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PROF. DR. FRANCISC SCHNEIDER
PO BOX 135
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e-mail: carmen.tatu@umft.ro

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IN MEMORIAM IOAN HAULICA

He was born October 29, 1924, in the village of Ipatele, from the county of Iasi, in a numerous family and attended high school in Iasi.

After graduating from the Faculty of Medicine in 1949, he worked for three years as a junior assistant in the departments of Pharmacology and Pathophysiology of the Institute of Medicine and Pharmacy Iasi. Being dismissed, he moved to the Institute of Normal and Pathological Physiology of the Romanian Academy in Bucharest, where he worked for 12 years as a researcher under the guidance of academics D. Danielopolu and Grigore Benetato.

After obtaining a doctoral degree in medical science in the field of physiology, he returned to Iasi in 1964, when he became associate professor of physiology in the same department of the Institute of Medicine in Iasi.

After obtaining the title of docent and obtaining the position of professor in 1968, he took over the coordination of the scientific and teaching activity of the department, where he continued working until his retirement in 1994.

Received training courses in USA, Austria, Poland, USSR, Germany, and France he worked as a "visiting professor" at University Village Connecticut (U.S) during the school year 1969-1970.

In 1972 he was able to organize in Iasi an international workshop of "Synaptic Transmission" for a period of three weeks, sponsored by IBRO/UNESCO and attended by professors from the U.S. and 12 European countries.

During his half of a century of relentless pursuit of the secrets of the human body he made significant contributions to important fields of physiology and human biology among which are worthy of mention a wide range of topics of normal and pathological physiology, making original contributions to some of the issues under study. He carried out fundamental research on the physiology and pharmacology of autonomic organs, functional biochemistry of brain, extrarenal origin renin-angiotensin system, neuroendocrinology of acute and chronic stress, neurohumoral bases of pain and stress analgesia, functional implications of free radicals and endogenous antioxidants, role of endothelium and nitric oxide in vascular reactivity, etc. Much of the experimental research were confirmed and quoted by various authors in the country and abroad. The discovery of the intrinsic renin-angiotensin system in the pituitary and pineal glands in mammals and humans and the identification of the role of the purinergic nerve fibers from the myenteric plexus, spinal ganglia, spinal cord, brainstem, cerebellum and hypothalamus are internationally recognized contributions.

In collaboration with the "Max Delbrück" Center for Molecular Medicine in Berlin he discovered a new active angiotensin II-forming enzyme in the human brain and endocranial glands (pituitary, pineal) called chimase, which predominates over the angiotensin converting enzyme in the brain.

He also brought cognitive contributions to the study of the heart and vascular renin-angiotensin system, eye, uterus and ovary, and the neuro-humoral basis of pain and the role of free radicals in the central neurochemical imbalances caused by stress. During the last years, he gave a special attention to the role of vascular endothelial reactivity, obtaining experimental evidence for the involvement of nitric oxide (NO) both as endothelial relaxing factor as well as neural messenger involved in nociceptive sensitivity.

He has published over 300 papers in specialized journals in the country and abroad. He elaborated and published as first author or in collaboration three treatises of human physiology and eight monographs, out of which three received the Romanian Academy Award ("Autonomic Nervous System" in 1975, "Local Hormones" in 1983 and "Synaptic Transmission" in 1999).

He was author or coauthor of 10 patents. He participated in numerous scientific events in the country and abroad, reports, communications, posters and roundtables. He was Dean of the Faculty of Medicine (1972-1976) and pro-rector of the Institute of Medicine and Pharmacy Iasi (1981-1988).

He was a member of several Romanian and international scientific societies: International Society of Physiological Sciences, FEPS, Society of Physicians and Natural Scientists from Iasi, International Society for the Study of Brain (IBRO), European Society for Pineal Studies, European Federation of Biological Sciences, Society for Neuroscience, Psychoneuroendocrinology Society and others.

He was deputy chairman of the Academy of Medical Sciences and president of the Society of Physiological Sciences in our country until 2004. He was president of the Society of Physicians and Natural Scientists from Iasi between the years 1990-1994. He was elected Fellow of the Romanian Academy in December 1991 and he became a full member in 1994.

In 1999 he was granted the title of Doctor Honoris Causa by the University of Medicine and Pharmacy, Tg. Mures and in 2003 by the University "Ovidius", Constanta. In 2002 he received the Order "Star of Romania" in the rank of Knight.

In 1997, three years after his retirement, he founded the Laboratory of Experimental and Applied Physiology subordinate to the Iasi branch of the Romanian Academy where he continued to pursue research on the renin-angiotensin system, biological properties of deuterium depleted water the influence of radical species on vascular reactivity and central nervous activity.

Until the very moment of his death, Professor Ioan Haulica continued to study and research as honorary head of the Laboratory of Experimental and Applied Physiology, to write and try to impart to the others out of the richness of his knowledge and experience. His last book, a treaty on the autonomic nervous system was finalized mere months before his untimely death, thus denying him the joy of seeing it published.

"The Professor" will remain forever in the memory of thousands of former students, PhD students, colleagues, collaborators and friends, upon whose lives he would have left an ineffaceable impression.

Physiology Department, UMF Iasi
Prof. Simona Slatineanu, MD, PhD

HEALTH SYSTEMS IN THE EUROPEAN UNION. AN OVERVIEW

CRISTIAN BUSOI

Public Health Department, "Victor Babes" University of Medicine and Pharmacy Timisoara

ABSTRACT

An analysis of the existing healthcare systems of the EU Member states shows a significant degree of diversity while all were inspired by the two major healthcare system models known as the Beveridge and the Bismarck models. The EU healthcare systems vary according to the financing of the system, to the proportion of the private healthcare sector in the overall system, the coverage of the insurance package, the more or less freedom to choose the service provider, the presence of guidance mechanisms towards a certain type of care etc. The Romanian healthcare system has suffered a series of transformations since 1989, a process that is still ongoing and which is to be related to the struggle for democratization and modernisation of Romania society.
Key words: healthcare system, EU.

MATERIAL AND STRUCTURE OF HEALTHCARE SYSTEMS

Beveridge system

With regard to the structure of healthcare systems, there are two worldwide known models that have become the source of inspiration for the majority of the European national health care systems.

One of them is the so called Beveridge system which was adopted by some EU Member States, namely the United Kingdom and the Kingdom of Sweden. It was named after its founder, Lord Beveridge and it is a healthcare system financed by the general tax system, where the entire population is granted access to healthcare services. The total amount of payments to which the patient is entitled is not linked in any way to the amount of the wages earned and health care services are offered to everybody without any formal obligation of prior contribution to the healthcare system. The responsibility for the management of Beveridge healthcare systems lies with a professional administration under parliamentary scrutiny. Member States such as Italy, Spain, Greece and Portugal have been more or less influenced by the British model.

Bismarck model

The second important social protection model which is to be found in Germany as well as in the Benelux countries is the Bismarck model, which also bares the name of its founder, the former Prussian Chancellor, Otto von Bismarck. The insurance contributions are established as a percentage of the salaries and are managed by independent health insurance funds which are to be chosen by each person. Thus, unlike the Beveridge system, the Bismarck system is not controlled by the state and the activity and management of the contributions by the healthcare insurance funds is not at all scrutinized by the parliament. The healthcare services package to which each insured person is entitled is established in the framework contract which is negotiated by medical practitioners and healthcare insurance funds representing the interests of the insured patients. Moreover, the compulsory social insurance system is designed to cover people depending on their membership of a socio-professional group.

One of the most representative healthcare systems within the EU is the French one, which is characterized by the universality of access to medical care, the freedom of medical practitioners and the free choice of the provider as well as by the fact that the management of the social security is done by social partners. The system depends on the monopoly power that insurance funds have on users as well as on the monopoly of the professional trade unions which are the only authorized to represent the insured within the insurance funds boards.

An original case is the Italian one due to its hybrid character combining the Anglo-Saxon model of healthcare system organization with the partial financing by patients. As in Germany and France, the Italian social protection system has developed through a plurality of professional regimes.

When it comes to choosing a healthcare system model, one ought to take into account the role of the state through its different agencies. At this point, the so-called ideological factor comes into play as those political parties which are generally supportive of an active role of the state in economy and the society level on a larger scale, will also be the supporters of state-controlled healthcare systems and vice versa. For the time being, there is no entirely privatized or nationalized healthcare system in the European Union. The most frequent situation is where state-controlled healthcare systems borrow some of the features of healthcare systems based on marked mechanisms, and the other way round. This happens because there is a risk

Received May 2010. Accepted June 2010. Address for correspondence: Busoi Cristian, MD, Public Health Department, "Victor Babes" University of Medicine and Pharmacy Timisoara, 2A Eftimie Murgu Square, Timisoara 300041, Romania, e-mail: cristian.busoi@gmail.com

of market failures and healthcare is a sector where the state intervenes in order to correct such failures and to make sure that the population has broad has an as broad as possible access to medical care. Therefore, the principles of state control and market mechanisms may be complementary (2). This is the idea lying behind the classification made by some authors who refer to three large groups of healthcare systems: liberal systems, nationalized systems and intermediary systems.

IN-PATIENTS CARE

Regarding in-patient care, a high degree of diversity can be noticed within the EU with a varying weight of the public and private sectors within healthcare systems. Thus the role of the private sector varies from maximum (in the Netherlands) to minimum. In the French system, for instance, 63% of the total amount of hospital beds belongs to the public sector, whereas only the remaining 37% have private owners. On the other hand, out-patient medical care in France is provided by medical doctors who work in private practices. There are also other cases that deserve full attention, namely the case of Belgium and Germany, where the private sector covers more than 50% of the overall healthcare system, even if this figure is slightly lower that in the Netherlands. Denmark and Sweden are on the opposite side, with an almost absent private sector (1).

As a general rule, in countries with intermediary healthcare systems, out-of-pocket payments for medical care are lower then in those with liberal healthcare systems and higher then in those countries that have chosen a nationalized system. France and Germany are good illustrations of this, as fees raise from 74% and 64% respectively.

FINANCIAL ISSUES IN EU MEMBER STATES

If we study carefully the healthcare system of EU Member States, diversity can be noticed, not only at the organizational level but also at a financial one.

Countries finance their healthcare system through taxes or social insurance contributions, or even a combination of the two. The system based on budgetary resources which come from contributions is highly used in Great Britain, Ireland, Denmark, Finland, Spain and Portugal. Other countries like Greece and Italy have chosen a mixed system where a part of the resources come from contributions and the rest come from the social healthcare insurance budget. Also, countries such as Belgium, Germany, Luxembourg and Austria have expressed their preference for a funding predominately based on social healthcare insurance contributions. One example is Luxembourg where 60% are from social healthcare insurance fees and the rest of 40% of the expenses for healthcare are financed by state. The extreme examples are France and the Netherlands which have systems that are exclusively financed by the social insurance budget, to which the population give their contribution.

Countries which have a system financed by the contributions to social healthcare insurance budget have different social security schemes. In some countries, the citizens have the freedom of choosing their insurance provider, having at their disposal many social healthcare insurances funds, such a way is found in Germany, Belgium and the Netherlands. In other countries like France, Austria or Luxembourg, the citizens have no saying in the matter, affiliation being made on the basis of their professional activity.

In countries where there is a national healthcare system, the population is regularly covered by that insurance, in comparison with countries which have chosen a social insurance system (on a contribution basis) like France, Austria, Luxembourg or Belgium, where self-employed and employees are required to take an insurance, and thus to contribute to the healthcare insurance fund. In the Belgian case, self-employed are only required to choose the state healthcare insurance system for hospital care, while for primary care or secondary care, they may as well opt for private health insurance. The French system is also an interesting case with a basic insurance system which doesn't cover the whole treatment, but which is complemented by a system of co-payment that may be covered by supplementary insurance ("mutuelle"). Another element that deserves attention is the total exclusion of the complementary insurance scheme of people with very high incomes, as it is the case in the healthcare system of Germany or the Netherlands. The difference between the two cases is that in Germany, these groups of citizens have the obligation to contribute to a private healthcare system, in comparison with the Netherland where the signing of this kind of insurance is left at the judgment of the person concerned, with no obligation like the case of Germany.

After a detailed analysis of healthcare systems structure in the EU, we are able to conclude that EU healthcare systems have a certain number of features in common, despite their obvious diversity. Under the institutional and financing aspect, these countries may be grouped in six different categories identified in an OCED report, which proved to be extremely pertinent, empirically speaking (1). The first group is composed by countries which rely mainly on market mechanisms regarding both the insurance system as the provision of medical care services (this is the case especially in Germany, the Netherlands and Slovakia). Two other groups are relatively close because of their public basic insurance system combined with offering medical services based on market mechanisms. These two groups differentiate themselves through the mechanisms of guiding the patient towards a certain type of medical care (primary, secondary or tertiary without a large freedom of choice for the patient), as well as through the existence, more or less pronounced, of private health insurance schemes as a complement to basic insurance (in the first group we have Belgium and France, while in the second, we find Austria, Greece, Luxembourg or the Czech Republic). The fourth group of countries, of which Sweden stands out as most representative, is the one in which the patient has a very large freedom of choice and where there is no patient guidance mechanism towards a certain type of medical care, but in which private health services represent a very low proportion. The last two groups are those which are composed by public healthcare systems extremely well regulated, but which differentiate themselves through the level of constraint exerted by patient guidance mechanisms towards

different types of medical care and also through the intensity of budgetary constraint in the healthcare sector. In the first of these two groups we may find Denmark, Finland, Portugal and Spain, whereas in the second and last group, countries such as Italy, Ireland, Great Britain, but also Hungary and Poland from the new Member States of the EU.

ROMANIAN HEALTHCARE SYSTEM

Romania has operated major reforms after December 1989. The communist system, of a totalitarian and statist type, was replaced by institutions of a democratic and pluralistic society. Right after the Revolution the main democratic institutions, the Parliament and political parties, were built and gradually all the institutions of the Romanian society suffered changes through the modernization and democratization process.

The transformations in the healthcare sector were not less significant. The healthcare system in the communist period, under-financed, statist and centralized was replaced with a health insurance system, which combines some of the free market mechanisms, a sector of public hospitals currently in a process of decentralization, as well as private initiatives in out-patient of in-patient care systems. The transformation was yet slow and filled with hesitations, uncertainties and controversies.

With all the changes produced, some influences of the communist period have still remained - the illusion of total freeness of medical services, a modest budget given to the healthcare system which led to a chronic under-financing, a lot of the aspects in hospital system structure, the relations within hospitals especially those in university centers, the patient-doctor relation or informal payments.

Despite these influences and reminiscences, the system underwent significant changes affecting a lot of its essential features. The introduction of healthcare insurance system, following the German model, creating professional organizations (Medical College, Pharmacist College etc), the method of financing services (per capita, DRG, through services), private initiatives in health, family medicine, reforms of the healthcare management were key elements for the system.

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SISTEME DE SANATATE IN UNIUNEA EUROPEANA. PREZENTARE DE ANSAMBLU

REZUMAT

Analiza sistemelor de sanatate existente in statele member ale UE a aratat un mare grad de diversitate, chiar daca toate acestea sunt inspirate din cele doua sisteme de sanatate model, cunoscute sub denumirile de Beveridge si Bismarck. Sistemele de sanatate ale UE difera in functie de structura de finantare a sistemului, de gradul de implicare al sectorului de sanatate privat in ansamblul sistemului de sanatate, de acoperirea pachetului de asigurare, de gradul de libertate in alegerea furnizorilor de servicii, de prezenta mecanismelor de ghidare spre un anumit tip de ingrijire, etc. Sistemul de sanatate din Romania a suferit o serie de transformari incepand cu anul 1989, un process care este inca in desfasurare si care este relationat cu lupta pentru democratizare si modernizare a societatii romanesti.

Cuvinte cheie: sistem de sanatate, UE

CONTEMPORARY MAN IN HIS BIO-PSYCHO-SOCIO-ECOLOGICAL DIMENSION

SORIN RIGA[1], DAN RIGA[1], AUREL ARDELEAN[2], FRANCISC SCHNEIDER[3]

[1] Department of Stress Research & Prophylaxis "Al. Obregia" Clinical Hospital of Psychiatry, Bucharest, RO
[2] "V. Goldis" Western University, Arad, RO
[3] Center for Applied Physiology and Molecular Biology "Vasile Goldis" Western University, Arad, RO

ABSTRACT

Nowadays civilization, globalization and capacity of phenomena understanding make it necessary and imperious redefinition of human notions, concepts and actions. Under the circumstances bio-psycho-social concept of human (Engel - Branzei - Bekhterev) should be defined in a different manner, namely bio-psycho-socio-ecological dimension of human being (S. Riga - D. Riga - A. Ardelean - F. Schneider). Consequently bio-psycho-social concept, dimension and determinants of human will be completed with ecological ones. This tetrad will have to be present in legislation, human actions and beneficial effects on civilization and environment. Acting in a positive and reasonable manner on geo- and bio-sphere, man will definitely contribute to homeostasis recovery of Terra system.

Key words: human civilization, redefinition of notions, bio-psycho-social concept of human, new bio-psycho-socio-ecological dimension of human being

INTRODUCTION

In these days, capability of understanding phenomena makes it imperative and necessary reevaluation of human knowledge, behaviour and actions. This is why human being should be redefined concerning his concept, significance and determinants for his future evolution and activity.

Accordingly we surveyed, classified and developed the understanding of human being in its complex structure and build, needs and aspirations. In the course of time, *Homo sapiens* received other Latin-based names for a better description of human features and characters. We reviewed the biologic, bio-social, bio-psycho, bio-psycho-social dimensions of humans and introduced a new concept, namely bio-psycho-socio-ecological significance of human being.

HUMAN BIOLOGIC DETERMINANT

Human species is defined from Latin as *Homo sapiens* with significance of "wise man" or "knowing man". Scientific classification affiliates the human being to Domain *Eukaria;* Kindom *Animalia;* Phylum *Chordata;* Class *Mammalia;* Subclass *Theria;* Order *Primates;* Suborder *Haplorrhini;* Infraorder *Simiiformes;* Parvorder *Catarrhini;* Superfamily *Hominoidea;* Family *Hominidae;* Subfamily *Homininae,* which contain Genus *Homo* (humans, human beings), together with Genera *Gorilla* (gorillas) and *Pan* (chimpanzees).

Carl von Linné (Carolus Linnaeus, 1707-1778), Swedish naturalist, botanist and physician, was the founder of modern systematics. He elaborated binomial classification system (binomial or binary nomenclature). In naming an organism, Carl von Linné chose to utilize a two-world naming system (genus name and specific name or epithet, which together form the species name), instead of using the full seven-category system (kindom-phylum-class-order-family-genus-species). Therefore, human becomes *Homo sapiens* as binomial name, in his monumental monograph *Systema Naturae,* 10th edition, 1758.

Homo sapiens sapiens (trinomial name introduced by Linnaeus also in 1758 - Genus *Homo,* Species *sapiens,* Subspecies *sapiens*) is the last representative from his species. He created human history and civilization, and has the possibility towards a fascinating evolution and unforeseeable achievements.

HUMAN BIO-SOCIAL DIMENSION

Aristotle-Aristoteles (384 BC - 322 BC) denominated human being as *Zóon politikón (Political animal, Homo politicus, Political man).* Man's political concept entails man's social determinant: *The political partnership must be regarded, therefore, as being for the sake of noble actions, not for the sake of living together* (Aristotle, *Politics*).

During the time humans received other bio-social names: *Homo socius, Social man,* man as social being (Peter Berger, Thomas Luckmann, 1966) and *Homo sociologus, Social man* (Ralf Dahrendorf).

HUMAN BIO-PSYCHO CHARACTERIZATION

Bio-psycho determinants of human being are very specific, special and important. They should be divided in features regarding human faculty of reasoning and traits concerning human emotion and behaviour.

Reason characteristic has multiple significations and denominates *Homo sapiens sapiens* as:

- *Animal rationabile,* animal capable of rationality (Carl von Linné 1760, Immanuel Kant 1798);

Received March 2010, Accepted May 2010. Address for correspondence: Dr. Sorin Riga, Department of Stress Research & Prophylaxis, "Al. Obregia" Clinical Hospital of Psychiatry, 10 Berceni Road, Sector 4, RO-041914 Bucharest, Romania, e-mail: D_S_Riga@yahoo.com

- *Homo investigans, Investigating man,* human capability to learn by deduction (Werner Luck 1976);
- *Homo imitans, Imitating man,* human capability to learn by imitation (A. N. Meltzoff 1988, Jürgen Lethmate 1992);
- *Homo discens, Learning man*, human capability to learn and adapt (Heinrich Roth, Theodor Wilhelm);
- *Homo loquens, Talking man,* (G. Herder 1772, J. F. Blumenbach 1779);
- *Homo faber, Toolmaker man* (Benjamin Franklin, Karl Marx, Kenneth P. Oakley, 1949);
- *Homo creator, Creator man,* human creativity (Michel Landmann 1955, W. E. Mühlmann 1962);
- *Homo metaphysicum, Metaphysical man* (Arthur Schopenhauer 1819);
- *Homo religiosus, Religious man* (Alister Hardy).

Emotion and behavour describe human being as:

- *Homo sentimentalis, Sentimental man,* man born to a civilization of sentiment (Milan Kundera 1990, Eugene Halton 1995);
- *Homo ludens, Playing man,* characterization of human culture as essentially learning the character of play (Friedrich Schiller 1795, Johan Huizinga);
- *Homo ridens, Laughing man* (G. B. Milner 1969);
- *Homo pictor, Man the artist,* human sense of aesthetics (Hans Jonas 1961);
- *Homo aesthetics, Aesthetic man,* capability to appreciate art and beauty (Ellen Dissanayahe 1992);
- *Homo generosus, Generous man* (Tor Nørretranders).

HUMAN BIO-PSYCHO-SOCIAL MODEL

Modern dimension of human associates *Homo sapiens sapiens* with bio-psycho-social concept. This integrative characterization was elaborated for the first time by V. M. Bekhterev in 1907 (1, 6), then by P. Branzei in 1970 (2, 3) and more recently by G. L. Engel in 1977 (4, 5). P. Branzei described this human feature as a dynamic and unitary structure respecting psychical processes in normal and pathological conditions. Right from the start, G. L. Engel published his new medical - biopsychosocial model in international renowned and well-known reviews: *Science* (1977), *Gen. Hosp. Psychiat.* (1979) and *Am. J. Psychiat.* (1980).

HUMAN BIO-PSYCHO-SOCIO-ECOLOGICAL INTEGRATION

With this paper the authors introduce a new and original concept about *Homo sapiens sapiens,* namely humans are and should be bio-psycho-socio-ecological beings. This integrated characterization of humans is the result of our critical examination concerning previous human definitions taking into account the present stage of human civilization (7, 8). In addition, our definition opens new prospects and possibilities for human future evolution. Practically, now and in the near future, humans should be realized a better bio-psycho-social integration associated with a civilized behavior regarding the nature.

