ISSN 0254-2307

Romanian Journal of Gerontology and Geriatrics

Prof. Acad. Ana Aslan M.D.

ESTABLISHED 1980

Volume 5, No. 1, 2016, New Series

Contents

Cuprins

THE EFFECT OF GEROVITAL H3 UPON CARDIAC CONTRACTILITY IN PATIENTS WITH MITRAL VALVE INSUFFICIENCY

***Revnic** Cristian Romeo [1], **Pena** Cătălina, **Prada** Speranța [2], **Revnic** Flory [2], **Prada** Gabriel [2,3]*

[1]Ambroise Pare Hospital, Pierre et Marie Curie University,Paris VI,France
[2] Ana Aslan National Institute of Gerontology and Geriatrics, Bucharest, Romania
[3]University of Medicine and Pharmacy"Carol Davila,Bucharest ,Romania

Corresponding author: Flory Revnic f_ revnic@yahoo.com

Abstract. Objective: The aims of our study were related with investigation of: 1) Mg ATP ase activity in left ventricle and atrium from patients with mitral valve insufficiency (MVI) versus controls. 2) Uptake of 3H Ouabain in left ventricle papillary muscles from Controls, MVI and MVI incubated with different Gerovital H3 (GH3) concentrations in order to see the effect of GH3 upon glycosidic receptors affinity, for 3H Oubain. 3) Effect of GH3 upon 3H ATP uptake in left ventricle pappilary muscle from Controls, MVI and MVI incubated with GH3. 4) The ionic behaviour as well as the change density upon contractile proteins from left ventricle papillary muscle from MVI versus Controls in Rigor (Ri), Contraction (Co) and Relaxation (Re) medium in the presence of Na^{22} radionuclid. Material and method: Biopsies from left ventricle pappilary muscle and from left atrium have been taken during surgery on open heart for MVI and from Controls free of cardiovascular pathology that died on road accidents. Biological material has been used for: assaying Mg ATP-ase activity in left ventricle and atrium from MVI patients and Controls using standard biochemical methods and left ventricle fragments for 3H Oubain, 3H ATP and Na^{22} radioisotope uptake studies using standard biochemical and radioisotope uptake methods. Results: A significant reduction in Mg ATP-ase activity and in content of myofibrilar proteins has been recorded in heart fragments from MVI patients in presence of 1mM $MgCl_2$. The depressed activity suggests that the interaction between thick and thin filaments within myofibrilar lattice may be abnormal. The decrease in charge density upon contractile filaments from MVI accounts for the fact that a certain proportion of cross bridges are no longer functionally efficient. Conclusions: Our data concerning the effect of GH3 have pointed out an effect upon glycosidic receptor increasing the permeability of membrane for Na^+. Also GH3 seem to act upon ATP-ase activity increasing its affinity for its substrate. These data are encouraging us in the attempt of using GH3 therapy in improving heart contractility in patients with MVI admitted in our Clinique for rejuvenation treatment.
Key words: mitral valve insufficiency, myofibrilar proteins, Mg ATP-ase, Gerovital H3, 3H Ouabain, 3H ATP, Na^{22} uptake

EFECTUL GEROVITALULUI H3 ASUPRA CONTRACTILITĂȚII CARDIACE LA PACIENȚII CU INSUFICIENȚĂ MITRALĂ

***Revnic** Cristian Romeo [1], **Pena** Cătălina, **Prada** Speranța [2], **Revnic** Flory [2], **Prada** Gabriel [2,3]*

[1]Spitalul Ambroise Pare, Universitatea Pierre si Marie Curie, Paris VI, Franta
[2]Institutul Național de Gerontologie și Geriatrie "Ana Aslan", București, Romania
[3]Universitatea de Medicina si Farmacie Carol Davila, București, Romania

Autor corespondent: Flory Revnic f_ revnic@yahoo.com

Rezumat. Obiective: Scopul studiului nostru a fost acela de a investiga: 1) Activitatea Mg ATP azei in fragmentele tisulare de atriu si ventricul stang de la pacientii cu insuficienta valvulara mitrala (MVI) comparativ cu martorii sanatosi. 2) Captarea 3H Ouabainei in mushiul papilar ventricular stang de la Martori, MVI si MVI

incubat cu diferite concentratii de Gerovital H3(GH3) in scopul obtinerii de date privind efectul GH3 asupra afinitatii receptorilor glicozidici pentru 3H Ouabaina. 3) Efectul GH3 asupra captarii de 3H ATP la nivelul biopsiilor de muschi papilar din ventriculul stang de la martori, de la pacientii cu MVI, precum si fragmente tisulare de la MVI incubate cu GH3. 4) Comportamentul ionic ca si densitatea de sarcini electrice de la nivelul filamentelor contractile glicerinate din muchiul papilar ventricular stang de la MVI comparativ cu martorii incubate in mediu de Rigor (Ri), Contractie (Co) si Relaxare (Re) in presenta radioizotopului Na^{22}. Material si metoda: Biopsiile recoltate de la nivelul ventriculului si atriului stang au fost recoltate intraoperator de la pacienti cu MVI iar cele de la martori au fost recoltate post mortem de la pacienti decedati in accidente rutiere. Materialul biologic a fost utilizat pentru: determinarea activitatii Mg ATP-azice in ventriculul si atriul stang de la pacientii cu MVI si de la martori, iar alte fragmente din ventriculul stang de muschi papilar au fost folosite pentru experimentele radioizotopice de captare a 3H Oubainei, 3H ATP si a radioizotopului Na^{22} utilizand metode biochimice si radioizotopice standard. Rezultate: S-a constatat o reducere in activitatea Mg ATP-azei de la nivelul atriului si a ventriculului stang si o reducere in continutul proteinelor miofibrilare de la pacientii MVI in prezenta 1mM $MgCl_2$. Activitatea scazuta a MgATP azei sugereaza faptul ca interactiunea dintre filamentele groase si cele subtiri in reteaua miofibrilara poate fi anormala. Reducerea in densitatea de sarcini la nivelul filamentelor contractile de la pacientii cu MVI este explicat prin faptul ca o anumita proportie de punti miozinice incrucisate nu mai sunt eficiente din punct de vedere functional. Concluzii: Datele noastre au evidentiat un effect al GH3 la nivelul receptorilor glicozidici,crescand permeabilitatea de membrana pentru Na^+. De asemenea, GH3 pare a actiona asupra activitatii ATP-zice crescandu-i afinitatea pentru substrat. Aceste date ne incurajaza in incercarea de a folosi terapia cu GH3 in imbunatatirea contractilitatii cardiace la pacientii cu MVI dependenti de medicatia glicozidica, admisi in Clinica de Geriatrie pentru tratamentul eutrofic .

Cuvinte cheie: insuficienta valvulara mitrala, proteine miofibrilare, Mg ATP-aza, Gerovital H3, captarea de 3H Ouabaina, 3H ATP, Na^{22}

INTRODUCTION

In normal adults, the mitral valve orifice is 4 - 6 cm^2, in the presence of significant abstruction i.e., where the orifice is less than about 2 cm^2, blood can flow from the left atrium to the left ventricle only if propelled by an abnormally elevated atrio-ventricular pressure gradient [1].

The depressed cardiac output in patients with mitral valve insufficiency (MVI) is related primarily to the obstruction of the mitral orifice but also may be due to the impairment of the function of either ventricle.

The role of sarcomere length

In all striated, including cardiac muscle, the force of contraction depends on initial muscle length. The sarcomere associated with the most forceful interaction is approximately 2.2 μm. At this length the two sets of myofibrils of the sarcomere are situated to provide the greatest aria for their interaction. In support of the sliding filament hypothesis force development diminishes in direct proportion to the decrease in overlapping between thick and thin filaments and the resultant reduction in the number of the active sites. The length of the sarcomeres appears to regulate the extent of activation of the contractile system i.e. sensitivity to Ca^{2+} which is also greatest at approximately 2.2 μm. When sarcomere length is increased to 3.65 μm, the thin filaments are entirely withdrawn from A band and no tension can be developed. Similarly, when the sarcomere are shorter than 2.0 μm, the thin filament bypass are another, doubly overlapping each other and so, reducing both the sensitivity of the contractile sites to Ca^{2+} and the capacity for force development remains constant in length. The relation between the initial length and the muscle fibre and the developed force is of the prime importance for the function of heart muscle. This forms the basis of the Frank-Starling law of the heart [2]. As muscle length decreases to the point at which sarcomere length approaches zero, the I bands at first narrow, then disappear while the A band remains constant in length.

This present study is concerned with molecular studies of cardiac contractility in patients with MVI, in order to improve our understanding of this condition and to learn how to deal with it.

Biophysical study of contraction cannot be achieved without understanding the ionic

transformations which undergoes in its activity. Contraction phenomenon in accordance with swelling theory is achieved by lateral expansion of hexagonal lattice of myofibrils [3]. Repulsive forces which appear from the ionic redistribution in diffusion layer when a filament is approaching another one are known as electrostatic forces [4]. These depend not only on the fixed charge upon filament but also on the radial position of charges. These forces are responsable for filament shortening. The large radius of the electric charges necessary to be acquired if the sub fragment 2 region of myosin tail (which carries ~ 1/3 of the negative charge) is tilted at 45^{o} angle toward filament axis [5].

Our previous studies [6] have pointed out a decrease in the active shortening capacity of heart sarcomere from patients with heart failure due to MVI. The aims of our study were related with investigation of: 1) Mg ATP ase activity in left ventricle and atrium from patients with MVI versus controls. 2) Uptake of 3H Ouabain in left ventricle papillary muscles from Controls, MVI and MVI incubated with different Gerovital H3 (GH3) concentrations in order to see the effect of GH3 upon glycosidic receptors affinity, for 3H Oubain. 3) Effect of GH3 upon 3H ATP uptake in left ventricle pappilary muscle from Controls, MVI and MVI incubated with GH3. 4) The ionic behaviour as well as the change density upon contractile proteins from left ventricle papillary muscle from MVI versus Controls in Rigor (Ri), Contraction (Co) and Relaxation (Re) medium in the presence of Na^{22} radionuclid.

DESIGN AND METHODS

Our study has been conducted on 30 subjects aged between 25 - 78 years. 15 patients with MVI admitted in Emergency Hospital for surgery on open heart and 15 controls free of cardiac pathologies that died in road accidents.

Left ventricle papillary muscle and atrial biopsies (50-100 mg) each have been collected from patients with MVI during surgery on open heart and from controls in the first 30 minutes after death. The fresh tissue has been used to assess Mg ATP-ase activity of heart sarcomeres.

The radioisotopes experiments of 3H Ouabain, 3H ATP and Na^{22} uptake have been done on glycerinated muscle. Glycerination procedure has been done in accordance with the published method [6] using 50% glycerol solution.

Fresh Ventricular muscle was freed of connective tissue and minced. The minced muscle was washed extensively with 0.05 M KCl 0.01 M potassium phosphate 1mM EDTA in a virtis hemogeniser for 60 seconds. The residue was suspended in 10 volumes of 0.05 M KCl 0.01 M phosphate pH 6.8. The triton washed myofibrils were extracted with 10 volumes of 0.47 M KCl - 0.02 M Na pyrophosphate 0.01 M phosphate pH 6.8 for 20 minutes.

