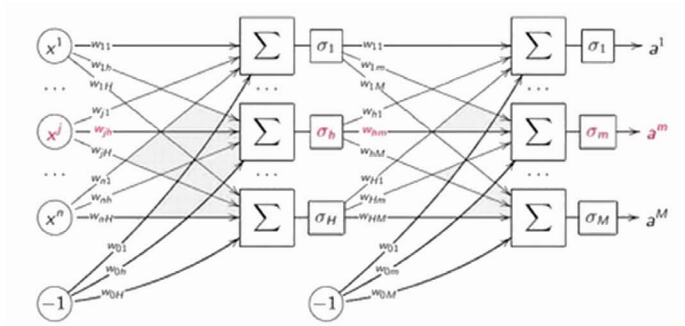

Fig. 3. Structure of two-layer type of network

A two-layer type of network is being considered here in form:

$$a^m(x,w)=\sigma_m\left(\sum_{h=0}^{H} w_{hm}\sigma_h\left(\sum_{j=0}^{n} w_{jh}f_j(x_i)\right)\right).$$

The input data of model represent a vector of interpulse intervals obtained by using of the input block of electrophysiological information.

The initial time series of interpulse intervals are divided by parts, each

of which comprises two measurements signal. Each pair defines a temporal component namely: so-called zero correction, accelerating and decelerating heart rate correction.

The random quantity X is introduced into consideration. The value of this quantity is associated with the duration and with the sign of correction.

All observed values belong to the intervals (1,55; 1,55) that were passed in increments of 0.05.

Thus, the alphabet of the system includes 61 classes of differential histogram microstructure patterns of heart rate variability. Computations are performed from an array of 500 interpulse intervals.

Frequencies of interval variation series are entered to an input of ANN X.

Input network x_j, $j=\overline{1,61}$, have been subjected to a process of normalization by means of subtracting the sample mean and dividing by the correcting sample standard deviation.

The activity of neurons in the hidden layer y_k^c, $k=\overline{1,10}$, is calculated by the formulas:

$$y_k^c = f\left(\sum\nolimits_{j=1}^{61} w_{kj}x_j\right), \quad (1)$$

Where w_{kj} is the weight ratio between the j -input and the k -hidden layer neuron.

As an activation function has been selected the hyperbolic tangent.

The activity of neurons in the output layer y_i, $i=\overline{1,6}$, is calculated by the formulas:

$$y_i = f\left(\sum\nolimits_{k=1}^{10} v_{ik}y_k^c\right), \quad (2)$$

Here v_{ik} the weight ratio between k -hidden layer neuron and i -output layer neuron.

The number of neuron of the output layer having a maximum activity is a marker of the class to which the network classifies the input sample.

The physician determines the position of the output class in the training set.

The position of the correct class is marked on the target vector with value of the 0.5. The rest coordinates of the target vector have of the value: -0.5.

Recognition of the class determined by the maximum output level of the neuron, which represents one of the six classes: a sharply pronounced predominance of the sympathetic nervous system (SPP SNS), pronounced predominance of the sympathetic nervous system (PP SNS), a moderate predominance of the sympathetic nervous system (MPP SNS), the equilibrium state between the sympathetic and parasympathetic nervous systems (Norm), a moderate predominance of the parasympathetic nervous system (MP PNS), pronounced predominance of the parasympathetic nervous system (PP PNS).

Results and discussion

In the experimental part of the work were carried out the studies on the adequacy of the developed models to real electrophysiological processes.

Computer modeling of artificial neural network trained by combining gradient and stochastic method was carried out in the programming environment Lazarus.

To evaluate the clinical effectiveness of the classification were analyzed records of the interpulse intervals from 139 healthy students of Belgorod State national research University. All of them were part of a social and age groups of 17 to 24 years.

Data analysis shows that the algorithm of training ANN on base of the back propagation of error has correctly recognized 96.0% of the samples.

Incorrectly detected cases were only in 4.0%. And all of them were attributed to cases hypodiagnostics. Cases of hyperdiagnosis the algorithm has not allowed.

All recognized cases (100%) are distributed as follows: these were 70% of the true positive cases and 26% these were true negatives cases, including 4% hypodiagnostics.

The sensitivity of the recognition algorithm was 100.0% (70.0/70.0 + 0.0), the specificity differential diagnosis – 86.7% (26.0/26.0 + 4.0).

Performed special research on a sample of examining showed that 93.0% of all cases have been correctly identified. Incorrectly detected cases were 7.0%. Of these cases the hyperdiagnosis has been marked in 5.0% of cases and only 2.0% were marked as cases of hypodiagnosis.

The sensitivity of the recognition algorithm was 97.1% (68.0/68.0 + 2.0), the specificity differential diagnosis – 83.3% (25.0/25.0 + 5.0). The neural network has overpriced the degree of class activity of ANS in only 5% of cases and it has understated only in 2% cases.

Analysis of the clinical effectiveness of combined algorithm back-propagation with stochastic training Cauchy showed that the training and control samples were correctly detected 100% of the examples. Classification of errors made 0%.

Resume

Based on our research, we found that best options of informational medical systems for classification degree of activity autonomous nervous system of person may be presented by means of artificial neural networks. It should also be emphasized that the revealed facts may be used for recognition different functional states of patient. This in its turn opens up a wide road for developments

a new medical technologies in region non invasive cardiovascular monitoring.

In this research were obtained the following scientific results characterized by novelty:

1. A biotechnical system, comprising an input unit of electrophysiological information, as well as a full-featured application designed for the classification of the current state ANS of man according to the pattern microstructure of heart rate variability.
2. A model of a two-layer artificial neural network (ANN) was designed to solve the classification problems of the degree activity of autonomic nervous system (ANS).
3. An effective a training algorithm of ANN based on a combination of gradient and stochastic methods of teaching was formed.
4. The clinical efficacy of the neural network algorithm classification of the degree activity of ANS was analyzed. The 100% cases were recognized correctly. The errors of classification amounted 0%.

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Kristina F. Biotechnical system of automatic classification scattergrams and evaluation of atrial fibrillation outcomes. *International Journal Of Pharmacy & Technology (IJPT) June-2016 | Vol. 8 | Issue No.2 | 14129-14136*

Neuromuscular and Psycho-Emotional Aspects of Marfan Syndrome in Pediatrics

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B428C0033

The clinical picture of Marfan syndrome described by McKusick 50 years ago. Most experts perceive MS as a rare condition associated with dolichostenomelia, subluxation of the lens, and aortic aneuryMS. However, in European populations of the syndrome (disease) Marfan occurs with a frequency of from 1:10 000-1:20 000 to 1:5000 of the population, which indicates a large number of erased forms that do not have the classic triad of symptoms: dolichostenomelia, ectopia lentis, aortic aneuryMS.

Erased form MS (Marfan syndrome) are explained by the multiplicity of molecular mechaniMSs of pathogenesis and heterozygosity. The syndrome is caused by a change in a gene on chromosome 15 (15q15–21), responsible for the synthesis of fibrillin – the most important part of microfibril elastic fibers. One gene already described more than 500 mutations, this explains the variability of the symptoms and in some cases, difficulties in the diagnosis.

Genetic diagnosis of MS is not possible in all cases, it is difficult and costly.

One of the first methods of diagnostics, the so called "omnibus": diagnosis of the syndrome at appearance of the patient and the results of morphometry.

Among children and adolescents with MS are often encountered patients with "myopathic" features: low weight muscle hypotonia, scoliosis, contractures of the joints. In rare cases described centrolobular myopathy.

Characteristic of MS adverse changes of the heart (expansion of the aorta, rhythm disorders, non-rheumatic mitral valve insufficiency), Hyper-mobility and/or joint contractures lead to a significant reduction of motor activity and compound muscle change. The number of messages provides information about the pleiotropic manifestations of muscle syndrome with a hereditary pathology of the connective tissue, but adequate studies of the muscles with the assistance of modern techniques was not performed. Information about the mental abilities of people with MS and their emotional status is not always straightforward.

It is considered that the index of intelligence (IQ), defined according to the standard techniques adapted to the age of the patients, not different from that of the population, although in other publications MS is associated with learning difficulties, even with normal IQ and predisposition to psychosis.

Keywords: Marfan syndrome, Condition of muscles, cognitive and emotional sphere, children and adolescents

The purpose of the study

was to examine the state of the muscles and nervous-emotional sphere by children and adolescents with MS.

Materials and methods of research

Examined 95 patients ranging in age from 8 to 20 years: boys 45, girls – 40 (table. 1). Among all children 65 (68,4%) were in the family of a relative with MS, in 5 cases in the family were likely. Thus, in 73% of cases, we observed a family MS. Under our supervision there were children mostly puberty, when signs dolichostenomelia manifested most clearly. Phenotypic MS in children during the first years of life are revealed rarely, only in very severe cases.

The diagnosis of MS is based on the clinical picture and is installed by the Ghent criteria.

1. General characteristics

A. the Face is triangular with a MSall chin, high nose bridge (bird's face), the eyes are set deep, close to each other. The expression of the eyes sad. The patient recalls the characters from the paintings of El Greco.
B. the Sky is high (Gothic). Improper growth of teeth, malocclusion, prognathia. Voice high.
C. Dolichostenomelia* (long thin limbs). Austenitnoi body type. The thumb laid across the palm and supports its ulnar edge (Steinberg sign). The little finger and the thumb of the patient freely covers your wrist (a sign of Mardaga, Fig. 1). The lower segment of the body more than the upper, arm span prevyshaet growth.
D. high Growth exceeds the average growth of healthy relatives 1st degree relatives (not target population).
E. "Chicken breast"*, or "the breast of the shoemaker"* often asymmetric.
F. Flat back, kyphoscoliosis*.
G. Hypermobility of the joints (Fig. 2).

H. hypotrophy of the muscles.
I. Hernia, often recurrent.
J. Velvety soft skin with sparse subcutaneous fatty tissue, and stretch marks stretching (20%) not associated with obesity.

Table 1

The age of examined patients			
Figure	**The median**	**Min**	**Max**
Age	**14**	**8**	**20**

K. Cysts of the tops of the lungs, recurrent pneumothorax.
L. ectasia of the Dura mater in lumbosacral Department, arteriovenous aneuryMS in the brain and spinal cord. The extension of the Cysterna Magna.
M. Protrusio acetabuli.

2. Eyes changing

A. Dislocation/subluxation of lens* more often bilateral, upwards, and outwards, in 60% of cases develop before age 4 years (Fig. 3). Timely diagnosis is important for the study using slit lamp on the background of mydriasis. The clinical symptom of the dislocation of lens – shake the inner edge of the iris.
B. Myopia caused by stretching of the capsule of the eye, and a spherical lens (rarely), megalocornea, flat cornea.
C. Coloboma of the iris, glaucoma, retinal detachment.
D. Narrow pupils (underdevelopment of the M. dilatator pupillae), Arcus senilis.
E. the Membrane of the pupil.

3. Cardiovascular changes (99%)

A. Progressive expansion of the aorta* and/or sinus of Valsalva*.
B. Dissecting aortic aneuryMS and/or rupture.
C. Insufficiency of the aortic valves.
D. mitral valve Prolapse, it's not enoughprecision, mixomatosis degeneration of the valves, calcification of the mitral annulus.
E. Violations of heart rhythm.