This new complex concept of human being, with his dimensions, features and determinants should be rapidly introduced in legislation (local, Romanian, regional and European), as well as known and accepted by the population. Then perceptions and actions of humans regarding the concept will reflect major modifications in their behaviors and beneficial effects on the environment and nature.

In the 20th century, the civilized (?) humans had an uncivilized (!) behaviour respecting the nature, with serious negative consequences, i.e. lack of legislation for nature protection, severe pollution, massive forestry clearances, acid rains etc.

In our days and near future (2000-2030) humans must act as ecological men, in ecological dimensions for an ecological civilization (10, 11). People and countries from the Carpathian-Danubian area (Austria, Czech Republic, Poland, Slovakia, Hungary, Ukraine, Serbia and especially Romania) should have a civilized behaviour regarding the nature.

In this respect, Arad city represents a pattern by "Vasile Goldis" Western University (rector prof. dr. Aurel Ardelean). This university is a model for Euro-regional civilization, an example which should be followed by others in Romania and Europe. Here you are three important achievements:

- creation and annual organizing of the DKMT (Danube-Kris-Mures-Tisa) Euro-regional
- Conferences on *Environmental Health and Protection;*
- national review *Fiziologia-Physiology,* Timisoara and Arad (editor-in-chief prof. dr. Francisc Schneider), with many published papers on ecology sciences (9, 13);
- present international conference UECDA (Unconventional Energies in the Carpathian and Danubian Area), entitled *Technical and Economical Ecology Issues and the Medical and Social Aspects* (7).

Topics of this UECDA conference are very relevant and important because show and indicate solutions and results for:

- "Green" energy (unconventional and non-pollutant energies, creating energy using biomass, intelligent hydro-energetic systems);
- "Green" industry-production-economy (non-pollutant units, ecological associations and holdings, intelligent hydro-energetic systems);
- 3R concepts (Reduce, Reuse, Recycle) - waste management hierarchy - EPR (Extended Producer Responsibility).

Contemporary man is an educational project (12) and his psycho-ecological dimension can be more easily implemented on human being (14).

CONCLUSIONS

Ecological concept is an important field of globalization, civilization and human evolution. Therefore, humanity must urgently acts in ecological dimension. The results will be beneficial for manking and for the Earth too.

Earth Day (April 22) started from 1970 is a good example to mobilize and unify the ecological forces. Earth Day 2009 is entitled "Green generation". In this respect ecological dimension

of human being is welcome and should be rapidly introduced in the behaviour of every person.

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OMUL CONTEMPORAN IN DIMENSIUNEA SA BIO-PSIHO-SOCIO-ECOLOGICA

REZUMAT

Etapa actuală a civilizatiei umane, globalizarea si capacitatea de intelegere a fenomenelor fac necesara si imperioasa redefinirea notiunilor, conceptelor si actiunilor umane. In acest context, conceptul bio-psiho-social al omului (Engel - Branzei - Bekhterev) trebuie definit intr-o alta perspectiva si anume dimensiunea bio-psiho-socio-ecologica a fiintei umane (S. Riga - D. Riga - A. Ardelean - F. Schneider). Astfel, conceptul, dimensiunea si determinantii bio-psiho-sociali ai omului vor fi completati cu cei ecologici. Aceasta tetrada va trebui sa se regaseasca in legislatie, actiuni umane si efecte benefice asupra civilizatiei si mediului. Actionand pozitiv si cu discernamant asupra geo- si bio-sferei, omul va contribui decisiv la recapatarea homeostaziei sistemului Terra.

Cuvinte cheie: civilizatie umana, redefinire notiuni, concept bio-psiho-social al omului, noua dimensiune bio-psiho-socio-ecologica a fiintei umane

REVIEW

INFECTIONS ASSOCIATED WITH PREGNANCY AND CHILDBIRTH

CAMELIA BUDISAN, CONSTANTIN ILIE
Department of Neonatology, "Victor Babes" University of Medicine and Pharmacy Timisoara, Romania

ABSTRACT

Prematurity is the cause of 85% of neonatal morbidity and mortality. Subclinical ascending infections through the lower female genital tract are predominant worldwide. Important micronutrient deficiencies may prevail in low-income countries where these infections are much more common than in high-income countries. Important morbidities related to poor perinatal outcome both for the mother and for the fetus and newborn comprise preterm birth, prelabor rupture of membranes, placental abruption (predelivery detachment of the placenta), postpartum sepsis and maternal anemia. In the fetus, sepsis and intrauterine growth retardation are suspected to be consequences of ascending maternal infections. In the newborn, septicemia and respiratory disorders as well as some neurological disorders seem to be consequences of such ascending genital infections in the pregnant woman. It is concluded that much more attention should be given to efforts to elucidate the host defense mechanisms and antimicrobial barriers from the vagina through the cervix, fetal membranes and amniotic fluid including the early fetal immunocompetence in the second and the third trimester of pregnancy.

Key words: fetal diseases, maternal morbidity

INTRODUCTION

Infections associated with pregnancy and childbirth have caused concern for women and their caregivers for centuries. Much attention therefore has been focused on understanding these infections. Although the clinical approach to infections has improved markedly in the past few years, infections continue to pose a problem in pregnancy, particularly in low-income countries (1– 4).

Infections are implicated in the pathogenesis of miscarriage, preterm labor and prelabor rupture of membranes, all of which are common events (4). Miscarriage is common worldwide and is the outcome of approximately 15% of all clinically diagnosed pregnancies. If syphilis and certain vaginal infections are common, this figure may reach significantly higher levels, including an increase in miscarriage in the second trimester. Preterm labor may occur in 10–20% of pregnancies in low-income countries whereas prelabor rupture of membranes and postpartum septicemia may occur in 5–10% in such settings. All these in turn are associated with neonatal infections and morbidity. Both the direct effect of the infection and the maternal immune response contribute to these eventualities (3, 4). For example, infections that trigger T-helper-1 response can lead to the release of cytokines such as interferon (IFN)-γ, tumor necrosis factor (TNF)-α and interleukin (IL)-2 with activation of killer cells and initiation of preterm labor (3).

Systemic infections and genital infections due to many different microorganisms including mycoplasmas, *Chlamydia trachomatis* and *Trichomonas vaginalis* are reportedly involved in initiating preterm labor (3,5-9). A wide variety of bacteria present in the normal vaginal flora of pregnant women such as anaerobes and *Escherichia coli* can also cause ascending infections, usually after rupture of membranes, resulting in intra amniotic infection (10). Chorioamnionitis resulting from such infections can lead to preterm labor and maternal and fetal morbidity (10). Recent data show that *Candida* sp. may also be important in causing preterm labor and neonatal morbidity. intra amniotic infection due to bacteria in the vaginal flora not only initiate labor but can also cause infections such as septicemia and meningitis in the newborn (10, 11).

Several host defense mechanisms operate against ascending infections; these include vaginal acidity, cervical mucus, intact membranes and antibacterial activity of amniotic fluid (12,13). One study in India demonstrated that all samples of amniotic fluid inhibited *Candida albicans* and *Clostridium perfringens* whereas 50%, 42% and 18%, respectively, inhibited *Staphyllococcus aureus*, *E. coli* and *Bacillus fragilis* (14). The inhibitory activity could be due to polymorphonuclear leucocytes, lysozyme, beta lysin, transferrin, immunoglobulins and other bacterial inhibitory factors such as polypeptide-zinc complexes in amniotic fluid (10).

Intra amniotic infection is difficult to diagnose on the basis of any single criterion and so diagnosis depends on a set of criteria, the most important clinically being maternal fever and tachycardia and fetal tachycardia(10). The use of laboratory methods for diagnosis is still not practical. The infection may be polymicrobial, but collecting amniotic fluid samples without contamination with normal vaginal flora is cumbersome and may require invasive procedures. Also, after membrane rupture many bacteria may enter the amniotic cavity without having caused the rupture. Because of these circumstances, cultures are not usually attempted, especially in Romania. Recent literature shows that detection and estimation of surrogate markers such as C-reactive protein (CRP), cytokines and fetal fibronectin help in diagnosing intra amniotic infection and in predicting and diagnosing early-onset neonatal infections (15-18).

Received December 2009. Accepted June 2010. Address for correspondence: Dr. Camelia Budisan, Department of Neonatology, Victor Babes University of Medicine and Pharmacy Timisoara, 2A Eftimie Murgu Square, e-mail: cbudisan@rdslink.ro

Levels of CRP rise when there is a microbial infection or an inflammation without microbes (19). Studies in pregnant women showed that CRP is elevated at the onset of labor even in normal pregnancies and reaches very high levels during the immediate postpartum period (20). Whether CRP levels are higher than normal in subclinical infections is not clear and the usefulness of this marker to diagnose intra amniotic infection remains to be established. However, several studies have shown the usefulness of CRP to predict and diagnose neonatal infections (16-18).

Diagnosis of neonatal septicemia remains a major challenge. Sepsis can develop in infants with and without risk factors. Clinical signs are nonspecific and the laboratory criteria are also not fully reliable. Although a combination of clinical and laboratory criteria are required to make a diagnosis, antibiotic treatment is often initiated on the basis of clinical suspicion alone. Because an infected neonate can have a negative blood culture, the initiation of antibiotic therapy without supporting hard evidence of infection is currently justified; in addition, results from blood culture are not available until several days after the harvesting of blood for culture. Treatment on the basis of clinical symptoms alone leads to considerable overuse of antibiotics in the nurseries. Although laboratory data may not be of much use in preventing initiation of therapy, such data could at least help in stopping unwarranted use of antibiotics.

The tests currently used to diagnose neonatal infections include total and differential counts, absolute neutrophil count and the ratio of immature to total white cells. The sensitivity and specificity of these tests are low. In recent years, CRP estimation has been found to be useful in diagnosis. One of the pitfalls is that, as mentioned, CRP can be positive when there is no infection (i.e., the positive predictive value is very low). To make the predictive values better, a more appropriate cutoff level has to be established. Consensus on the cutoff level does not exist at present. In true infection, the test may become positive after 12 h, so estimation of CRP at presentation may not be of much value in diagnosis. Serial determinations may be required and may have a better predictive value than static single estimates (21). This test may be valuable for making decisions about discontinuing therapy. The test can be done using automated systems and a latex agglutination test, which is widely available in Romania.

Over the years several proinflammatory cytokines have been tested for their use in diagnosing IAI and neonatal infections. These cytokines include IL-2, IL-6, IL-8 and IFN-*γ*. Maternal, cord and neonatal blood IL-6 levels have been found to correlate with chorioamnionitis and neonatal sepsis (16-18).

IL-6 stimulates the production of CRP. Therefore, IL-6 levels should rise before CRP levels rise. Several studies have confirmed that IL-6 is an early and sensitive marker of sepsis in newborns and in adults. IL-6 levels are found to be better predictors of mild sepsis (22). Combined use of IL-6 and CRP is found to give better predictive values than the use of either alone. However, more studies in different settings are required to confirm these findings and to evaluate their applicability as routine diagnostic tests.

TNF-*α* is responsible for organ injury. Although the levels of this cytokine also increase in infection, this is a less sensitive marker than IL-6. Combined use again increases sensitivity (22). IL-1β is a soluble protein released by macrophages in response to infection and inflammation. With IL-6 and TNF-*α* it also can initiate acute phase responses such as fever and synthesis of acute phase hepatic proteins such as CRP. However, estimation of levels of this cytokine in infections has yielded conflicting results and it is not considered important for diagnosis (22). Another widely studied marker is fetal fibronectin. Elevated levels of fetal fibronectin in vaginal fluids is highly predictive for preterm labor. This marker is detected with the use of monoclonal antibodies (19, 20).

Prelabor rupture of membranes.

The term "prelabor" should be used rather than "premature" or "preterm" because the latter two relate neither to gestational age nor to the weight of the fetus or neonate. The membrane rupture itself should be characterized as preterm (occurring before 259 completed days) or term (occurring after 259 completed days).

Several studies have shown that in patients with prelabor rupture of membranes in the preterm period, prophylactic antibiotics are of value in prolonging the latent period between rupture and onset of labor and in reducing the incidence of maternal and neonatal infection (32). The most extensively tested antibiotic regimen used for prophylaxis includes erythromycin either alone or with ampicillin (32). There is no evidence that antibiotic therapy prevents prelabor rupture of membranes. Bacterial vaginosis in early pregnancy has been found to be associated with prelabor rupture of membranes in the preterm period (31).

Considerable attention has been given to ILs as predictors of prelabor rupture of membranes. Lewis et al. (37) found that IL- 6 in maternal plasma was a predictor of neonatal infectious complications in patients with prelabor rupture of membranes even when the data were stratified for patients receiving and not receiving corticosteroids. The neonatal infectious complications examined included respiratory distress syndrome, necrotizing enterocolitis, intraventricular hemorrhage, IAI, presumed neonatal sepsis, neonatal sepsis and congenital pneumonia.

Reactive oxygen species, which are generated by the body's response to diverse insults such as infection, have also attracted attention. Such insults may activate collagenolytic enzymes and impair fetal membrane integrity (38). This impairment is then inhibited by antioxidants like vitamin E and possibly vitamin C (38). Damage by reactive oxygen species that impairs fetal membrane integrity and reduces midgestation levels of vitamin C is associated with prelabor rupture of membranes in the preterm period (38). Vitamins E and C can be safely and effectively absorbed and delivered to gestational tissues, which opens the possibility of intervention trials (38).

Fetal morbidity

Fetal sepsis - studies on cord blood in women with clinical suspicion of having infants subject to IAI have shown that cord blood cytokines may predict neonatal outcome. Cord blood from neonates with intrauterine infections had more IFN-*γ*. – producing CD3+T cells than did cord blood from uninfected neonates (45). The percentage of these cells in the infected neonates correlated with a duration of membrane rupture before the onset of labor but not with the level of CRP. The infected neonate born the longest time after membrane rupture had an increased percentage of IL-4–producing

CD3+T cells. The result suggests that the increase of cord blood IFN-γ – and IL-4 – producing T cells is part of the immune system's reaction to perinatal intrauterine infections (45).

Intrauterine growth retardation - most of the literature available linking infection with intrauterine growth retardation focuses on malaria. Some evidence shows that cytomegalovirus infections may play a role in this context. Cytomegalovirus immunoglobulins were given to pregnant women with primary cytomegalovirus infection to inhibit viral activity; the authors concluded that this treatment may prevent fetal cytomegalovirus infection (46). A study from India did not substantiate any relationship between cytomegalovirus infection and intrauterine growth retardation (47).

Neonatal morbidity

Neonatal sepsis. As discussed above, neonatal blood IL-6 levels have been found to correlate with chorioamnionitis and neonatal sepsis (16-18).

Neonatal respiratory disorders. Several studies now correlate intrauterine infection and neonatal respiratory disorder. The link between chorioamnionitis and intrauterine lung injury with subsequent development of bronchopulmonary dysplasia has been substantiated (48). Exposure to pro-inflammatory cytokines is implicated in this impairment of the fetal lung. Hitti et al. (49) demonstrated that in amniotic fluid infection, elevated TNF - is associated with respiratory distress syndrome, multiple organ dysfunction and various intracerebral disturbances.

Neonatal neurological disorders squeals (49), such as intraventricular hemorrhage and multiple organ dysfunctions. Similar results were shown in other studies and evidence now exists of a relationship between intrauterine infection and the development of neonatal intraventricular hemorrhage, possibly by the ventricular leukomalacia with subsequent cerebral palsy (48, 50). The intraventricular hemorrhage is thought to be mediated through the generation of pro-inflammatory cytokines by the fetus.

CONCLUSION

Only partially understood host defense mechanisms operate against infections affecting maternal and fetal morbidity. Subclinical ascending infections through the lower female genital tract are predominant worldwide. Proinflammatory cytokines have been tested for their use in diagnosing such infections, and promising leads indicate that affordable kits may soon be available for serological diagnosis of the mother. Important morbidities related to poor perinatal outcome both for the mother and the fetus and newborn comprise preterm birth, prelabor rupture of membranes, placental abruption, postpartum sepsis and maternal anemia. Fetal sepsis and intrauterine growth retardation are suspected to be consequences of ascending maternal infections. Neonatal septicemia and neonatal respiratory disorders as well as some neurological disorders seem to be consequences in the newborn of such ascending genital infections in the pregnant woman.

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INFECTII ASOCIATE CU SARCINA SI NASTEREA

REZUMAT

85% dintre cauzele de morbiditate si mortalitate sunt asociate cu prematuritatea. In intreaga lume sunt predominante infectiile subclinice ascendente de la nivelul tractului genital feminin. In tarile subdezvoltate pot sa predomine deficitele nutritive importante, aceste tipuri de infectii fiind mult mai raspandite comparativ cu tarile dezvoltate. Intre morbiditatile importante relationate cu evolutie perinatala deficitara, atat a mamei cat si a fetusului si non-nascutului, se afla ruptura de membrane inainte de travaliu, nastere inainte de termen, detasarea placentei, sepsis post-partum si anemia materna. La fat, sepsisul si retardul in dezvoltarea intrauterina sunt suspectate ca fiind consecinta infectiilor ascendente materne. La nou-nascuti, septicemia si afectiunile respiratorii, precum si dereglarile neurologice par sa fie tot consecinta unor astfel de infectii genitale materne, in perioada sarcinii. Se poate concluziona ca trebuie acordata atentie sporita eforturilor de elucidare a mecanismului de aparare si barierelor antimicrobiene de la nivelul vaginului pana la cervix, membranelor fetale si fluidului amniotic, incluzand imunocompetenta fetala precoce, in trimestrele doi si trei de sarcina.

Key words: afectiuni fetale, morbiditate materna

C REACTIVE PROTEIN IN DIABETES MELLITUS TYPE 2 COMPLICATED WITH DIABETIC NEPHROPATHY

MATEI D[1], MOLDOVAN C[1], MURESAN M[1], MICLE L[1], MICLE O[1], BURTA O[1], OROS R[1], MURESAN A[2]

[1]Faculty of Medicine and Pharmacy Oradea

[2]Physiology Department, University of Medicine and Pharmacy I. Hatieganu, Cluj- Napoca

ABSTRACT

Aims: In subjects with diabetes mellitus type 2 complicated with diabetic nephropathy, there is an increase of markers of inflammation, generated by multiple mechanisms. Chronical subclinical inflammation is a finding observed in diabetes mellitus type 2. This study was designed to establish the correlation between the level of inflammatory marker C-reactive protein (CRP) with the duration of diabetes, the degree of glycemic control and the stage of diabetic nephropathy. **Method:** The study was performed on four groups of subjects suffering from diabetes mellitus type 2 complicated with nephropathy and a control group. The first group included 47 patients with diabetes mellitus type 2 and normoalbuminuria (UAE < 30mg/d), the second group included 54 subjects with diabetes and microalbuminuria (UAE = 30 – 300mg/d), the third group had 49 subjects with diabetes type 2 and macroalbuminuria (UAE> 300mg/d). In the forth group were selected 45 patients with macroalbuminuria and chronic renal failure. In the control group were included 25 subjects. For all individuals included in the study we assessed the following parameters: glycemia, glycosylated hemoglobin A1c, creatinine, urea, uric acid, glomerular filtration rate, C reactive protein and urinary albumin excretion/day. **Results:** We obtained a significant increase of these elements in study groups by comparison with control group, except the glomerular filtration rate, which decreased. CRP was significant increased in experimental groups comparatively with the control group ($p<0.001$). We found a positive significant correlation between the level of CRP and UAE ($r = 0.49$, $p< 0.001$). The correlation of HbA1c with CRP was $r = 0.25$, $p < 0.001$ and the correlation between the concentration of CRP and the duration of diabetes $r = 0.31$, $p<0.001$, both positive and significant statisticaly. **Conclusions:** The level of CRP was significantly elevated in diabetic patients complicated with nephropathy and it correlates positively with the urinary excretion of albumin, the duration of diabetes mellitus and the degree of glycemic control. In addition to metabolic and hemodynamics factors, inflammation can play a role in pathogenesis of diabetes mellitus type 2, complicated with nephropathy.

Keywords: diabetes mellitus type 2, diabetic nephropathy, C reactive protein, urinary excretion of albumin.

INTRODUCTION

In the recent years numerous studies have shown that low-grade inflammation is associated with the risk of developing type 2 diabetes mellitus and its vascular complications (1, 14). Patients with diabetes type 2 and nephropathy exhibit high levels of different acute phase markers of inflammation: C-reactive protein (CRP), fibrinogen, serum amyloid A and IL-6. High level of glycemia contributes to the glycation of proteins and lipids, resulting in formation of advanced glycated end products (AGE). The receptors for AGE are expressed in different tissues and cells, including endothelial cells, smooth muscle cells and macrophages. The binding of AGE to their specific receptors leads to intracellular generation of reactive oxygen species (ROS), which in turn activates the nuclear factor-kappa B (Nf-kB). A consequence of this fact is the increase of expression of multiple cytokines (Tumor necrosis factor alpha and beta, Interleukins 1, 6, 8, 18 and gamma interferon). Especially IL 6 and TNFα stimulate the synthesis of CRP, complement, serum amyloid A, fibrinogen, von Willebrand factor, cortisol and plasminogen activator inhibitor-1(9, 17, 18). Subjects with kidney failure or uremia manifest evidence of chronic inflammation (17, 22). Chronic inflammatory process is thought to play an important role in development of micro and macrovascular complications in type 2 diabetes mellitus (1). The inflammatory marker C-reactive protein (CRP) has been found in most prospective studies to be associated with future cardiovascular outcomes (11).

The aim of this study was to establish the correlation existing between the level of inflammatory biomarker C- reactive protein and the duration of diabetes type 2, the degree of glycemic control and the stage of diabetic nephropathy. We hypothesized that in patients with diabetes mellitus type 2, complicated with nephropathy, the urinary excretion of albumin might be related to the marker of chronic inflammation (CRP) and there is a positive correlation between the duration of diabetes, the quality of glycemic control and the concentration of CRP.

MATERIAL AND METHOD

We performed the study on four groups of patients suffering of diabetes mellitus type 2 with diabetic nephropathy admitted at The Municipal Hospital from Oradea and one control group of healthy subjects.