The viscous mixture was centrifuged at 13.000 g for 30 minutes. The supernatant was diluted with 10 volumes of cold distilled water to precipitate crude myosin. The precipitate was collected at 2000 g for 30 min. and dissolved in KCl pyrophosphate buffer with 5 mM $MgCl_2$. Crude myosin solution was centrifuged at 100.000 g for 2 hours. The supernatant from this step was fractionated with a saturated solution of ammonium sulphate containing 10 mM EDTA. Myosin was dissolved in 0.5 M KCl, pH 7.0 and dialysed against 0.3 M KCl - 1 mM EDTA until it was free of sulphate ions.

ATP-ase activity was usually determined in the presence of 50 mM Tris maleate (pH 7.6), 10 mM $CaCl_2$ and 5 mM ATP in a final volume of 1.0 ml for 10 min. at $30^{o}C$. Tris chloride was the buffer for the determination of Ca^{+2} requirants [7,8].

Fresh and glycerinated heart fragments have been processed for radioactive uptake of 3H Oubain, 3H ATP and Na^{22}, have been processed in accordance with the published methods [6,9] and radioisotope studies have been performed using a Beta Berthold Scintillation Counter.

The radioisotopes used in our experiments have been purchased from Amersham.
3H oubain - specific activity 15 - 50 Ci / mmol.
3H ATP - Adenosine 5'triphosphate, tetrasodium salt (2, 8, 3, 4) - specific activity 30-50 Ci / mmol.
Na^{22} in aqueous solution - specific activity 100-1000 Ci / mg Na.

RESULTS

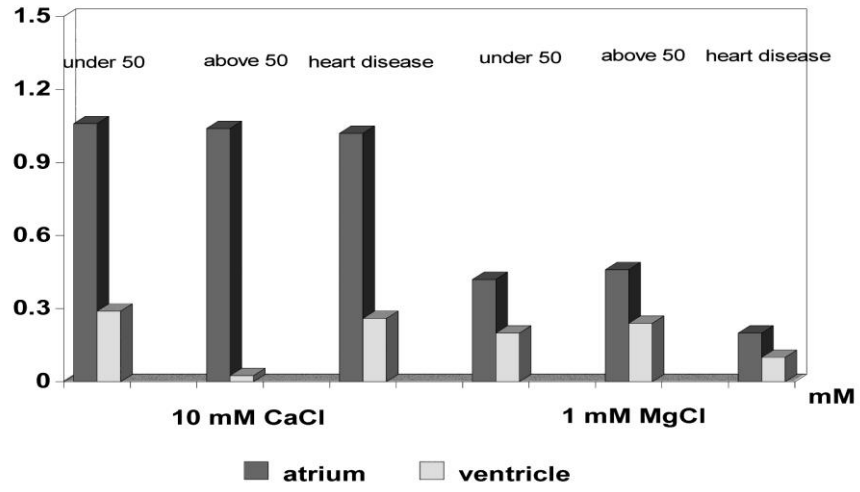

Fig. 1. Mg ATP ase activity in atrium and ventricle from controls and heart disease

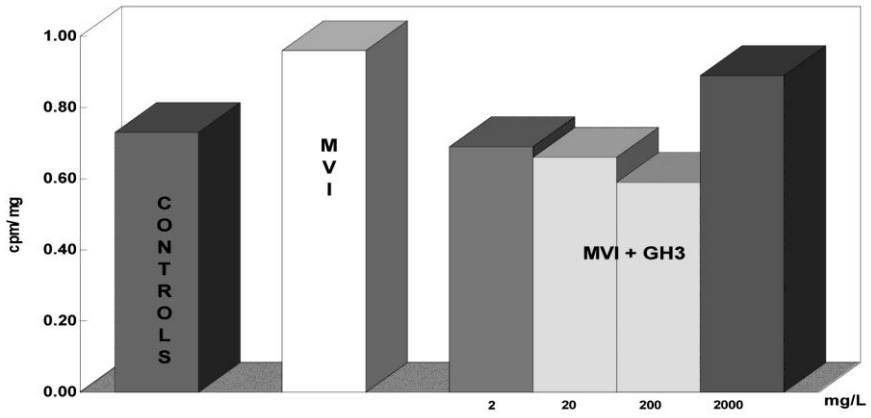

Fig. 2. 3H oubain uptake by the heart fragments from Controls, MVI and MVI +Gerovital H3 in different concentrations

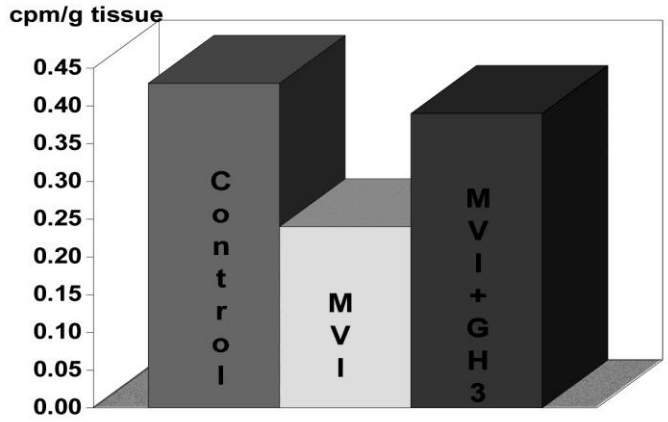

Fig. 3. The uptake of 3H ATP by heart fragments from controls, MVI and MVI + GEROVITAL H3

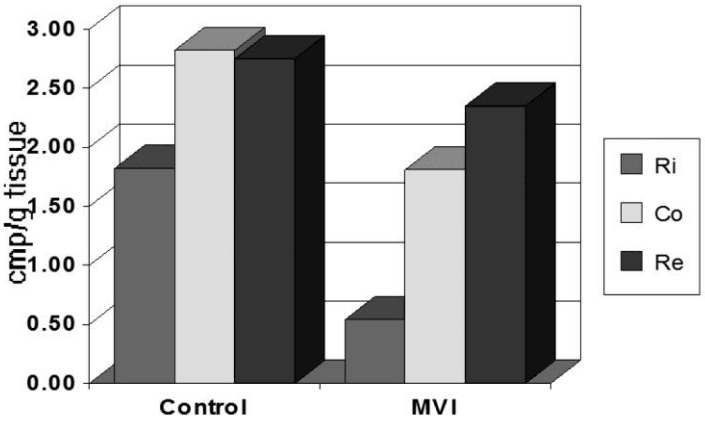

Fig. 4. Na^{22} uptake in pappilary glycerinated muscle from Control and MVI patients incubated in Rigor (Ri), Contraction (Co) and Relaxation (Re) medium

DISCUSSION

In Fig. 1 a significant reduction in Mg ATP-ase activity has been recorded in diseased myocardium in comparison with ageing hearts from controls. The depressed activity suggests that the interaction between thick and thin filaments within myofibrilar lattice may be abnormal even though the enzymatic activity of myosin is normal. A significant correlation has been made between Mg ATP-ase activity and sarcomere shortening capacity in diseased heart [10].

In ageing hearts, there is no modification in ATP-ase activity even a drop SH group content has been recorded. It seems possible that the loss does not imply S1 and S2 regulatory types of SH residues [9].

We can conclude that myosin from ageing heart is not affected from structural and functional point of view likes that from diseases heart [10].

In Fig. 2 there are presented the results of 3H oubain uptake by the heart fragments incubated in vitro with Gerovital H3 in the following concentrations: 2mg/l, 20 mg/l, 200 mg/l, 2000mg/l Gerovital H3 in comparison with controls. A decrease in 3H oubain uptake has been recorded when muscle fragments have been incubated with 200 mg/l GH3 versus controls.

This can be accounted for an increase in membrane permeability for Na^+.

These results may have some clinical implications for ageing people with heart failure dependent on glycosidic medication

admitted in our Clinique for rejuvenation cure.[6].

Fig. 3 presents an increase in 3H ATP uptake in heart muscle incubated with Gerovital H3 versus controls.

Gerovital H3 may have a stimulatory effect upon the contraction mechanisms in increasing the affinity of ATP-ase for ATP substrate [9].

Fig. 5 presents the uptake of Na^{22} in (Ri), (Co) and (Re) state in heart fragments from MVI in comparison with controls.

Investigation of charge density upon contractile proteins in contraction by means of Na^{22} uptake experiments, has pointed out a decrease in its uptake which accounts for a reduction in contractile capacity of heart sarcomeres from MVI patients [11].

CONCLUSIONS

A significant reduction in Mg ATP-ase activity and in content of myofibrilar proteins has been recorded in heart fragments from MVI patients.

The depressed activity suggests that the interaction between thick and thin filaments within myofibrilar lattice may be abnormal.

The decrease in charge density upon contractile filaments from MVI accounts for the fact that a certain proportion of crossbridges are no longer functionally efficient.

Our data concerning the effect of Gerovital H3 have pointed out an effect upon glycosidic receptor increasing the permeability of membrane for Na^{+}.

Also GH3 seems to act upon ATP-ase activity increasing its affinity for substrate.

These data are encouraging us in the attempt of using GH3 therapy in improving heart contractility in patients with MVI admitted in our Clinique for rejuvenation treatment.

Acnoweledgements. This study has been supported by Medical Academy Research Grant 1995-1998: Experimental and paraclinical studies regarding the effect of Gerovital H3 upon functional capacity of cardiomiocytes in patients with/without cardiac pathology, in cooperation with V.Babes Institute, Laboratory of Molecular pathology and Radiopathology.

REFERENCES

[1] *Harrison's Principles of Internal Medicine*. 13th ed., 1994, vol.1, p.1020, Isselbacher, Braunwald.

[2] Nejad N.,Xu Y (). *Effect of muscle length on cross-bridge kinetics in intact cardiac trabeculae at body temperature.* J Gen Physiol, 2013.,Jan; 141(1):133-9.

[3] Collins, E. W., and C. Edwards.*Role of Donnan equilibrium in the resting potentials in glycerol-extracted muscle.* Am. J. Physiol., 1971,221:1130-1133.

[4] Bie Q-M, Togashi A, Sasaki TN, Takano M, Sasai M, et al. *Coupling of Lever Arm Swing and Biased Brownian Motion in Actomyosin*. PLoS Comput Biol, 2014,10(4): e1003552. doi:10.1371/journal.pcbi.1003552

[5] Elliott, G. F., G. R. S. Naylor, and A. E. Woolgar. *Measurements of the electric charge on the contractile proteins in glycerinated rabbit psoas using microelectrode and diffraction effects. In Ions in Macromolecular and Biological Systems.* (Colston Papers No. 29.) D. H. Everett and B. Vincent, editors. John Wright & Sons Ltd.,Bristol, 1978, 329-339.

[6] Revnic F., N., Teleki N., Zeana C. *New trends in management of cardiac insufficiency in elderly patients.* Geriatria, 1996, Suppl.vol.VIII,nr.5,Sett./Ottobre, 229-230.

[7] Alpert A. *Myofibrillar adenosine triphosphatase activity in congestive heart failure.* Am. J. of Physiol., 1962, 202, 940.

[8] Fiske C. *The colorimetric determination of phosphorus*. J.of Biol.Chem., 1925, 66, 375.

[9] Dumitru M, Floarea Revnic, S.Hulea. *Quantitative determinations of contractile proteins and myosin SH groups in human heart from young and old patients The influence of eutrophic treatment with Gerovital H3*. Rom.J.Geront.&Ger. ,1988,Suppl.,nr.1,Tome 9, pag 42.

[10] Farid Moussavi-Harami, Maria V. Razumova, Alice W. Racca, Yuanhua Cheng, April Stempien-Otero. *2-Deoxy adenosine triphosphate improves contraction in human end-stage heart failure*. Journal of Molecular and Cellular Cardiology, 2015,79, 256–263.