4. Family history

Frequency of occurrence in the relatives 1st degree relatives, 75% for sporadic cases, 25%. You must have at least two main features marked with an asterisk (at least one of the four above-mentioned groups), and several additional. Electromyographic studies were performed to exclude benign muscular dystrophy in the presence of complaints of patients to muscle weakness, difficulty in climbing stairs. Sensorineural (somatosensory) evoked potentials provide an objective conclusion about the body's response to irritation of the peripheral nerves. In the presence of appropriate indications to exclude a primary muscle in the process (myopathy) histological study of muscles. Biopsies were taken during surgical corrective interventions of a straight back muscles during surgery about ectasia of the Dura of the spinal cord (7 patients) or pectoralis major muscles during surgery for funnel chest (12 patients). For light microscopy, the biopsy was placed in buffered solution paraformaldehyde-glutaraldehyde.

Fig. 1. *Mardoch characteristic – the patient's ability to embrace your wrist with the little finger and thumb*

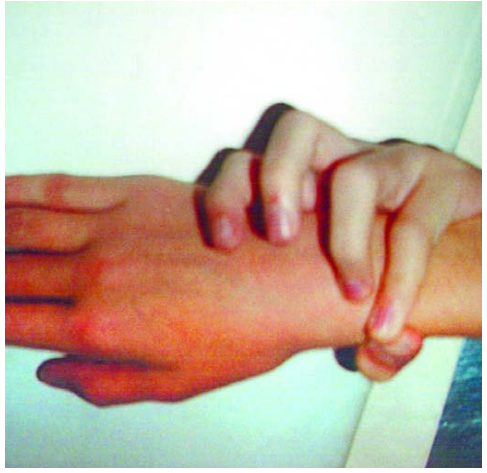
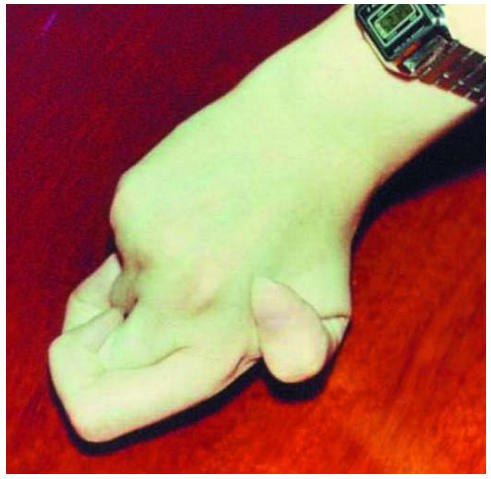


Fig. 2. *Joint hypermobility: a teenage girl is capable of braid your fingers are literally in a pigtail*



After pouring paraffin sections were prepared and stained them with hematoxylin-eosin, Mallory 3 rum and pas reaction and were studied with magnification 200 and 400 times. In the process of General survey of children we used the results of the evaluation of the index of intelligence (IQ) test Kattell, made by professional psychologists. The degree of deficiency of attention with or without hypermobility was determined according to the recommendations of the American psychiatric Association.

To obtain more objective conclusions about psycho-emotional disorders in

18 children studied, the excretion of noradrenaline in the urine in rest and during mental stress (solving mathematical problems at school). The concentration of noradrenaline in urine was determined by the method of D. Becker.

The results were processed mathematically using the nonparametric criterion Mann-Whitney, Wilcoxon, Matched Pairs Test, "Theory the differences against the theory there is no difference". Differences were taken for statistically significant at p=0.05 or less.

The results of the study and their discussion

Of the 95 surveyed children and adolescents 39 (41,1%) complained of headaches. Headaches in children with MS were detected significantly more often than in the population of students (10%) without the syndrome dolichostenomelia. Among the additional features that the researchers usually do not record, it should be noted malocclusion

(78 children 82,1%), clicks in the temporomandibular joint, and pain in the temporomandibular joint, Orofacial pain. Ectasia of the Dura of the spinal cord was in 8 patients. General overt clinical picture allowed to establish their diagnosis MS to 7 years of age. Patients with ectasia of the Dura of the spinal cord complained of headaches (7/8). They intensified in the upright position and decreased in the supine position, which might indicate a decrease in intracranial pressure redistribution

CSF in the upright position. In addition, it was noted back pain (5/8), usually in the lower thoracic – upper lumbar spine, pain in the lower abdomen (4/8) without signs of cystitis, radiculopathy (1/8). All children marked disturbance of posture and flat feet; 70 (73,7%) – pain in various muscle groups. 17 children in the first year of life recorded a loss of muscle tone.

Further observed hypotrophy of the muscles, reduction of muscle strength (14 children), difficulty in climbing stairs. 9 children was a violation of fine motor skills.

Fig. 3. *Subluxation of the lens upwards (arrow)*

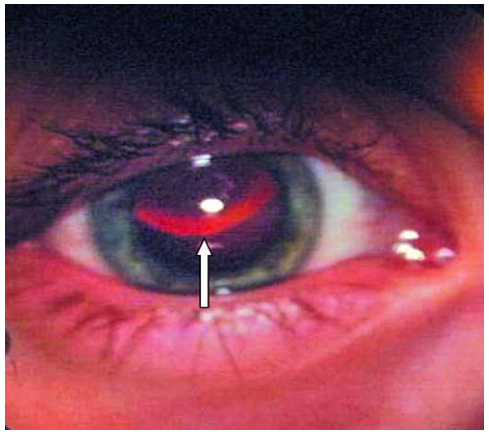
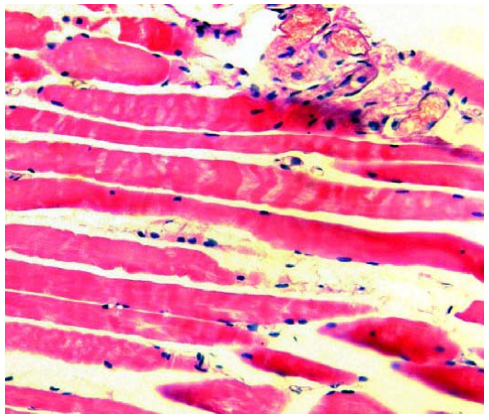
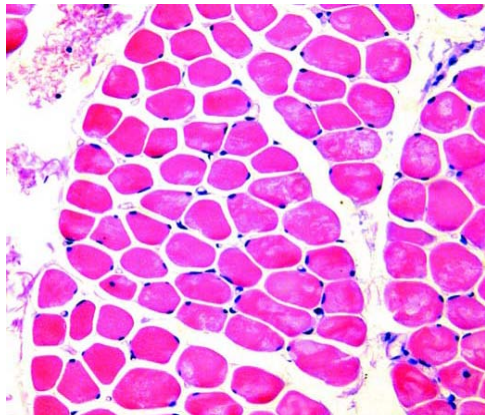


Fig. 4. *Echogram of muscle in Marfan syndrome: development of connective tissue image resembles a featherbirds*

Fig. 5. *Visible muscle fibers of different sizes, inside of which are determined by the nucleus (A). Edema of the interstitial tissue, perimysium (B)*



A

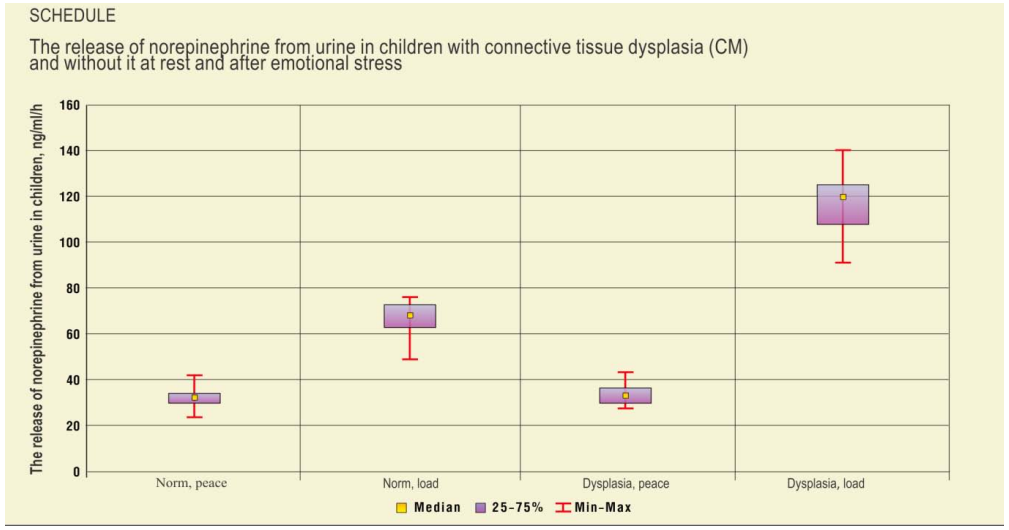


B

Table 2. The release of norepinephrine from urine in children without dysplasia, with dysplasia of connective tissue at rest and under emotional-mental load, ng/ml/h

Figure	n	The median	Min	Max
Norm, peace	**20**	**33,0**	**24,0**	**41,0**
Norm load	**20**	**68,0**	**49,0**	**76,0**
Dysplasia, peace	**20**	**34,5**	**27,0**	**42,0**
Dysplasia, load	**20**	**120,0**	**90,0**	**140,0**

Schedule



Upon further study of the condition of the muscles in all children and adolescents with the signs of hypotrophy of muscles, weakness, difficulty climbing lesti TSE noted changes in the EMG and muscle echograms.

Among 15 patients without clinical signs of myopathy syndrome in 10. irawan myogenic level of the lesion according electromyography, which was manifested quantitative ing a decrease in electrical activity. In the study of somatosensory evoked potentials with N. tibialis signal delay was found in 6 patients with ectasia of the Dura of the spinal cord and 3 patients without ectasia. The relationship between expressed completely ectasia and changes in somatosensory potentials in this patient group was not detected.

Echographies in patients with MS revealed a moderate increase in acoustic density of the muscles was visualized by many layers of connective tissue, resembling a drawing of a quill pen (Fig. 4).

Histologically, along with the polygon portage us have met different round dies in size from 10 to 65 microns in diameter. In some beams the number of fibers was MS aller. While rarely found MS all bundles of thin fibers (5 microns in diameter). In single fibers was observed migration of nuclei inside fibers, increased connective tissue in the endo and primizie various Noah, width, density, ripeness, the swelling of the interstitial tissue and peremisyally.

In perimysium IU showed a slight lymphocytic infiltration around the decaying fibers. Recorded a decrease and uneven races the position of the granules of glycogen in single fibers – coarse glycogen granules in the periphery of the fibre and between fibrils, basophilia, sometimes some Raya metachromatically cytopla MS of myocytes. In the nerve fibers marked edema, dystrophic and sclerotic changes.

The histological changes confirm the echo graphic findings, deviations, electromyograms (Fig. 5, A, B). Changes of nerve fibres can be one possible reason we found conduction disorders.