The first study group included 47 patients with diabetes

Received March 2010. Accepted May 2010. Address for correspondence: Matei Daniela, University of Oradea, Faculty of Medicine and Pharmacy, Department Preclinic I, no.10 P-ta 1 Decembrie St., 410037, Oradea, Romania, phone + 40(259)415680, Fax. +40(259)418266, e-mail: danielacernau@yahoo.com

mellitus type 2 and normoalbuminuria (UAE< 30 mg/d). The characteristics of this group were: the mean age – 53.53 ± 8.77 years, the distribution on sexes- 34.04% female and 65.96% male, mean duration of diabetes 4.83 ± 2.66 years, BMI (body mass index) = 29.54 ± 4.62 kg/m^2, 55.31% of them were insulin-dependent and 44.69 % non insulin-dependent (treated with biguanides and sulhonylureas). In the second group were admitted 54 patients with diabetes mellitus type 2 and microalbuminuria (UAE = 30 – 300 mg/d). In this group 44.44 % of patients were female and 55.56% male, the mean age was 61.37± 7.67 years, the BMI = 30.96 ± 4.72 kg/m^2, the mean duration of diabetes 8.07 ± 3.17 years, 62.96 % insulin dependent and 37.04 % non insulin-dependent. The third group included 49 subjects with diabetes mellitus and macroalbuminuria (UAE> 300 mg/d), having the following characteristics: the mean age: 64.86 ± 7.69 years, mean duration of diabetes 10.24 ± 4.45 years, BMI = 29.86 ± 3.96 kg/m^2, 42.86% female and 57.14% male, 95.91% insulin dependent. In the last group were included 45 subjects, with macroalbuminuria and chronic renal failure (GFR < 60 mL/min/1.73m^2), having the age of 65.22 ± 6.74 years and a mean duration of diabetes of 13.84 ± 3.87 years, 42.22% were female and 57.78% male. Their BMI was 29.40 ± 5.47 kg/m^2, all insulin dependent.

In the control group we selected 25 subjects matched for age, sex and BMI with the patients of study groups (mean age = 49.32 ± 3.97 years, 40% female and 60 % male and BMI= 28.16 ± 4.20 Kg/m^2).

Blood samples were collected in the morning, after 8-12 hours of overnight fasting. We analyzed glycemia, glycosylated hemoglobin A_{1c} glomerular filtration rate, serum creatinine, urea, uric acid, C reactive protein (CRP), complete blood count, urine analysis. Two 24 hours samples of urine were collected from the subjects, from where UAE was confirmed and the mean value was calculated. Glomerular filtration rate was calculated with MDRD formula with four variables: creatinaemia, age, sex, and race.

Urinary albumin excretion was assessed by Micro-Albumin ELISA test (DRG Diagnostics), glycosilated hemoglobin by a chromatographic- colorimetric method (Biogamma) and CRP by Turbilatex method (AI Instruments KFT).

According to our inclusion criteria, patients with urinary tract infection or other infections, inflammatory disease, pyrexia, malignancy, active immunological diseases, were excluded from the study. All of the subjects participating in this study provided the written informed consent.

The statistical analysis of data was performed by SPSS 13.0. The correlation between the mentioned parameters was tested by Pearson`s correlation test. Differences in the parameters between the groups were analyzed by Student`s test.

RESULTS

We obtained a significant increase of all assessed parameters in study groups by comparison with control group.

The subjects of experimental groups had higher values of glycemia, glycosilated hemoglobin A1c, uric acid, serum creatinine, urea and C reactive protein, except the glomerular filtration rate which was lower in these groups than in control group.

The values of glycosylated hemoglobin A_{1c} was 7.32 ± 1.32 % in group 1 (normoalbuminuria), 7.84 ± 1.36% in the second group (microalbuminuria), 8.33 ± 1.69 % in the third group (macroalbuminuria) and 8.05 ± 1.89 % in the last experimental group (macroalbuminuria and chronic renal failure). The mean value of glycosylated hemoglobin in control group was 5.23 ± 0.64%.

C Reactive Protein presented a similar variation, with higher values in experimental groups compared with control group. It was 4.40 ± 1.19 mg/dL in group 1, 6.48 ± 2.75 mg/dL in group 2, 7.36 ± 3.50 mg/dL in group 3 and 13.40 ± 13.00 in the group 4. The control group had a mean value of 2.98 ± 0.61 mg/dL. This inflammatory marker had a statistically significant increase related to the control group ($p < 0.001$) for all experimental groups (Figure 1).

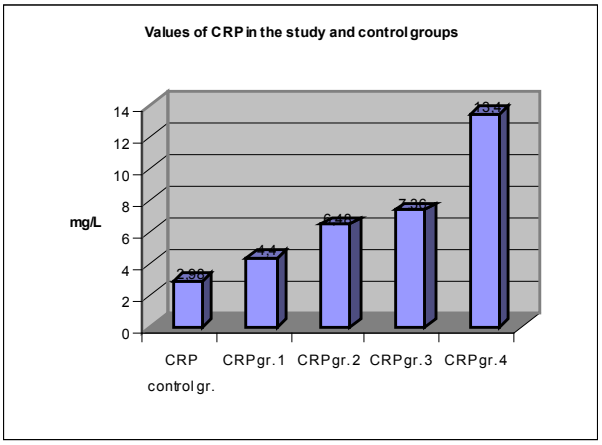

Fig. 1. Values of CRP in the study and control groups

The correlation between the duration of diabetes mellitus and the concentration of CRP was r = 0.31, p< 0.001 (Figure 2). The concentration of glycosylated hemoglobin A_{1c} had a positive significant correlation with CRP, r = 0.25, p = 0.001 (Figure 3). Between the level of CRP and urinary albumin excretion there was also a positive significant correlation (r = 0.49, p < 0.001) (Figure 4).

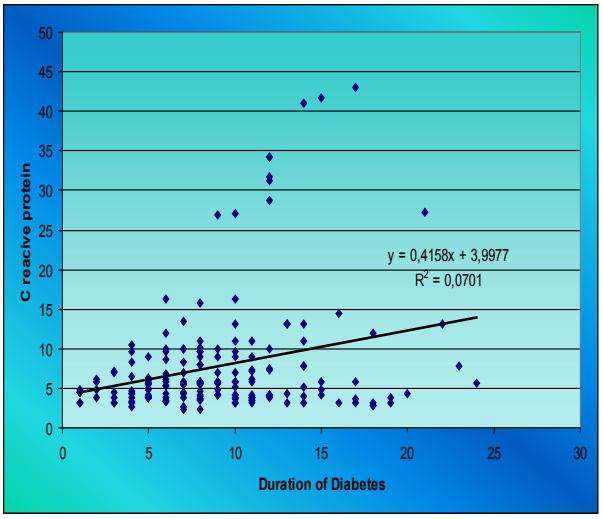

Fig. 2. Correlation of the duration of diabetes with CRP

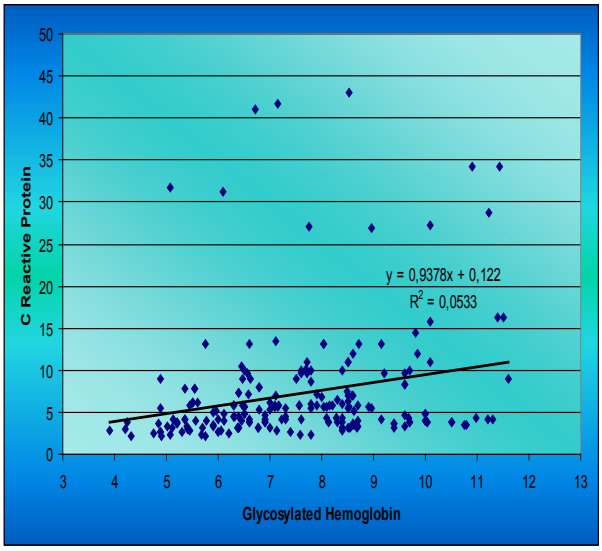

Fig. 3. Correlation of Glycosylated hemoglobin A_{1c} with CRP in diabetic subjects

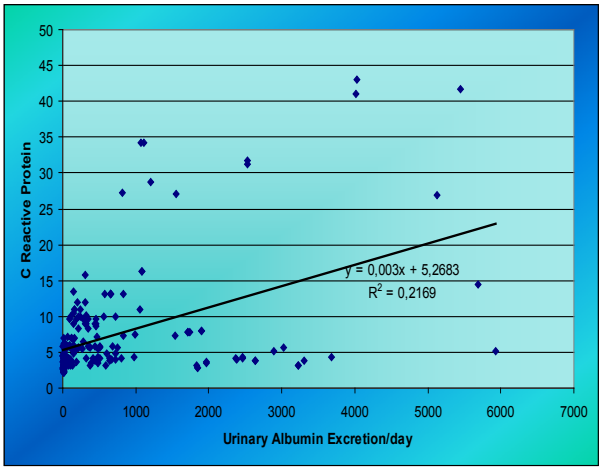

Fig. 4. Correlation of CRP with UAE in diabetic subjects

DISCUSSION

Diabetic nephropathy is considered a progressive glomerular disease, having a predominant inflammatory nature, which evolves in diabetic medium. It has been suggested that long- term innate immune system activation, resulting in chronic inflammation, elicits disease instead of having a benefic effect, which can lead to development of insulin resistance syndrome and diabetes mellitus type 2. Pro-inflammatory cytokines and acute phase reactants are involved in multiple metabolic pathways relevant to insulin resistance, including insulin regulation, reactive oxygen species, lipoprotein lipase action and adipocyte function (7).

Certain clinical and experimental studies have demonstrated that in diabetic nephropathy there are signs of inflammation, which play a significant role in pathogenesis of this disease. Chow et al. (6) showed that db/db mice, a model of type 2 diabetes and diabetic nephropathy, exhibited an increased expression of intracellular adhesion molecule-1 (ICAM-1), which stimulates inflammation by increasing leukocyte infiltration and adherence in glomeruli and tubules, along with macrophage infiltration. Hasegawa et al. (12) demonstrated that macrophages incubated with glomerular basement membranes from diabetic rats produced greater levels of IL-1 and TNF-α, than macrophages incubated with membranes of normal rats. IL-1 increases vascular endothelial permeability and has been involved in the proliferation of mesangial cells and matrix synthesis, as well as in the development of intraglomerular microcirculatory abnormalities.

Markers of inflammation, a well- recognized manifestation of oxidative stress have been observed to increase in response to elevated glucose levels (26). It has been proposed that type 2 diabetes mellitus is a disease of immune system, involving a cytokine-mediated acute phase inflammatory response (20). In this disease there is an accelerated rate of atherosclerosis, which is though to be due in part to the irreversible formation and deposition of molecules known as advanced glycation end products (AGEs). Augmented blood levels of glucose contribute to the glycation of proteins and lipids, resulting in the formation of AGEs (2). Reaction of AGEs with the receptors for AGEs (RAGEs) generates reactive oxygen species (ROS), responsible for the activation of signal transduction cascade (PKC, MAPK and JAK/STAT) and transcription factors (NF-kB, AP-1) and upregulate TGF-β1 and fibronectin in renal cells. ROS-regulated signaling pathways lead to extracellular matrix (ECM) deposition in diabetic kidney. In addition to upregulation of ECM synthesis, ROS play an important role in ECM degradation and epithelial-mesenchymal transition in tubular epithelial cells leading to glomerular mesangial and tubulo-interstitial expansion (16).

Increase of acute –phase markers are associated with increase cardiovascular risk, because chronic inflammation is one of the pathogenetic mechanisms of atherosclerosis. In contrast, the relationship between low-grade inflammation and diabetic microangiopathy is still unclear. As regard the diabetic nephropathy several studies have examined the relationships with inflammation, leading to conflicting results (20, 22, 25). Most studies have reported an increase in acute-pahse biomarkers in patients with diabetes and microalbuminuria. The coexistence of an inflammatory condition with diabetic nephropathy could explain in part the tremendously increased cardiovascular risk among these patients (15, 21, 24). Dalla Vestra et al. (8) showed that patients with diabetic nephropathy exhibit high level of diverse acute-phase markers of inflammation, such as CRP, fibrinogen, serum amyloid, interleukin 6 and these are correlated with the thickening of glomerular basement membrane.

In our study we found that there was an increase of level of inflammatory marker – CRP in patients with diabetes mellitus type 2 than controls ($p< 0,001$); furthermore the values of this parameter increased significantly as nephropathy progressed. CRP had a positive significant correlation with the urinary excretion of albumin ($r= 0.49$, $p < 0.001$). Similar results were obtained by other researchers. Navarro et al. found a correlation between CRP and UEA of $r = 0.68$, $p < 0.001$. They also established a positive correlation of CRP with duration of diabetes and glycated hemoglobin level (19). Streja et al. indicated the same positive

correlation between these parameters (25). The prospective Irbesartan Diabetic Nephropathy study concluded that CRP was positively associated with female sex, BMI, serum creatinine, hemoglobin A_{1c} and inversely correlated with pyridoxal 5`-phosphate and folate. But it revealed that in patients with nephropathy, CRP does not add predictive information above and beyond that provided by traditional established risk factors. A finding of a link between CRP and congestive heart failure needs further confirmation (5).The Insulin Resistance Atherosclerosis Study demonstrated a significant association between inflammatory markers (CRP and fibrinogen) and urinary albumin excretion in type 2 diabetic patients with microalbuminuria (10). Choudhary et al. (2008) found a positive correlations between the level of CRP and urinary albumin excretion ($r = 0.781$, $p < 0.001$) and CRP and glycosylated hemoglobin ($r = 0.750$, $p < 0.001$) .

In patients with diabetes mellitus the incidence of macroangiopathy is at least twice higher than in nondiabetic subjects. Although the concept of atherosclerosis as an inflammatory disease is now well established, line of evidence suggests that chronic inflammation may be involved in the pathogenesis of insulin resistance and diabetes mellitus type 2. This leads to the hypothesis that inflammatory changes may be considered as common pathogenetic step in all these conditions. Oxidative stress may explain the presence of inflammation in all these conditions. The pathways that generate the mediators of inflammation, such as adhesion molecules and interleukins are all induced by oxidative stress. (4) Elevated concentration of inflammatory markers may be the result of preexisting atherosclerosis in patients with abnormal albuminuria. On the other hand, the inflammatory markers may alter the glomerular function and thus be causally involved in the development of albuminuria. In diabetic nephropathy there is an infiltration of glomeruli and interstitium with macrophages, which can responsible for increased level of inflammatory biomarkers (5).

Higher level of inflammatory parameter (CRP) in diabetes type 2 complicated with nephropathy indicates that inflammation might be a pathogenetic mechanism in this condition, but further studies are necessary to confirm the intra-renal production of inflammatory cytokines. Treatment with anti-inflammatory drugs might be beneficial in preventing the progression of diabetic nephropathy from its initial stage towards the renal failure, due to the new vision of pathogenesis of diabetes mellitus.

CONCLUSIONS

1. The concentrations of CRP was increased in patients with diabetes mellitus type 2 complicated with nephropathy and its value was higher as long as diabetes progressed

2. There was a positive significant correlation between the level of CRP and urinary excretion of albumin

3. There was a positive correlation between the concentration of CRP and duration of diabetes mellitus

4. CRP showed a positive correlation with the level of glycemic control (glycosylated hemoglobin A_{1c})

5. Inflammation could be a pathogenetic mechanism responsible for alteration of glomerular function, involved in the development of albuminuria

It is possible that in future new therapeutic strategies based on anti-inflammatory drugs to be effective in treatment of diabetic complications

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PROTEINA C REACTIVĂ ÎN DIABETUL ZAHARAT TIP 2 COMPLICAT CU NEFROPATIE DIABETICĂ

REZUMAT

Obiective: La subiecţii cu diabet zaharat tip 2, complicat cu nefropatie dibaetică există o creştere a markerilor inflamatori, generaţi prin mecanisme multiple. Inflamaţia cronică subclincă a fost observată în diabetul zaharat de tip 2. Scopul studiului a fost de a stabili corelaţia dintre nivelul markerului inflamator - Proteina C reactivă (PCR) cu durata diabetului, gradul controlului glicemic şi stadiul nefropatiei diabetice.
Material şi Metodă: Studiul a fost efectuat pe patru grupe de subiecţi cu diabet zaharat tip 2 complicat cu nefropatie şi un grup de control. Primul grup experimenatal a cuprins 47 de pacienţi cu diabet zaharat tip 2 şi normoalbuminurie (UAE < 30mg/zi), al doilea grup a inclus 54 de subiecţi cu diabet zaharat şi microalbuminurie (UAE = 30- 300mg/zi), cel de al treilea grup 49 de subiecţi cu diabet şi macroalbuminurie (UAE >300 mg/zi). În al patrulea grup au fost selectaţi 45 de pacienţi cu macroalbuminurie şi insuficienţă renală cronică. În grupul martor au fost selectaţi 25 de subiecţi. Pentru toţi subiecţii incluşi în studiu am determinat următorii paramentri: glicemia, hemoglobina glicozilată A_{1c}, creatinina, urea, acidul uric, rata de filtrare glomerulară, proteina C reactivă şi excreţia urinară de albumină/24 ore. Rezultate: Am obţinut o creştere semnificativă a acestor paramentri în grupele de studiu în comparaţie cu lotul martor, cu excepţia ratei de filtrare glomerulară, care a scăzut. PCR a fost semnificativ crescută la loturile experimentale faţă de lotul martor (p <0,001). Am stabililit o corelaţie pozitivă şi semnificativă între concentraţia PCR şi UAE (r = 0,49, p< 0,001). Corelaţia HbA_{1c} cu PCR a fost de r = 0,25, p < 0,001, iar corelaţia dintre nivelul PCR şi durata de evoluţie a diabetului zaharat r = 0,31, p< 0,001, ambele pozitive şi semnificative statistic. Concluzii: Nivelul PCR a fost semnificativ crescut la subiecţii cu diabet zaharat tip 2 complicat cu nefropatie şi s-a corelat pozitiv cu excreţia urinară de albumină, durata şi evoluţia diabetului zaharat, cât şi cu gradul controlului glicemic. Pe lângă factorii metabolici şi hemodinamici, inflamaţia ar putea deţine un rol în patogeneza diabetului zaharat tip 2 complicat cu nefropatie.
Cuvinte cheie: diabet zaharat tip 2, nefropatie diabetică, proteina C reactivă, albumină urinară

AUTOIMMUNE ENDOCRINE ASSOCIATIONS IN ADULT PATIENTS WITH AFFECTED GLYCEMIC METABOLISM AND THYROID DISORDERS

ADRIANA GHERBON[1], ROMULUS TIMAR[2], IOANA ZOSIN[3]
[1]Department of Physiology, UMF "Victor Babeş" Timişoara
[2]Department of Internal Medicine III, County Hospital Timişoara
[3]Department of Endocrinology, County Hospital Timişoara

ABSTRACT

Autoimmune pathology appears by almost all endocrine glands and affects them in a variable incidence. Type 1 diabetes associated with certain endocrine disorders, pictures the autoimmune polyglandular syndromes. The purpose of this study is to determine the prevalence of the combination of different kinds of autoimmune disorders in adult patients with deterioration of glycemic balance and thyroid diseases. The studied group was of 650 cases with an age between 17-79 years.

The group of adults was subdivided according to the type of changes in the glycemic balance in 4 subgroups: DM group with type 1 represented by 60 cases (9.23%), DM group with type 2 represented by 290 cases (44.61%), a group with IGT represented by 183 cases (28.15%) and a IFG group of 117 cases (18%).

Keywords: diabetes mellitus, autoimmune thyroid disease, autoimmune polyglandular syndromes

INTRODUCTION

The autoimmune process is the most frequent cause of endocrine disorders. Autoimmune thyroid diseases tend to associate among themselves or with other autoimmune disorders. Polyglandular syndrome associates multiple autoimmune endocrine disorders with other nonendocrine disorders at the same person and their families (19).

DM type 1 is commonly associated with endocrine disorders and systemic autoimmune pathology such as: Graves-Basedow disease, Hashimoto thyroiditis**,** Addison's disease, Celiac disease, Pernicious anemia, Myasthenia gravis, Vitiligo. (5).

1 in 100 patients with DM type 1 will develop Graves's disease and 1 to 20 patients are affected by hypothyroidism (7). The incidence of type 1 DM association with hyperthyroidism and hypothyroidism varies from 3.2% to 4.6%, respectively from 0.7% to 4% (15).

1 to 200 patients with type 1 DM (7) will develop Addison's disease which is extremely rare in the general population.

Celiac disease appears at 1 in 20 patients with type 1 DM, Pernicious anemia in 1 of 50 patients with type 1 DM (7).

A particular combination of type 1 with DM and hypo-or hyperthyroidism is characteristic in the polyglandular autoimmune syndromes. There are 3 autoimmune polyglandular syndromes.

The autoimmune polyglandular syndrome type 1 (PAS - 1) is an autosomal recessive disorder caused by a mutation in the short arm of chromosome 21, characterized by the triad: muco-cutaneous candidiasis, hypoparathyroidism and Addison's disease.

The symptoms and signs appear in childhood; candidiasis is usually the first sign, followed usually by hypoparathyroidism and Addison disease (2. 14). DM type 1 occurs in less than 4% of affected children, but increases to 12% by adults.

Autoimmune polyglandular syndrome type 2 (PAS - 2) (4. 17) is the most common endocrinopathy. Occurs in adult life and affects mostly women. The same patient has two or more of the following conditions: Addison's disease, Graves's disease, autoimmune thyroiditis**,** DM type 1, primary hypogonadism**,** Myasthenia gravis and Celiac disease. Most disorders are associated with the following HLA: A1, B8, DR3 (DQA1 * 0501, DQB1 * 0201) and DR4 (DQA1 * 0301, DQB1 * 0302). The autoimmune syndrome disorders present usually a long prodromal phase and the antibodies are present prior to the development of the disorder.

Autoimmune polyglandular syndrome type 3 (PAS - 3) (3) is a **PAS 2** syndrome, but without the adrenocortical involvement. It comprises a group of autoimmune disorders characterized by severe glandular insuffiency. A quarter of the patients with hypo functional glands present other endocrine diseases as well. This syndrome is associated with diseases as: celiac disease, hypogonadism**,** myasthenia gravis, sarcoidosis**,** Sjogren syndrome, rheumatoid arthritis, gastric neoplasia, malabsorption, and may be classified in 3 subcategories:

- PAS III A - autoimmune thyroiditis with DM type 1
- PAS III B - autoimmune thyroiditis with pernicious anemia
- PAS III C - autoimmune thyroiditis with vitiligo and / or alopecia and / or other autoimmune diseases

DM is associated although with other endocrine disorders and autoimmune polyglandular syndromes (6) faced with a much lower frequency.

Insulin resistance Syndrome Type B is caused by the presence of the insulin antibodies. A third of these patients have associated autoimmune disorders, as systemic lupus erythe-

Received February 2010. Accepted May 2010. Address for correspondence: Adriana Gherbon, Md, PhD, Physiology Department, "Victor Babes" University of Medicine and Pharmacy Timisoara, 2A Eftimie Murgu Square, RO-300041, Phone/Fax: +40256490507, e-mail: adrianagherbon@yahoo.com

matosus, autoimmune thyroid pathology. In spite of elevated levels of plasma glucose secondary to the insulin resistance, cetoacidosis is not characteristic. Patients may present spontaneous remission and severe hypoglycemia (secondary effects of insulin-like antibodies, effects demonstrated in vitro).

Poems syndrome includes DM, primary hypogonadism, sensitive and motor neuropathy, bone lesions, hyper pigmentation.