[11] Revnic S, Revnic F. *Structural and enzymatic comparison of human cardiac myosin from young and old patients with or without MVI*. J.Muscl.Res.&Cell Motil., 1992,13,2, pp.62.

LIFESTYLE AND FRAILTY PREVENTION IN ELDERLY

***Roditis** Doina [1], **Lupeanu** Elena [1], **Bârsan** Monica [1], **Tronaru** Maria [2]*

[1] "Ana Aslan" National Institute of Gerontology and Geriatrics, Bucharest, Romania
[2] "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

Corresponding author: Doina Roditis elena.roditis@gmail.com

Abstract. Frailty is a potentially reversible clinical syndrome through targeted therapeutic actions if detected early. The study aims to evaluate factors contributing to frailty and point out healthy lifestyle elements in a NIGG inpatients sample. Material and method: A sample of 214 patients (185 women and 29 men), $\overline{X}$ =68.8 years, is evaluated with a test battery using: ▪ Groningen Frailty Index (GFI); ▪ Mini Nutritional Assessment (MNA); ▪ Simple Lifestyle Indicator Questionnaire (SLIQ); ▪ADL and IADL; ▪ the Lubben Social Network Scale–6(LSNS-6) and ▪ income size. Results: The sample has a18.7% frailty prevalence. Using a global health assessment, the obtained variables are correlated with the frailty score. The resulting significant correlations match the classification of the factors contributing to frailty (after J Puxty). The most important correlations, ranked after the correlation intensity, are: **1**- polypharmacy (r=0.350/p=0.000); **2**- nutritional risk (r=0.341/p=0.001); **3**-slow gait (r = 0,329/ p=0.000) and **4**-psychiatric illnesses (r=0.229/p=0.001). On the **5**-th place is the correlation between frailty and "Lubben friends-subscale", which reveals marginal friendship ties percents (of 45%) in both groups: the pre-frails and the frails. So, we can say that the isolation tendency represents an (early) risk factor for frailty. Conclusions: the best suited lifestyle habits for preventing frailty are: avoiding polypharmacy, healthy eating, active physic life; also very important are: avoiding stress, rest, harmonious family and society relationships that have a protective role against frailty, and a beneficial effect on psycho-cognitive problems.
Key words: prevention, frailty, lifestyle, social support

STILUL DE VIAȚĂ ȘI PREVENIREA FRAGILITĂȚII LA VÂRSTNICI

***Roditis** Doina[1], **Lupeanu** Elena[1], **Bârsan** Monica[1], **Tronaru** Maria[2]*

[1]Institutul Național de Gerontologie și Geriatrie "Ana Aslan", București, Romania
[2]Universitatea de Medicina si Farmacie Carol Davila, București, Romania

Autor corespondent: Doina Roditis elena.roditis@gmail.com

Rezumat. Fragilitatea reprezintă un sindrom clinic potențial reversibil prin intervențiile terapeutice țintite, dacă este detectat precoce. Studiul are ca obiectiv evaluarea factorilor favorizanți ai fragilității și evidențierea unor elemente ale stilului de viață sănătos, într-un lot de pacienți INGG. Material și metodă: un lot de 214 pacienți (185 femei și 29 bărbați), $\overline{X}$ =68.8 ani, se evaluează cu o baterie de teste: ▪ Groningen Frailty Index (GFI); ▪ MNA(Mini Nutritional Assessment-Long Form); ▪ Simple Lifestyle Indicator Questionnaire; ▪ADL si IADL; ▪ Lubben Social Network Scale–6 și ▪mărimea venitului. Rezultate: Prevalența fragilității a fost de 18,7%. În urma evaluării globale a sănătății, variabilele obținute au fost corelate cu scorul de fragilitate. Prin corelațiile semnificative rezultate, s-a ajuns la clasificarea factorilor favorizanți ai fragilității (dupa J Puxty). Cei mai importanți, în ordinea intensității au fost: 1- polipragmazia (r=0.350/p=0.000); 2-riscul nutrițional (r=0.341/p=0.001); 3-mersul încetinit (r = 0,329/ p=0.000) și 4-afectarea psihiatrică (r=0.229/p=0.001). Locul 5 l-a ocupat corelația între fragilitate și "subscala–prieteni". Legăturile la limită cu prietenii apar în ponderi mari atât în rândul prefragililor, cât si fragililor (45%). Prin urmare, tendința de izolare în comunitate reprezintă factor (precoce) de risc pentru fragilitate. Concluzii: Stilul de viață indicat pentru contracararea apariției fragilității constă în: evitarea polipragmaziei alimentație rațională, evitarea sedentarismului; de asemenea foarte important:

evitarea stresului, odihna şi relaţiile armonioase în societate (ultimele recomandări protejează vârstnicii şi împotriva tulburărilor psiho-cognitive).
Cuvinte cheie: prevenție, fragilitate, stil de viață, suport social.

INTRODUCTION

The concept of "frailty" has being discussed in the geriatric literature for more than 40 years, but a unique operational definition has not been accepted yet. The current debate is focused on defining frailty by using only the biomedical aspects or together with the psychosocial ones (1).

During the International Conference in Montreal (2006), a group of experts proposed the following definition: "Frailty is a multidimensional clinical syndrome, differentiated of disability, a syndrome which increases vulnerability and can cause functional impairment, at minimal stressors. The syndrome might be reversible or attenuated by interventions and health workers would detect it as soon as possible"(2). In 2012, another conference from Orlando, Florida aimed at obtaining an operational definition and framing aspects for frailty screening and treatment. An important agreement of this group was to recognize the distinction between the broader definition of frailty, which is a general state of an individual, and a more specific medical syndrome, the **physical frailty**. The physical aspects are more easily diagnosed using objective criteria and can be addressed with a specific therapy. For this reason, many clinicians prefer to diagnose frailty exclusively on a physical basis rather than to include also the psychosocial aspects (3).

Some simple measurements were proposed as robust indicators for identifying physical frailty: Gait Speed, Up and Go Test, Handgrip-Strength. Other simple tests focus more on the physical frailty: Frail Phenotype (Fried) or FRAIL Scale. Also, more complex tools targeting psychosocial aspects and brief sensory (sight and hearing) assessments are used: Groningen Frailty Index (GFI) and Tilburg Frailty Indicator (TFI). At the Florida Conference, a consensus of experts also stated that the definitive frailty diagnosis must be made by geriatricians and based on well-validated models. The traditional geriatric approach considers the psychosocial and environmental aspects as essential components for assessing the elder patients' vulnerability (3).

Canadian geriatrist J. Puxty supports this approach and classifies risk factors of frailty in three categories: 1) physical factors (advanced age, weight loss, fatigue/inactivity, reduced muscle strength, slow gait); 2) co-morbidity factors (impaired cognition or mood, polypharmacy, multiple chronic diseases) and 3) socio-economic ones: isolation, caregiver gaps, poverty (4).

Initially, some researchers considered frailty a static concept, stating that there is a certain functional reserve that diminishes in time, reaching a limit below which people become frail. Others (Witten, Brocklehurst, Stuck) pointed out the importance of dynamic models with interacting key factors. A recent dynamic model for frailty belongs to J. Puxty (2013, Fig 1). In this model, the decline in functional capacity is determined by both chronic and acute pathological risk factors as well as human behaviour. The recovery of the functional capacity can be difficult if it falls below a certain level, especially in the presence of mental disorders or an unhealthy lifestyle such as malnutrition or sedentariness (4).

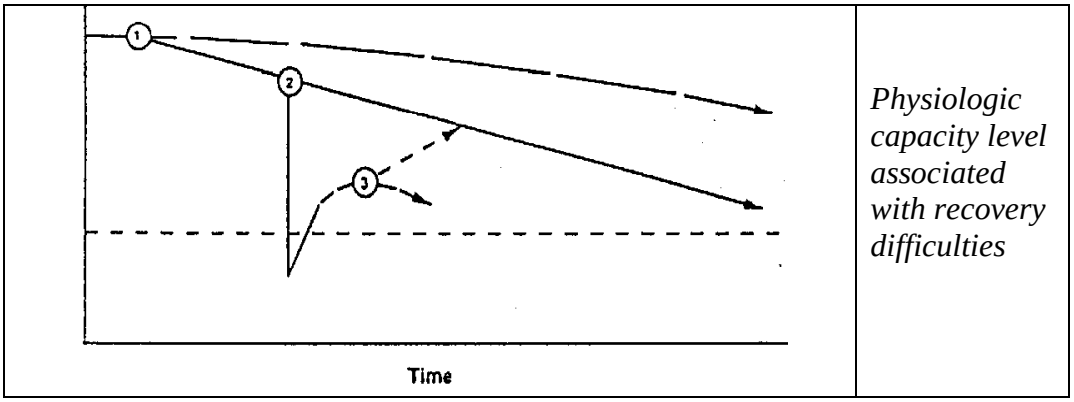

Fig. 1. Conceptual model regarding frailty risk factors (John Puxty)

Legend:

(1): Risk factors for chronic accelerated decrease of physiologic capacity
e.g. - diseases, inactivity.
(2): Risk factors for acute/sub acute physiologic capacity
e.g. - flu, hip fracture.
(3): Risk factors for blocked recovery after losing the physiologic capacity
e.g. - depression, malnutrition, fear of falling, misbelieve that "rest is healthy".

The concept of "lifestyle" defines man as a whole in interaction with his environment and represents the individual life pattern resulting from economic, social and cultural conditioning. Abraham Maslow was one of the first psychologists who ranked needs and motivations, two aspects that determine human behavior. His pyramid places at the bottom the physiological needs such as: food, water, air, sleep, shelter. The second level is represented by the safety needs: physical security, health, family security and also, stability needs. On the third level, the social needs are tied to the social nature of man (friendship, family, pleasant relationships with others). The upper levels (fourth and fifth) are represented by self-respect and self-assertion. They are achieved by meeting the lower-level needs (5).

In recent years, a particular concern is for assessing lifestyle in order to prevent the chronic diseases outbreak. Several socio-medical instruments were created for evaluating lifestyle. One of them is „The Simple Lifestyle Indicator Questionnaire", which targets at subjects with various ages (6). This questionnaire focuses on physical activity level, healthy eating, avoiding smoking and alcohol consumption. Also, it briefly assesses the psychological stress level, an important aspect of modern life. We emphasis this aspect, as our study focuses on *frailty, which at "minimal stressors**,** increases vulnerability and can cause functional impairment".*

In recent years, the elderly nutrition is frequently assessed using MNA (Mini Nutritional Assessment) (7). The test takes into account the food consumption, as well as anthropometric data, physical and psychological functionality aspects. Practically, this is also an assessment of the subjects' lifestyle.

OBJECTIVES

The study evaluates the factors contributing to frailty and aims to identify essential healthy lifestyle habits in a presenescent and senescent inpatients sample.

MATERIALS AND METHODS

The sample is represented by 214 patients (185 women and 29 men), selected from NIGG „Ana Aslan", with average age $\overline{X}$ = 68,9 years. The chronic pathology was drawn from inpatient medical records.

The medico-social investigation was made using a questionnaire with a test battery for evaluating the following aspects:

▪ subjects' frailty, using the Groningen Frailty Index (GFI) (8);

▪ nutritional status using Mini Nutritional Assessment- Long Form (MNA-LF);

▪ lifestyle- Simple Lifestyle Indicator Questionnaire (SLIQ);

▪ basal physical functionality -ADL (Barthel Index) and instrumental functionality -IADL (M.P. Lawton & E.B.Brody scale);

▪ social status: -Geriatric Assessment Wizard (R.Kleindlenst-2002, (Version 1.3), -the Lubben Social Network Scale–6 (LSNS-6) and the individual and household income (9).