According to the results of a dedicated survey 37 children psychologists average index IQ amounted to 110.2±12,7 (fluctuation limits 75-133).

IQ was borderline (75, 77 and 79) have three children, all of them left – handed. In the group of children with enough high Kim IQ of left-handed had one child, and one hidden left-handed.

Neuropsychological problems registered in 24 (64,9%) children, including 7 (41%) girls and 17 (85%) boys. 12 (32,4%) patients had learning difficulties (three of them border IQ). Children with low IQ came from financially disadvantaged families. In 10 out of 12 children with school failure was a syndrome, articular hypermobility, including 5 – severe. Articular hypermobility in children without her replicarolexes problem was roofing to two cases, in both moderate ($p<0.01$). De children with MS was characterized by high anxiety, school fears. General anxiety was aggravated by low self-esteem, especially characteristic of children with visual impairments, deformity of the chest. A lot of anxiety brought appearance – not that coy as peers.

To assess the possible factors influencing the emotional reactions of children with MS, dysplasia of connective tissue, we determined the excretion but adrenaline of urine in children without generalized connective tissue dysplasia in children with dysplasia. It turned out that the alone allocation noradrenalin with urine in children with MS was 27.7±4,9 ng/ml/hour (norm-34,0±7,7 ng/ml/hour). After emo tional load, the excretion of norepinephrine was increased to 125,3±10,1 vs 69,8±10,9 ng/ml/h in children without MS (table. 2, chart).

Thus, in children with MS, identified increased sympathetic activation.

Summary

The study showed that patients with MS may be changes in the muscles and features of the psychoemotional sphere. Muscle pathology is manifested in violation of the contractility and elasticity of the skin. ness of the muscle, its degeneration, the growth of the United of legislative fabric. Pathology can exacerbate time position of nerve fibers, increased secretion of SIM of Pecatonica.

Features organ pathology when the MS associated with the presence fibrillin, immunotec efficiency which differs from that of healthy out of people, and the presence of specific mutations Peculiarities of psychoemotional sphere can be due to genetic reasons, and in some cases, family factors. Biochemical analysis display cal studies have shown that alone the allotment tion of norepinephrine in children with MS, dysplasia legislative Conn tissue without dysplasia is almost the same. Increased release of norepinephrine during emotional load inherent in all children, this reflects a General response to stress. But special but dramatically the secretion of norepinephrine is increased in children with MS, which may explain their anxiety, difficulties in the perception of educational material, hypermobility.

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Saccadic Eye Movements During a Prognosis Activity in Patients with Early Stages of Parkinson's Disease

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B428C0046

Abstract

Parkinson's disease (PD) is a slowly grow progressively neurodegenerative disease of

The nervous system. It manifests tremor, hypokinesias, muscular rigidity, postural instability. In most cases, patients with PD have violations of cognitive functions. The process of prognosis the appearance of the events is one of the cognitive brain functions underlying of the human intellectual activity.

Numerous studies have shown that cognitive processes (attention, memory, thinking) accompanied by saccadic eye movements. Moreover, the cognitive processes are often complicated without those movements. Functional and anatomical overlapping of brain structures (frontal and parietal cortex areas, basal ganglia) provides, on the one hand, the process of planning, programming and decision making, on the other - the control of saccades generation [1].

Keywords: Parkinson's disease, cognitive functions, saccadic eye movements, the prognosis

Introduction

Cognitive functions are closely linked with saccadic eye movements.

Early, we assumed the saccades are needed to "glue" the "cognitive fragments" into a single idea, leading to a decision-making, to receive, process and store information in the memory during the interval between saccades [2].

Parallel impaired of motor and cognitive functions in Parkinson's disease is found to be changed. The oculomotor system is highly sensitive to functional brain changes and therefore the saccadic movement's disorders can be observed in the early Parkinson's disease stages. In view of above, the aim of the present study is to investigate the possible parallel changes in the cognitive

and oculomotor systems in the early stages of Parkinson's disease.

Methodology

Two group of subjects were involved into the study. One - Parkinson's disease (PD) (stage I-II) in age 61.1 years (n=17) without treatment included.

The latter (control) (n=14) healthy adults (in age 57.6 years) included. Saccadic eye movements were registration continuously in both groups in various states of calm wakefulness: with eyes, open to perform cognitive tasks of increasing complexity. To assess the prognosis, attention and memory effectiveness the original psychological method Prognosis 2.5 were used in adult subjects [3].

The program offered three sets of cards in three tests. The sets contain cards red and black suit, arranged in a certain a constant sequence (unknown subject).

The one set installed a sequence of two cards (red-black or black-red), were repeated 10 times (n=20). The latter test contained a block of three cards also repeated 10 times (n=30) and random rotation (the program has several options for alternation of red and black cards, one of which was a randomly offered by computer to a particular subject).

The third, the most complex test included a block of five cards, repeated 12 times (n=60). According to instructions, the subject is asked to choose a card by pressing the key corresponding to the black or red card. Different kind of sounds evidenced whether it was truth or falls of choice, and the word "correct" or "incorrect" appeared on the screen. On the computer screen, the correct card and proper sound were shown in the case of an incorrect choice the correct card was demonstrated in addition to the low sound and visual card. Before fulfill the received tasks, the detailed instructions were shown to the subject.

The task was to prognosis each successive card; the subject has to determine the order of cards connection. The order was considered to be detected if the subject accurately predicted every next card in three blocks in a row. After all the sets was showed subject was asked by memory to reproduce the cards order in each of them. After the sets finish the total number of saccades produced by subject in each test on a computer screen was amounted visually.

Since the duration of each test was different the number of saccades per 1 sec (saccade/s) in each test was analyzed. Data of saccade number was statistical conducted by Mann-Whitney and Kruskal- Wallis methods.

Results

After finding average values saccade/s for each subject under the background and in cognitive testing, we counted the average values for these indicators for each group. The statistical results processing average number of the saccade/s under the background and in cognitive testing in a healthy subject and in patients with Parkinson's disease are presented in tables 1,2 (descriptive statistics).

Then a comparison between groups on different statistical methods was conducted. In healthy subjects the average number of the saccade/s representatively increased in prognosing and reproducing compared to quiet wakefulness with open eyes (table 3). In additionally, complicated increase, however, these differences were not statistically representative both under tests conducting and reproducing (criterion Kruskel – Willis, $p=0.1078$, $p>0.05$).

The number of saccade/s under the background in the group of patients did not differ in healthy subjects (table 5). In patients, the number saccade/s under prognosing and reproducing comprised with background did not changed (table 4). The differences of this index in the two groups during cognitive tests carring out were representative (table 5). Conducting cognitive tasks the average number of saccade/s in patients increased, but to some lesser extent than in the healthy. As the complication of test number the saccade/s not increased, but decreased (table 2).

Patients to carry out tests required more time than healthy subjects and they made more mistakes, but indicators of attention were higher in this group.

This phenomenon can be explained by the fact that patients were motivated more to correct conduction nevertheless significant difficulties in its realization.

We observed a decrease in the number of saccades/s in this group with task in during becoming complexity of the task, although significant statistical level not reaching. Saccades suppress are known the cognitive processes that requiring attention [4]. Therefore, we can suggest that in group of patients it is need to keep a good attention level that leads to a decrease in saccade with the task becoming more complexity. Attention decrease in a healthy group may be associated with the aging. The results of conducting the tasks in healthy subjects differed significantly (Shapiro - Wilk criterion) from those in patients (prognosing - $p=0.01$, reproduction, $p=0.00$).

Conclusions

The detected interrelationship between complication of the cognitive tests and the number of saccades can be used not only for studies of cognition

processes, but also in clinical practice for early diagnosis of pathologies associated with parallel impairment of motor and cognitive functions.

It was demonstrated that cognitive processes, including visual and spatial attention, working memory, planning, and decision making are considerably impaired in these pathologies [5], the parameters of saccades are also disturbed (increased saccade latency and duration, multisaccades) [6].

Thus, in this study we used two methods – a) check the brain prognosis abilities using computerized method "Prognosis -2.5", and b) the registration of saccadic eye movements. Taking into account the statistically significant differences in two groups of subjects, this approach may be used in the complex diagnosis of diseases, associated with concurrent disease of motor and cognitive functions, in particular Parkinson's disease.

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Tables

Table 1. Average number of saccades/s in a group of 14 healthy subjects under the background and in cognitive tests

	Average	Median	Minimum	Maximum	Lower quartile	Higher quartile
Background	1,1	1,0	0,5	1,7	0,9	1,5
Inst.	2,3	2,1	1,4	2,7	1,6	2,5
Set. 1	2,8	2,7	1,3	3,8	2,0	3,1
Set. 2	3,1	2,9	1,6	3,8	2,4	3,5
Reprod. 1	2,6	2,4	1,1	3,3	2,2	3,2
Reprod. 2	2,8	2,6	1,5	4,2	2,2	3,3
Reprod . 3	2,9	2,7	1,6	3,8	2,3	3,4

Note: here and further - Installation (inst.), Set (set.), Reproduction (reprod)

Table 2. Average number of saccades/s in 17 patients with Parkinson's disease of I - II stages under the background and in cognitive tests

	Average	Median	Minimum	Maximum	Lower quartile	Higher quartile
Background	0,8	0,8	0,41	1,23	0,53	1
Inst.	1,3	1,4	0,7	1,7	0,9	1,6
Set. 1	1,1	1,0	0,5	2,2	0,6	1,3
Set. 2	0,9	0,8	0,5	1,3	0,8	1,1
Reprod.1	1,0	1,1	0,6	1,4	0,9	1,2
Reprod. 2	0,9	1,0	0,3	1,4	0,8	1,1
Reprod. 3	0,9	0,8	0,5	1,5	0,7	1,1

Table 3. Comparison of average number of saccades/s in a healthy under the background and in cognitive tests (Mann – Whitney criterion)

Pairs compared	Value p	Level p	Reliability of p differences (yes/not)
Background – inst.	0,000	<0,05	yes
Background – set. 1	0,001	<0,05	yes
Background – set. 2	0,000	<0,05	yes
Background – reprod. 1	0,001	<0,05	yes
Background – reprod. 2	0,001	<0,05	yes
Background – reprod. 3	0,000	<0,05	yes

Table 4. Comparison average number of saccades/s in a patient with Parkinson's disease of I -II stages under the background and in cognitive (Mann – Whitney criterion)

Pairs compared	Value p	Level p	Reliability of p differences (yes/no)
Background – inst.	0,016	>0,05	no
Background – set. 1	0,176	>0,05	no
Background – set. 2	0,305	>0,05	no
Background – reprod. 1	0,086	>0,05	no
Background – reprod. 2	0,181	>0,05	no
Background – reprod. 3	0,279	>0,05	no

Table 5. Comparison average number of saccades/s in a healthy and in patients with Parkinson's disease of I - II stages under the background and in cognitive tests (Mann – Whitney criterion)

healthy and in patients	Value of p	Level of p	Reliability of p differences (yes/no)
Background	0,199	>0,05	no
Inst.	0,001	<0,05	yes
Set. 1	0,000	<0,05	yes
Set. 2	0,000	<0,05	yes
Reprod. 1	0,000	<0,05	yes
Reprod. 2	0,000	<0,05	yes
Reprod. 3	0,000	<0,05	yes

Consolidated Pelvic Fractures in Pregnant Women

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B428C0054

Abstract

The problem of the consequences of pelvic fractures in pregnant women remains understudied. An orthopedic research of 72 pregnant women who experienced pelvic fractures was performed. An average period from the moment of the trauma to conception was equal to 4.6 years. Out of 72 clinical observations 25 (34.72%) cases of pelvic fractures were treated operatively; 47 (65.28%) cases were treated with conservative methods. The main clinical symptoms of the consequences of pelvic fractures in pregnant women were subject to examination. The most unfavorable consequences of the past pelvic fractures are the pelvic ring deformity manifesting itself in the following symptoms of orthopedic pathology: pain (93.05%), soft tissue (36.11%) and bone (19.44%) pelvic asymmetries, shortening of one leg (18.05%), contracture of coxofemoral joints (15.27%) and limping while walking (13.88%).