DIDMOAD syndrome is an autozomal recessive disorder, which includes diabetes insipidus, DM, optic atrophy, deafness. Diabetes mellitus is usually the first manifestation in children.

Down syndrome is relatively common associated with the presence of DM type 1 and thyroiditis. This suggests that chromosomal abnormalities affect autoimmune processes or that the susceptibility to develop autoimmune diseases may be associated with chromosomal disorders.

For diagnosis of autoimmune disorders is important to determine for each gland their specific antibodies. Specific antibodies are represented by ICA, GAD, IA2 for DM type 1, the antithyroid antibodies, and 21-OH antibodies for Addison's disease (20).

In case of diagnosis of an autoimmune endocrine disorder the patient should be investigated for other endocrine disorders that can be in the latent phase. The second step is to find specific biological parameters for each gland.

METHOD

INVESTIGATED POPULATION

The group of subjects included 650 people, young adults, adults and elderly people, aged between 17 and 79 years (Tab. I). The subjects were with diabetes and in time developed thyroid disorders as well or subjects with thyroid disorders who developed in time anomalies in the process of glycoreglation or diabetes mellitus.

Table I. Distribution by age and sex in the group of adults

Age	Number of cases		Sex F		Sex M	
	n	%	n	%	n	%
18 – 19	11	1.7	10	90.9	1	9.1
20 – 29	29	4.46	27	93.1	2	6.9
30 – 39	48	7.38	43	89.58	5	10.42
40 – 49	168	25.84	141	83.93	27	16.07
50 – 59	219	33.7	209	95.43	10	4.57
60 – 69	118	18.15	112	94.91	6	5.09
70 – 79	57	8.77	46	80.7	11	19.3

The group of subjects was subdivided according to the type of change in the glycemic balance in 4 subgroups (fig. 1):

-group DM type 1 represented by 60 cases (9.23%)
-group DM type 2 represented by 290 cases (44.61%)
-group IGT represented by 183 cases (28.15%)
-group IFG of 117 cases (18%)

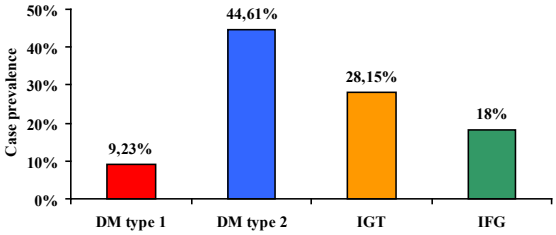

Fig.1. The allocation of cases based on changes in the levels of plasma glucose

METHODS OF INVESTIGATION

The methods of investigation were represented by clinical data - case history, current status, imagistic- thyroid ultrasonography, biochemical - carbohydrate metabolism parameters: fasting plasma glucose, glycosuria, HbA1; thyroid hormones and some immunological parameters.

Determination of plasma glucose was performed by enzyme technique with glucosooxidasis. Normal values were taken between 70 - 110 mg%; diabetes mellitus - values over 126 mg%, impaired glucose tolerance - values between 110 - 126 mg% and the OGTT at 2 h between 140 - 200 mg% and impaired fasting glucose - values between 110 - 126 mg% and OGTT at 2 h under 140 mg%.

Determination of HbA1c was achieved through the DiaStat for measuring HbA1c reported to the total HbA.

To determine the TSH level in plasma, the free fraction of triiodotironin (FT3), and the plasma free fraction of thyroxin (FT4) were performed a quantitative method ARCHITECT; witch is an immunological method, (CMIA) Chemilumnescent Microparticle Immunoassay. Normal values were following: TSH = 0465-4.68 Miu / ml, FT3 = 3.69 -10.4 pmol / l, FT4 = 10-28.2 pmol / l.

To obtain the level of cortisol was performed the technique IMMULITE / IMMULITE 1000, an imunometric method, in solid phase, competitive, of chemiluminescent, ICEM (Immuno Chemilumino Enzymometric assay). It was considered normal: a.m. 5-25 microgram / dl.

FSH level was measured quantitatively by the ARCHITECT method; a Chemilumnescent Microparticle Immunoassay. Reference values: determined with ARCHITECT test.

Table II. The reference values for FSH

Population field	mIU/ml
Women:	
- Follicular phase	3.35 – 21.63
- Ovulating phase	4.97 – 20.82
- Luteal phase	1.11 – 13.99
- Postmenopausal	2.58 – 150.53
Men	1.37 – 13.58

The immunological parameters were represented by autoimmune thyroid markers - antibodies (antiTPO and antiTg antibodies).

To determine serum levels of antiTPO antibodies it was used the kit AxSYM an immunological method (Microparticle Enzyme Immunoassay). Normal values: antiTPO antibodies (<35 IU / ml).

To determine serum levels of antiTg antibodies it was used the kit AxSYM antiTg, a MEIA method as well (Microparticle Enzyme Immunoassay). Normal values:AntiTg antibodies (<55 IU / ml).

To determine 21-hidroxilaza (anti 21-OH antibodies) antibodies level it was used the radioimunodetermination method combined with a technique of imunoprecipitation, based on human 21-OH marked with I 125 reacting with the antibodies anti 21-OH from the samples test and forming immune complexes that precipitated with the solid-phase of protein A. Normal range: <1 IU / ml

Thyroid ultrasonography was performed in all cases and allowed us to measure thyroid volume, thyroid study and the changes in parenchyma's density. An increased density, uniform, characterizes normal thyroid parenchyma easily distinguished from the neck muscles that are hypo dens.

Inflammatory processes and autoimmune pathology appears hypo dens**.** The scale was assessed as being discreet +, moderate ++ and marked +++.

In the autoimmune thyroid disease the parenchyma of the gland appears hypo dens.

In Graves's disease is seen an increased volume of the thyroid, hypoecogenity of different intensities with variable homogeneity in the thyroid parenchyma.

Chronic autoimmune thyroid disorder appears with a hypoecogenity of the parenchyma and normal or increased thyroid volume.

RESULTS AND DISCUSSION

In the group of adults 9.23% had type 1 DM, 44.61% had DM type 2, IFG 28.15% and 18% ITG**.** The main endocrine immune combinations were represented by DM type 1, Addison's disease, autoimmune thyroid disorder (TCA), autoimmune ovarian insufficiency and the nonendocrine main disorders were vitiligo, alopecia, Biermer anemia, rheumatoid arthritis (Tab. III)

Table III. Prevalence of endocrine autoimmune disorders in the studied group

Associations	Subject group	
	No.	%
DM tip 1 + TCA	31	4.76
DM tip 1 + Graves-Basedow disease	5	0.76
DM tip 1 + TCA + decalvant pelad	2	0.3
DM tip 1 + TCA + vitiligo	3	0.46
DM tip 1+ TCA + Addison disease	7	1.07
DM tip 1 + TCA + autoimmune ovarian failure	6	0.92
DM tip 1 + Graves-Basedow disease + vitiligo	1	0.15
TCA + Addison disease	4	0.61
PR + Graves –Basedow disease	2	0.3
TCA + vitiligo	3	0.46
Graves-Basedow disease + vitiligo	1	0.15
DM tip 1 + TCA + vitiligo + Biermer anemia	1	0.15

In the group of adults the main endocrine autoimmune associations are represented in Tab. IV:

Table IV. Prevalence of the main endocrine autoimmune associations in the studied group

Endocrine autoimmune associations	No.	%
DM tip 1 + TCA	37	5.69
DM tip 1 + Graves – Basedow disease	6	0.92
DM tip 1 + TCA + autoimmune ovarian failure	6	0.92
DM tip 1 + TCA + Addison disease	7	1.97
TCA + Addison disease	4	0.61

In the group of adults with DM type1 the first imunopathy was DM type 1, present in 33 of the cases. TCA was associated in 31 cases and Basedow disease in 2 cases. In 4 cases, thyroid disorder and DM type 1 were detected at the same time (2 cases with TCA and 2 cases of Graves-Basedow disease). In 19 cases thyroid disorder preceded the DM type 1 (17 cases with TCA and 2 cases of Graves-Basedow disease) (Tab. V).

To track the association with Addison disease it was determined the plasma level of cortisol and 21-OH antibodies by 39 adults taken in the study. The values of 21-OH antibodies were significantly increased in 7 cases.

Tracking the association with autoimmune ovarian insufficiency led to determine the levels of FSH, which was increased > 25 IU / l in 6 cases. Primary ovarian insufficiency (early menopause) usually occurs before the age of 40 years (in the absence of iatrogenic causes) and it s clinical signs are secondary amenorrhea and hypergonadotropism with hypoestrogenemia.

Autoimmune ovarian insufficiency (AOI) is usually associated with other autoimmune pathology such as diabetes mellitus type 1, Addison's disease, TCA, vitiligo, etc.; its diagnosis is difficult and it is usually based on the exclusion of other possible causes of primary ovarian insufficiency and the notice of autoimmune etiology (3).

In our group of subjects the patients had organ-specific autoimmune endocrine disorders shown in Fig. 2.

Endocrine immunopathys may be linked to a variable incidence of systemic organ-specific nonendocrine disorders.

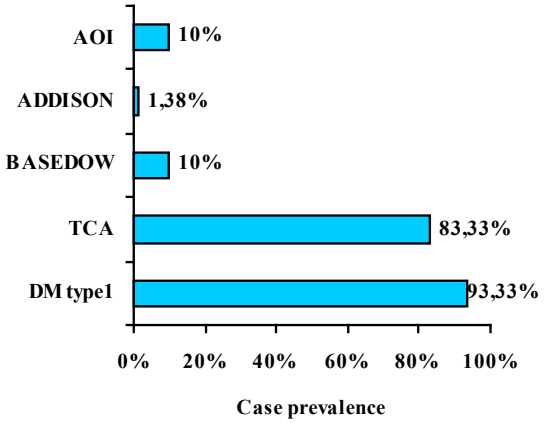

Fig 2. Endocrine imunopathys distributed on the number of cases

In 8 cases it was associated vitiligo, which occurred before the onset of endocrine imunopathys. Pelade decalvant appeared in 2 cases and also preceded the onset of autoimmune endocrinopathys. Rheumatoid arthritis appeared in one case. One case in the group of adults with autoimmune endocrine diseases had associated more than one nonendocrin autoimmune disorder respectively Biermer anemia and vitiligo.

Table V. Range (years) between the onsets of imunopathys in adults with type 1 diabetes

Period of time	No. of cases	Media ± SD (age)
Onset DM – Onset TCA	31	22.29 ± 12.42
Onset TCA - Onset DM	17	2.47 ± 1.94
Onset DM – Onset Basedow	2	9.5 ± 4.94
Onset Basedow - Onset DM	2	5.5 ± 6.36
Onset Addison – Onset TCA	4	16

In adults, the average time between the onset of type 1 DM and immunopathys was 22.29 ± 12.42 years. In 17 cases the first immunopathy was TCA, followed at a distance of 2.47 ± 1.94 years by DM type 1. By Basedow-Graves disease: 2 cases were initially with DM, followed at a distance of 9.5 ± 4.94 years of Graves-Basedow disease and 2 cases were initially with Basedow-Graves disease, followed at intervals of 5, 5 ± 6.36 years of DM type 1. By Addison disease associated with TCA the first immunopathy was Addison's disease followed at a distance of 16 years by TCA. In adults with type 1 DM PAS type 3 has appeared in 49 (81.66%) cases and PAS type 2 in 7 (11.66%) cases.

The average interval of time between the onset of DM and thyroid disease was 22.29 ± 12.42 years for TCA and 9.5 ± 4.94 years for Graves Basedow disease.

The average interval of time between the onset of thyroid disease and the appearance of DM type 1 was 2. 47 ± 1.94 years for TCA and 5.5 ± 6.36 years for Graves Basedow disease.

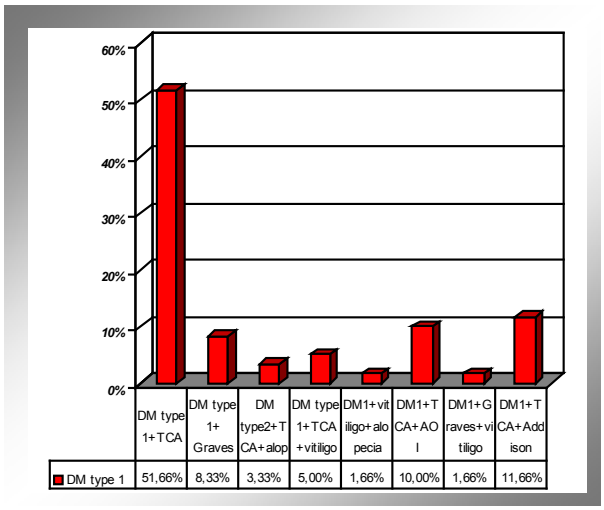

Fig.3. Association of endocrine and non-endocrine immune diseases by adults with DM type 1

In adults with type 2 DM PAS type 3 C was found in 2 (0.69%) cases, of which 1 case (50%) Graves Basedow disease + rheumatoid arthritis and 1 case (50%) TCA + vitiligo. Both cases were females. The median age was 55 ± 14.14 years and the average age of onset was 53.5 ± 12.02 years. It was no thyroid familial history disease found.

PAS type 2 was found in 4 cases (1.34%) TCA + Addison disease. The average interval of time between the onset of Addison disease and TCA was 16 years. All 4 cases were females. Their average age was 61.75 ± 7.32 years and the mean age of onset of thyroid disease was 60.75 ± 8.38 years. It was no thyroid familial history disease found (Fig.4).

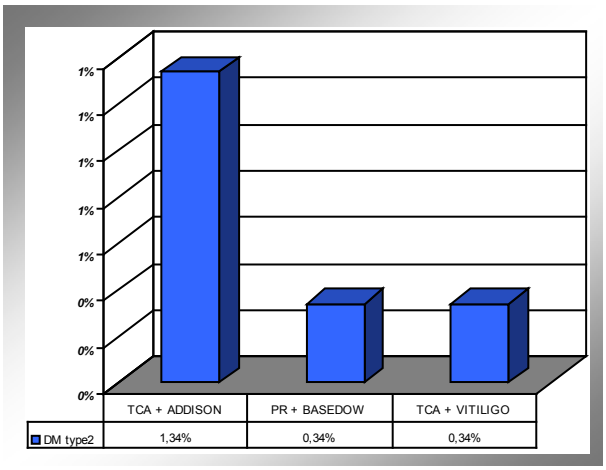

Fig.4. Association of endocrine and non-endocrine immune diseases by adults with DM type 1

In adults with IGT, PAS type 3 C was found in 2 cases (1.09%): 1 case (50%) TCA+ vitiligo and 1 case (50%) Graves Basedow disease + rheumatoid arthritis (Fig.5).

Both cases were females. The median age was 60 ± 4.24 years and the average age of onset of thyroid disease was 59 ± 2.82 years. It was no thyroid familial history disease found.

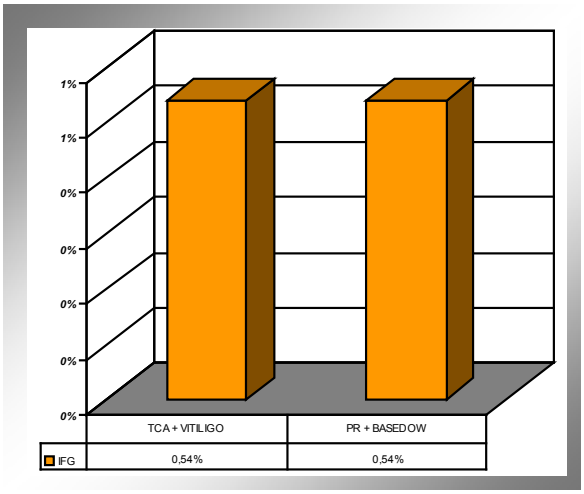

Fig.5. Association of endocrine and non-endocrine immune diseases by adults with IGT

In adults with IFG, PAS type 3 C was found in 2 (1.7%) cases:

1 case (50%) Graves' disease + vitiligo and 1 case (50%) TCA + vitiligo (Fig.6). Both cases were females. The median age was 55 ± 5.65 years and the average age of onset of thyroid disease was 51 ± 11.31 years. Thyroid familial history disease was present in one case.

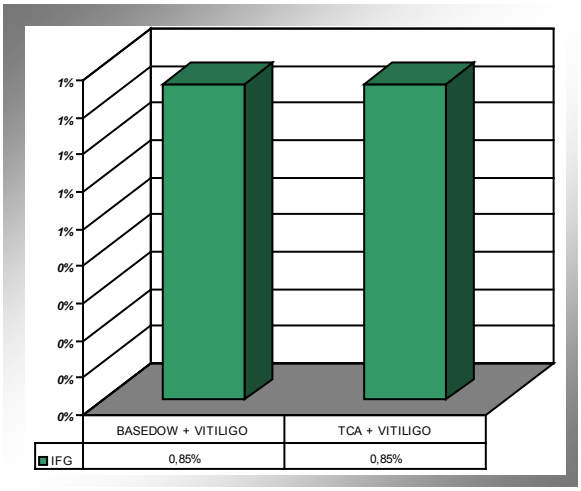

Fig.6. Association of endocrine and non-endocrine immune diseases by adults with IFG

PAS 1 is a very rare disorder. The largest number of patients was reported in Finland, where the prevalence was estimated at 1 in 25,000 subjects. The ratio is F / M 0.8 - 1.5 / 1, and in 1998 the ratio was 2.4 / 1. The onset is in the childhood with 3 - 5 years old or in the early adolescence, but always before the third decade of life. Death is determined by the individual components of the syndrome. (2)

PAS 2 is quite often in the U.S. about 14-20 to 1 million people are affected by this syndrome. The ratio of F / M is 3 - 4 / 1 and the onset is in the third and fourth decade of life. So far, the mortality and morbidity rate hasn't been assessed clinically, but it is believed that it equals the mortality and morbidity rate by individual components (17).

The prevalence of **PAS 3** is unknown. It is more often met in women than by men; usually by middle-aged women but it can occur in people of any age. Death is determined by the individual components of the syndrome (3).

In general, in the first stage of PAS antibodies levels are elevated. In the second stage the disease is sub clinical and in the third stage becomes clinically manifested.

In the study group, all patients had type 1 DM clinically manifest, all being treated with insulin in different therapeutic schemes. By patients with DM type 2, and IGT, IFG the treatment was diet.

If Graves - Basedow disease was associated, all had hyperthyroidism in treatment with antithyroid synthesis drugs.

By TCA, 112 cases were with euthyroidism and 96 cases with hypothyroidism (76 clinical cases and 20 cases sub clinical). 36 cases did not require treatment; the remaining 172 had substitution treatment with thyroid hormones. AntiTPO antibodies were present by 27 of cases of TCA and DM type 1, a total of 23 cases presenting insignificant values. All 4 cases with Addison disease were symptomatic and the diagnosis was on the basis of the clinical symptoms.

If Addison disease is associated the substitution treatment can cause imbalance of DM, especially of type 1 DM (13).

Also estrogen therapy in autoimmune ovarian insufficiency may increase the risk of cardiovascular disease (12).

The treatment with methotrexat used in rheumatoid arthritis, proved to have toxic effects on liver and lungs (1).

Ideal is to determine the presence of antibodies, especially in DM type 1, because they may be present by subjects without clinical symptoms. If their levels are raised, it is good to monitor annual the TSH level and if it is normal it is recommended to doze antithyroid antibodies by intervals of 2-3 years (8,18).

Also, if the disease is autoimmune, the patient should be investigated for other autoimmune associations of endocrine or non-endocrine nature.

A study in Czech Republic on 51 patients with type 1 DM showed that it is associated with autoimmune thyroid diseases, with Addison's disease and Celiac disease.

The authors recommend finding the specific antibodies for each disease, to diagnose the disease in the initial phase, and to prevent the complications that will affect the quality of the patients' life (9).

If DM type 2 is present it is recommended to evaluate TSH levels, and if it is normal, to repeat this evaluation every 5 years.

If pre-existing thyroid pathology is present it is recommended to evaluate plasma glucose levels annually.

CONCLUSIONS

In the lot of adults with various changes in the glycemic balance and thyroid disorders, the main PAS encountered was **PAS 2** (1.68% cases), **PAS 3** (7.5% cases) and **PAS 3 C** (0.91% cases) (Fig.7). The most common was **PAS 3 A** (7.5%) because of the association of type 1 DM with TCA (both likely autoimmune diseases).

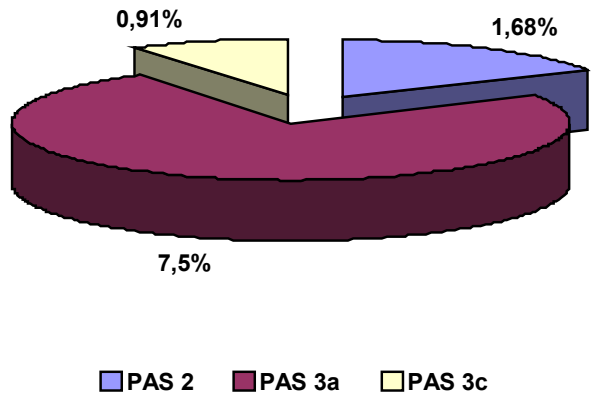

Fig.7. Prevalence of PAS in the group of adults

Many disorders involved in PAS present a long prodromal

phase, characterized by the presence of characteristics antibodies for each disorder in part, before the clinical manifestations.

The treatment of patients with PAS involves early identification of all components.

The treatment of PAS is currently the treatment of each component of endocrine disorder (usually through hormone substitution therapy). Iso-hormonal therapy has "immunomodulatory" capacities (hormone produced by the target organs may be able to influence autoimmunity).

Associations of specific autoimmune endocrinopathys requires specific management:

- substitution treatment with thyroxin may precipitate adrenal insufficiency by untreated Addison's disease

- hypoglycemia or decreased insulin necessity in patients with type 1 DM may signify the onset of a adrenal insufficiency

- asymptomatic forms of Addison's disease should be treated carefully under acute stress

- in Addison's disease associated with ovarian insufficiency, substitution therapy with steroid hormones can prevent severe osteoporosis

- controversial discussions are described in the literature on the effectiveness of thyroxin in patients with positive antibodies, but with euthyroidism or sub clinical hypothyroidism (10)

Some show a significant reduction of the TSH and of the anti-TPO antibodies in patients with autoimmune thyroiditis and euthyroidism after 1 year of treatment with thyroxin (10).

The PAS classification is not final. This may change over time, with the onset of new endocrine disorders or associations with new autoimmune determination. .