RESULTS

The evaluation of the subjects' frailty using the Groningen Index reveals that 18.7% patients are frail and 24.8% are vulnerable.

By analyzing the overall health data, a complex picture resulted, including: chronic diseases, nutritional status, physical and psychosocial functionality, lifestyle aspects. The correlation of these elements with the frailty score (GFI) assesses the existence of many factors contributing to frailty from J.Puxty's classification.

Grouped in three categories, these factors are found in our study as follows:

(1). Physical factors are ordered after the intensity of correlation with frailty:

• slow gait (evaluated by Up and Go Test, r = 0,329 / p= 0,000),

• poor grip strength (r = 0,317/ p= 0,000),

• advanced age (r= .293 / p=0,000),

• inactivity (evaluated by the item „frequency of leaving home": r= 0,291 / p=0,000) and •weight loss (r= 0,153 / p=0,025).

Table I and Fig. 2 shows the meaning of the previously enumerated correlations through some percents.

Table I. The percentage distribution of physical factors related to decreased muscle strength, grouped by health level: robust, vulnerable, frails

Physical factors	The robusts	The pre-frails	The frails
• Slow gait - The fall risk assessed using "Up and Go Test"	10.7 %	15.3 %	**45.0** %
• Poor grip strength – Handgrip Test	30,0 %	49,0 %	**72.5** %

• **Slow gait** is evaluated using „Up and Go Test", which is used in population studies for assessing the fall risk. (The test evaluates gait speed and balance when transferring oneself to a chair). The fall risk of the subjects, represented by the calculated weights, slightly increases between the robust and the pre-frails group, but it triples between the pre-frails and frails group.

• The **muscle weakness** (Handgrip test) increases with 20 percents between the robust and pre-frails, as well as between the pre-frails and frails. Further, as Fig. 1 points out, **inactivity** is four times higher in the frails compared to the robust group (inactivity is evaluated by "the frequency of leaving home").

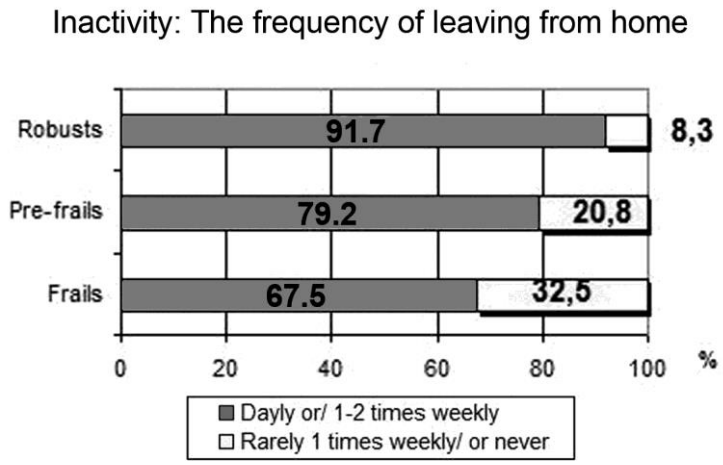

Fig.2. The percentage distribution of the "inactivity" factor in health-level groups

(2). Co-morbidity factors including polypharmacy (especially sedatives use), impaired cognition and/or affective state and multiple chronic diseases, are presented in Tab. II.

Tab. II. Statistically significant correlations between the co-morbidity factors and frailty, ordered by the decreasing correlation values

Frailty score correlated with:	r	p
Polypharmacy	**0,350** (**)	0,000
No. of psychiatric diseases	0,229 (**)	0,001
Cognitive impairment	0,227 (**)	0,001
No. of heart diseases	0,213 (**)	0,002
No. of chronic pathologies	0,200 (**)	0,004
Urinary incontinence	0.209 (**)	0,002

** Correlation is significant at the 0.01 level (2-tailed).

Polypharmacy is important both for high co-morbidity level and for iatrogenic effects which are frequent especially in elderly. Impaired cognition and/or emotional state have a great influence in developing frailty. Also, cardiac pathology determines a higher percent of frails. So, from data presented, we can emphasize in our sample the five "giants of geriatrics": iatrogenesis (caused by polypharmacy), immobility, instability, cognitive impairments, and urinary incontinence.

(3). Socio-economical factors contributing to frailty: isolation, absence of support persons and poverty are presented in Tab. IIIA. In our study, it must be mentioned from the correlation analysis: the variables "daily life interest", "educational level" and "marital status" represent important elements for influencing the social support and the poverty.

Daily life interest is a sensitive psycho-social indicator, used both alone and as an item in some depression scales. Because its prominent place in Tab. IIIA, we see it as a determinant factor in the subjects' social relations with friends and family.

Scores for Lubben Social Network Scale–6 range from 0 to 30. The cutoff of 12 signifies "risk for social isolation". Similarly, on the three-item subscales *(for family or friends)* the cutoff of 6 signifies "risk for marginal ties" (with family or friends) (5).

In the Tab. IIIA, we see Friends Network and Friends Subscale on the second and respectively the third places. That indicates that frequent relation with friends, good communication with them and their practical help represent an important protective factor against frailty. Also, in Tab. IIIB, we see high weights for marginal friendship ties (45%), both for the pre-frails and for the frails. So, we could consider Lubben Friends subscale as a true marker of vulnerability.

The significant correlation between frailty and education level suggests that higher pensions according to better professional qualifications are a protective factor against frailty.

The negative correlation between the gender and pensions level shows a poorer socio-economic status in women. Our subjects are in majority women, 47.7% of them are widows and 49% of widows live alone. For these reasons, the positive correlation between frailty and marital status suggests that widowhood and loneliness represent risk factors for frailty. On the other hand, the household income – frailty negative correlation reveals the economic risk factor that contributes to the appearance of fragility. As seen in Tab. IIIB, the percentage of subjects who received help when needed (Dartmouth Questionnaire) suggests that the frails are disadvantaged compared to the vulnerable ones.

Tab. IIIA. Statistically significant relations between social variables and frailty score, ordered by correlation strength

Groningen Frailty Index (GFI) correlated with:	r	p
Daily life interest	0,295 (**)	0,000
Social network formed by friends	-0,252 (**)	0,000
Friends Subscale (Lubben)	-0,217 (**)	0,001
Education level **(**high school graduation)	-0,216 (**)	0,002
Lubben score	-0,174 (*)	0,011
Marital status	0,164 (*)	0,016
Household income (Poverty)	-0,161 (*)	0,019
Family Subscale (Lubben)	-0,143 (*)	0,036

** Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed).

Tab. IIIB. Percentage distributions of the analyzed social factors - sample was grouped by health status using the GFI score

Socio-economic factors:	The robusts	The pre-frails	The frails
• Isolation			
Social isolation risk (Lubben score)	11,7 %	22,6 %	27,5 %
Marginal family ties (from Family Subscale)	5,8 %	17,0 %	15.0 %
Marginal friendship ties (from Friends Subscale)	22.5 %	45.3 %	45 %
• Caregiver gaps (Dartmouth Questionnaire) „During the last month, did you have someone close to help you?"	The robusts	The pre-frails	The frails
Yes, always when in need	7,4	3,8 (ns)	10,0 (ns)
Yes, quite enough	25,6	**28,3**	22,5
Yes, a little	43,8	47,2	**62,5**
Not at all	23,1	20,8	5,0 (ns)

As we initially stated, the study also aims at emphasizing some healthy lifestyle elements. By correlating frailty with health risk factors, as presented in Tab. IV, we see that the first place belongs to the nutritional risk, assessed by MNA. Tab. IV contains elements from the bottom of the Maslow's hierarchy of needs: nutrition, rest, activity. Also, stress belongs to pyramid, but to the second level, corresponding to the security and stability needs.

Tab. IV. The relation between the analyzed lifestyle elements and the frailty score, ordered by correlation intensity (n=214)

GFI correlated with:	r	p
Nutritional risk	-0,341 (**)	0,001
Sleep hours	0,286 (**)	0,000
STRESS level	-0,260 (**)	0,000
Overall lifestyle score	-0,253 (**)	0,000
Physical exercises score	-0,235 (**)	0,001

** Correlation is significant at the 0.01 level (2-tailed).

In addition to the correlations in Tab. IV, we also investigated the relation between the previously detected factors contributing to frailty and the „Simple Lifestyle Indicator Questionnaire" scores. We obtained significant correlations with the family relationships Lubben (r=0.187/ p=0.006) and with mental disorders (depression, impaired cognition: r= 0.155/ p=0.024). The need for socialization, for affection towards family and friends is human nature (the third level in Maslow's Pyramid). For this reason, maintaining healthy social connections improves well-being, mitigating psychological problems. And also, it represents an essential protective factor against the development of frailty.

CONCLUSIONS

Within the model which presents the contributing factors towards frailty, **inactivity** is found both among the factors leading to functional capacity decrease in chronic and acute pathologies, and also among the behavior-related factors. After the correlational analysis, all enumerated factors in Puxty's model as well as those classified in the three frailty risk groups were covered by our research.

The five "giants" of geriatrics appeared in this order: iatrogenesis (due to polypharmacy), immobility, instability, cognitive impairment, urinary incontinence.

The study pointed out the presence of some essential healthy lifestyle elements ranked as follows: rational nutrition, avoiding inactivity, appropriate rest and avoiding stress; (the last two factors are important protective factors against impaired cognition/or affective state, frequently associated with frailty).

Another important factor was the maintenance of good relationships with family and friends. Moreover, a decent pension level can be certainly regarded as a beneficial condition for a healthy lifestyle.

Acnoweledgements. The study was performed under the contract no.335/2014(PN-II-PCCA-2013-4-1686) and it was funded through the PARTNERSHIPS UEFISCDI – Collaborative Applied Research Projects - Competition 2013.

REFERENCES

[1] Lally F., Crome P. *Understanding frailty*. Postgrad Med J., 2007, January; 83(975): 16–20.

[2] Bergman H. *Frailty - An Emerging Clinical and Research Paradigm?* 2nd International working meeting on Frailty and Aging, Montreal, March 2006.

[3] . Morley JE, Vellas B, Abellan van Kan G, et al. *Frailty Consensus: A Call to Action*. J Am Med Dir Assoc, 2013; 14:392-7.

[4] Puxty J. *Frailty and Aging – Managing from a Community Perspective*. 6th Annual Falls Prevention Conference "End Falls This Fall", 2013. Retrieved from http://docs.communityconnection.net/Falls%20Docs/Falls%20Presentations%206/Microsoft%20PowerPoint%20-%20DrPuxtyFrailtyAndAgingCommunityPerspective.pdf

[5] Maslow A. H. *A Theory of Human Motivation*. Psychological Review, 1943, 50, 370-396.

[6] Godwin M. *Testing The Simple Lifestyle Indicator Questionnaire*. Can Fam Physician, 2008, V.54(1); Jan.

[7] Guigoz Y. *The Mini Nutritional Assessment* (MNA®) Review of the literature – What does it tell us? The Journal of Nutrition, Health & Aging© 2006, Volume 10, number 6.

[8] Metzelthin S.F., Daniëls R., Rossum E., et al. *The psychometric properties of three self-report screening instruments for identifying frail older people in the community*. BMC Public Health. 2010; 10: 176.

[9] Lubben, J., Blozik, E., Gillmann, G., et al. *Performance of an abbreviated version of the Lubben Social Network Scale among three European Community–dwelling older adult populations*. Gerontologist, 2006, 46(4), 503–513.