Pregnant women after healed pelvic bones fractures should be consulted by an orthopedist in early pregnancy.

Keywords: findings of the orthopedic pelvic study, consolidated pelvic fractures, pregnant women

Introduction

In recent decades, a continuously growing number of pelvic fractures have been registered in the population of the industrially developed world countries [3, 6]. The main causes of fractures are motor vehicle related injuries and falls from heights [4, 7, 11]. The age and sex profile of the injured people shows that women of active childbearing age constitute a considerable part of the victims [2, 5].

Treatment and rehabilitation of the patients suffering from pelvic fractures, as a rule, take a lengthy period of time and such fractures often result in complications [8, 9, 10]. Regarding women planning pregnancy after the

injury, such complications can have negative effects during gestation in the form of a pain syndrome, limitation of hip joint movement, and limping while walking.

Post-traumatic pelvic ring deformities can have a negative impact on fetal development such as utero-placental and fetal-placental blood flow disorders.

Besides, in case of anatomical changes in the pregnant woman's pelvis, there is a greater possibility of fetal pathologies in terms of head presentation, position of the spinal cord and extremities, which will require significant efforts after birth to correct them [1]. Severe posttraumatic deformities of the pelvic ring can become a relative indication for operative delivery [2].

Few scientific publications tackling the issues of the consequences of pelvic fractures in pregnant women prove relevance and timeliness of the performed study.

Objective

Study the patterns and frequency of the consequences of pelvic fracture in pregnant women.

Materials and methods

We have accumulated more than 20 years of experience of dynamic monitoring and treatment of 72 pregnant women after healed pelvic fracture.

The age of the patients ranged from 17 to 39 years old, with an average of 27.6.

The average period from the day of the pelvic injury and until the conception date ranged from 1.5 to 17 years, with an average of 4.6 years.

Out of 72 pregnant women, 18 (25.0%) women experienced a fracture of one pelvic bone; 24 (33.33%) women had a fracture of two pelvic bones and 30 (41.67%) women had a fracture of three and more pelvic bones and joints.

In total, the women under study had fractures of 72 pubic, 50 ischiac and 14 iliac bones (including 8 cases of fractures of the acetabulum).

Moreover, 19 patients were diagnosed with consolidated sacral fracture, 12 patients – with pubic symphysis fracture and 6 women had a fracture of one of the sacroiliac joints.

The causes of the injuries included a motor vehicle related trauma in 56 (77.79%) cases; previous delivery involving injury of the pubic symphysis – 8 (11.11%); falls from height – 7 (9.72%); occupational trauma – 1 (1.38%) 17 (23.61%) pregnant women experienced pelvic fractures accompanied by

fractures of other bones of the skeleton, in some cases multiple. Fractures of the femoral bones were most frequent (10 cases). More rarely women suffered from fractures of the collar bone (4 cases); heel and shoulder bones, spine (3 cases of each), ribs (2 cases), brachial and shin bones (1 case of each).

In addition, 10 (13.88%) women experienced injuries of other body systems, apart from the skeletal system. The craniocerebral trauma was the most common pattern of multisystem injuries (5 cases). Urinary bladder and kidney ruptures were diagnosed in 4 patients; the spleen rupture – in 1 woman. 6 (8.33%) women experienced traumatic shock I-III degrees.

25 (34. 72%) patients underwent operative treatment of pelvic fractures; 47 (65.28%) women underwent conservative treatment.

We reviewed the course of labor and delivery of 30 (41.66%) women out of 72. 19 (63.33%) cases required Caesarean section. 11 (36.67%) women had a spontaneous vaginal delivery.

To diagnose pelvic fractures in the women under study, the analysis of their complaints, anamnestic data and orthopedic examinations were done.

The most reliable sources of information were the medical documents (discharge summaries from trauma care departments, X-rays and computed tomography images of pelvic bones) confirming the fact of the received trauma and medical treatment methods.

Results and discussion

The conducted clinical research study of pregnant women after healed pelvic fractures has allowed to establish the character and frequency of the main consequences of such fractures.

The pain syndrome, causing women the most trouble, dominated in the clinical picture. Pains localized mainly in the sacral region, sacroiliac joints, pubic region, groin, greater trochanters and buttocks. 67 (93.05%) pregnant women reported pains in the abovementioned regions. 12 (16.66%) women reported a pain syndrome in the projection of the pelvis before pregnancy.

According to the assessment criteria of the visual analogue scale, severity of the pain syndrome in the women under study ranged between 1 – 6 points, with an average of 4 points.

The intensity of pain depended on two factors: severity of the healed pelvic fractures and gestational age. In all the cases there was a direct interrelation: the more severe the fracture (especially multiplanar fractures with dislocations which were not completely repaired) and the bigger the gestational age, the stronger and more long lasting the pain syndrome was.

Establishment of the asymmetries of the pelvic bones and soft tissues is of great clinical importance in the process of assessment of the patient's orthopedic status. The presence of such asymmetries signifies deformities of the pelvic ring which has an adverse effect on pregnant women.

During the examination, we assessed the location of the wings of the ilium, anterior and posterior superior iliac spines, contours of the greater trochanters, regularity of the sides and angles of the Rhombus of Michaelis. Assessing the symmetry of the paired pelvic bones, we paid attention to the surgical scars left on the skin after the installation of metal structures and, in some cases, after removal of such structures during operative treatment of the fractures.

14 (19.44%) women were found to have pelvic deformities due to asymmetry of the paired organs. Among these women, 11 women had plain radiography images of their pelvis which confirmed the fact of asymmetry of the halves of the pelvic ring in all the cases.

Apart from the asymmetry of the paired bones, 26 (36.11%) pregnant women had asymmetry of soft-tissue organs, first of all, gluteal folds. The domination of the soft-tissue asymmetries over the bone ones is a sign of frequent muscular hypotony, primarily, of the greater and medial gluteal muscles.

The most reliable symptom of the indicated pathology is the positive Trendelenburg's sign which was firmly established in 13 (18.05%) pregnant women.

In 9 (69.23%) of 13 cases, this symptom corresponded to that half of the pelvis which was injured. In 4 (30.77%) clinical case studies, the positive Trendelenburg's sign was diagnosed in women with consolidated fractures of both right and left halves of the pelvis. In all cases, the positive Trendelenburg's sign was registered in women with healed lateral mass of the sacrum which was synthesized during operation with a cannulated screw which, among other things, leads to sacroiliac joint blockage.

The clinical examination of the pregnant women in a horizontal position was performed on a device excluding pressure on the uterus. Moderate painfulness in the wings of the ilium upon pressing was diagnosed in 58 (80.55%) women.

At that, pains were mainly localized in the contact area of the doctor's hands and the patients' wings of the ilium as well as in the region of the sacrum and sacroiliac joints.

The functional condition of the sacroiliac joints in pregnant women was of particular interest as normally its sufficient movement amplitude ensures the necessary and sufficient extension and flexion of the ilium bone in relation to the sacrum. It is known that limited movement in one of the sacroiliac joints accounts for 30% of lumboischialgia (lower back pain irradiating down the leg). In cases of injured articulation surfaces of the ilium bones and sacrum we registered a pain syndrome and algesic contracture in the projection of the joint, especially in the cases of operative sacroiliac screw fixation and if the screw was not removed after consolidation of the fracture.

27 (37.5%) pregnant women pointed out pains in the projection of one of the sacroiliac joints. In the course of the examination, the pain syndrome always increased during palpation in the region of the joint. Palpatory diagnostic procedures, which were used in relation to these 27 pregnant women, confirmed limited joint mobility in 24 (88.88%) clinical cases.

Palpation in the projection of the pelvic joint was accompanied by palpation of the gluteal muscles. Special attention was paid to the projection of the greater sciatic foramen. It is well-known that pain and painful palpation in this anatomic region is one of the manifestations of the syndrome of the piriform muscle (piriformis syndrome). Different degrees of the painful limitation of the internal rotation of the hip confirmed this syndrome. During the examination, 12 (16.66%) women admitted clinical signs of the piriformis syndrome.

In 8 cases pathology was localized on the left of the pelvis; in 4 cases – on the right. We did not manage to find any correlation between the localization of the manifestations of the piriformis syndrome and the localization of the injured bones. Probably, the main pathogenetic link in the formation of this pathology in pregnant women is compression of the fibers of the sciatic nerve in the foramen infrapiriforme due to development of lumbar hyperlordosis and piriformis muscle contracture in case of degenerative-dystrophic diseases in the lumbar functional spinal unit.

Another confirmation of this is the positive therapeutic effect resulting in complete cure of the pain syndrome or significantly lower degree of its manifestation during postisometric relaxation of the muscles in question in all 12 pregnant women.

Examination of the pregnant women in the horizontal position, lying flat on their backs, gave us two more crucial facts characterizing the orthopedic status of the women after healed pelvic traumas.

Thus, 13 (18.05%) pregnant women were diagnosed with limb-length difference. The frequencies of shortening of the left and right legs were the same. Herewith, analysis of the severity of the healed pelvic fractures showed that fractures of one half, especially combined with the iliac wing fracture and lateral sacral mass fracture and not completely fixed dislocation, resulted more often in leg shortening. The difference in the limb length ranged from 0.7 – 0.8 mm to 3.5 cm with an average shortening of 1.5 cm.

Such leg shortening caused limping while walking; the more the limb-

length difference the more noticeable the limping was. Distinct limping was diagnosed in 10 (13. 88%) women.

The most important consequences of the healed pelvic fractures in the women under study were contractures in the coxofemoral joints.

During the examination, 11 (15.27%) women were diagnosed with limited range of motion in the coxofemoral joints indicating their contractures.

In all the cases, we registered limited bending and external rotation of the hips, i.e. such directions of movement which are essential for vaginal delivery.

The average amplitude of the leg bending in the hip joint equaled to 73 and the amplitude of the external rotation – 22.

Thus, the performed orthopedic study helped us garner information about the character and frequency of the main symptoms of pelvic pathologies remained after healed fractures: pain (93.05%), soft-tissue (36.11%) and bone (19.44%) asymmetries of the trunk, shortening of one leg (18.05%), contractures of the coxofemoral joints (15.27%), and limping while walking (13.88 %).