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ASOCIERI ENDOCRINE AUTOIMUNE ÎNTÂLNITE LA PACIENȚII ADULȚI CU ALTERĂRI ALE ECHILIBRULUI GLICEMIC ŞI AFECȚIUNI TIROIDIENE

REZUMAT

Procesele autoimune interesează aproape toate glandele endocrine, care sunt afectate într-o incidenţă variabilă. Diabetul zaharat tip 1 asociat cu anumite endocrinopatii realizează tabloul poliendocrinopatiilor autoimune. Scopul acestui studiu este de a stabili prevalenţa asocierii a diferitelor afecţiuni de natură autoimună la pacienţii adulţi cu alterări ale echilibrului glicemic şi afecţiuni tiroidiene. Lotul general studiat a fost reprezentat de 650 cazuri, cu vârste cuprinse între 17-79 ani.

Lotul de adulţi a fost subîmpărţit în funcţie de tipul modificării echilibrului glicemic în 4 subloturi: lotul cu diabet zaharat tip 1 reprezentat de 60 cazuri (9,23%), lotul cu diabet zaharat tip 2 reprezentat de 290 cazuri (44,61%), lotul cu scăderea toleranţei la glucoză reprezentat de 183 cazuri (28,15%) şi lotul cu scăderea toleranţei la glucoză a jeun reprezentat de 117 cazuri (18%).

Cuvinte cheie: diabet zaharat, boala autoimună tiroidiană, poliendocrinopatie autoimună

LONG-TERM IMPLICATIONS OF CARDIOVASCULAR AND RESPIRATORY MEDICATIONS IN ELDERLY ASTHMATICS

SIMONA MACESANU, ELENA ARDELEAN
Pneumology Ambulatory Care, Emergency County Hospital, Timisoara
"Victor Babes" University of Medicine and Pharmacy Timisoara

ABSTRACT

Asthma in the elderly, but also in other age groups, shows a high incidence in last years. Frequent co-morbidities, especially in cardiovascular pathology, in the elderly, require combined treatment, often it has side effects or contraindication, for other diseases associated.

Often remove from treatment the asthma medication or the other an important drug from cardiovascular medication as a precaution or unjustified reasons, produce from exhaustive policy of selling drugs and sometimes unjustified targets in our country. The goal of the present paper is to raise and to define these issues, to demonstrate that we need medication every disease that is separately, considering that the side effects or the existing contraindications, without forgetting the treatment of asthma in the elderly, main target for elderly patients being maintaining a good quality of life.

Key words: cardiovascular medication, asthma, elderly

INTRODUCTION

Asthma is one of the most common chronic diseases in the world, and WHO estimated that prevalence is increase. In Romania asthma prevalence is around 7-8% in the population, that is over one million people is asthmatic (1).

Late-onset asthma (in patients over 50) is more common than previously thought, and its prevalence rate is constantly increasing to around 5% in the population.

Currently asthma in patients over 65 years creates additional problems in terms of diagnosis, treatment, chronic disease monitoring, comorbidities, and additional costs of disease and changes over time. In the patients with undiagnosed asthma, symptoms are not perceived as being similar to those of asthma, the elderly may develop atypical asthmatic symptoms and nocturnal cough may be their only symptom. In these patients, symptoms may be falsely assumed to belong to other diseases or to other trigger factors such as heart failure or other cardiac distress, thyroid disease, old age, pollution, smoking, or reduced tolerance to effort, and in fact, these morbidities can coexist with asthma (2).

Nowadays, in Romania is a continuous increase in prevalence of cardiovascular diseases, especially hypertension, coronary heart disease, stroke and last but not least, heart failure. The cardiovascular diseases represent the main cause of premature death in Europe and are a significant cause of disability, significantly contributing to the rising cost of health care (3).

Frequent co-morbidities, especially in cardiovascular pathology, in the elderly, require combined treatment, often it has side effects or contraindication, for other diseases associated.

Often remove from treatment the asthma medication or the other an important drug from cardiovascular medication as a precaution or unjustified reasons, produce from exhaustive policy of selling drugs and sometimes unjustified targets in our country.

When cardiovascular diseases and asthma coexist, medications to treat cardiovascular disorders may cause an exacerbation of asthma and viceversa.

Asthma in the elderly may either be due to late onset (over 65 years) disease, or part of long-standing asthma which had persisted into old age, while elderly asthmatics had significantly more near-fatal episodes. The factors, more prevalent in the elderly, which predispose asthmatics to near fatal attacks are: 1) delay in diagnosis and treatment,2) poor cardiorespiratory reserve, 3) impaired perception of increasing airways obstruction, 4) blunted hypoxic ventilatory drives, 5) psycho-social and cognitive problems (4).

MATERIALS AND METHODS

A. Cardiovascular medications and their implications for comorbid respiratory diseases

Received May 2010. Accepted June 2010. Address for correspondence: S.Macesanu, MD, Ambulatory Care, Emergency County Hospital, Timisoara, e-mail: macesanusimona@yahoo.com

A.1. Betablockers

The effectiveness of beta-blockers has been verified not only for common indications (coronary heart disease, hypertension and certain forms of heart failure), but also in arrhythmias, pheochromocytoma, dissecting aneurysm, portal hypertension, hyperkinetic syndrome, glaucoma (in local administration).

Propranolol, one of the most frequently used antiarrhythmic in the recent past, is now being replaced by selective antiarrythmics as metoprolol, betaxolol, nevibolol, but caution is recommended when prescribed (5).

Antiarrhythmic sotalol is a broad spectrum drug, useful for various supraventricular and ventricular arrhythmias. Sotalol may increase susceptibility to allergens and severity of anaphylactic reactions due to its effect of blocking beta-adrenergic receptors. In patients with a history of hypersensitivity reactions or in those under desensitization treatment there are high risks of severe anaphylactic reaction (6). The findings of SENIORS study showed superiority of nebivololum (other commonly used beta-blocker) compared to placebo in reducing mortality and hospitalization rates in elderly patients with heart failure (7).

For patients with obstructive airway disease and concomitant congestive heart failure or ischemic heart disease it is common practice to give ß-2 agonists while withholding ß- blockers. There are now several case-control studies that have documented an association between ß-2 agonist use and the development of congestive heart failure, acute myocardial infarction, or cardiac death. This association may be explained by ß-agonist stimulation causing tachycardia, hypokalemia, and arrhythmia, especially in the presence of cardiac comorbidities or hypoxemia. Cardioselective β-blockers do not produce clinically significant adverse respiratory effects in patients with mild to moderate reactive airway disease. The results were similar for patients with concomitant chronic airways obstruction (8).

A.2.Other antiarrhythmic medication

Amiodarone has the following side effects when taken on a long-term basis: development of corneal pigment deposits, hypothyroidism and diffuse pulmonary fibrosis.

A.3.Calcium channel blockers

Verapamil is an antiarrhythmic and bradicardizant and produces vasodilation, and it is indicated in coronary patients with supraventricular arrhythmias because it slows the ventricular rhythm and in junctional tachycardia. Verapamil has numerous side effects, it exerts negative inotropism in heart and can cause cardiac decompensation (9). It should be *cautiously coadministered with* Propranolol (because summation of myocardial depression can occur), Digitalis (there is a high risk of heart block), Diltiazem it has verapamil-like properties, and is used as antiarrhythmic and as antianginal treatment.

A.4.Cardiovascular medication that might *induce* or aggravate asthma or chronic cough in elderly patients (Table I) (10).

Table I. The influence long term of cardiovascular drugs in chronic obstructive respiratory diseases

Medication	Comorbidities at risk of being treated	Side effects	Comments
Beta-adrenergic β blockers	Hypertension Tremors Glaucoma	Asthma exacerbation Bronchospasm Reduced responsiveness to bronchodilators	To avoid as much as possible; When recommended selective β blockers will be chosen
Non steroidal anti-inflammatory drugs (NSAIDs)	Arthritis Musculoskeletal disorders	Asthma exacerbation Bronchospasm Aspirin, fenofibrat may cause pulmonary eosinophilia	Though not all elderly asthmatics develop intolerance to NSAIDs these drugs should still be avoided as much as possible
Non–potassium-sparing diuretics	Hypertension Cardiac insufficiency	Worsening of cardiac function / arrhythmias induced by hypokalemia	Produce additive effects in combination with asthma medication:- $ß_2$-agonists have an additive effect with oral or injected steroids and may cause potassium loss -coadministration with cardiac glycosides may cause hypokalemia
Anticholinergic agents (ipratropium bromide, tiotropium bromide)	Urinary retention Glaucoma	Bronchospasm Bronchorrhea	Ephedrine-based medication may aggravate urinary retention and glaucoma
ACE Inhibitors	Cardiac insufficiency Hypertension	Increase in cough incidence	
Fenofibrat,amiodarone, metisergida, acebutolol		Interstitial pneumonitis, alveolitis, pulmonary fibrosis	In the long administration
Procainamide, metildopamina, quinidine, metisergida, hidralazine, phenytoin		Disseminated lupus erythematosus with pulmonary determinations	In the long administration
Hidralazine, quinidine, phenothiazines		Systemic polyarteritis with lung determinations	In the long administration

A.5.ACE inhibitors and statines

Angiotensin-converting enzyme inhibitors (ACE) are widely used in the management of cardiovascular diseases and systemic hypertension. Statins are the most commonly prescribed lipid-lowering agents and have proven benefit in primary and secondary prevention of coronary heart disease. There is increasing awareness that the broader pharmacologic properties of ACE and statins encompass the abilities to modulate local fibroproliferative pathways in a variety of organ systems. In the lung, angiotensin II is emerging as a potentially important profibrotic mediator via induction of alveolar cell apoptosis and as a fibroblast mitogen. Some of studies was demonstrates that ACE and/or statins are not associated with improved survival in IPF (11).

Patients who develop chronic cough in association with angiotensin-converting enzyme inhibitor therapy should be switched to an agent from another drug class. If cough persists, a chest radiograph and other investigations should be ordered to rule out malignancy and other serious conditions. ACE inhibitors cause a nonproductive cough in 5 to 20 percent of people (more often women than men). This side effect is not dose related, and the cough may begin one week to several months after ACE inhibitor therapy is initiated. The cough should subside in a few days to several weeks after the ACE inhibitor is stopped (12).

B.Coexisting asthma and heart disease- the **treatment implications** of asthma in the elderly

Asthma medications may have increased side effects in elderly patients with coexisting cardiovascular disease, so a treatment plan

should be adjusted. Response to bronchodilator medication may change with age. Elderly patients, particularly those who have associated ischemic heart disease, may develop tremor or tachycardia after beta-agonists or theophiline administration. Concomitant use of beta-agonists and anticholinergics is recommended in elderly asthmatics only if cardiac function is closely monitored (13).

B.1. Methylxanthines (aminophylline, theophylline)

Theophylline(THY) has been used the management of asthma for more than 50 years and remains a widely prescribed anti-asthmatic agent worldwide.

Theophylline clearance may be reduced by concomitant diseases prevalent in elderly patients, and , which further impair clearance of this drug and have the potential to increase THY blood levels, age appears to *be* an important independent iatrogenic risk factor in high doses administration.(Shannon and Lovejoy 1990).The efficacy and toxicity of THY are closely related to the serum drug concentration.In patients receiving theophylline as monotherapy for chronic asthma, doses providing peak serum concentrations between 10 and 20 μg per milliliter are the most likely to prevent symptoms and decrease the need for rescue therapy. However, bronchodilatory, antiinflammatory, and immunomodulatory effects occur for THY lower serum concentrations, which may be adequate for elderly (14).

Methylaxanthines have a number of drug interactions which require some precautions in their administration on elderly. Some of side effects may be present in up to 50 percent of patients when serum concentrations of 10 to 20 μg per milliliter are rapidly attained, but they are much less frequent when the initial dose is low and the dose is increased at intervals of no less than three days so that these serum concentrations are reached gradually. For THY serum concentrations >35 mcg/mL, both the frequency, and severity of adverse reactions are increased, while cardiac arrhythmias, hypotension, hyperglycemia, and intractable seizures which can be lethal, brain damage, peripheral vascular collapse, or death may occur during treatment with theophylline (15). Shin et al., Vestal et al. showed that total clearance (bound plus unbound theophylline) in the elderly was 33%less than in younger groups. These observations indicate that both renal and metabolic THY elimination processes are less active in the elderly (16).

Theophylline is currently a less preferred option than inhaled corticosteroids recommended as a second-line choice of controller in management of patients with asthma at Step 2 of the Global Initiative for Asthma Guidelines (GINA) 2002 guidelines (17). Although long-acting inhaled $ß_2$-agonists are more effective as an add-on therapy at Steps 3 and 4 of the GINA 2002 guidelines, theophylline is considerably less expensive and may be the only affordable add-on treatment when the costs of medication are limiting (18).

B.2. $β_2$-agonists therapy as bronchodilator medication in asthma

B.2.1.Rapid-acting inhaled $ß_2$-agonists

This is the medication of choice for relief of bronchospasm during acute exacerbations of asthma and for prevention of exercise-induced bronchoconstriction. Rapid-acting inhaled $ß_2$-agonists should be used only when they are strongly required and at the lowest dose required. Increased use of rapid-acting inhaled $ß_2$-agonists is a warning of deterioration of asthma control and indicates the need to reasses treatment.

Adrenaline has been used in the treatment of asthma since the beginning of the 20th century. While adrenaline is an effective bronchodilator, it also causes anxiety, profound stimulation of the heart, increase of blood pressure and excessive stimulation of the brain. These unwanted side effects led the researchers to search for an adrenaline analog that would retain the bronchodilating quality without the cardiovascular and central nervous system liabilities. A close analogue of adrenaline, isoprenaline is free from vascular side-effects, but may cause an increase in the force and rate of contraction of the heart.

Salbutamol was found to be both a highly selective and potent $ß_2$-adrenoceptor agonist, with an adequate metabolic stability that made it a highly effective bronchodilator than fenoterolum or terbutalina. The most common side effects of Salbutamol are cardiac tachyarrhythmias, QT-interval prolongation, ST-segment depression, some caution is recommended in patients with heart disease, coronary heart disease, arrhythmias or hypertension (19).

Metaproterenol, although not entirely free of $beta_1$-receptor effects, also improved specificity relative to both epinephrine and isoproterenol and provided a somewhat longer duration of action.

Oral preparations can be useful in elderly patients unable to administer inhaled therapy, but with a much higher frequency of adverse effects, particularly tachycardia or elevated blood pressure.

B.2.2 Long-acting beta-agonists (LABA)

Long-acting beta-agonists (LABA) are recommended for partially controlled or uncontrolled persistent asthma in adults and in the elderly, usually in combination with inhaled corticotherapy, depending on the stage of severity according to GINA.

Pharmacological studies in vivo and in vitro show selectivity on beta2-adrenergic receptor and its similarity with isoproterenol, which has approximately equal effect on beta1-and beta2-adrenoreceptors. Studies show that salmeterol is 50 times more selective than isoproterenol on beta2-adrenergic receptors that prevail in bronchial smooth muscle and beta1-adrenergic receptors that predominate in the smooth muscle of the heart (10-50% of beta2-receptors are found in the heart muscle). The high selectivity of salmeterol on beta2-adrenergic receptors does not exclude the possibility of cardiac adverse effects.

Drug interactions are reported at concomitant use of beta-blockers that may decrease bronchodilating, and vasodilating effects of beta-agonists such as salmeterol; concurrent administration with methyldopa may increase pressor response, ECG changes and hypokalemia resulting from diuretics may worsen when coadministered with salmeterol. Long-acting beta-agonists are contraindicated in patients with documented hypersensitivity, angina, tachycardia, cardiac arrhythmias associated with tachycardia. Castle W. et al. show in asthmatic patients who require regular bronchodilator treatment, the overall incidence of serious events from all causes

(4% for salmeterol versus 41% for salbutamol) was unremarkable, the incidence of those suspected as being related to the drugs (1.19% versus 1.15 %) was low, and importantly there was no evidence of any previously unrecognised side effect of salmeterol or salbutamol (20).

B.2.3.Combination therapy (CSI and LABA)

The combination preparation fluticasone and salmeterol used in the management of asthma and chronic obstructive pulmonary disease (COPD).

Whilst the use of inhaled combination steroids and LABA, are recommended in asthma guidelines for the resulting improved symptom control, concerns have been raised that the use of long-acting inhaled bronchodilators such as formoterol and salmeterol may increase the risks of asthma-related deaths and this additional risk is not reduced with the additional use of inhaled steroids. The Salmeterol Multicenter *asthma* research *Trial* (SMART study) that was a multicentre, randomized double-blind, placebo-controlled, observational study initiated in 1996, and finished in 2003, had positive findings. The SMART study has concluded that salmeterol or other long-acting β_2-agonists (LABA) do not increase the risk for asthma-related deaths but it found a higher incidence of death in African Americans with salmeterol therapy compared to placebo (African Americans presented high incidence of asthma symptoms, deterioration in baseline PEF, more emergency departments visits and more frequent hospitalizations, these facts being reported prior to their study participation). The authors speculated on possible genetic causes, mentioning β-receptor polymorphisms (21).

The long-term use of inhaled formoterol and budesonide could causes systemic adverse effects that include coetaneous allergic reactions, severe shortness of breath, arterial hypertension, tachycardia or arrhythmia, may significantly prolong the QTc interval in a dose-dependent and gene-dependent manner, induce hypokalemia and *raise glycemia* levels, tremor, anxiety, increased incidence of respiratory infections, eye-related ailments (cataracts and glaucoma), and may decreased bone mineral density.

Co-administration of combination steroids and LABA with other beta-blockers (eg, Propranolol) may block the pulmonary effect of formoterol, in addition to producing severe bronchospasm in asthmatic patients but no other drug interactions have been found between budesonide and medication used to treat bronchial asthma. Thus, budesonide and formoterol combination should not be administered in association with beta-adrenergic antagonists (including those in ophtalmic drops), except for well-justified cases.

B.3.Oral steroidal anti-inflammatory drugs

Long-term use of orally inhaled corticosteroids may affect normal bone metabolism, resulting in loss of bone mineral density .The decrease in bone mineral density is dosage-dependent especially when it is associated with the use of inhaled corticoids, even though high doses or medium doses are not associated with potential for major adverse effects. The elderly are at high risks if they also have pre-existing osteoporosis, changes in estrogens level that affects calcium absorption, or if they have a sedentary lifestyle. Patients with asthma who are on long-term systemic glucocorticosteroids in any form should receive preventive treatment for osteoporosis. Intake of calcium, D vitamin supplements and estrogens has additional therapeutic enjoying osteodensitometry assessment should be performed every six months.

Co-administration with digoxin may cause digitalis toxicity secondary to hypokalemia, phenobarbital, phenytoin, and rifampin may increase metabolism of glucocorticoids (consider increasing maintenance dose) and it is useful to monitor hypokalemia with coadministration of diuretics (22).

B.4. Inhaled corticosteroids

Inhaled corticosteroidal medication has no adverse reactions or side effects in cardiac patients, due to the low systemic dose absorbed through gastrointestinal tract. Inhaled corticosteroid therapy both improve clinical control and reduce exacerbations. Some recent studies have found that the use of inhaled corticosteroid may increase the risk of developing cardiovascular diseases and the risk of death. Other numerous studies indicated that inhaled corticosteroid therapy is associated with no risk of developing cardiovascular diseases and no risk of death (23).

B.5.Leukotriene modifiers:

Clinical studies have demonstrated that leukotriene modifiers provide clinical benefit, reduce asthma symptoms including cough, and improve lung function in older adult patients but not to the same extent as in the case of young patients with associated atopy. Leukotriene modifiers are well tolerated and no cardiovascular adverse reactions after long-term therapy with montelukast have been reported.

B.6.Inhaled anticholinergic medication

Inhaled anticholinergics are rapid-acting bronchodilators with therapeutic role that are less effective reliever medications in asthma than rapid-acting inhaled ß$_2$-agonists and are recognized as an alternative bronchodilator for patients who experience major adverse effects from rapid-acting inhaled ß$_2$-agonists.

Tiotropium is a long-acting, anticholinergic bronchodilator used in the management of COPD and his side effects are concentration-dependent. In the elderly plasma drug concentration increases and reduces renal function, thus tiotropium bromide should not be administered in patients with moderate-severe renal insufficiency only if clinical benefits overweigh potential risks. Long-term data on the efficacy of tiotropium administration in asthma patients with severe renal insufficiency is lacking. The occurrence of adverse drug effects inhalation of tiotropium can cause respiratory tract infections, tachyarrhythmias, urinary retention.

B.7. Nonsedative antihistamines (terfenadine and astemizole) used in allergic rhinitis can produse worsening of cardiac function or ventricular arrhythmias, induced by prolongation of QTc interval. We found increase incidence asthma and allergic rhinitis in elderly in our area, involving attention both disease diagnosis and treatment (24).

B.8. Inhaled aerosol terapy

According to the respiratory tract anatomy, pharmacokinetics and dynamics of the flow characteristics of inhalation therapy was recommended as the best prevention and treatment of chronic asthma treatment methods. Using inhaled corticosteroids steroids aerosols(Budesonide), β2-aerosol receptor agonists (Salbutamolul ampoules), aerosol anticholinergic drugs (ipratropium), have fewer side effects in elderly and do a better penetration of aerosol (25).

DISCUSSIONS AND CONCLUSION

Asthma management in the elderly follows the same national and international guidelines for asthma treatment and control that are used in young adults, additionally psycho-emotional and social changes associated with ageing should be also considered, addressed and treated as appropriate.

Elderly asthmatics have different clinical characteristics from youths and we are required to understand these differences.Bronchial asthma has more severe clinical forms in the elderly than in adult or young patients and it is associated with significant morbidity and mortality (26). Therapeutic management of bronchial asthma in the elderly requires close attention to the person's adherence/ compliance to the medication plan, to achieve and maintain control of asthma symptoms, to prevent exacerbations, main target for elderly patients being maintaining a good quality of life.

Asthma treatment and attainment of control in the elderly are also complicated by: refusal to use aerosol devices for asthma, lack of family support, poor medical knowledge, high costs of asthma medications, denial of asthma diagnosis, the occurence of adverse drug reaction that are not perceived by elderly patients, lack of confidence in the efficiency and necessity of asthma medications, poor self-monitoring of athma treatment and control (27,28).

Bronchial asthma raises frequent problems in terms of differential diagnosis, and is associated with numerous comorbid conditions that complicate the overall choice for a medication plan, it is also associated with risks of polypragmasy, and with high probability of adverse drug reactions.

The lack of asthma management in cases of uncontrolled asthma with frequent exacerbations in order to protect the asthma patients with cardiovascular co-morbidities against the risk of cardiogenic shock induced by anti-asthma medication has no proven benefits if patients maintain their mobility. Older asthma patients with cardiovascular comorbidities and with well-controlled asthma are less likely to experience exacerbations and have a good quality of life.