OXIDATIVE STRESS INVOLVEMENT IN FRAILTY

***Borșa** Claudia [1], **Grădinaru** Daniela [2], **Ionescu** Cristina [1], **Prada** Gabriel Ioan [1,3], **Pena** Cătălina Monica [1]*

[1] "Ana Aslan" National Institute of Gerontology and Geriatrics, Bucharest, Romania
[2] "Carol Davilla" University of Medicine and Pharmacy, Faculty of Pharmacy, Bucharest, Romania
[3] "Carol Davilla" University of Medicine and Pharmacy, Department of Geriatric Medicine, Bucharest, Romania

Corresponding author: Claudia Borsa, cldbrs@yahoo.co.uk

Abstract. The elderly frailty represent an increasing medical, social and research challenge with growing emphasis on identifying its underlying pathophysiology, risk factors and mechanisms, and new prospects for early intervention. Dysregulation of cellular responses to endogenous and exogenous stressors: oxidative stress, cell injury/insult, free radicals, DNA, protein and lipid damage, redox imbalance; as well as deficit in cellular and tissue repair mechanisms contribute to frailty. Recent studies underlined the markers of multiple systems dysregulation in frailty, such as: high levels of oxidative tress biomarkers, elevated cytokines and chemokines, reduced hormones, perturbed neutrophil, monocyte and white blood cell distribution. The aim of this review is to underline the role and the mechanisms of oxidative stress in elderly frailty, the state of art in experimental and clinical research and the important therapeutic targets in development of alternative, complementary or novel therapies for prevention and treatment of frailty. Inhibition of excessive reactive oxygen species generation and their signaling pathways, diminishing oxidative damages as well as influencing the redox balance in muscle cells in favor of antioxidant status could represent useful approaches in development of novel therapies for prevention and early treatment of frailty and its consequences.
Key words: biomarkers, oxidative stress, mechanisms, frailty

IMPLICAREA STRESULUI OXIDATIV ÎN FRAGILITATE

***Borșa** Claudia [1], **Grădinaru** Daniela [2], **Ionescu** Cristina [1], **Prada** Gabriel Ioan [1,3], **Pena** Cătălina Monica [1]*

[1]Institutul Național de Gerontologie și Geriatrie "Ana Aslan", București, Romania
[2] Universitatea de Medicina si Farmacie "Carol Davilla", Facultatea de Farmacie, Bucuresti, Romania
[3] Universitatea de Medicina si Farmacie "Carol Davilla", Departamentul de Medicina Geriatrica, Bucuresti, Romania

Autor corespondent: Claudia Borșa, cldbrs@yahoo.co.uk

Rezumat. Fragilitatea varstnicului reprezinta o provocare crescanda din punct de vedere medical, social si de cercetare, cu accentuare marita in identificarea patofiziologiei, a factorilor de risc si a mecanismelor implicate si noi perspective pentru interventia timpurie. Dereglarea raspunsurilor celulare la stresori endogeni si exogeni: stres oxidativ, lezare celulara, radicali liberi, lezarea ADN-ului, proteinelor si lipidelor, imbalanta redox, ca si deficitul in mecanismele de reglare celulara si tisulara contribuie la fragilitate. Studii recente au subliniat markerii dereglarii sistemelor multiple in fragilitate, cum sunt: nivele ridicate ale biomarkerilor de stres oxidativ, citokinelor si chemokinelor, reducerea nivelelor hormonilor si perturbarea distributiei celulare a neutrofilelor, monocitelor si celuleler albe. Scopul acestui review este prezentarea rolului si a mecanismelor de actiune ale stresului oxidativ in fragilitatea varstnicului, a statusului actual al cercetarilor clinice si experimentale in domeniu, precum si a tintelor terapeutice importante pentru dezvoltarea de terapii alternative, complementare sau noi in preventia si tratamentul fragilitatii. Inhibitia generarii excesive a speciilor reactive de oxigen si a cailor lor

de semnalizare, diminuarea lezarilor oxidative ca si influentarea balantei redox in favoarea statusului antioxidant in celulele musculare ar putea reprezenta abordari utile in dezvoltarea de noi terapii pentru preventia si tratamentul timpuriu al fragilitatii si a consecintelor ei.
Cuvinte cheie: biomarkeri, stres oxidativ, mecanisme, fragilitate

INTRODUCTION

Frailty, a physiological syndrome characterized by decreased reserve and diminished resistance to stressors, resulting from cumulative decline across multiple physiological systems, determines vulnerability to adverse outcomes and high risk of disabilities, diseases and even death. (1).

Conceptualizing frailty through the four main underlying processes: changes in body composition, energetic imbalance, homeostatic dysregulation, and neurodegeneration, recognizes that the processes that underlie frailty are age-related and progress more rapidly later in life, with a higher degree of heterogeneity between individuals (2,3).

Frailty is hypothesized to be a phenotype of accelerated aging and has been associated with changes in several physiological systems, including: metabolism, musculoscheletal, immune, endocrine and autonomic systems. Evidence suggests that these physiological changes are evident ever since in a preclinical stage of frailty, named prefrailty, and more predominant in the frailty status. The alterations in these physiological systems are responsible for the characteristics of frailty: weight loss, muscle weakness, low activity level, exhaustion, and slow gait.

Recent studies underlined the markers of multiple systems dysregulation in frailty, such as: high levels of oxidative stress biomarkers, elevated cytokines and chemokines, reduced hormones, perturbed neutrophil, monocyte and white blood cell distribution (1,4,5,6).

Dysregulation of cellular responses to endogenous and exogenous stressors: oxidative stress, cell injury/insult, free radicals, lipid, protein and DNA damage; as well as deficit in cellular and tissular repair mechanisms contribute to frailty.

The etiology of frailty is not well understood, and it has been suggested that the identification of blood markers that distinguish at-risk frail older adults would be useful for the prevention, treatment and novel therapy (3).

Accumulating evidence suggests that age-related diseases are associated with increased oxidative stress, impaired antioxidant defense system, and deregulated immune and inflammatory responses. Fragile adults affected by severe chronic diseases also exhibit an altered oxidative status and impaired antioxidant defense system (7,8,9).

The aim of this review is to underline the role and the mechanisms of oxidative stress in elderly frailty, the state of art in experimental and clinical research and the important therapeutic targets in development of alternative, complementary or novel therapies for prevention and treatment of frailty.

Oxidative stress association with frailty

Numerous experimental and clinical studies have suggested that oxidative stress may be related to age-associated frailty. Progressive and irreversible accumulation of oxidative damage associated with aging contributes to impaired physiological function and increased incidence of disease and frailty (2,6,10).

By “ex vivo” experimental research, Baptista et al. (2012) have demonstrated that superoxide anion production by NADPH oxidase in whole blood cells of frail older subjects was greater than in non-frail (11).

High oxidative stress modifies the activity of proteins and makes them more susceptible to oxidation, glycoxidation and degradation. Thus, biomarkers of protein oxidation are representative for systemic oxidative damages in humans.

Protein carbonylation evaluated by protein carbonyl groups represent a major systemic biomarker of protein oxidation. Numerous

cross-sectional studies have demonstrated that higher protein carbonyl levels were associated with components of frailty, such as: poor grip strength, gait speed decline or frailty phenotype established by Fried criteria. Protein carbonyl levels are associated with low grip strength and have recently been proposed as a biomarker of severe dependence. (2,12,13,14)

Lipid peroxidation is also implicated in aging and frailty. Biomarkers of lipid peroxidation or oxidation were correlated with elderly frailty. Thus, circulated levels of malondialdehyde (MDA), hydroxy-nonenal (HNE), 4-HNE-adducts, MDA-protein adducts, conjugated dienes or trienes, and isoprostanes were independently associated with frailty phenotype: slower gait speed, greater frailty odds, or higher risk of frailty in elderly (5,10,15,16).

Serum 8-hydroxy-2- deoxyguanosine (8-OH-dG), biomarker of DNA oxidative damage was also independent associated with frailty phenotype (2). The strong interrelations between oxidative DNA damage and frailty suggest the important role of DNA damage and DNA responses like DNA repair mechanisms, cell senescence and apoptosis in frailty.

Numerous correlative human studies using different markers of oxidative stress consistently showed that increased oxidative stress independently predicts frailty. Table I presents the important clinical studies underlining the associations of oxidative stress biomarkers with frailty.

Table I. Clinical studies underlining the oxidative stress associations with frailty

Population under study	**Oxidative stress biomarkers**	**Oxidative stress association with frailty**	**References**
1919 participants in Framingham Offspring Study	8-epi-FGF-alfa isoprostanes	Higher isoprostanes levels were associated with greater frailty odds and slower gait speeds	Liu C. K. Et al, 2016 (16)
Pilot study (15 frail elderly)	Conjugated dienes and trienes MDA bound to plasma proteins	- Higher levels of conjugated dienes and trienes and MDA-bound proteins were associated with higher risk of frailty in elderly - Conjugated dienes correlated with frailty score of Rockwood - MDA-bound proteins correlated with risk profile score	Pereira M.C. et al, 2016 (5)
2518 participants in ESTHER Cohort Study	Biological antioxidant potential (BAP) Derivate of reactive oxygen metabolites (d-ROM) Total thiol levels (TTL)	Significant positive association between frailty status with d-ROM and significant negative one with TTL were found	Saum KU et al, 2015 (4)
742 participants (aged 65-95) in Toledo Study for Healthy Aging	MDA Protein carbonylation	MDA and protein carbonylation are related to frailty	Inglés M et al, 2014 (15)
280 participants (aged ≥ 60) in Cross-sectional study	Whole blood cells superoxide anion production by NADPH oxidase	Persons within highest tertile of superoxide anion production had higher adjusted odds ratio of being frail compared with those in the lower 2 tertiles	Baptista et al**,** 2012 (11)
62 participants (aged ≥ 65) in Cross-	MDA- and 4-HNE-adducts GSH	Redox balance **(**GSSG/GSH ratio) and MDA-adducts	Serviddio G. et al, 2009 (10)

sectional study	GSSG	strongly correlated with frailty	
90 participants (aged ≥ 65) in Cross-sectional study	8 hydroxy-2-deoxyguanosine	High serum 8-OHdG levels were independently associated with frailty	Wu et al, 2009 (2)
545 participants (aged ≥ 65) in longitudinal study (3 years)	Protein carbonyl	Serum protein carbonyl levels independently associated with decline in walking speed and slow walk speed	Semba et al, 2007 (17)
672 participants (aged ≥ 65) in Cross-sectional study	Protein carbonyl	Serum protein carbonyl levels negatively correlated with grip strength	Howard et al, 2007 (14)

MDA, malondialdehyde; GSH, Glutathione; GSSH, oxidized glutathione; HNE, hydroxynonenal; 8 OHdG, 8 hydroxy- 2 deoxyguanosine

Oxidative stress, cellular mechanisms and frailty

Multiple underlying biological factors such as dysregulation of inflammatory processes, genomic instability, oxidative stress, mitochondrial dysfunction, and cellular senescence and apoptosis appear to contribute to frailty.

In human aging the levels of reactive oxygen species (ROS) are increased, oxidative stress and inflammatory processes are intensified with individual age, resulting in enhancing catabolic effects and reducing anabolic effects on muscle (9).

Oxidative stress may directly injure muscle by oxidative damage to protein, lipids and DNA (protein carbonylation; protein, lipid and DNA oxidation, protein advanced oxidation and glycoxidation), thus contributing to sarcopenia and frailty (2,5,14,16,17).