Conclusion

The pelvic ring and the located in its cavity uterus of the pregnant woman are those natural “reservoirs” in which the fetus develops during the whole gestation period. Perinatal outcomes depend mainly on how comfortably first the embryo and then the fetus develop in the maternal pelvis. The consequences of pelvic traumas, especially their wrongly consolidated fractures, are the most critical pathogenetic situations which can result in serious complications of the act of delivery itself (for example, baby’s intranatal neck or spinal cord injury) as well as neurological and orthopedic diseases of the child after its birth (for example deformities of the head, neck, trunk and limbs).

The quality and appropriateness of the pelvic trauma management in relation to women of childbearing age influence not only on the woman’s condition but also health of her future child which she is likely to carry after the healing of the trauma.

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Gynecological Care and Rehabilitation of Patients with Chronic Pain Syndrome Related to CPID

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B428C0062

Abstract

In the present survey, the topographical and anatomic position of the uterus and the pathogenesis of pain of patients with CPID have been studied.

The mechanism for stopping the pain syndrome in case of CPP has been examined with the help of LLLT. It is shown that SLLLT restored the uterine tone and function of the nerve centers. The uterine contractions with the help of SLLLT led to resorption of adhesions and scar formations in the uterus.

The normalization of topographical and anatomic position of the uterus provided the pelvic pain relief.

Keywords: Pelvic pain syndrome, chronic inflammation of the uterus, lasers

Relevance

Today, chronic pelvic inflammatory disease (CPID) often leads to the positional disruption of the uterus and its appendages (uterine tubes and ovaries), as well as the compression of nerves and chronic pelvic pains (CPP) [1, 2, 3, 4]. CPP is one of the most serious problems in modern gynecology, and its therapy should be based on pathogenetic principles. According to Russian and European recommendations, it is necessary to approach the treatment of CPP in an integrated manner [5, 6, 7].

However, the effects of existing methods of therapy and rehabilitation are insufficient. In recent years, low-intensity laser radiation therapy has been widely used in gynecological practice. But while many researchers prefer treating the pathological area with a scanning laser beam, a mechanism for dealing with the pain syndrome, in the case of CPP, has not been studied yet.

The purpose of the work was to make a comparative study of the influence of low-level laser radiation (LLLR) when scanning and using traditional techniques on the topographical and anatomic condition of a uterus and its appendages among the patients with CPID, suffering from CPP.

The objective is to study the topographical and anatomic position of the uterus of patients with CPID, to investigate the pathogenesis of pain of people with CPID and to consider methods of alleviating pain (CPP) when carrying out LLLT.

Material and methods of the research work

351 women of genesial age with CPID have been investigated. Examination of the women was conducted using the same constants, including passport data, medical history, a standard laboratory and special methods of research.

Special attention was given to the ultrasonic methods of the research by studying the morbidity of vegetative nervous formations of abdominal and pelvic cavities and the responsive reactions from the uterus after laser treatment. The topographical and anatomic position of the uterus and its appendages were made by bimanual vaginal investigation and the ultrasonic devices “Aloka 650” and “Toshiba 140A” (Japan). The devices were supplied with a transabdominal convex transformer with a frequency of 3,75 MHz and the vaginal sensor with an oscillation frequency of 6 MHz. The study of the solar plexus morbidity was carried out while the patient was lying down.

Two fingers on the right hand ran a palpation to the point located between the middle and lower third of the line connecting the xiphoidal shoot to the navel. The hypogastric nervous plexus was palpated to the point located 2,0-2,5 cm below the navel. The uterine nervous plexus, which is located in the side surfaces of the uterus, was palpated through the fornices of the vagina during bimanual vaginal examination. The ovarian nervous plexuses, which are localized at the top external angle of the wide uterine ligament, were also studied bimanually from both sides using palpation. The pelvic areas of the sympatic nervous trunk and its branches were also investigated through the vagina. The knots of the boundary nervous trunk are located on the surface of the sacrum, with its branches going to the left and right hypogastric nervous plexuses. This is located on the posterolateral wall of the basin and was palpated using two fingers of the right hand, entered into the vagina.

148 patients suffering from pain had been given laser treatment (SLLLT) through the vagina, using “Luch-200” (radiation wavelength: 0,89 microns) scanning with a frequency of 1-10 Hz, and “Ulf-01” (radiation wavelength:

0,63 microns) via the 1.5 meters long, flexible quartz light waveguide shown in a sterile plastic test tube in Fig. 1 below. The therapy involved 8-14 sessions, each one lasting 9-12 minutes. The emissive output power of the light waveguide varied from 1,0 to 5,0 mW. 92 patients received treatment with these laser devices using a traditional technique, through the vagina, but were carried out with the fixed light waveguide (TLLLT).

The dose of laser influence in both groups was identical.

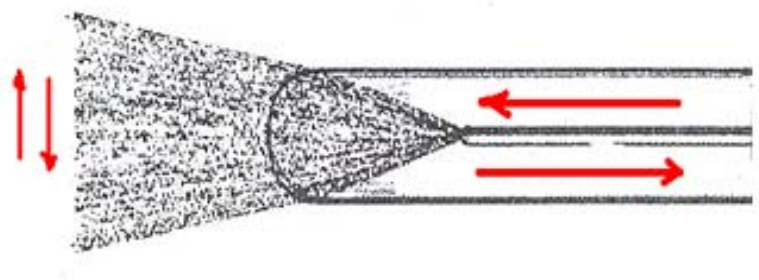

Fig. 1. The device for carrying out SLLLT

We assumed that the scanning laser beams emerging due to the difference of light pressure in the irradiated tela would cause more active resorption, loosening of adhesions and scarring in the uterus than the fixed laser beams from TLLLT. All women received at least one course of laser therapy.

142 (40.5%) patients went through two courses of treatment, 89 (41.2%) patients using SLLLT and 53 (39.3%) patients using TLLLT. 33 patients went through three courses of treatment, 17 (7.9%) women using SLLLT and 16 (11.8%) women using TLLLT.

Results of the study

Pain in the lower abdomen was found among 240 (68.4%) women. Retroflexion-version of the uterus was discovered among 74 (21.1%) and uterine lateroposition among 166 (47.3%) women. Limited mobility of the uterus was detected among 241 (68.7%) patients, and the fixity of the uterus - 70 (19.9%) patients (Fig. 2).

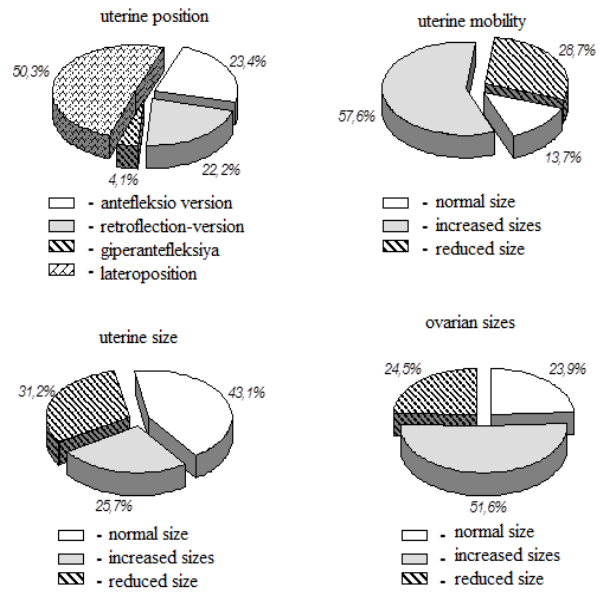

Fig. 2. Characteristic topographic anatomical condition of the uterus and appendages.

Along with pain, abdominal tenderness of the vegetative nerve plexuses was revealed among the patients: solar, lower hypogastric, uterine, ovarian plexuses and pelvic walls, where nodes of sacral parts of the sympathetic nerve trunk are placed. Thus, in the investigation, most patients had a severe algic reaction of the upper hypogastric and the solar plexuses (49.6% and 29.9%).

Less patients (19.4% and 24.5%) suffered pain in the sacral parts of the sympathetic trunk and uterine plexuses. Normally, the peripheral autonomic nerve structures are painless [1, 2, 8]. Therefore, we interpreted the nerve plexuses pain, arising when palpation, as their dysfunction, which is the attribute of CPP related to CPID. During the SLLLT, 3 levels of muscle contractions stimulation (MS) of the uterus were given: one weak (first), one moderate (second) and one strong (third). Muscle contractions of the uterus were identified in the survey and confirmed by ultrasound. These responses in the uterus have not been revealed to the patients treated with TLLLT.

The patients' pain stopped most often at the third level of MS stimulation.

The pain relief when using the third level of MS stimulation was 1.3 times more effective than the second level of stimulation, and two times more effective than the first level of MS stimulation. During the second level of MS stimulation pain relief was 1.6 times more effective than during the first level stimulation.

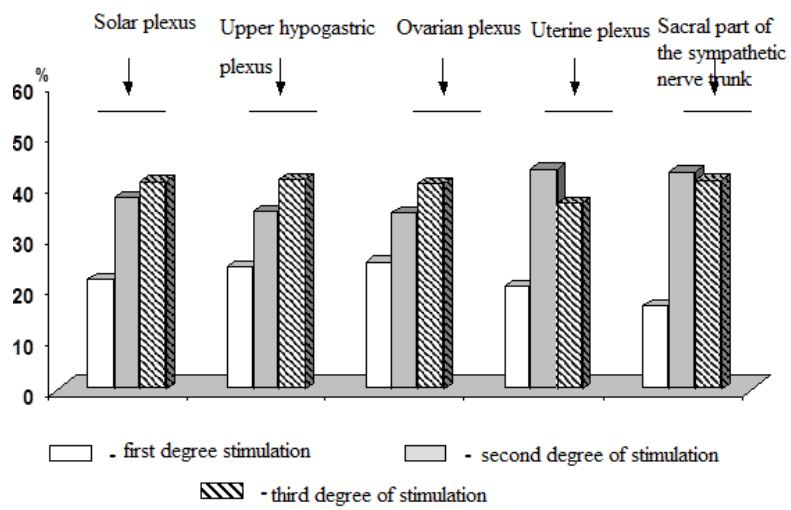

Fig. 3. The effectiveness of pain relief on autonomic nerve formations in the abdomen and pelvis based on the level of MS stimulation

The most effective pain relief of the autonomic nerve formations in the abdomen and pelvis occurred during the second and third levels of MS stimulation (Fig. 3). Thus, using the first and second levels of MS stimulation on solar pain and upper hypogastric and ovarian plexuses were insufficient for almost half of the patients (respectively, 41.3%, 41.0%, 38.9% of patients).

The morbidity of such plexuses took place at the second level of MS stimulation among one-third of the women, and the first level among one quarter of the women. Pain in the uterine nerve plexus and the sacral part of the sympatic trunk was stopped among most patients (respectively, from 80.5% to 84.6%) using the second and third levels of MS stimulation. The first level of MS stimulation was effective among a minority of the women. Therefore, the intensity of MS stimulation can be used to estimate the severity of violations of protective and adaptive mechanisms of the genital system, the dynamics of their recovery, adequacy of the conducted laser therapy, and predicting the effectiveness of pain relief.