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IMPLICAȚIILE PE TERMEN LUNG ALE MEDICAȚIEI CARDIOVASCULARE ŞI RESPIRATORII LA VÂRSTNICII ASTMATICI

REZUMAT

Astmul bronşic la vârstnici, dar şi celelalte grupe de vârstă, prezinta o incidenţă crescută în ultimii ani. Comorbidităţile frecvente, în special în patologia cardiovasculaă, la vârstnici impun un tratament combinat, de multe ori cu reacţii adverse sau contraindicaţii pentru celelalte boli asociate. De cele mai multe ori, se renunţă la medicaţia astmului ori la un medicament important din terapia cardiovasculară din precauţie sau din considerente nejustificate, determinate şi de o politică prea exhaustivă şi uneori eronată a vânzării medicamentelor la noi în ţară. Scopul lucrării este de sensibiliza şi delimita aceste aspecte, de a demonstra că este necesară medicaţia fiecărei afecţiuni în parte, ţinând cont de reacţiile adverse sau contraindicaţiile respective, fără a omite tratarea astmului la vârstnici, ţinta principală la această categorie de pacienţi, fiind menţinerea unei calităţii bune vieţii.

Cuvinte cheie: medicatie cardiovasculara, astm, varstnici

STROMAL CELLS - TUMOR MICROENVIRONMENT INTERACTIONS - Part II

FLORINA BOJIN[1], CALIN TATU[2,3], OANA GAVRILIUC[1], ALEXANDRA GRUIA[3], GABRIELA TANASIE[1,3], CARMEN TATU[1,3], ANGHEL SIMONA[1,3], MIRABELA CRISTEA[3], FELICIA CIUCULESCU[1], ALEXANDRU TOCUT[1], DANIELA CRISNIC[1,3], ADRIAN CARABINEANU[4], DACIANA NISTOR[1,3], CARMEN BUNU[1], VIRGIL PAUNESCU[1]

[1]Physiology-Immunology Department, "Victor Babes" University of Medicine and Pharmacy Timisoara
[2]Biology Department "Victor Babes" University of Medicine and Pharmacy, Timisoara
[3]Regional Center for Transplant Immunology, Emergency Clinical County Hospital Timisoara
[4]Oncologic Surgery Department, Municipal Emergency Hospital Timisoara

ABSTRACT

The complex cellular tumor (micro)environment comprises immunocompetent and inflammatory cells, endothelial cells and fibroblasts. All of these cell types may critically influence the multi-step process of carcinogenesis and malignant phenotype. Fibroblasts are known to take part in immune reaction during tissue damage and injury by modulating local cellular and cytokine milieu, adjusting the kinetics and components of the inflammatory infiltrate, and by modulating the functional status of the immunocompetent cells. Purpose of our study was investigation on tumor-associated fibroblasts (TAFs) role in immune suppression, thus profoundly affecting tumor cells progression.

Human TAFs were isolated from breast cancer surgical pieces, cultivated and expanded in vitro as monolayer cell culture. Supernatants of cell cultures at different passages were collected and presence of IL-4, IL-10, IL-13, TGF-β1, TNF-α, INF-γ and VEGF was detected by ELISA method. Specific mouse anti-human fluorochrome-conjugated antibodies identified in flow-cytometric analysis surface markers of TAFs, such as CD14, CD117, CD90, CD106, CD44, CD29, CD73, HLA-DR, CXCR4. Expression of cytoskeleton and extracellular matrix proteins was revealed by immunocytochemistry (vimentin, α-smooth muscle actin).

Secretion of cytokines with direct immunosuppressive effect, such as IL-10 and IL-13, and other cytokines, IL-4 and TNF-α, was increased in cultured TAFs at all passages. Enhanced production of TGF-β1 by TAFs may also be a critical factor in tumor homeostasis. Expression of MHC class II molecules (HLA-DR) on activated TAFs contribute to an additional level of immunosuppression and pro-tumoral effect. High level of VEGF production suggests that TAFs provide not only structural support, but also pro-angiogenetic molecules for tumor vascularization. α-smooth muscle actin expression in fibroblasts is variable and associated with an activated status.

The complex network of effects TAF transduced by enhanced expression of various immune cell deactivating/suppressing factors and neoangiogenesis molecules should stimulate consideration of TAFs-based therapeutic designs.

Key words: MSCs, TAFs, microenvironment

INTRODUCTION

Cancers may arise from malfunctions in the cell control segment, that lead to the dysregulation proliferation of cells. The underlying causes of cancers are mutations and other alterations in DNA, and attendant innappropriate expression level, of genes encoding proteins, that either promote growth or restrain it, or direct the apoptosis machinery, or are responsible for DNA damage repair and signaling, and chromatin remodeling. The mutations can be inherited (present in the germ cells), or they may be produced in somatic cells (1).

In metastasis, malignant cancer cells break away from where they are immobilized, enter the circulatory system, and invade other organs. They form colonies, or secondary tumors, at the new locations and cause damage to their neighbors by appropriating sources of nutrition (1).

Cancers derived from epithelial cells are the most common type of cancer. In order for the cancerous epithelial cells to break away from the initial location, they must detach from other epithelial cells and from the ECM (2). To do so they must disrupt the cell-to-cell adhesive contact established by E-cadherins, and cell-to-ECM contacts maintained by the integrins. Once they have detached from one another and from the ECM they hace to pass through the basement membrane to reach and enter the circulatory system.

Growth factors ad growth factor receptors support the development of malignant tumors in several ways. One proeminent contributor to the onset of malignancy is the vascular endothelial growth factor (VEGF). In order for a solid tumor to grow and thrive it must have an adequate blood supply. In response to this need for vascular expansion, tumor angiogenesis take place. The expression of RNA for VEGF ligands is enhanced in most human tumor cells. Increased VEGF mRNA are present in rapidly growing glioblastoma multiform brain tumors, and in cancers of the lung, breast, gastrointestinal tract, female reproductive organs, thyroid gland, and urinary tract (3).

Alterations in the mix of cell adhesion molecules being ex-

Received January 2010. Accepted February 2010. Address for correspondence: Florina Bojin MD, Physiology-Immunology Department, "Victor Babes" University of Medicine and Pharmacy Timisoara, 2A Eftimie Murgu Square, RO-300041, Phone/Fax: +40256490507, e-mail: florinabojin@umft.ro

pressed accompany tumor progression. The altered expression patterns occur not only during metastasis, but also during solid tumor growth. Most of the cancer develop from epithelial cells, and loss of E-cadherins is a common occurrence. A consequence of the growth factor receptor-adhesion molecule clustering is the strengthening of signals that would otherwise be too weak to elicit a cellular response if conveyed by one or other alone (4-6).

Once the tumor cells are reaching the appropriate milieu, in different organs and tissues, depending on the homing abilities of the cells, an interaction between stromal compartment and tumor cells is established, which could activate the entire arsenal of changes in tumor cells and stromal cells behavior, starting with adhesion molecules connections, up to dysregulation of the entire gene profile, for both tumor cells, and stromal cells compartments (7).

We investigated modification induced during stromal cells interaction with tumor cells on phenotypical behavior and gene expression pattern, in coculture models developed *in vitro* conditions.

MATERIAL AND METHODS

1. Isolation and culture of MSCs, TAFs and bulk tumor cells

All cellular types were isolated, cultured and further expanded as previously described by Paunescu V et al. (8) and Bojin F et al. (9).

2. Immunophenotyping

MSCs and TAFs in coculture with bulk tumor cells were trypsinized (Trypsin-EDTA, Sigma) at the end of the experiment, at 14 days, centrifuged 7 min at 1500 rpm, and washed twice with PBS. Cells were then re-suspended in PBS, 100 µl of cellular suspension togheter with 4 µl of each antibody (mouse anti-human fluorochrom conjugated). Cells are then vortexed and put to rest at room temperature, in the dark, for 30 minutes. After incubation, cells are washed with 2 ml Cell-Wash Solution (BD Biosciences), centrifuged 1 min at 1500 rpm, supernatant is removed and cells are re-suspended in 500 µl Cell-Wash. Flow cytometric analysis was performed using FACSCalibur (Becton-Dickinson), 2 lasers acquisition system, capable to differentiate 4 colors simultaneously. The antibodies used for flowcytometric assay were conjugated with the following fluorochroms: PE - CD14 (BD Pharmingen™), CD117 (BD Pharmingen™), α-SMA (BD Biosciences), CD29, CXCR4, Nestin, E-Cadherin (R&D Systems), and FITC - CD34, CD44, CD45, CD73, CD90, CD106, HLA-DR, (BD Pharmingen™), Cytokeratin (R&D Systems) şi APC - CD31 (BD Pharmingen™). Part of the cellular markers (E-Cadherin, Cytokeratin) have an intracellular location, recquiring a supplementary permeabilization step, using FACS Permeabilizing Solution 2 (10x; BD Biosciences), followig a protocol presented by the supplier. Acquisition and data analysis were performed using CellQuest Pro software (Becton-Dickinson).

3. Characteristic gene expression of MSCs and TAFs in coculture system

Total RNA was extracted from MSCs and TAFs, at different passages, as well as from cells in coculture, at the end of 2 weeks period, from all cocultures types. From cultured cells, after trypsinization and washing with saline solution (PBS) followed by centrifugation, total RNA was extracted using GenElute™ Mammalian Total RNA Miniprep Kit (Sigma). Concentration of each sample was measured using Nanodrop ND-1000 spectrophotometer (Wilmington, DE, USA). For RT-PCR we used concentrations of 100 ng RNA/reaction and we amplified fragments of 554 bp (CXCR4), 584 bp (VEGF), 220 bp (IL-4), 424 bp (IL-10), 315 bp (TGF-β1) and 486 bp (Thy1). The following program was used for amplification: 50°C - 31', 95°C - 15', (94°C - 1', 55°C - 1', 72°C - 1') x 35 cycles, 72°C - 10'; samples can be thus stored at 4°C for indefinite time period. For gene amplification we used primer pairs whose sequences were valideted in GENEBANK. Thermal cycles were adjusted for each primer pair. Table I presents the sequences of primers used for amplification, while Table II enumerates the samples analyzed by RT-PCR. For data analysis, the amplification products are submitted to 2 % agarose gel electrophoresis, visualized with Fluor-S™ MultiImager (BIO-RAD) UV system, and analyzed with GelDoc software, which allows a semi-quantitative analysis of the results.

Tabelul I. Genes and primers sequences used for RNA amplification and analysis using RT-PCR

Gene	Primer pairs
CXCR4	F: 3'-CAGCAGGTAGCAAAGTGACG-5' R: 3'-AGACGCCAACATAGACCACC-5'
VEGF	F: 3'-CTACCTCCACCATGCCAAGT-5' R: 3'-TGGTGAGAGATCTGGTTCCC-5'
IL-4	F: 3'-TGCCTCCAAGAACACAACTG-5' R: 3'-ACTCTGGTTGGCTTCCTTCA-5'
IL-10	F: 3'-TTACCTGGAGGAGGTGATGC-5' R: 3'-TGGGGGTTGAGGTATCAGAG-5'
Thy1	F:3'-ATGAACCTGGCCATCAGCATCGC-5' R:3'-TCACAGGGACATGAAATCCGTGG-5'
TGF-β1	F: 3'-GACTGCGGATCTCTGTGTCA-5' R: 3'-CCTCCCTTAACCTCTCTGGG-5'

Tabelul II. Samples analyzed for gene expression

No..	Sample type	RNA conc. (ng/µl)
1.	TAF cocultures-TAF medium	269.4
2.	TAF cocultures-tumor medium	50.7
3.	TAF P5	1656
4.	MSC cocultures-MSC medium	204.3
5.	MSC cocultures-tumor medium	58.4
6.	MSC P4	574.7

4. Statistic analysis

Statistic analysis was performed using Excell Microsoft Office 2003 (Microsoft Corporation) software. The central

tendencies of the variables were expressed as a mean (M), and the dispersion ones as standard deviation (sd). In order to perform the statistic comparisons, „t"-Student test and the variance analysis (ANOVA) were used for continuous variables. Differences were considered significant for $p < 0.05$.

RESULTS

1. Characteristic gene expression of MSCs and TAFs in coculture model with tumor cells

Gene expression was evaluated for both the cellular substrate MSCs and TAFs, as well as for tumor cells put in coculture with them. The samples' order is presented in Table II, from left to right.

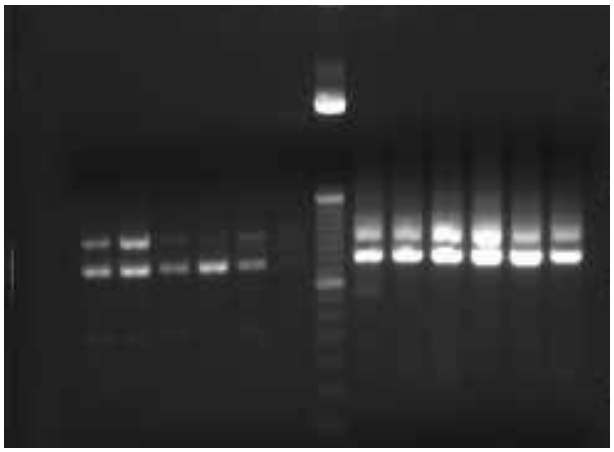


Fig.1. VEGF expression (left) and CXCR4 (right). Notice the increased expression of VEGF in cocultures having TAFs as cellular substrate, while MSCs present a decreased expression in coculture, independently of the culture media. MSCs cultured in appropriate medium do not present expression of this molecular marker. CXCR4 is present in all cellular types.

VEGF expression is increased in TAFs (also confirmed by immunocytochemical analysis); presence of this pro-angiogenic factor in coculture model could be suggestive for TAFS' role in supporting tumor cells. MSCs do not posses the same molecular characteristics as TAF, VEGF not being expressed in these cells; but, MSCs cocultures with tumor cells do present a comparable expression of this marker, probably do to presence of tumor cells characteristics (Figure 1).

CXCR4 is present in all cellular types, both independently cultured or in cocultures. CXCR4 is a cellular homing marker, MSCs and TAFs expressiong this receptor type on the cellular surface.

IL-4 gene expression is very week, the results being correlated with the other analysis and tests performed for determination of this cytokine (ELISA) (Figure 2). IL-10 is a molecular marker present for all samples, while TGF-β1 is especially expressed in TAFs and cocultures using this cellular substrate, MSCs laking expression of this marker (Figure 3).

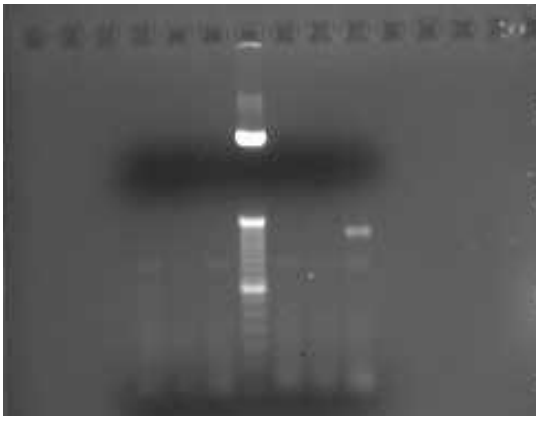


Fig.2. IL-4 expression. Decreased expression of IL-4 for MSCs at P4

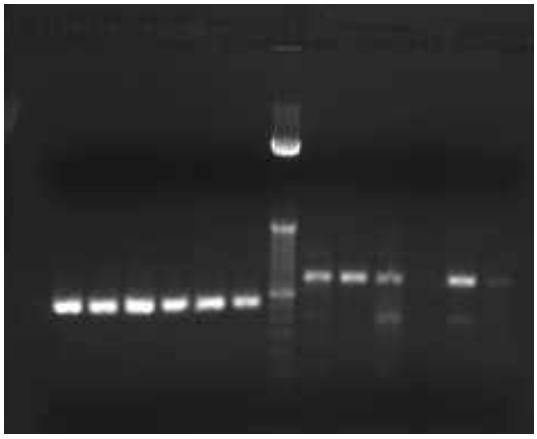


Fig.3. IL-10 (left) and TGF-β1 (right) expression. Increased expression of IL-10 for all samples, but decreased / absent for TGF-β1, in MSCs in coculture or single culture, in presence of specific medium

Thy1, also known as CD90, is expressed in all samples. The resultant protein is CD90 cell surface marker, which is present on the surface of mesenchymal stem cells and tumor-associated fibroblasts. Expression of this marker even in coculture is significant for MSCs and TAFs ability to retain their initial characteristic features (Figure 4).

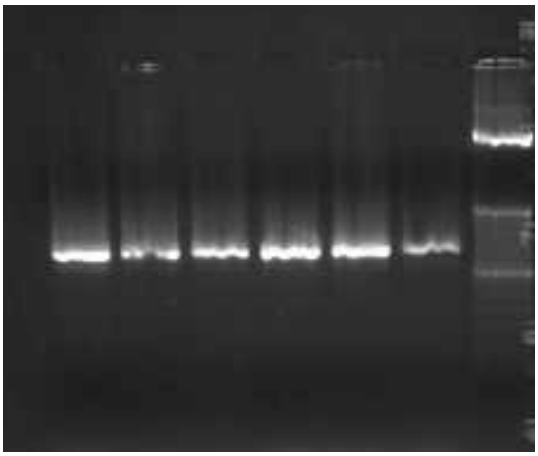


Fig.4. Increased and constant Thy1 (CD90) expression in all samples, significant for cellular ability to retain initial characteristics

3.4. Phenotypical analysis of cells in coculture

We used specific markers of MSCs and TAFs for phenotypical analysis of cells in coculture; identification markers of tumor cells were also used. We could not find any significant differences in markers expression in MSCs coculture systems, fo both medium types used – MSCs specific medium and tumor cells medium (Table III, Figures 5 and 6). TAFs cocultures with tumor cells presented significant differences in markers expression when cultivated in fibroblasts specific medium, compared with tumor cells medium. Cytokeratin, CXCR4, HLA-DR and α-SMA expressions were decreased, while CD44 and CD90 expression presented a significand increased compared with TAFs cultures alone. When we compared the two cellular types in coculture with complementary media, significant differences were revealed in CD45 expression (hematopoietic stem cells marker), mening that the cocultures using MSCs as cellular substrate presented an overexpression of CD45, for both medium types, when compared with correspondent cocultures using TAFs as cellular substrate. CXCR4, which was detected as marker for TAFs coculture systems, presented a lower expression comparative with MSCs cocultures, especially when we used tumor cells specific medium for cultivation (Table III, Figures 7 and 8).

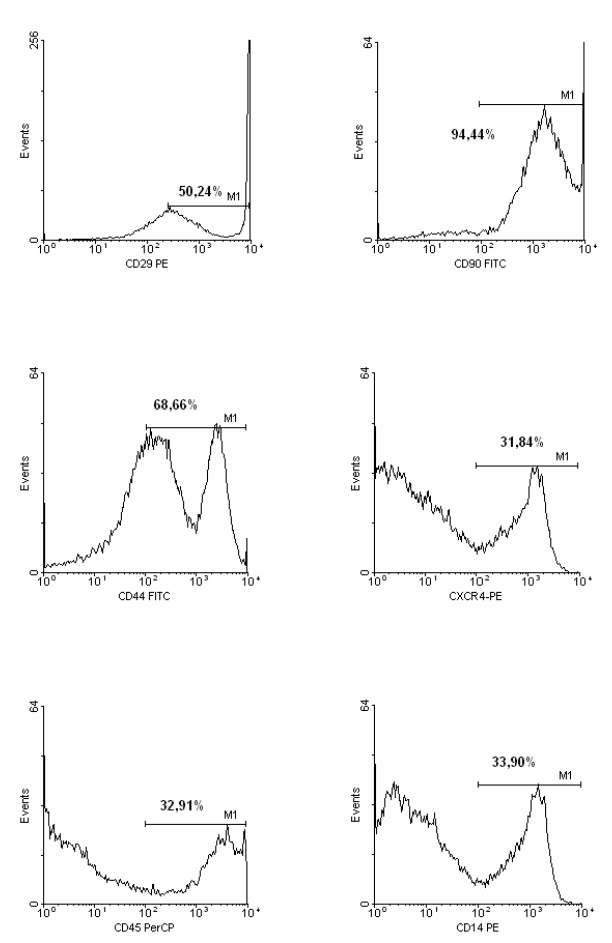


Fig.5. Phenotypical surface markers of MSCs-tumor cells cocultures in MSCs' growth medium

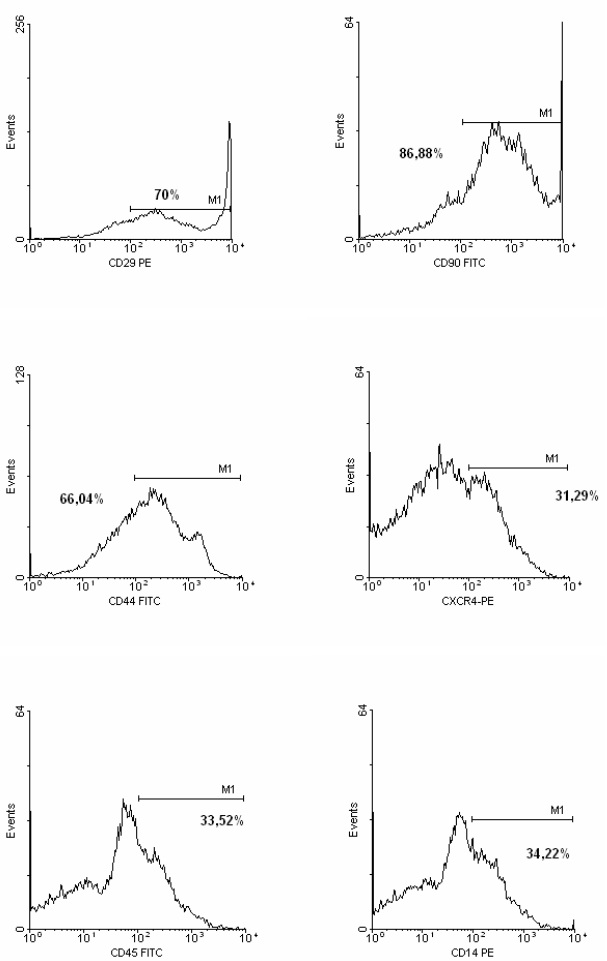
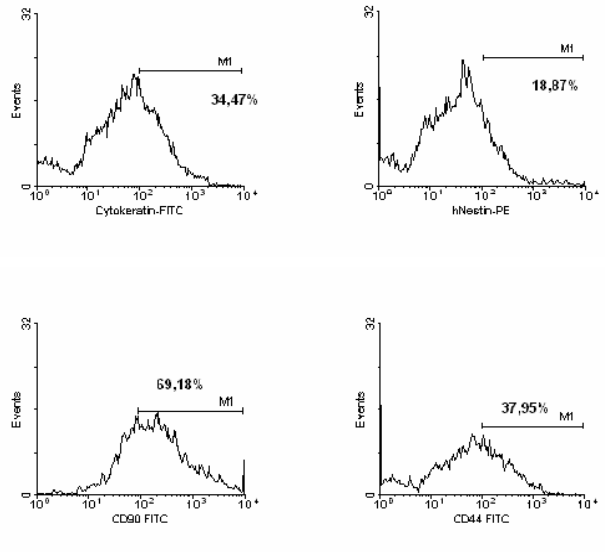