Also, oxidative stress could action indirectly by inflammatory pathways stimulation (IL-6, IL-1b interleukines, inflammatory modulators), by activating nuclear factor kappa B (NF-kB) and other transcription factors, enhancing inflammation and over-expression of specific proteins in inflammatory and proliferative responses (9).

Oxidative stress can cause frailty by the following cellular mechanisms:

- mitochondrial dysfunction
- damage to proteins critical for maintaining homeostasis and muscle function
- endoplasmic reticulum stress
- cellular senescence
- cellular apoptosis
- abnormal cellular signaling

The involvement of oxidative stress in the pathologic pathways of frailty can be reflected by three important interrelated mechanisms: causing oxidative damage, promoting cellular senescence and apoptosis, and generating inappropriate cellular signaling.

Mechanisms linking oxidative stress- oxidative damages- frailty

Oxidative stress is implicated in poly-pathologies and frailty by generating oxidative damages. ROS generated by mithocondria imply age-related accumulation of mithocondrial oxidative damages, leading to mithocondrial dysfunction and raise ROS production, which lead to further oxidative damage in other cellular components. Mithocondrial dysfunction can also lead to altered cellular bioenergetics, insulin resistance, adiposity, sarcopenia and frailty (2).

Oxidative damages of proteins, DNA and lipids can cause mithocondrial dysfunction, which has detelerious effects on cells and tissues, affecting energy-dependent cellular activity and raising intracellular levels of metabolites. Through activating protein kinase C, these metabolites can increase serine phosphorilation of insulin receptor substrate-1 (IRS-1) and block insulin signaling (18).

Mithocondrial dysfunction can further increase the mithocondrial production of

ROS, leading to a vicious cycle. Excessive production of free radicals could also activate the nuclear factor kappa B (NF-kB) pathway and lead to inflammation, which has also been linked to frailty. (6)

By modifying protein function, oxidative protein damages can impair the receptors function, signal transduction and transport proteins. Also, oxidative damage to endoplasmic reticulum (ER) proteins or accumulation of misfolded proteins trigger the ER stress response, which inhibits insulin signaling leading to insulin resistance through activating the Jun N-terminal kinase (JNK) (19). Oxidative protein damage can also impair the cytoplasmic Ca^{2+} homeostasis leading to sarcopenia and frailty (16).

Mechanisms linking oxidative stress- cell senescence/apoptosis – frailty

Oxidative DNA damage can induce stress responses involving single- or double-stranded breaks by activating a signaling cascade to promptly repair the damage and maintain cell cycle arrest. Severe DNA damage can lead to cellular senescence or apopthsis. Cell senescence can cause dysfunction and age-related pathologies, by diminishing the replicative capacities of neural, hematopoietic, skeletal muscle, pancreatic beta-cells (20,21).

Recent studies have suggested that senescent cells actively secrete molecules that alter the structure and function of local tissue microenvironments and organs (22), like are pro-inflammatory cytokines, especially IL-6 which are implicated in inflammation, an important process contributing to frailty. Inhibition of cell senescence can ameliorate pro-inflammatory cytokines expression.

Baker et al. (23,24) provided direct evidences showing the causal roles of cellular senescence in frailty, demonstrating that BubR1 protein (a mitotic checkpoint protein) insufficiency causes premature aging phenotypes because of accelerating senescence. The clearance of senescent cells can block premature skeletal muscle aging resulted in preserved muscle mass and function and a decreased expression of IL-6 (24).

Mechanisms linking oxidative stress- cellular signaling- frailty

The mechanisms involving oxidative stress, cellular signaling and frailty is complex.

ROS, acting as signaling molecules, can actively participate in intracellular and intercellular signaling, as well as in regulation of organism responses to stress. Excessive production and accumulation of ROS can trigger aberrant signaling, leading to multiple pathologies and frailty (25).

Redox signaling, involving reversible oxidation of sulfhydryl (–SH) groups can alter or inactivate the activity of target proteins, such as: protein phosphatases, especially tyrosine (Tyr) and serine/ threonine (Ser/Thr) phosphatases, p 53 or redox-sensitive transcriptional factors. This phosphatases inactivation leads to increased phosphorilation of specific kinases, especially mitogen-activated protein kinases and thus the activation of signaling cascade, with important effects on the growth factor signaling networks (26).

Thus, oxidative stress-induced inactivation of phosphatases can activate c-jun N-terminal kinases (JNK kinases) and lead to serine phosphorilation of IRS-1, interfering with insulin signaling and contributing to insulin resistance, an important characteristic of human aging and frailty (26).

The phosphatases inactivation by oxidative stress is also implicated in increased inhibitor protein-kB (IkB) phosphorilation on two serine residue by IKK1 (IKK1, inhibitor of nuclear factor kappa B kinase subunit alpha) and IKK2 (IKK2, inhibitor of nuclear factor kappa B kinase subunit beta) kinases, causing the dissociation of IkB bound to transcription factor NF-kB (nuclear factor-kB) in IkB – NF-kB complex. Free NF-kB increases the expression of some cytokines, such as: IL-2, IL-6, IL-8 and tumor necrosis factor alpha (TNF-alpha); as well as some acute-phase proteins (27). TNF-alpha bound to

its receptor can further increase oxidative stress and also activate NF-kB, causing a positive feedback loop that amplifies inflammation and oxidative stress (28,29).
These complex mechanisms underline that the age-related or oxidative stress – inflammation – related sarcopenia and frailty are close dependent to the increased activity of nuclear factor NF-kB, which lead to a metabolic picture of increased catabolic and decreased anabolic forces exerted within muscle cells.

CONCLUSIONS

Oxidative damage to macromolecules critical for maintaining homeostasis and muscle function imply cellular pathogenic states which in turn may result in multiple system pathologies leading to a vulnerable state of old age and frailty.
High levels of oxidative stress biomarkers are associated with frailty phenotype and could predict the development of frailty in older adults.
The interrelated cellular mechanisms linking oxidative stress to frailty status imply oxidative damages to DNA, proteins and lipids, mitochondrial dysfunction, cellular senescence and apoptosis and inappropriate cellular signaling. These complex mechanisms are close dependent to the increased activity of nuclear factor NF-kB, leading to a metabolic picture of frailty status.
Inhibition of excessive reactive oxygen species generation and their signaling pathways, diminishing oxidative damages as well as influencing the redox balance in muscle cells in favor of antioxidant status could represent useful approaches in development of novel therapies for prevention and early treatment of frailty and its consequences.

REFERENCES

[1] Walston J, Hadley EC, Ferrucci L, et al. *Research agenda for frailty in older adults: Toward a better understanding of physiology and etiology: Summary from the American Geriatrics Society/National Institute on Aging Research Conference on Frailty in Older Adults.* J Am Geriatr Soc., 2006, 54:991-1001.

[2] Wu IC, Shiesh SC, Kuo PH et al. *High oxidative stress is correlated with frailty in elderly Chinese*. J Am Geriatr Soc, 2009,57:1666–1671.

[3] Mitnitski A., Collerton J., Martin-Ruiz C., et al. *Age-related frailty and its association with biological markers of ageing.* BMC Medicine, 2015,13:161.

[4] Saum K.U., Dieffenbach A.K., Jansen E.H., et al.*Association between Oxidative Stress and Frailty in an Elderly German Population: Results from the ESTHER Cohort Study*. Gerontology**,** 2015, 61(5):407-15.

[5] Pereira M.C., Miralles R., Serra E., et al. *Lipid peroxidation in the lymphocyte membrane and protein oxidation in the serum of elderly people. Are they potential markers of frailty and dependence? Preliminary results*. Rev Esp Geriatr Gerontol., 2016, 51(1):25-8.

[6] Hubbard RE, and Woodhouse KW. *Frailty, inflammation and the elderly*. Biogerontology, 2010, 11: 635–641.

[7] Mulero J., Zafrilla P., and Martinez-Cacha A. *Oxidative stress, frailty and cognitive decline.* The Journal of Nutrition, Health&Aging, 2011, 15, 9, 756–760.

[8] Ferrucci L, Hesdorffer C, Bandinelli Set al. *Frailty as a Nexus Between the Biology of Aging, Environmental Conditions and Clinical Geriatrics*. Public Health Reviews, 2010, 32:475-88.

[9] Ershler W. B. *A gripping reality: oxidative stress, inflammation, and the pathway to frailty*. J Appl Physiol, 2007, 103: 3–5.

[10] Serviddio G, Romano AD, Greco A et al. *Frailty syndrome is associated with altered circulating redox balance and increased markers of oxidative stress*. Int J Immunopathol Pharmacol, 2009, 22:819–827.

[11] Baptista G, Dupuy AM, Jaussent A, et al. *Low-grade chronic inflammation and superoxide anion production by NADPH oxidase are the main determinants of physical frailty in older adults.* Free Radic Res.**,** 2012, 46(9):1108-14

[12] de Gonzalo-Calvo D, de Luxan-Delgado B, Rodriguez-Gonzalez S et al. *Oxidative protein damage is associated with severe functional dependence among the elderly population: A principal component analysis approach*. J Gerontol A Biol Sci Med Sci, 2012, 67A:663–670.

[13] Darvin K., Randolph A., Ovalles S., et al. *Plasma protein biomarkers of the geriatric syndrome of frailty*. J Gerontol A Biol Sci Med Sci., 2014, 69 (2):182-6.

[14] Howard C**,** Ferrucci L, Sun K, et al. *Oxidative protein damage is associated with poor grip strength among older women living in the community*. J Appl Physiol, 2007, 103(1):17-20.

[15] Inglés M., Gambini J., Carnicero J.A., et al. *Oxidative stress is related to frailty, not to age or sex, in a geriatric population: lipid and protein oxidation as biomarkers of frailty*. J Am Geriatr Soc., 2014, 62(7):1324-8.

[16] Liu C.K., Lyass A., Larson M.G., et al. *Biomarkers of oxidative stress are associated with frailty: the Framingham Offspring Study.* Age (Dordr), 2016, 38(1):1.

[17] Semba RD, Ferrucci L, Sun K, et al. *Oxidative stress and severe walking disability among older women*. Am J Med., 2007, 120(12):1084-9.

[18] Petersen KF, Dufour S, Befroy D, et al.*Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes*. N Engl J Med., 2004, 12; 350(7):664-71.

[19] Martindale JL and Holbrook NJ. *Cellular response to oxidative stress: signaling for suicide and survival.* J Cell Physiol., 2002, 192(1):1-15.

[20] Krishnamurthy J, Ramsey MR, Ligon KL, et al. *p16INK4a induces an age-dependent decline in islet regenerative potential*. Nature, 2006, 443(7110):453-7.

[21] Molofsky AV, Slutsky SG, Joseph NM, et al. *p16INK4a expression decreases forebrain progenitors and neurogenesis during ageing*. Nature, 2006, 443(7110):448-52.

[22] Campisi J, *d'Adda di Fagagna. Cellular senescence: when bad things happen to good cells*. FNat Rev Mol Cell Biol., 2007, 8(9):729-40.

[23] Baker DJ, Jeganathan KB, Cameron JD, et al.*BubR1 insufficiency causes early onset of aging-associated phenotypes and infertility in mice.* Nat Genet., 2004, 36(7):744-9.

[24] Baker D J, Wijshake T, Tchkonia T, et al. *Clearance of p16^{Ink4a}-positive senescent cells delays ageing-associated disorders*. Nature, 2011, 479, 232–236

[25] D'Autréaux B.and Toledan M. B**.** *ROS as signalling molecules: mechanisms that generate specificity in ROS homeostasis.* Nat Rev Mol Cell Biology, 2007, 8, 813-824.