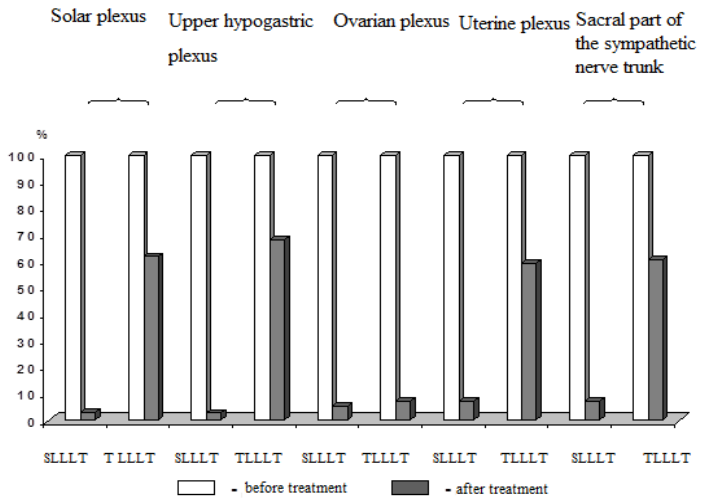

Fig. 4. The dependence of the efficiency of nerve plexus pain relief in abdominal and pelvic cavities from laser exposure mode

As a result, (Fig.4) the number of women with uterine anteflexion after SLLLT increased by 2.8 times, and after TLLLT only half as much.

The number of women with uterine retroflexion decreased by 3.7 and 1.3 times respectively, and with uterine lateroposition it decreased by 3.3 and 1.3 times. The number of patients with movable uterus was greater at 6.6 times after SLLLT, and TLLLT 4.7 times. Pain was stopped among 142 (96.0%) patients who received SLLLT and 60 (65.5%) patients treated with TLLLT.

The effectiveness of the pain relief of autonomic nerve formations of pelvic and abdominal cavity is shown in Figure 4. As is shown in the data, SLLLT showed better results than TLLLT. SLLLT efficiency was higher three times during the pain of lower hypogastric plexus and the sacral part of the sympathetic trunk. The results were higher (2.7, 2.5 and 2.3 times respectively), during ovarian, uterine and solar plexuses pain. The long-term results of the treatment were followed among 298 (84.9%) women, including 181 (83.8%) patients who received SLLLT and 117 (86.7%) patients who received TLLLT.

The duration of the follow-up was two years. The number of women who complained about lower abdominal pain and/or lower back pain was 5.3 times lower. (SLLLT: 11.4 times, TLLLT: 2.9 times).

Conclusion

The study has revealed the pathogenesis of pain in CPP - wrong topographic

and anatomic position of internal genitals, caused by adhesions, cicatricial process in the uterus connected with the change of the normal state of autonomic nerve centers of the pelvic and abdominal and uterine tone weakening.

Pain during palpation of the nerve plexuses can be interpreted as their dysfunction, which is an inseparable attribute of CPP related to CPID. SLLLT, due to the difference of the laser light pressure in the irradiated tela, caused greater resorption and loosening of adhesions, as well as scar formations in the uterus. Also, SLLLT, through the restoration of uterine tone and function of the nervous centers, stimulated the of muscle fibers in the uterus, with the levels of intensity acting as a predictor of the effectiveness of rehabilitation of patients with CPP, related to CPID. Uterine contractions normalized its topographic and anatomic position in the pelvis and eventually stopped the CPP.

As the results of the investigation have shown, the method of pain relief in the case of CPID consisted of the following units: resorption of adhesions and scar formations in the uterus; restoration of the function of autonomic nerve centers of the pelvic and abdominal cavity; restoration and activation of uterine tone; normalization of topographic position of the uterus. Thus, the developed technique of laser therapy among patients with CPID, aimed at restoring the topographical and anatomical position of the uterus scanned with low-intensity laser beams, significantly improves the results of the rehabilitation of this group of gynecological patients.

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The Effect of the Sodium Dichloroacetate on Metabolic Indices in Rats Suffering from the Alloxan Diabetes Mellitus

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B428R9007

Abstract

In the article the results of the research in the effect of the sodium dichloracetate on the development and the clinical course of experimental diabetes mellitus are presented. It has been shown that the sodium dichloracetate possesses the evident hypoglycemic effect, i.e. the glucose concentration has decreased by 36% (p<0,05) in comparison with the group of rats suffering from the diabetes mellitus without correction. In addition, the low hypolipidemic effect has been detected which has manifested itself as the lowering of the general cholesterol concentration by 23% (p<0,05). The less evident imbalance in functioning of the thiol link of the antioxidant system confirmed by the concentration maintenance of the reduced glutathione in erythrocytes on the normal level has been revealed as well. The received data allow speaking about the prospective researches in the possible usage of the sodium dichloracetate in complex therapy of the diabetes mellitus.

Keywords: diabetes mellitus, sodium dichloroacetate

Introduction

The diabetes mellitus (DM) is an urgent issue of the modern health care, its prevalence allows speaking about the epidemic of the disease.

The experts of the World Diabetic Federation predict that the number of patients suffering from the DM will reach 642 mil to 2040. The danger is hidden in the development of the severe incapacitating late complications; so annually over 1 mil of the worldwide amputations of lower limb are performed in patients

suffering from the diabetic foot, over half a million of patients lose their vision as a result of the diabetic retinopathy, about 500 thousand suffer from the developing chronic renal insufficiency as a result of the diabetic nephropathy [1]. The development of late complications of DM is mostly associated with the chronic hyperglycemia and the development of the oxidative stress in such patients [2]. Therefore the medical preventive measures must be aimed at the elimination of these two factors. The action mechanism of the sodium dichloracetate (DCA) consists in the activation of the pyruvate dehydrogenase mitochondrial complex which provides the formation of reduced coenzymes that can be finally used by the enzymes of the antioxidant system (AOS), in addition the energy exchange normalizes which influences the carbohydrate metabolism and the development of DM [3].

The purpose of the present study is the research in the influence of the sodium dichloracetate on the development and the clinical course of the alloxan DM in rats for the reason of evaluation of the glycemic level, the lipidic profile, the state of the antioxidant system and the level of the endogenous intoxication.

Methodology

To carry out the set purpose 3 groups of white non-linear male rats with the mass about 200-230 g have been involved into the experiment. The 1st group (control) has been made up of 15 intact rats kept in the same conditions that the rest of animals. The other groups have undergone the DM modeling by means of intraperitoneal injections of the alloxan monohydrate by 10 mg/100 g of the animal weight three times with an interval of 1 day against the background of starvation [4]. The 3rd group has been made up of 15 rats taking DCA orally in the dosage of 15 mg/100 g every day with the drinking water within one month before and after the DM modeling. The comparison group (2nd group, n=15) has been made up of the laboratory animals that have also undergone the modeling of the alloxan DM but without the experimental correction by means of DCA. All the laboratory animals have been kept in the vivarium of FSBEI HE KubSMU on the standard ration and with the free access to water in accordance with the "Protection Rules of Vertebrate Animals Adopted by the European Convention" (Strasbourg, 1986). One month after the first alloxan injection the animals have been removed from experiment under the general anesthesia by means of the Zoletil 100 («Virbac», France) at 10 mg/kg intramuscularly; besides they have undergone the blood sampling for further laboratory studies. In the blood plasma of animals the concentration of glucose, the general cholesterol (GCS), high-density lipoproteins (HDLP), low-density lipoproteins (LDLP) and triglyceride (TG) have been determined by means of the assay kit of «Vital Development Co» (St. Petersburg, Russia).

To evaluate the state of the AOS of erythrocytes the activity determination of enzymes of the thiol link notably glutathione peroxidase (GPO) and glutathione reductase (GR) has been performed as well as the concentration determination of the reduced glutathione. To reveal the development level of the endogenous intoxication the content of substances with the medium and the low molecular weight (MM&LW) has been determined in accordance with the Malakhova method under which the optical density has been measured by 238-298 nm of the blood plasma solution after the protein precipitation by the trichloracetic acid [5]. The results of the study have been statistically processed by means of the R system for the statistical analysis (R Development Core Team, Austria, 2008). With a glance on the Mann-Whitney criterion the index changes have been registered, the difference of $p<0,05$ has been considered to be the correct one.

Results

By determining the glucose concentration in the blood plasma of rats after one month of alloxan injections it has been revealed that in all groups the evident DM has developed. In the comparison group, which has not undergone correction the glusoce content exceeded the control indices 6,9 times (Tab. 1).

The lipidic metabolism has evidently changed in rats suffering from the DM. In the comparison group the GCS concentration has increased by 66% mainly due to the CS-LDLP which content increased by 62% while the level of HDLP has remained on the initial level. The TG concentration has increased by 55% in comparison with the control group. The DCA injection had the evident hypoglycemic effect and the low hypolipidemic effect. Thus the glucose level of the blood plasma has decreased in comparison with the group 2 index by 36%. The hypolipidemic effect has manifested itself as the concentration lowering of the GCS by 23% though the LDLP content has not decreased.

The TG level has even increased by 30% in comparison with the 2nd group.

Tab. 1 Glucose content and lipidic profile of rats suffering from the aloxan diabetes mellitus

Group, N	Indices, mmol/l (M±SD)				
	Glucose	GCS	LDLP	HDLP	TG
1 (control)	5,03± 0,76	3,14± 0,45	1,50± 0,21	0,91± 0,14	0,42± 0,05
2 (comparison)	34,67± 4,83*	5,22± 1,04*	2,43± 0,42*	0,87± 0,24	0,65± 0,15*
3 (DCA)	22,18± 2,49*^	4,02± 0,84*^	2,97± 0,72*	0,85± 0,20	0,85± 0,09*^

*Note: * - p <0,05 in comparison with indices of group 1 (control), ^ - p <0,05 in comparison with indices of group 2.*

To evaluate the AOS functioning the functioning of the thiol link as the one of the most sensitive to the oxidative damage of the system links has been studied. While modeling the DM (2nd group) the increase in the GR activity by 65% has been revealed, the activity of GPO erythrocytes has practically not changed while the content of the reduced glutathione has decreased by 11% (Tab. 2). The DCA injection for rats suffering from the DM has led to the activity increase of GPO by 33,3046,9% in comparison with the 1st and the 2nd group. The GR activity has increased in comparison with the control group but has been not so evident as in the 2nd group – only 28,5%. The content of the reduced glutathione has reliably not changed in comparison with the 1st group. Thus in the 3rd group the less evident imbalance in functioning of the AOS has been detected which on the one hand has manifested itself in the GPO stimulation and the more active neutralization of free radicals and xenobiotics but the less evident GR activity by which even the little increase in its activity is sufficient for the maintenance of the normal GSH concentration.