Fig.6. Phenotypical surface markers of MSCs-tumor cells cocultures in tumor cells growth medium



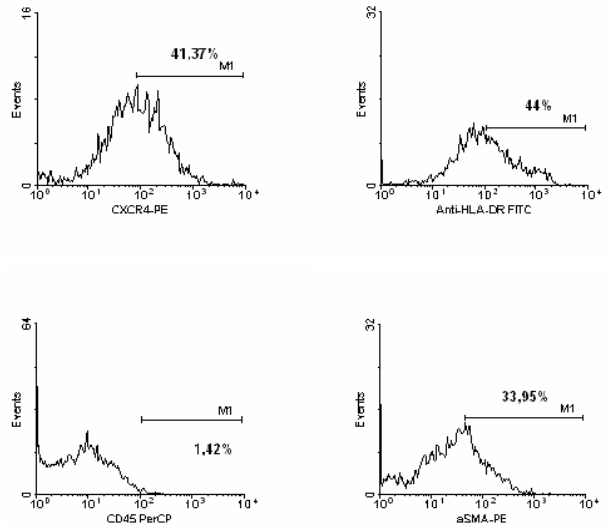


Fig.7. Phenotypical surface markers of TAFs-tumor cells cocultures in TAFs' growth medium

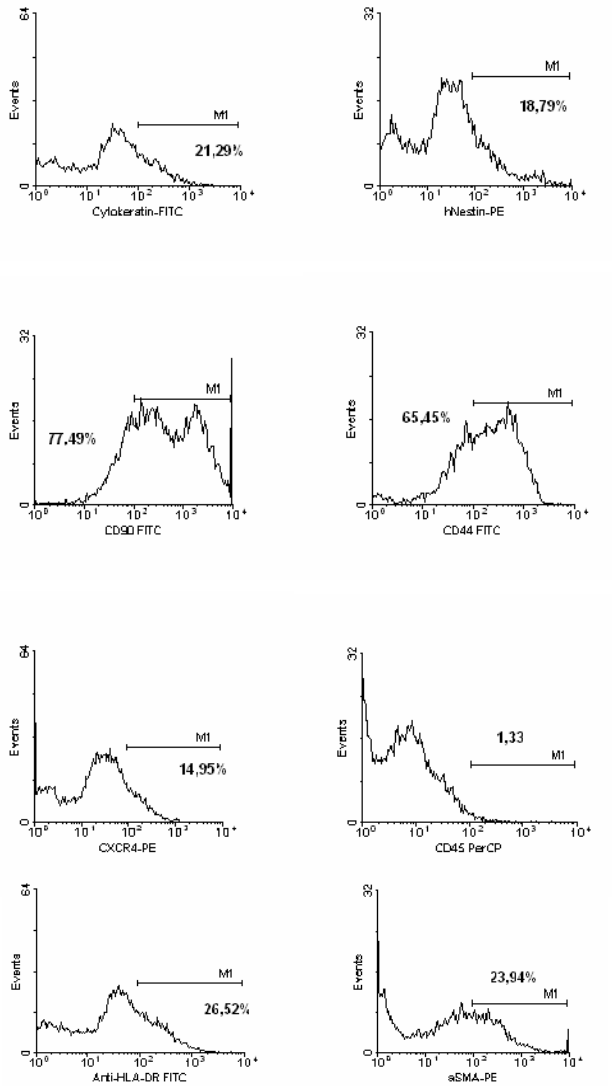


Fig.8. Phenotypical surface markers of TAFs-tumor cells cocultures in tumor cells' growth medium

Tabel III. Synopsis of flowcytometric markers in MSCs/TAFs – tumor cells cocultures

Medium type	MSCs medium (%)	Tumor cells' medium (%)	TAFs Medium (%)	Tumor cells' medium (%)
CD14	33.90	34.22	-	-
CD29	50.24	70	-	-
CD44	68.66	66.04	37.95	65.45
CD45	32.91	33.52	1.42	1.33
CD90	94.44	86.88	69.18	77.49
CXCR4	31.84	31.29	41.37	14.95
HLA-DR	-	-	44	26.52
Citokeratina	-	-	34.47	21.29
Nestina	-	-	18.87	18.79
α-SMA	-	-	33.95	23.94
Cellular type	**MSCs**	**MSCs**	**TAFs**	**TAFs**

DISCUSSION AND CONCLUSIONS

The in vitro model of cocultures between MSC / TAF and bulk tumor cells proves to be a functional model for analysis of tumor cells behavior, similar to the conditions encountered in vivo. MSCs and TAFs, used as cellular substrate in the coculture models exhibit a profound alteration of morphological aspect, induced by culture conditions. Morphological changes are associated with alterations of phenotypical markers expression of cells in coculture, as well as modifications in sectretor profile of these cells. MSCs retain the initial characteristic of stem cells (CD90, CD44 expression), while TAFs present a decresed expression of these markers when cultured with tumor cells, in coculture conditions. Several cytokines are secreted within the culture medium, but the results converge to the conclusion that they caould be responsible for decreased cellular proliferation, for both cellular substrate components, as well as for tumor cells.

The hypotesis of tumor-fibroblast interactions to critically depend on fibroblast and tumor cell type, as well as on the proportion of cocultured cells was elaborated by Olumi et al. since 1998 (10). A tumor-promoting effect of TAFs is supported by the more recent *in vivo* and *in vitro* observation of Spaeth et al. (11), who demonstrated tumor-derived fibroblasts to suppress tumor cell apoptosis. Although being inconsistent, the literature data clearly imply the source of fibroblasts (normal, embryonic, tumor-derived) as a critical determinant in tumor cell response. Both cell-cell and cell-matrix interactions, as well as paracrine factors are found to be involved in this dynamic process.

According to the expression profile depicted in our previous paper, TAFs produce a multitude of paracrine immune-modulators, including chemokines, cytokines and peptide growth factors (8,9), while the correspondent gene expression seem to be increased for almost all these factors, while TAF/MSC are put in contact with tumor cells. Lack of deactivation of fibroblasts contributes to persistence of inflammatory infiltrate and prolonged inflammation. Accordingly, stromal cells are considered santinels in chronic inflammation as they essentially contribute to leukocyte migration to the site of damage and influence the local immune response (12,13). It is well known that chronic inflammation and malignancies show some molecular and cellular similarities with the regard to cytokine profile (14,15).

MSCs and TAFs could induce an increase of the *in vitro* proliferation of tumor cells, but their effect could be different in vivo, due to intervention of the immune system. They profoundly contribute

to the creation of a tumor microenvironment that inappropriately promotes expansion and dissemination of neoplastic cell population by determining a tumor-specific profile.

Far from being complete, our present study gives an overview and discusses how MSCs and TAFs directly and indirectly affect tumor growth and propagation in order to stimulate consideration of stromal cells as novel therapeutic target in solid tumors. Recapitulation the modified expression profile of MSCs and TAFs in coculture with normal corresponding cells, some examples are given to stress the potential impact of stromal cells, especially TAFs on tumor progression, i.e. TAF-derived signals resulting in angiogenesis and immune dysfunction.

Acknowledgements

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INTERACTIUNEA DINTRE MICROMEDIUL TUMORAL SI COMPARTIMENTUL STROMAL – Partea II

REZUMAT

Micro-mediul celular tumoral complex include celule imunocompetente si inflamatorii, celule endoteliale si fibroblaste. Aceste tipuri celulare ar putea avea o influenta critica in procesul stadial al carcinogenezei si inducerea fenotipului malign. Se stie ca fibroblastele participa la reactiile imune din timpul injuriei tisulare prin modularea mediului local celular si citokinic, ajustand cinetica si componentele infiltratului inflamator, precum si prin modularea statusului functional al celulelor imunocompetente. Scopul acestui studiu a fost investigarea rolului fibroblastelor tumorale (TAF) in imunosupresie, cu efect important asupra dezvoltarii celulelor tumorale.
TAF umane au fost izolate din piese de rezectie chirurgicala provenite din cancere mamare, fiind cultivate si expandate in vitro in culturi celulare monostrat. Supernatantele culturilor celulare la diferite pasaje au fost colectate si determinata prezenta IL-4, IL-10, IL-13, TGF-β1, TNF-α, INF-γ si VEGF prin metoda ELISA. Pentru analize flowcitometrice au fost folositi anticorpi specifici conjugati cu fluorocromi, care sa identifice expresia markerilor de suprafata fibroblastici - CD14, CD117, CD90, CD106, CD44, CD29, CD73, HLA-DR, CXCR4. Expresia proteinelor citoscheletice si ale matricei extracelulare (vimentina, α-SMA) a fost detectata prin imunohistochimie.
Secretia citokinelor cu efect imunosupresor direct, IL-10 si IL-13, precum si a altor citokine, IL-4 si TNF-α, a fost crescuta pentru TAF in cultura, indiferent de pasaj. Cresterea producerii de TGF-β1 in supernatantul TAF ar putea fi un factor important in homeostazia tumorala. Expresia moleculelor CMH clasa II (HLA-DR) pe suprafata fibroblastelor activate contribuie suplimentar la imunosupresie avand un efect pro-tumoral. Nivelul crescut de VEGF sugereaza ca fibroblastele tumorale nu confera doar support, ci si molecule pro-angiogenice necesare neovascularizatiei tumorale. Expresia α-SMA este variabila si este asociata cu un status fibroblastic activat si agresiv. Complexa retea de efecte induse de TAF prin cresterea expresiei unor factori care dezactiveaza sau suprima celulele imune ar putea fi considerata pentru strategii terapeutice viitoare bazate pe TAF.

Cuvinte cheie: MSC, TAF, micromediu

THE ANALYSIS OF STATE OF TENSION AND STRAIN AT THE LEVEL OF CERVICAL VERTEBRAE (ATLAS) THROUGH FINITE ELEMENT METHOD

MACIOVAN ADINA
Family doctor, Soc.Civ.Med. DUO-MED, Timisoara, Romania

ABSTRACT

Objective: the analysis of state of tension and strain at the level of cervical vertebrae (atlas) through finite element method.
Method and material: We started with CAD modelling of biomechanic structures, realising a model of sustainability of the cervical C1 vertebrae, after which we performed the analysis states tension and strain at the level of cervical C1 vertebrae using finite element method. For elaboration of the solid CAD model was used tomography scanning of a real human atlas. The model developed was subjected to different loads through successive iterations similar to those governing the spine in a car accident.
Results: Higher or lower incidence of certain injuries is not random, and can not be charged solely to the conditions of the accident occurrence, but is implicitly determined by the structural characters of the vertebrae and the size of the loading forces.
Key words: cervical vertebrae C1, tension, deformation, resistance

INTRODUCTION

The achievement of CAD models of the cervical vertebrae, which covers the four basic phases of reverse-engineering in the geometric modelling namely: data acquisition phase, stage of data pre-processing, the stage delimiting the areas and construction of the surfaces, construction phase of the solid geometrical model CAD.

The phase of data acquisition treats the imagistic of the vertebrae transverse sections through the process of tomography scanning. The phase of data pre-processing sets in detail the geometric reconstruction of the vertebrae surfaces starting from cross section images obtained by tomography scanning. Reconstructed surfaces of the vertebrae form the triangulation network, obtained by the technique of bevelling modelling, this technique being described in full in the introductory chapter.

MATERIALS AND METHODS

In this stage appear the itinerary realising the triangulation network using the program of MATERIALISE Mimics 10.01. It is important to mention that physical object surface detail fidelity, whose geometric reconstruction was performed after its tomography scanning, depends on the accuracy of the cross sectional images of self-performance and implicit on the performances of the tomography scanning equipment. Tomography equipment of the last generation is capable of very high performance in terms of achieving cross-sectional images of any physical object.

Therefore the use of performant tomography equipment in conjunction with the adoption of appropriate programs of geometric reconstruction leads to impressive results in the field. In general, following completion of the triangulation network, there may be defects of *discontinuities* (gaps) type, which necessarily must be removed by filling operations. These operations were performed under the Rapidform XOR program. After carrying out the operations of filling gaps and refining the triangulation networks it results a number of 374,314 facets for the cervical vertebrae, the average size of 0.2 mm sides. With in the phase of *areas delimitation and surfaces construction,* triangulation network is divided into a multitude of areas (regions) taking into account the variation of the curves as there are oriented the facets in space. Operation of delimitation of areas or regions is also called *segmentation.* On the basis of regions resulting from segmentation operation is performed the3D curved surfaces that mimic the geometry of the scanned object 3D, such areas are interconnected by criteria of continuity and tangency. The 3D curved surfaces may be of Bézier or NURBS type. In other words, within the stage, it is realised the transition from the multitude of flat triangular surfaces (facets) of the triangulation network to the multitude of 3D curved surfaces. In the case of the C1 vertebrae, the stage of areas delimitation and creation of surfaces was conducted throughout the Rapidform XOR program. Basically, were obtained 2.165 surfaces for the cervical vertebrae. In the future compatibility of Bézier surfaces with CAD platforms, they are saved in an IGES file.

The CAD solid geometrical model construction phase realises the compatibility at the level of representation and consistency of Bézier surfaces with CAD platforms. This compatibility depends entirely on the type of the file that stores information about the Bézier surfaces, file made at the completion of the earlier phase. Therefore, generally the surfaces of

Received May 2010. Accepted June 2010. Address for correspondence: Maciovan Adina, MD, PhD student, Family doctor, Eftimie Murgu Square No. 2A, 300041 Timisoara, Romania, Phone: +40256215584

Bézier type are saved in IGES files, these files having 100% compatibility with most CAD platforms. Also during this phase it is realised the ''hardening'' of the geometrical model by taking into account the volume defined by the multitude of the Bézier surfaces and the possibility of linking this volume of some mass properties specific to a considered material. In case of the cervical vertebrae, the operations afferent to the phase were realised in the CAD SolidWorks 2005 program. Analysis of the stress and strain states at the level of the cervical vertebrae with the help of the Finite Element Method (FEM) begins with a brief description of the method. The proximate character of the FEM results pursuant to the fact that the real geometry is always replaced with a network of finite elements seeking the real form, but it can not be exactly reproduced only for some particular geometry, due to finite number of elements, and the unknown measures of the problems are calculated only in structure nodes. It follows the conclusion that the accuracy of calculation increases with the increase of the number of finite elements.

In general, structures of resistance, whose geometric shape present a plane of symmetry (i.e. the structures may be considered at geometric level as two ''halves'' identical mirrored),it is analysed with FEM at the level of a single ''half'' of the considered structures. As to the conditions applied in the FEM analysis, one can say that the CAD solid geometric model on half of a symmetrical structure resistance is embedded in the absent part (the half in the mirror) not represented in the model. For these reasons, CAD models of the two vertebrae were analyzed with FEM taking into account only half of their geometry.

As main objective of the analyses with FEM it consists the distribution method of the total tensions throughout each vertebra in part, tensions due to forces developed in various impact situations on the vertebrae, based on the fact that maximum values of tension during spread are known, respectively compression, supported by bone material. Also, as the main objective is to determine the values of the critical forces of impact that can cause fractures at the level of the C1 vertebrae.

The Software package with which the analyses with FEM were made, within the CAD SolidWorks 2005 program, is entitled COSMOSWorks 2005. Initially it is specified the type of study to be conducted. The type „Static Analysis'' was chosen because it is considered that the forces that are developed on the vertebrae are applied in a static regime. Once the study defined, the material of which the assembly is made, is declared. Because the SolidWorks 2005 and COSMOSWorks 2005 package do not include in the database of the material types the characteristics of the bone matter, a new sheet of material was created with the required characteristics (maximum tensions during stresses - compression, the longitudinal modulus of elasticity and the coefficient of Poisson's transverse contraction). It is noted that it was considered perfectly homogeneous and isotropic material, i.e its mechanical properties of resistance do not vary with the directions of application of forces. This fact was used as a simplifying assumption.

After declaring the type of study and specification of the material characteristics, it was chosen the type of discretization in the finite elements (operation called *meshing*). In this case it were used finite elements of the tetrahedral type with four nodes per element – TETRA 4 adopted for the FEM analysis of solid bodies. It was adopted a high accuracy of discretization of the two CAD models in half of the vertebrae, the average size of finite elements was 1 mm. The discretization of the cervical vertebrae model gathered 100,141 finite elements with a total of 19,479 nodes. Introducing the *boundary conditions or conditions on the outline* are materialized by declaring the *bearings* imposed on CAD models of the vertebrae (those areas considered fixed, that do not suffer of displacements or who suffer certain blocked displacements - displacements being targeted to the three fundamental axes: x, y and z). Also during this phase, over the CAD models of the vertebrae are applied *external charges* (external forces – direction of the force marked with the mauve shade on the figures) that take place after the appliance of some shocks (strokes) as effect of some situations encountered in various road accidents.

RESULTS

A series of FEM analysis were made, each analysis taking into account a possible scenario as a result of a road accident. In each analysis, the adopted value of the external force applied was determined by successive iterations, so that the total voltage σvonMises to reach the maximum allowable value (133 MPa), case in which it is considered the danger of fracture occurrence – the red shade on the figures show the risk areas for fracture occurrence. The possible scenarios considered for the cervical vertebrae are:

I) *frontal bump* (*anterior*) *horizontally* (Fig.1, 2, 3a, and 3b),

II) *back bump on vertical plan* (Fig.4, 5),

III) *side bump on horizontal plan* (Fig.6, 7, 8) and

IV) *vertically blow* as effect of the lateral compression of the cervical vertebrae (Fig. 9, 10, 11).

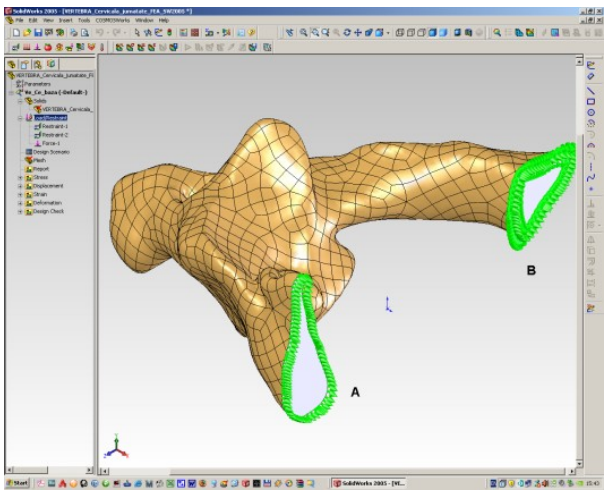

Fig.1. Cervical vertebrae - frontal bump (anterior) horizontally – leaning

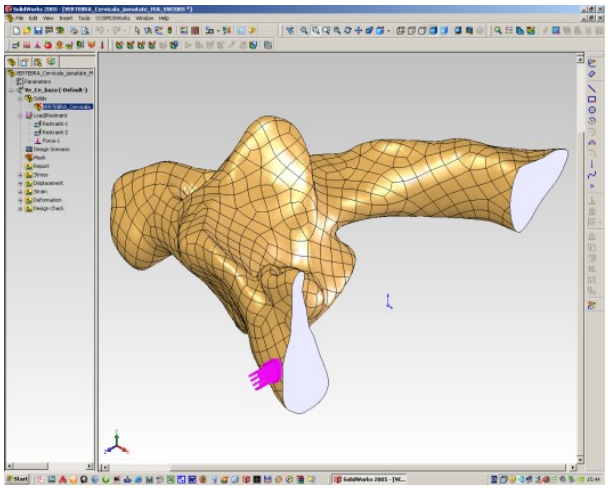

Fig.2. Cervical vertebrae - frontal bump (anterior) horizontally – charges

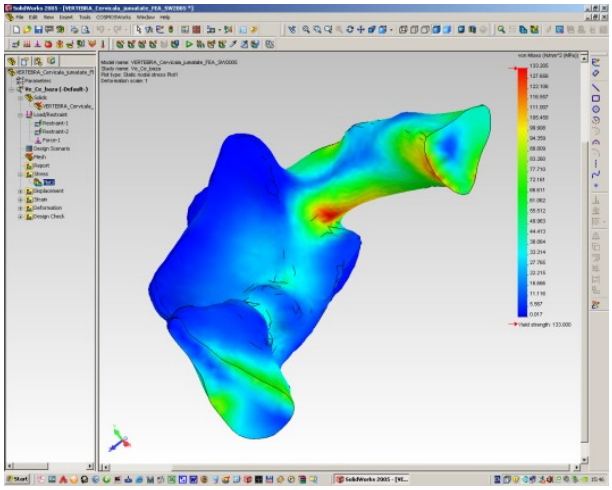

Fig.3. a) Cervical vertebrae - frontal bump (anterior) horizontally – deformations effects the red shade on the figures show the risk areas for fracture occurrence

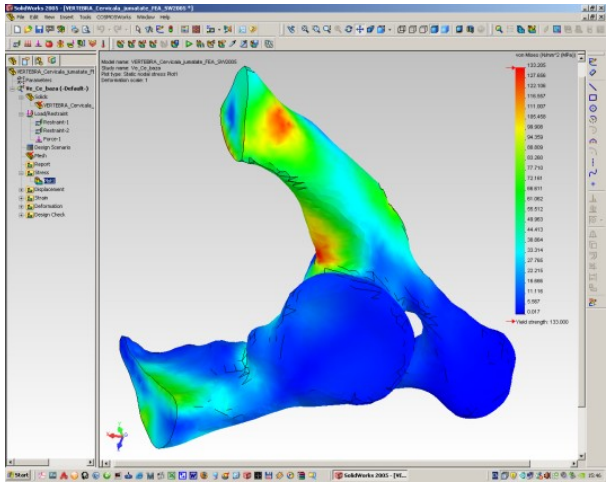

Fig.3. b) Cervical vertebrae - frontal bump (anterior) horizontally – deformations effects the red shade on the figures show the risk areas for fracture occurrence