[26] Kaneto H, Nakatani Y., Miyatsuka T., et al. *Possible novel therapy for diabetes with cell-permeable JNK-inhibitory peptide.* Nature Medicine, 2004, 10, 1128 – 1132.

[27] Finkel T. *Redox-dependent signal transduction*. FEBS Lett., 2000, 476(1-2):52-4.

[28] Haddad. J J. *Pharmaco-redox regulation of cytokine-related pathways: from receptor signaling to pharmacogenomics*. Free Radical Biology and Medicine, 2002,33, 7, 1: 907–926.

[29] Wu I.C., Hsiung C. A., Hsu C.C.,et al. *Oxidative Stress and Frailty: A Closer Look at the Origin of a Human Aging Phenotype. Book: Aging, Oxidative stress and Dietary antioxidants*. Ed.by Victor R. Preedy, Academic Press Elsevier, 2014, pg. 3-15.

CURRENT APPROACHES IN TYPE 2 DIABETES

***Ionescu** Cristina[1], **Grădinaru** Daniela [2], **Borșa** Claudia[1], **Răducanu** Ileana [1]*

[1]National Institute of Gerontology and Geriatrics Ana Aslan, Bucharest
[2]University of Medicine and Pharmacy Carol Davila Bucharest-Faculty of Pharmacy

Corresponding author: Cristina Ionescu, cristinaionescucristina@gmail.com

Abstract. Diabetes mellitus continues to be a major public health problem due to its increasing prevalence and also a social problem as recent studies have pointed to the association of diabetes with cognitive decline and impulse control disorders. There is still a large heterogeneity in describing roles of insulin resistance and insulin deficit in diabetes mellitus. Considering that for type 2 diabetes certain genetic determinants, environmental triggers (endocrine disruptors, viruses, food advanced glycation end products, gut biome) and role of inflammation have been precised, interpreting higher fasting insulin concentrations in plasma of diabetic patients as hyperinsulinemia is at present insufficient. Also, if high insulin concentrations are interpreted in the context of concurrently elevated glucose levels in diabetic patients, an insulin deficit rather than hyperinsulinemia becomes apparent. Moreover, it has been stressed that data regarding fat distribution are superior to the BMI in predicting type 2 diabetes. Changes in insulin sensitivity involve both beta-cell secretory function modification and variations of beta-cell mass. There is no means to establish the extent to which the decrease of beta cell function is due to its decline and the extent to which the above decrease is due to a beta-cell mass deficit. The contribution of beta-cell deficit versus that of beta-cell dysfunction to the pathogenesis of type 2 diabetes are noteworthy also as they vary among human populations.
Key words: insulin deficit, fat distribution, beta-cell mass

ABORDĂRI CONTEMPORANE ÎN DIABETUL TIP II

***Ionescu** Cristina[1], **Grădinaru** Daniela [2], **Borșa** Claudia[1], **Răducanu** Ileana [1]*

[1]Institutul Național de Gerontologie și Geriatrie ”Ana Aslan”, București, Romania
[2] Universitatea de Medicina si Farmacie “Carol Davila”, Facultatea de Farmacie, Bucuresti, Romania

Autor corespondent: Cristina Ionescu, cristinaionescucristina@gmail.com

Rezumat. Diabetul continua sa fie o problema majora de sanatate publica din cauza prevalentei sale in crestere si totodata o problema sociala intrucat studiile recente au evidentiat asocierea diabetului cu declinul cognitiv si tulburarile impulsive. Exista inca o mare heterogenitate in descrierile privind rolurile rezistentei la insulina si defictului de insulina in diabet. Tinand cont ca anumiti determinanti genetici, factori de mediu (virusuri, factori de dereglare endocrina, microbiom) si rolul inflamatiei in aparitia diabetului sunt deja precizati, interpretarea concentratiilor crescute de insulina din plasma pacientilor diabetici doar ca hiperinsulinemie, in prezent este insuficienta. In cazul interpretarii unor concentratii crescute de insulina in contextul unor hiperglicemii concomitente, devine plauzibil mai degraba un deficit de insulina decat o hiperinsulinemie. Mai mult, se pune accent pe date privind distributia adipozitatii ca parametru superior indexului de masa corporala BMI in ceea ce priveste predictia diabetului de tip 2. Modificarile de sensitivitate la insulina implica atat modificarile functiei secretorii a celulelor beta cat si variatiile masei de celule-beta. Nu exista mijloace pentru a stabili masura in care scaderea functiei de secretie de insulina este determinata strict de declinul functiei celulelor-beta pancreatice si in ce alta masura, de deficitul de masa celulara beta. In patogeneza diabetului sunt importante contributia deficitului de masa de celule- beta fata de cea a disfunctiei celulelor beta si prin faptul ca aceste contributii variaza in randul populatiilor umane.
Cuvinte cheie: deficit de insulina, distributia tesut adipos, masa de celule-beta

INTRODUCTION

Diabetes mellitus continues to be a major public health problem due to its increasing prevalence and also a social problem as recent studies have pointed to the association of diabetes with cognitive decline and impulse control disorders [1,2]. Since 1980, age-standardized diabetes prevalence more than doubled in men and increased by 60% in women worldwide [3]. Correspondingly, there is a need to constantly incorporate current knowledge in studies that enable better understanding the disease.

Another aspect which was emphasized by recent research was that incidences of diabetic macrovascular and microvascular complications changed in the last two decades [4]. Between 1990 and 2010 decreasing incidences of diabetic complications have been the result of a strict control of glycemia, blood pressure and lipid levels. Control of aforementioned parameters presumed at first changes in organizing clinical care concerning risk factors medical and support for patients adopting changes in their lifestyles. Also, in the above mentioned period of time, new therapeutic interventions received approval and consequently, use of lipid lowering drugs has been extended together with coronary revascularization. To point out changes of incidences of lower-extremity amputation, end-stage renal disease, acute myocardial infarction, stroke, and death from hyperglycemic crisis, the research carried out by Gregg used age standardized to the U.S. population in the year 2000 and rates for the overall population, in which a change in prevalence also affects complication rates. The greatest absolute decline was in the number of cases of acute myocardial infarction and the smallest absolute decline was in the number of deaths from hyperglycemic crisis. As well, there was a clear reduction in the relative risk of complications associated with diabetes but not in rates of amputation, stroke, or end-stage renal disease [4].

In the specialized literature there is still a large heterogeneity in describing the role of insulin resistance and that of insulin deficit in diabetes mellitus. Most often, type 2 diabetes has been characterized by insulin resistance, deficit of insulin and glucagon hypersecretion, whereas pancreatic beta cell loss characterizes type 1 diabetes. There is a multitude of genetic determinants of insulin resistance, beta cell function and mass of islet beta-cells, and genomic researches identified more than forty loci statistically associated with parameters related with onset of type 2 diabetes. It has been already stressed that insulin resistance increases type 2 diabetes risk, independent of BMI [5]. Moreover, new treatment options will target more the beta-cell dysfunction and less the insulin resistance [6,7]. Some of the parameters enumerated below have been considered more potent:

- Decreased beta-cell responsiveness, leading to impaired insulin processing and decreased insulin secretion (TCF7L2)
- Lowered early glucose-stimulated insulin release (MTNR1B, FADS1, DGKB, GCK)
- Altered metabolism of unsaturated fatty acids (FSADS1)
- Dysregulation of fat metabolism (PPARG)
- Inhibition of serum glucose release (KCNJ11)
- Insulin resistance and increased adiposity (IGF2BP2and FTO)
- Control of the development of pancreatic structures, including islet beta-cells (HHEX)
- Transport of zinc into the beta-islet cells, which influences the production and secretion of insulin (SLC30A8)
- Survival and function of beta-islet cells (WFS1) [6].

Several important environmental factors leading to type 2 diabetes

As regards some of the environmental factors leading to type 2 diabetes, Hales and Barker advanced the hypothesis that adverse influences, mainly poor nutrition during foetal and early post natal development could permanently impair the size and structure of organs and tissues [8]. Poor nutrition of the foetus and infant leads to permanent changes of structure and function of certain organs and tissues. Timing and nature of nutritive deficiencies determine patterns of metabolic

and functional abnormalities, which are noticed in adults, including diabetes and hypertension and possibly some hyperlipidaemias and insulin resistance. The aforementioned poor intrauterine nutrition may lead either to generalised growth retardation, or growth of the brain may be protected at the expense of the viscera. In this context Hales and Barker proposed that one of the major long-term consequences of inadequate early nutrition is impaired development of the endocrine pancreas and a greatly increased susceptibility to the development of type 2 diabetes. Not only pancreatic cells may be altered but also islet vasculature and innervation may be abnormally developed. In support of Hales' hypothesis, Meier noted a remarkable variation in pancreatic beta -cell area (30-fold) in individuals of similar age-groups throughout the pre- and postnatal growth period [9].

Adipose tissue impact on insulin resistance and type 2 diabetes

Obesity is a risk factor for insulin resistance [10] and it has been stressed that in describing obesity, data regarding fat distribution are superior to the BMI in predicting type 2 diabetes.

Recent research has shown that according to both metabolic profiles and BMI, subjects may be classified in the following phenotypes: lean and healthy, lean and unhealthy (also known as thin outside, fat inside), obese and healthy (known as obese and insulin-sensitive) and obese and unhealthy [11]. Metabolically healthy obese patients have been characterized by absence of dyslipidemia, no symptoms of hypertension and high levels of insulin sensitivity. As described by McArdle, particular for the lean, metabolically unhealthy and less aerobically-fit subjects were higher percentages of body fat, lower physical energy expenditure, higher cholesterol levels, no changes in ghrelin, leptin and adiponectin levels and increased triacylglycerol and free fattty acid levels [12]. By contrast lipodystrophic patients who present partial or total loss of subcutaneous adipose tissue SAT (acquired or inherited) and more visceral adipose tissue VAT and ectopic fat, are more insulin-resistant and at high risk high for type 2 diabetes mellitus, atherogenic dyslipidemia and heart disease but also prone to non-metabolic comorbidities. Also, research reported by Castro showed that abdominal subcutaneous fat, as determined by magnetic resonance imaging and computed tomography, was a strong correlate of insulin sensitivity assessed by use of the euglycemic-clamp [11].

Despres also underscored that the ratio of waist-to-hip circumferences (WHR), as a simple index of regional body fat distribution, was more strongly correlated with metabolic complications and cardiovascular outcomes than the BMI. For instance, independently of the BMI, a high WHR was found to be predictive of an increased risk of dyslipidemia, hypertension, and cardiovascular disease and type 2 diabetes mellitus. Furthermore, it was suggested that the simple anthropometric index of total adiposity had to be accompanied by indices such as the waist circumference or the WHR [13].

Adipose tissue has expandability and plasticity. The capacity to extend reflects the ability of adipose tissue to store lipids, either by increasing adipocyte size (hypertrophy) or adipogenesis and pre-adipocytes differentiation (hyperplasia). Ethnicity is a variable accounting for differences in visceral adiposity deposition and in this sense; whites are susceptible to having more visceral adipose tissue. Visceral adipose tissue is characterized by a low rate of proliferation and differentiation capacity, which leads to growth mainly by hypertrophy. The consequence of this type of cell growth is that hypertrophic adipocytes have impaired functions.