Tab. 2 Functioning indices of the thiol link of the AOS in rats suffering from the alloxan DM (M±SD)

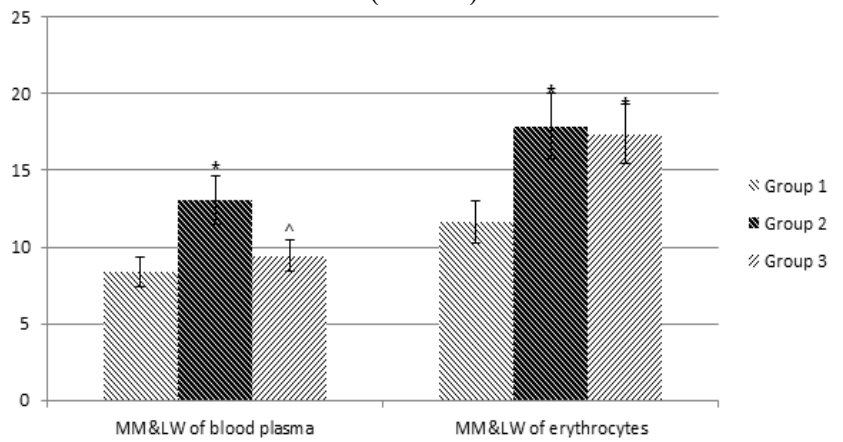
Group, N	Indices (M±SD)		
	GPO, micromol/(min×l)	GR, micromol/(min×l)	GSH, micromol/ml
1 (control)	31,48±4,87	589,73±63,82	2,74±0,27
2 (comparison)	34,69±4,03	970,99±67,64*	2,43±0,21*
3 (DCA)	46,25±6,44*^	757,30±73,27*^	2,60±0,45

*Note: * - p <0,05 in comparison with indices of group 1 (control), ^ - p <0,05 in comparison with indices of group 2.*

According to the research of the MM&LW content in rats suffering from the alloxan DM the increase of the endogenous intoxication has been revealed.

Thus in the comparison group the MM&LW content in the blood plasma has increased by 56% and in the erythrocytes by 54% (Fig. 1) which indicates the saturation of the erythrocyte membranes with endotoxins and the further free circulation of substances with the low and the medium molecular weight in the blood plasma. In the 3rd group where the DCA has been used the concentration increase of the MM&LW has been detected only in the erythrocyte fraction which proves the less evident intoxication notably the one in the compensation phase.

Fig. 1. MM&LW content in bioliquids of rats suffering from the alloxan DM (**M±SD**)



*Note: * - p <0,05 in comparison with indices of group 1 (control), ^ - p <0,05 in comparison with indices of group 2.*

Conclusion

Summarizing the above-stated the availability of the DCA usage in the complex therapy of DM can be registered. The long-term usage of DCA is restricted to its side effects but due to the necessary of its implementation against the background of the general therapy and the possible decrease in the frequency and the intensity of the undesirable effects by means of the usage of thiamine and lipoic acid remedies the further research of this substance can be reasonable.

The present research was carried out under the support of the Ministry of Public Health Care of the Russian Federation (28.01.2015, part 1, chapter 1) “Carrying out the applied scientific researches including pre-clinical trial of

the medicinal agents and clinical trials of drugs."

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The Research Osteoregenerative Properties of Various Types of Implants with Natural Calcium-Phosphate Coating in the Experiment

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B428C0048

Abstract

Background

The prevalence of osteoporosis and osteopenic conditions affecting different groups of the population, significantly complicates the treatment of the patients of trauma and orthopedic profile [3]. In Russia, 32.5% of the population older than 50 years faced with this problem. Already by age 30, 10-11% of women have osteopenic syndrome, whose prevalence increases to 50% with the onset of their menopause [2]. Clinical manifestations and complications in orthopedic profile associated with fractures, the most common and significant of which are fractures of the proximal femur, distal forearm and lumbar vertebral bodies.

In most cases, the only way to assist is to perform the surgical treatment with the use of different implants made of titanium alloys, but reduced mineral bone density in these patients does not allow for stable fixation of bone structures and to form a strong bone-metal block.

To improve the quality of surgical treatment of patients with osteopenic syndrome, it is advisable to use implants with bioactive calcium phosphate coating, having osteoinductive and osteoconductive properties [1].

Keywords: Innovation, technology, research projects, titanium implants, bioactive coating

Objectives

To explore osteoregenerative properties of implants made of titanium, covered with a natural calcium-phosphate-coated and porous titanium nickelide, highly filled natural calcium-phosphate complex in the experiment.

Materials and methods

There were conducted an experimental study on rabbits to explore osteoregenerative properties and rationale of clinical use of bioactive implants made of titanium, covered with a natural calcium-phosphate-coated and porous titanium nickelide, highly filled natural calcium - phosphate complex.

The research design was to conduct surgeries on rabbits. In the two sides of the iliac bones were fixed varying types of implants: the right side was fixed two implants: one made of titanium with natural calcium-phosphate coating, the second porous titanium nickelide, highly filled natural calcium phosphate complex. On the left side also two implants, one in titanium, the second of porous titanium nickelide, but both without a natural calcium-phosphate coating and complex. The implants were fixed in the holes with a diameter of 4 mm. The research involved a macroscopic, histological and radiographic assessments periimplantation zone in terms of 7, 14, 28 and 36 days.

All 16 animals were operated on, the removal from the experiment were exposed for 4 animals in the same timeframe. Macroscopic assessment was carried out visually and using a digital microscope with a maximum magnification up to 64 times. Histological examination included the study of structural changes of bone tissue in periimplantation zone.

Results

After one week of experiment, on the side of the fixed implant made of titanium with natural calcium-phosphate-coated and porous titanium nickelide, highly filled natural calcium-phosphate complex, the growth of beams of coarse-fibered bone tissue compared to implants that are installed on the opposite side, uncoated and without complex. At a later date (14, 28 and 36 days) area of coarse-fibered bone tissue increased, is formed bone beams, osteoblasts had become to osteocytes in periimplantation area of implants with natural calcium-phosphate coating and complex. In 28 days, there have been isolated osteoclasts. When carrying out histological examination it may be that the source of the cell populations of reparative regeneration are the stromal

cells of the bone marrow with further generation of osteoblastic differon.

In macroscopic research, after two weeks of implants fixation which made of titanium, covered with a natural calcium-phosphate-coated and porous titanium nickelide, highly filled natural calcium-phosphate complex in periimplantation area is found more distinct osteogenesis with moderate hyperplastic reaction of bone tissue, there is no zone of resorption and sites generations of cancellous bone with a low x-ray transparency. In the research of zones around the implants without coating and complex, reveals a less distinct osteogenesis manifested weak reparative reaction of bone, the presence of areas of resorption and generation sites of cancellous bone with increased x-ray transparency, which may indirectly indicate the absence of bioactive properties of the implant.

Conclusions

After analyzing the experimental results we can conclude about the presence of implants made of titanium, covered with a natural calcium-phosphate-coated and porous titanium nickelide, highly filled natural calcium-phosphate complex, distinct osteoregenerative properties, which confirms their influence on the regeneration of bone tissue in periimplantation area and expediency of its application in trauma and orthopedic practice with patients with osteopenic syndrome and risk group of osteoporosis.

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Electrophysiological Patterns of the Immune Status of the Future Firefighters

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B428C0059

Annotation

Students, who have chosen to become firefighters have been polled (n=75).

The immune status of the polled students was defined with the help of the acupuncture method "ROFES". According to the data of the electrophysiological testing three types of immune status were discovered: those without any special training for the endurance level; those with high and medium level of endurance to hypoxia. It was shown that the first group is different from the rest by the average indexes of the skin conductivity; the second from the third - by the nature of the self-organization of the physiological functions.

Keywords: immune status, skin conductivity, ROFES, self-organization of the physiological functions

Introduction

The modern stage of the human civilization development is being characterized by the creation of the artificial environment – techno sphere.

Its components (information, chemical and physical ones) can have a negative impact on the immune status of the human beings and become the cause of the secondary immune deficiency. This could refer to the workers of the atomic power stations and those engaged in oil and gas industry, as well as the inhabitants of the areas affected by the industrial electromagnetic smog.

The immune status of the firefighters, who deal with integrated ecological stress and psychological and emotional pressure on a constant basis, has been studied less thoroughly.

Today on the territory of the Russian Federation the reform of the existing fire and rescue units is taking place; aero-mobile units are being created, landing units of the State Fire Service are being upgraded [1]. The successful work of the units mentioned above can only be achieved through an appropriate selection of the candidates. In our opinion, assessment of the firefighters' immune status and its monitoring with the help of the computer express testing

in the near future will become a mandatory element for providing job access similar to the way it is being organized at the enterprises of high radiation risk.

Over a number of years, we have been monitoring the maladjustment syndrome referring to the students of the Ural Institute of the State Fire Service of EMERCOM (Emergency Control Ministry) of the Russian Federation.

The electrophysiological component of the following monitoring has been justified in our previous publications. Previously, we (G.V. Talalaeva) have shown that the effect of the complex stress can be adequately described across a skin conductivity value of biologically active acupuncture points, according to which people at the time of the survey can be assigned to one of the three phases of adaptation: satisfactory adaptation, environmental stress, social-environmental stress [2-3]. In the framework of this study thorough understanding of the ecological immunology will be provided as we will also be focused on the types of the students' immune status depending on their endurance to hypoxia. We suppose, that the endurance to work in the isolated breathing apparatus under super-heavy physical exertion accompanied by vital emotional stress appears to be not only the result of special trainings, but is closely linked to the genetic traits of the human body. The division of all humans into adaptive and functional types and a possibility to realize a number of evolution steps simultaneously in the men-made environment has been proved by N.A. Agadjanyan, V.P. Kaznacheyev, A.S. Severtsov, N.V. Timofeyev-Rysovsky. Our observations illustrate some well-known concepts and are based on the survey of the future firefighters.

The main goal of our study was to identify and formalize the predictive characteristics of the electrophysiological state of the students, which marks out their potentially high tolerance to the physical exertion under hypoxia conditions.

Data and methods

Electrophysiological testing of the immune status of the students of the Ural Institute of the Russian State Fire Service has been carried out.

The ROFES method has been used in order to describe the immune status.

This method enables us to measure the skin conductivity in biologically active spots (BAS).

Forty eight (48) measurements are conducted within one session, which lasts from five to seven (5-7) minutes. The skin conductivity indexes are being processed with the help of the computer program, and later displayed on the screen as figures and charts. The obtained data allows us to describe the type of the inter-systematic links in the human body, characterize its adaptive processes. Among the key measurements are electro-conductivity indexes

of the acupuncture channels of intestines, lungs, kidneys, cardiovascular and immune systems. The variation statistic methods were used in the following study to compare groups with high and low level of resistance to hypoxia.

The average indexes were calculated based on the BAS, average error, and difference reliability measurement with the help of the Student's criteria.

In order to formalize the process of self-regulation (types of inter-system links in the structure of adaptation syndrome) correlation method was used, which enables us to determine the level of interconnection among BAS conductivity indexes.

The ROFES method is a modern biophysical method of a personal examination, it belongs to a group of functional methods of research like electrocardiography, electroencephalography, myography etc. Along with the above listed diagnostic methods, it is based on registration of biophysical parameters concerned with personal vital activity.

There are some versions of ROFES - diagnostics differing from each other by the topography of representative acupuncture points where biophysical information is taken. It is a classic, su-djok, vertebrology variant of inspection.