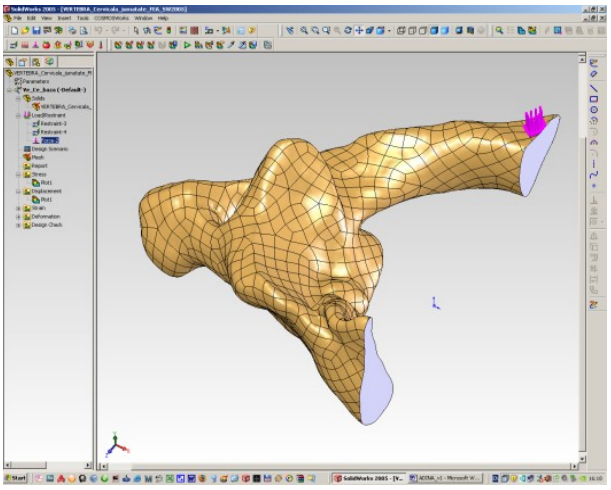

Fig.4. Cervical vertebrae - back bump on vertical plan – leaning

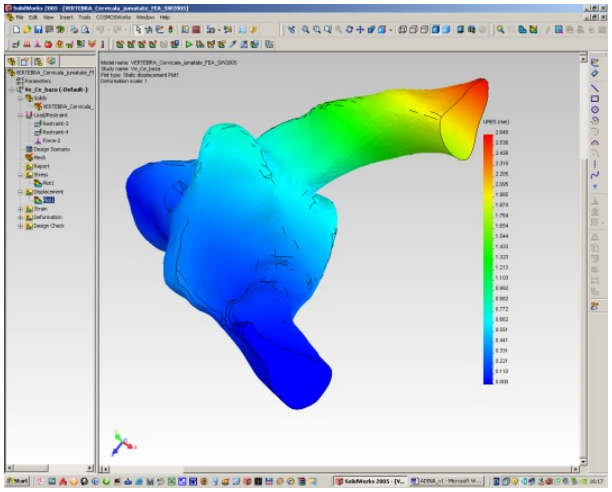

Fig.5. Cervical vertebrae - back bump on vertical plan

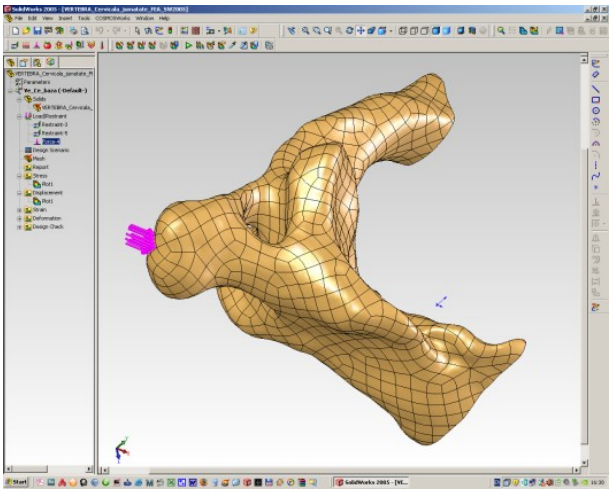

Fig.6. Cervical vertebrae - side bump on horizontal plan – leaning

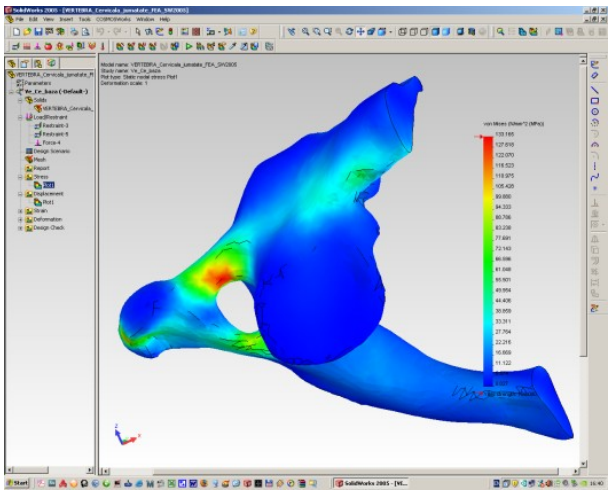

Fig.7. Cervical vertebrae - side bump on horizontal plan

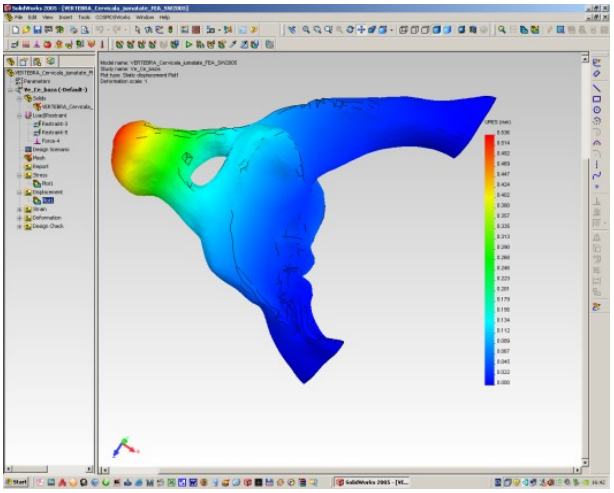

Fig.8. Cervical vertebrae - side bump on horizontal plan

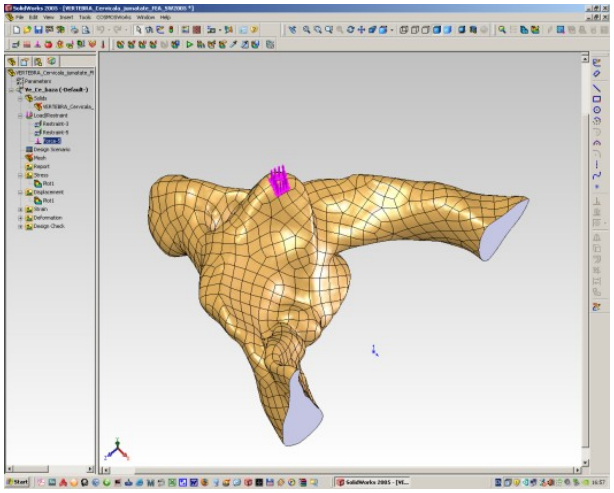

Fig.9. Cervical vertebrae - lateral compression – leaning

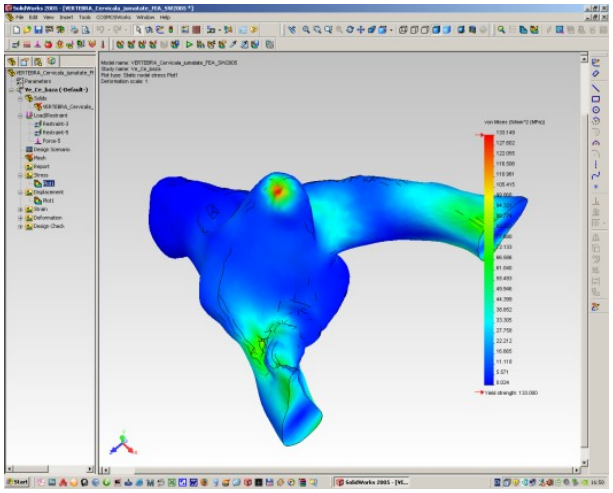

Fig.10. Cervical vertebrae **- side bump on horizontal plan vertically blow as ef-**fect of the lateral compression of the cervical vertebrae - deformations effects

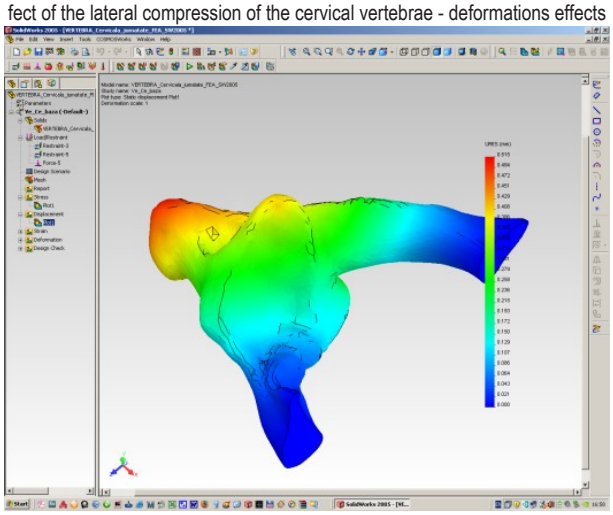

Fig.11. Cervical vertebrae **- side bump on horizontal plan vertically blow as ef-**fect of the lateral compression of the cervical vertebrae - deformations effects

CONCLUSIONS

Issue I): in case of an external bump applied to anterior side of the atlas body, the fracture risk is highest to the posterior spring and with maximum deformations to the anterior spring, the site of action of force (Fig. 2, 3a, and 3b) .

Issue II): in case of an external bump applied to the rear side of the atlas, to the posterior tubercle, the risk of fracture occurrence is highest to the anterior spring and the maximum deformations to the posterior spring, the site of action of force (Fig. 4, 5) . A similar force acts on the posterior spring direction movement hyperextension forced back by the head, following a forced flexion, given by frontal impact.

Issue III): in case of an external lateral bump applied to the transverses apophyses, the risk of fracture occurrence is highest to the lateral pedicles and the maximum deformations at the transverses apophyses, in the site of action of force (Fig.6,7, and 8). Such a force can be printed by forced flexion movement of the head, or by direct kick, side impact printer car accident.

Issue IV): in case of an external bump in vertical plan with

the lateral compression effect over cervical vertebrae to the transverses apophyses, the risk of fracture occurrence is highest to the shock place and increase to the vertebral spring; the maximum deformations is to the transverse apophysis (Fig. 9, 10, and 11). Such a mechanism is involved in producing Jefferson fracture type.

After conducting the analysis with MEF afferent to each scenario in hand, it can come off a first conclusion, perhaps the most important one, the one related to the resistance of the vertebrae, the maximum resistance being given by the highest value of the external load applied in various situations of possible scenarios. Thus, *the cervical vertebrae* presents the maximum resistance in case of a horizontal lateral bump (734 N) and the minimum resistance in case of a vertically back bump (260 N).

In terms of deformations (movements) for maximum results, we considered favourable the situations in which they recorded the minimum values. Thus, the cervical vertebrae presents the minimum resultant deformation in the case of a vertical stroke as effect of lateral compression of the cervical vertebrae (0.515 mm) and the maximum resulting deformation for in the case of vertically posterior bump (2.646 mm).

The results of this study demonstrating that higher or lower incidence of certain injuries is not random, and can not be charged solely to the conditions of the accident occurrence, but is implicitly determined by the structural characters of the vertebrae and the size of the loading forces.

This study was included into the PhD thesis entitled "**Clinico-statistical considerations regarding the incidence and emergency treatment of fractures of the cervical and lumbar vertebrae caused by car accidents",** developed by doctoral student under Maciovan Adina, scientific supervisor: university teacher dr. Pompiliu Petrescu, U. M.F. "Victor Babes " Timisoara, dec. 2009.

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ANALIZA STARII DE TENSIUNE SI DEFORMATIE LA NIVELUL VERTEBREI CERVICALE ATLAS CU AJUTORUL METODEI ELEMENTULUI FINIT

REZUMAT

Obiectiv: analiza starilor de tensiune si deformatie la nivelul vertebrelor cervicala C1 cu ajutorul metodei elementului finit.
Material si metoda: Am pornit de la modelarea CAD a structurilor biomecanice realizand un model experimental solid al vertebrei cervicale C1, dupa care am efectuat analiza starilor de tensiune si deformatie la nivelul vertebrei cervicale C1 cu ajutorul metodei elementului finit. Pentru elaborarea modelului solid CAD s-a apelat la scanarea tomografica a unui atlas real uman. Modelul realizat a fost supus prin iteratii succesive la diferite incarcari, similare cu cele la care este supusa coloana vertebrala intr-un accident de automobil.
Rezultate: incidenta mai mare sau mai mica a anumitor leziuni nu este intamplatoare, si nu poate fi pusa numai pe seama conditiilor de producere a accidentului, ci este implicit determinata de caracterele structurale ale vertebrelor si de marimea fortelor de incarcare.
Cuvinte cheie: vertebra cervicala C1, tensiune, deformatie, rezistenta

SEROLOGICAL IMMUNOPATHOLOGY OF PATIENTS WITH MYASTHENIA GRAVIS

BOGDAN ISTRATE[1,2], CRISANDA VÎLCIU[2]
[1]Department of Neurobiology and Molecular Physiology,University of Bucharest, Romania
[2]Department of Neurology,Clinical Institute Fundeni,University of Medicine and Pharmacy, Bucharest, Romania

ABSTRACT

Acetylcholine receptor (AChR) antibodies are present in sera from 80-90% of patients with generalized Myasthenia Gravis. Besides AChR antibodies, in serological immunopathology of myasthenia, other antibodies are incriminated, such as MuSK, anti-titin, anti-ryanodine receptor (RyR), anti striational antibodies. These antibodies are responsible of complement activation. In mysthenic sera complement components like C3 and C4 are detectable too in variable concentrations, depending by the medication and the clinical course of the disease. MG is treated with cholinesterase inhibitors or/and with immunosuppressive drugs. Surgical removal of the thymus (thymectomy) may result in permanent remission or reduced needs of drug therapy. These factors mentioned above could modify in a significant way the immunoserological profile of the myasthenic patients, allowing in the same time a good monitoring of the disease.

Key words: myasthenia gravis, serologic tests, immune markers

INTRODUCTION

Myasthenia gravis is a chronic autoimmune disease that results in progressive fatigue, loss of muscular tonus and increasing paralysis. These symptoms are caused by inappropriate activation of complement resulting in an immune response directed against the nicotinic acetylcholine receptor (AchR) which leads, to impairement of neuromuscular transmission. Myasthenia gravis may occur in association with other autoimmune diseases. Besides AChR muscle antibodies, with regards on serological immunopathology of Myasthenia Gravis, antibodies such as against titin (1), and ryanodine receptor(5), are present in many sera from patients with thymic involvement or late –onset Myasthenia. The impairment of neuromuscular transmission caused by AChR antibodies implies complement-mediated focal muscle membrane damage (2), with accelerated degradation of AChR (3).

Our goal in this study was to measure the levels of AChR, C4, and IgG (because anti-AChR antibodies are Ig G type) in mysthenic sera and to correlate these concentration in thymectomized and non-thymectomized M.G. patients according to complement involvement, Ig G and anti-AChR antibodies.

PATIENTS

Thirty (male/female: 26/4) MG patients were included. Sixteen were thymectomized, three years ago and the rest fourteen, were not thymectomized. Seven patients had additional autoimmune disorders: three female patients had autoimmune thyroiditis, another two female patients had systemic lupus erythematous, one female patient had chronic evolutive polyarthritis and one male patient had autoimmune inflammatory myophaty. All patients have received immunosuppressive therapy at the time when blood samples were collected.

METHODS

All sera were stored at -80°C and at -20°C before analysis,and treated in the same way. The same serum sample of each patient was used for determination of AChR antibodies, IgG and C4 complement component.

Sera were brought to room temperature prior to analysis for C4 complement factor by laser nephelometric assay on a Behring Nephelometry analyzer using rabbit polyclonal antiserum to purified human C4, according with manufacturer instructions (Dade Behring).

Ig G was measured by the immunonephelometric determination using reagents of Dade Behring (Marburg GmbH). AChR muscle auto-antibodies were measured by standard radioimmunoassay, using AChR, RRA kits (4).

Reference intervals for the parameters determined were the following: 10-40 mg/dl - for C4 complement compound, 700-1600 mg/dl - for IgG, and < 0.4 nmol/l - for anti-AChR antibodies.

RESULTS

For the patients included in this study, we have performed the immunoserological analysis including anti-AChR ab, C4 complement compound and IgG (Table I).

Received May 2010. Accepted June 2010. Address for correspondence: Bogdan Istrate, MD, Department of Neurobiology and Molecular Physiology, University of Bucharest, Romania, e-mail: bogdan_ellis@yahoo.com

Table I. Immunoserological values of thymectomized / non-thymectomized patients with myasthenia gravis

	Thym-ectomy	Anti-AChR ab (nmol/l)	C_4 (mg/dl)	IgG (mg/dl)
1.	Yes	126	32	803
2.	Yes	250	27	1419
3.	Yes	19	27	998
4.	Yes	120	32	954
5.	Yes	50	39	1314
6.	Yes	9.5	25	744
7.	Yes	125	43	607
8.	Yes	42	25	827
9.	Yes	22.47	44	1781
10.	Yes	12	29	828
11.	Yes	16.98	51	831
12.	Yes	11.33	38	701
13.	Yes	274	42	1631
14.	Yes	25	64	1210
15.	Yes	134	65	884
16.	Yes	211	34	872
17.	No	0.4	34	912
18.	No	0.4	30	747
19.	No	50	32	666
20.	No	9.5	25	744
21.	No	0.4	17	874
22.	No	0.4	17	905
23.	No	0.4	25	1385
24.	No	0.2	32	1111
25.	No	4.25	31	1901
26.	No	0.4	36	1515
27.	No	0.21	71	1612
28.	No	0.1	58	928
29.	No	0.8	61	318
30.	No	0.5	72	611

Except this, we tried to see the relationship and the concordance/correlation between anti-AChR ab, C4 and IgG, in sera of thymectomized and non-thymectomized patients with myasthenia gravis (Fig.1 and 2).

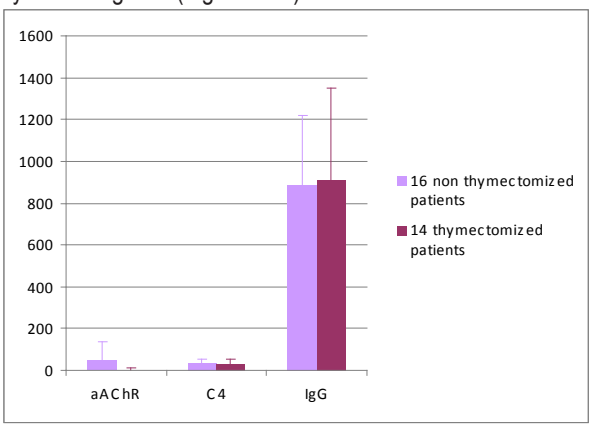

Fig. 1. Relevance of anti-AChR antibodies,C4 and IgG in myasthenic sera, for thymectomized and non thymectomized patients. The error bars, represents mean ± SD

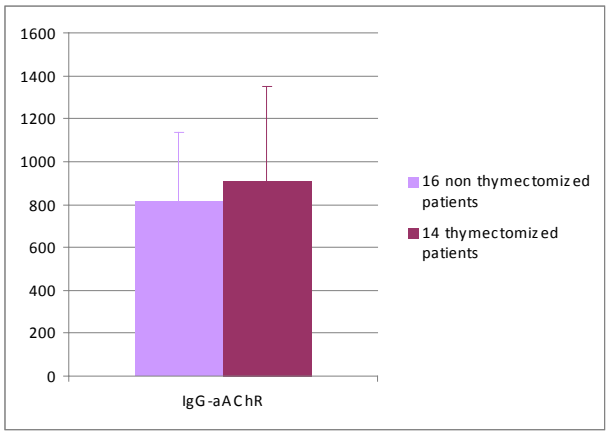

Fig. 2. Correlation between IgG and anti-AChR antibodies in myasthenic sera. The error bars, represents mean ± SD

Thymectomy can induce a considerable reduction in IgG concentrations and an increase in level of C4 (Fig.3).

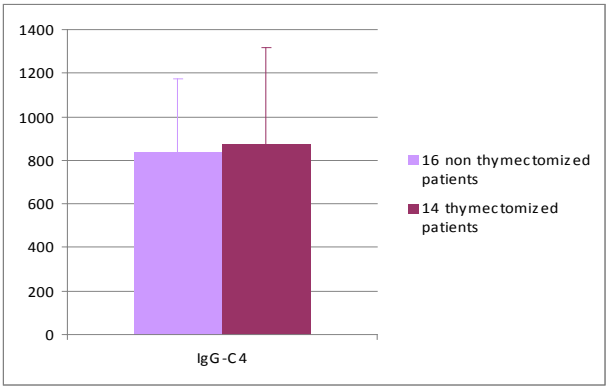

Fig.3. Correlation between IgG and C4 in myasthenia gravis sera. The error bars,represents mean ± SD

The ratio IgG-ANTI-AChR ab/IgG-C4, show a lower level in non-thymectomized patients, and a high level in thymectomized patients due to the other associate autoimmune disease and to the immunosuppressive therapy (Fig.4).

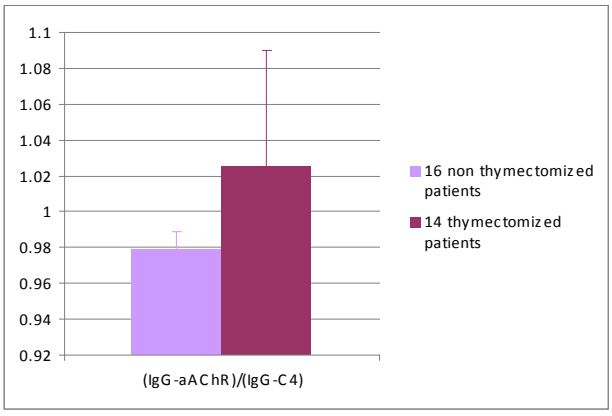

Fig.4. Ratio between IgG-anti –AchR antibodies and IgG-C4 in myasthenic sera.The error bars,represents mean ± SD

CONCLUSIONS

The important immunological dyscrasia between anti-AChR ab, IgG and C4 complement compound is due to the fact that all patients taken in this study have received immunosuppressive therapy and surgical treatment, which could significantly influence the humoral immune response.

The complement mediated cell lysis or phagocytosis could play a role in the pathogenesis of myasthenia gravis.

The anti-AChR antibody titer does not correlate with clinical course of the disease. The detection of anti-AChR antibodies helped us to establish the type of myasthenia: sero-negative or sero-positive.

Other immunological elements exist, in order to moderate the immune response in myasthenia gravis, such as: anti-MuSK ab, CIC, anti-striational antibodies, and anti-RyR antibodies.

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Abbreviations and defining terms

Anti-AChR ab. Anti-acetylcholine receptors antibodies
Anti-MuSK ab. Anti muscle specific tyrosin kinase antibodies
Anti RyR ab. Anti-Ryanodine receptor antibodies
CIC. Circulating immune complexes
C4. Compound of complement system
IgG. Immunoglobulin G
SD. Standard deviation

PATOLOGIA IMUNOSEROLOGICA A PACIENTILOR CU MIASTENIA GRAVIS

REZUMAT

Anticorpii anti-receptor pentru aceticolina (AChR), sunt prezenti in serul a 80-90% dintre pacientii cu forma generalizata a Miasteniei Gravis. Pe langa anticorpii anti-receptor pentru acetilcolina, in patologia imunoserologica a miasteniei sunt incriminati si alti anticorpi precum anticorpii anti-tirozinkinaza muscular specifica (MuSK), anticorpi anti-ryanodina (RyR), anticorpi anti-fibra musculara striata. Acesti anticorpi sunt capabili de a activa si sistemul complement. In serul pacientilor cu miastenie, componentele C3 si C4 ale sistemului complement sunt detectabile de asemenea in concentratii variabile, depinzand de medicatie si de evolutia clinica a bolii. Miastenia Gravis este tratata cu inhibitori ai colinesterazei si/sau cu medicatie imunosupresiva. Extirparea timusului (timectomia), poate avea ca rezultat remisiunea permanenta a bolii sau reducerea dozelor tratamentului medicamentos. Acesti factori, anterior mentionati, pot modifica intr-un mod semnificativ profilul imunoserologic al pacientilor cu miastenie, permitand in acelasi timp o buna monitorizare a bolii.

Cuvinte cheie: miastenia gravis, teste serologice, markeri imuni