Classically gauging is taken in key points of wrist and ankle joints, connected with twelve basic acupuncture channels, named on those organs or functional systems which activity they regulate directly and as feedback.

The sequence of gaugings reflects chronology of one-day activization of an organism functional systems and is presented as follows: the channel of lungs, thick intestines, gaster, pancreas and spleen, heart, thin intestines, urinary bladder, kidneys, pericardium, three heaters, gall bladder, liver. The results of inspection are represented in the form of digital values and of the schedule in circular coordinate system that is called "a rophogram". The treatment of the test results can be executed in several research paradigms. The rophogram data may be analyzed from the position of geographical, regional and functional norm; for specification of boundary values of adaptive norm corridor and its variations for various occupations and functional situations; from the position of expert analysis; of images theory; phase transitions of adaptation process; for definition of the person and his technogenic environment co-evolutional vector in artificial anthropogenous ecosystems. Depending on the applied methodological device the biophysical information received by the ROFES method, may serve as an instrument of medical diagnostics, ecological monitoring.

Two groups of students took part in our survey: control one (n=32) and the core one (n=43). The control group consisted of younger students, who had not gone special endurance trainings. The core group consisted of the two sub-groups: first one (n=24) and second one (n=19). The students of the first group while being under pre-dosed physical exertion demonstrated low level of

oxygen consumption. The students of the second group demonstrated medium level of oxygen consumption under physical exertion. The level of oxygen consumption under physical exertion was determined in accordance with the norms and regulations of the Ministry of Emergency Situations of Russian Federation [4]. The exercises were done while the students were completely fit-out, including garments, fire belts, and an isolated breathing apparatus, which contained compressed air. Pulse frequency was controlled remotely with the help of the pulse watches. The level of air consumption from the breathing apparatus was identified via pressure gauges, which were included in the breathing apparatus set.

Results and discussion

60% of the students from the control group showed signs of the skin conductivity disorder in the acupuncture reference points, which reflect the immune system activity. The students of the core groups demonstrated the similar phenomenon more often: 83,33% referring to the group with the higher resistance to hypoxia and 86,84% referring to the group with the medium resistance to hypoxia. The difference between core grouped was not that significant. The difference of the core groups from the control one reached the degree of statistical validity ($p<0,05$). We believe, that this proves that the level of special trainings appears to be a very powerful stress factor, which leads to significant pressure of the adaptive mechanisms of the students and affect their immune status.

Comparative analysis of average indicators of the BAS conductivity has shown that greater differences between the control and the core groups were concentrated in the five out of twelve reference points, that were a subject to study in the framework of the ROFES-diagnosistics. These points reflected the functional state of the lungs' channel (P), kidneys (R), spleen and pancreas, (RP), urinary bladder (V) and triple heater (TR). The last channel in accordance with the classic norms of acupuncture reflects the state of the immune system.

The conductivity indexes of the channels mentioned above referring to the control and the first core group are provided in the Table 1.

Table 1. Skin conductivity indexes of the acupuncture key channels referring to the students of two groups (M ± m, standard units)

Type of channel	Control group (n=32)	Core group (n=43)	Statistical significance
P	16,7 ± 1,4	8,1 ± 0,6	t = 4,18; **p < 0,05**
GI	12,1 ± 0,6	11,0 ± 0,7	t = 1,20; p > 0,05

E	26,2 ± 1,5	31,1 ± 1,7	t = 2,16; **p < 0,05**
RP	17,2 ± 1,1	10,6 ± 0,7	t = 6,57; **p < 0,05**
C	9,6 ± 0,5	8,7 ± 0,6	t = 1,15; p > 0,05
Ig	9,4 ± 0,7	8,3 ± 0,7	t = 1,11; p > 0,05
V	16,5 ± 1,0	10,0 ± 0,7	t = 6,53; p < 0,05
R	26,0 ± 1,6	33,7 ± 1,5	t = 7,73; **p < 0,05**
MC	11,5 ± 0,7	7,0 ± 0,8	t = 4,25; **p < 0,05**
TR	21,0 ± 1,2	16,2 ± 1,2	t = 4,85; **p < 0,05**
VB	20,0 ± 1,4	15,4 ± 1,3	t = 1,26; p > 0,05
F	24,0 ± 1,3	25,1 ± 1,6	t = 0,53; p > 0,05

Statistical significance between the control group and the core group marks out the presence oft he disctinctive differences in their functional status.

The following conclusion is applicable to the indicators of the skin conductivity referring to the acuptunture channel TR, which characterizes the immune system activity level. Statistical significance of the average indexes referring to the skin conductivity BAS among the first and the second sub-groups has not been identified. However, the type of interconnection between the separate acupuncture channels referring to the students of the first and second sub-groups was not the same. The greatest difference was indentified referring to the rate of the channel TR with the channels RP, C, IG, MC, which altogether characterize the vascular component of the adaptive reactions (Table 2).

Table 2. Correlation rate between the skin conductivity indexes of the acupuncture channels referring to the students of the core group

Type of connection	Control group (n=32)	The first core sub-group (n=24)	The second core sub-group (n=19)	Presence of the connection type inversion referring to the second sub-group in relation to the first sub-group
TR – P	0,75	0,28	0,37	-
TR – GI	0,60	0,48	0,66	-
RT – E	0,77	0,43	0,15	-
TR – RP	0,41	0,16	-0,06	+
TR – C	0,72	0,34	-0,14	+
TR – Ig	0,52	-0,02	0,25	+
TR – V	0,40	-0,10	-0,12	-
TR – R	0,40	0,30	0,18	-

TR – MC	0,65	0,15	-0,11	+
TR – VB	0,71	0,24	0,37	-
TR – F	0,62	0,19	0,40	-

In majority of cases the students of the first sub-group showed positive connections between skin conductivity indexes of the TR channel (immune system) and the indexes of the channels that characterize the state of the cardiovascular system and mycro-vasculature track, although the degree of these links is not that significant. The inversion of the connection type referring to the second group in comparison with the first one was marked out.

Conclusion

The following study has allowed us to identify three fundamentally different functional statuses of the future firefighters: referring to the those who have not gone through special training for endurance; referring to those with high and medium endurance level. The difference between the students who have gone through special training and those who have not been trained can be easily identified via average rate of the skin conductivity indexes.

The difference between the trained and not trained students is related to the different algorithms of self-organization minding the same level of average conductivity indexes.

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Assotiation Maternal Selenium Intake in Pregnancy and Wheezing Illnesses in Children at One Of Age

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B428R9047 - late arrival

Abstract

Background

Asthma and allergies are important public health problems. Human in utero exposure to heavy metals such as selenium can reduce the prevalence of childhood asthma and allergic diseases.

The objective of the study was to determine Selenium in Pregnant and newborn blood samples and evaluate their association with the development of wheezing.

Methods

Plasma selenium concentrations were measured in maternal blood on the 13-17 weeks of gestation in 32 pregnant women [among them 6 with sevier stress] and in the cord blood after delivery. During 1 year the frequency of wheezing evaluated by the specially adapted questionnaire. Cohort children were followed up from birth to 1 years using health questionnaires filled out by the parents Maternal plasma selenium was related to the childhood outcomes.

Results

Our results showed, that fetal stress positively and Selenium high level negatively associated with risk of wheezing in early childhood.

Conclusions

The results of this study suggest that the level of maternal and cord blood selenium and fetal tress could have an influence on the risk of wheezing in infancy and potentially on the risk of developing asthma later in life.

Keywords: Asthma, Selenium, wheezing

Background

Asthma and allergies are important public health problems Human in utero exposure to heavy metals such as selenium can reduce the prevalence of childhood asthma and allergic diseases. It has been suggested that human in utero exposure to heavy metals such as selenium can reduce the prevalence of childhood asthma and allergic diseases. No clear reasons are available for the increase in prevalence of asthma and allergies in developed countries, but it is likely that a changing environment and the behaviors associated with a "Westernized" lifestyle contribute to the problem. Selenium is an essential micronutrient that is important for various aspects of human health including proper thyroid hormone metabolism, cardiovascular health, prevention of cancer, and optimal immune responses. Most populations worldwide acquire dietary Se at levels that do not result in severe deficiency or toxicity, but there are important exceptions. For example, regions in China and New Zealand have low Se content in the soil, which may lead to insufficient Se in plants and livestock those results in low Se foods.

The objective of the study was to determine Selenium in Pregnant and newborn blood samples and evaluate their association with the development of wheezing.

Methods

Plasma selenium concentrations were measured in maternal blood on the 13-17 weeks of gestation in 32 pregnant women [among them 6 with fetal stress]and in the cord blood after delivery. During 1 year the frequency of wheezing diseases evaluated by the specially adapted questionnaire. Cohort children were followed up from birth to 1 years using health questionnaires filled out by the parents Maternal plasma selenium was related to the childhood outcomes.

The parents completed a questionnaire including questions on asthma and wheezing at the age of 1 and on doctor diagnosed atopic dermatitis. Asthma was defined as parental report of doctor-diagnosis of asthma plus either one or more attacks of wheeze or asthma medication in the last 12 months. Wheeze was defined as present if the parents answered "yes" to the question "Has your child had wheezing or whistling in the chest in the preceding 12 months?".

Allergic rhinitis was defined as sneezing, nasal congestion, or rhinitis, other than with respiratory infections, accompanied by eye itching and tearing during the previous 12 months. Finally, parents reported atopic dermatitis diagnosed by a doctor. The responses at age of 1 year were incorporated into calculated lifetime prevalence of wheezing, asthma, atopic dermatitis.

Results

Our results showed that fetal stress positively and Selenium high level negatively associated with risk of wheezing in early childhood. Categorical variables were expressed as the number (%) and continuous variables were expressed as the mean ± standard deviation (SD). The mean selenium concentrations were compared according to maternal educational level, BMI, age, socio-professional category and tobacco use during pregnancy by applying Student's test (for binary variables).

We used logistic regression models to investigate the associations between health-related variables and selenium exposure defined in tertiles. We estimated the odds ratio (OR) [95% confidence interval (CI)] for each health-related variable in the child by the age of 1.

Conclusions

An analysis of the associations between selenium levels and the health-related variables revealed a significant, negative association between a high maternal plasma selenium level and the risk of wheezing in the child at 1.

After multivariable adjustment, the previously observed significant inverse associations persisted (by age of 1: OR = 0.74 (95%CI = 0.47–0.92), $p = 0.03$).

Furthermore, an analysis of maternal selenium concentration as a continuous variable revealed in the unadjusted models, a significant negative association between plasma selenium concentration and the risk of wheezing by 1 year (OR = 0.94 (95% CI = 0.90–0.99), $p = 0.04$) and a trend toward a negative association by 3 years (Table 3). However, the latter results were borderline-significant ($p = 0.06$ by age of 1) after adjustment for potential confounding factors.

The results of this study suggest that the level of maternal and cord blood selenium and fetal tress could have an influence on the risk of wheezing in infancy and potentially on the risk of developing asthma later in life.

It is worked out in the framework of the project of Rustaveli Scientific National Fund (project # DO385/8-308/14)

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