By contrast subcutaneous adipose tissue (found out mainly at the lower body) grows mainly by hyperplasia. When the capacity to expand of both visceral and subcutaneous compartments is exceeded, lipids spill over to the liver, the skeletal muscle, the heart, the pancreas and the kidney, the phenomenon being termed ectopic fat deposition. Despres

mentioned possibly inherited defects of the subcutaneous adipose tissue that may limit its ability to expand through hyperplasia, so lipids spill over. Although the lipid spillover hypothesis raised controversy, considerable clinical and experimental data have supported it. For example, laboratory fatless mice lacking subcutaneous adipose tissue were characterized by ectopic fat deposition. On the other hand, ectopic fat deposition and insulin resistance are found out especially in patients with various forms of lipodystrophies. HIV patients with lipodystrophy were treated with peroxisome proliferator– activated receptor gamma agonists that induce growth of subcutaneous fat through hyperplasia and are likely to reduce ectopic fat deposition. However, some adverse cardiovascular side effects have compromised administration of PPAR gamma agonists in patients. Thus, Despres suggested in his study further experimental work on both this laboratory model and this class of drugs.

White adipose and brown adipose tissues, WAT and BAT, are taken into acccount as the two main types of adipose tissue in the human body. BAT and WAT can be localized together throughout the adipose tissue, the brown adipose tissue being most important for regulation of thermogenesis in response to food intake, cold and sympathetic activation. It has been suggested that human WAT due to its plasticity has the capacity to transdifferentiate into BAT and conversely, depending on conditions to which adipose tissue is exposed. During cold exposure, some WAT may be transformed into brown adipose tissue in order to increase heat production, whereas BAT exposed to a rich diet, is transdifferentiated into WAT [13]. Under conditions of excess energy, white adipose tissue stores lipids (triacylglycerides TAG) and mobilizes stored lipids in situations of nutrient deprivation.

Inflammation and cytokines involvement in endothelial dysfunction associated with obesity and insulin resistance

The adipose tissue is characterized by low grade inflammation and production of reactive oxygen species at this level and in the perivascular adipose tissue. These radical species along with adipokines deteriorate nitric oxide signaling pathways, abnormal production and activity of endothelin being hallmarks of obesity associated endothelial dysfunction.

Inflammation may produce endothelial dysfunction by impairing vasodilation, namely by increasing vasoconstriction or reducing endothelium-derived vasodilators. Cytokines induce vasoconstriction through synthesis of endothelin-1, decreased expression of endothelial nitric oxide (NO) synthase and decreasing bioavailability of NO. TNF-alpha has also been linked to endothelial dysfunction and insulin resistance. TNF-alpha mediates its biological activities through binding to two different membrane receptors, TNFR1 and TNFR2, activating different signalling cascades and thus mediating distinct cellular responses. After binding to these receptors, a proteolytic cleavage of the extracellular parts elicits the soluble sTNFR1 and sTNFR2 forms. SolubleTNFR1 and solubleTNFR2 concentrations are taken into account as reflecting previous effects of TNF alpha. Increased sTNFR1 expression and reduced TNF-alpha bioactivity have been considered as protecting the myocardium from infarction following ischemia and reperfusion. As well, sTNFR1 might have a protective role through stimulating endothelial cell growth. On the other hand, sTNFR2 levels have been linked to coronary artery disease, insulin resistance and hypertension [14]. Fernandez Real has provided an explanatory hypothesis according to which a low grade inflammation (evidenced by increased interleukin-6 in the adipose tissue) could account for a first injury that induces predominantly endothelial dysfunction. During a second aggression, insulin resistance caused by obesity would exert effects upon the endothelium through further vascular dysfunction that associates metabolic abnormalities. To better understand the mechanisms prospective studies taking into account at the same time inflammation,

insulin sensitivity and endothelial function have been considered necessary.

Glucotoxiticy, beta-cell loss and insulin resistance

Insulin resistance requires insulin hypersecretion in order to maintain normal glucose tolerance, whereas the effect of improvement in insulin sensitivity on islet beta cell is that the cell reduces insulin release in order to avoid hypoglycemia. So beta-cell function adapts to changes in insulin sensitivity. The aforementioned changes requiring adjustments of insulin output can occur rapidly or over a long time. According to Carrera and Meier, two major mechanisms are affecting insulin sensitivity and these are the functional decline of the beta-cells and the mass of pancreatic beta-cells, although in most instances it appears that functional decrease predominates (at least on short term) [15, 9]. A third mechanism would involve causes that lead to beta-cells' death. But there is no means to establish to what extent decrease in beta cell functions is due to impaired beta-cell mass, or simply due to declining functions. Further, Meier pointed out that the individual contribution of beta-cell deficit versus that of beta-cell dysfunction to the overall pathogenesis of type 2 diabetes varies among human populations.

Causes leading to beta-cell mass deficit have not been yet established. The common view is that of increased beta-cell apoptosis, which determines a continuous loss of beta-cells. In support of this theory, studies using either immunohistochemistry or Western blot analysis showed that apoptosis was increased in islets from patients with type 2 diabetes compared with nondiabetic subjects.

Protein misfolding in the endoplasmatic reticulum (ER stress) and glucotoxicity can result in induction of apoptosis. Glucotoxicity is caused by continuous overstimulation of the pancreatic beta-cell leading to depletion of insulin stores, beta-cell impairment and hyperglycemia. Correlates of glucotoxicity are high levels of reactive oxygen species as well as low antioxidant defences of pancreatic beta cells [15]. Meier noted possible difficulties in attempting to estimate which of these factors is most important for induction of beta-cell death in patients with type 2 diabetes.

Under in vitro conditions, beta-cell death has been induced by various factors of the type 2 diabetes phenotype, such as high glucose and free fatty acids concentrations, or human islet amyloid polypeptide IAPP. As described by Carrera, a remarkable change in the population of pancreatic cells of the Langerhans islets was found in the type 2 diabetes temporal sequence. This modification was mainly caused by accumulation of amylin fibers derived from the islet amyloid polypeptide. Hypersecretion of IAPP and amylin fibers deposition to the endoplasmic reticulum leads to its stress as caused by excessive biosynthesis

Accelerated beta-cell death accounting for the beta-cell deficit in type 2 diabetes was supported by clinical observations stating a progressive deterioration of insulin secretion in diabetic patients with type 2 diabetes. Autopsy studies have illustrated the contribution of beta-cell mass deficit in the pathogenesis of type 2 diabetes. Studies reported by Carrera utilized pancreatic tissue from 124 patients with type 2 diabetes mellitus and control subjects and showed that pancreatic beta-cell mass was approximately between 0-65% in diabetic subjects compared with the BMI matched nondiabetic controls. These studies also showed that low rates of replication and neogenesis of beta cells were not different between subjects with type 2 diabetes and controls and suggested that there was a progressive beta-cell loss and no concurrent formation of new beta-cells. Another way to address the impact of islet beta -cell loss is to study individuals with a deficit of pancreatic beta-cell due to causes other than type 2 diabetes. Meier reported that in a large group of patients who underwent partial pancreatectomy for various pancreatic diseases, type 2 diabetes occurred when beta-cell areas (as quantified in the resected pancreatic tissue) were reduced on average, also by 65%. The same study reported that an

acute hemipancreatectomy, namely a 50% reduction in pancreatic beta cell mass led to an abnormal glucose tolerance. In this case the acute loss of pancreatic beta-cells was examined in subjects who donated 50% of their pancreas for transplantation.

Considering the already identified genetic determinants, the environmental triggers such as endocrine disruptors, viruses, food advanced glycation end products, gut biome and the role of inflammation in type 2 diabetes [16], interpreting higher fasting insulin concentrations in diabetic patients simply as hyperinsulinemia is less acceptable at present. Moreover, if higher insulin concentrations are interpreted in the context of concurrently elevated glucose levels in patients with type 2 diabetes, an insulin deficit rather than hyperinsulinemia becomes apparent. In this context common investigations for patients with type 2 diabetes should be expanded.

REFERENCES

[1] Hackethal V. *Type 2 Diabetes Rates Quadruple Worldwide Since 1980* , 2016, http://www.medscape.com/viewarticle/861591?src=emailthis

[2] de Jonge P. et al. *Associations between DSM-IV mental disorders and diabetes mellitus : a role for impulse control and depression*. Diabetologia, 2014, http://www.diabetesincontrol.com/depression-and-impulse-control-disorders-associated-with-diabetes/

[3] Ezzati M. and NCD. *Risk Factor Collaboration (NCD-RisC). Worldwide trends in diabetes since 1980*: *a pooled analysis of 751 population-based studies with 4·4 million participants.* 2016, 387, 10027:1513–1530.

[4] Gregg E.W., Li Y., Wang J., Burrows N.R. et al. *Changes in Diabetes-Related Complications in the United States, 1990–2010*. N. Engl. J. Med. ,2014,370:1514-23.

[5] Scott R.A., Tove F., Pasko D. et al. *Common genetic variants highlight the role of insulin resistance and body fat distribution in type 2 diabetes, independently of obesity*. Diabetes, 2014, 63, 4378-43-87.

[6] Romesh Khardori; Chief Editor: George T Griffing. *Type 2 Diabetes Mellitus*. 2015, http://emedicine.medscape.com/article/117853-overview#a5

[7] Vetere A., Choudhary A., Burns S.M. and Wagner B.K.*Targeting the pancreatic beta-cell to treat diabetes.* Nature Reviews Drug Discovery, 2014, 13, 278-289.

[8] Hales C.N. and Barker D.P.J. *Type 2 (Non-Insulin-Dependent) Diabetes Mellitus The Thrifty Phenotype Hypothesis*. Int. J. Epidemiol. ,2013,42(5):1215-1222.

[9] Meier J.J., Bonadonna R.C. *Role of Reduced Beta-Cell Mass Versus Impaired b-Cell Function in the Pathogenesis of Type 2 Diabetes.* Diabetes Care, 2013,36, 2, S113-119.

[10] Prieto D., Contreras C. and Sanchez A. *Endothelial dysfunction, obesity and insulin resistance.* Curr. Vasc Pharmacol., 2014, 12(3), 412-26.

[11] Castro A.V.B., Kolka C.M., Kim S.P. et al. *Obesity, insulin resistance and comorbidities – Mechanisms of association*. Arq. Bras. Endocrinol. Metab, 2014, 58(6):600-9.

[12] McArdle M.A., Finucane O.M., Connaughton R.M. et al. *Mechanisms of obesity-induced inflammation and insulin resistance: insights into the emerging role of nutritional strategies*. Frontiers in Endocrinology Diabetes, 2013,vol. 4: 1-23.

[13] Despres J.P. *Body Fat Distribution and Risk of Cardiovascular Disease An Update*.Circulation. 2012, 126:1301-1313.

[14] Fernández-Real J.M. *Insulin Resistance and Atherosclerosis. The Impact of Oxidative Stress on Endothelial Function*. Rev. Esp. Cardiol., 2008,Supl. 8:42C-49C.

[15] Carrera Boada C.A. and Martínez-Moreno J.M. *Pathophysiology of diabetes mellitus type 2: beyond the duo "insulin resistance-secretion deficit"* Nutr. Hosp., 2013, 28 (Supl. 2):78-87.

[16] Schwartz S.S., Epstein S., Corkey B.E. et al. *The Time Is Right for a New Classification System for Diabetes Rationale and Implications of the Beta-Cell-Centric Classification Schema.* Diabetes Care, 2016, 39(2):179-186.

IMPACT OF OBESITY ON LIPOPROTEIN PROFILE AND CARDIOVASCULAR RISK AT ELDERLY PATIENTS

Opriș *Simona* [1]**,** ***Constantin*** *Gianina* [1]**,** ***Grigorie*** *Mihaela* [1]**,** ***Halici*** *Florentina* [2]**,** ***Valuch*** *Anton* [1]

[1] *"Ana Aslan" National Institute of Gerontology and Geriatrics Bucharest, Romania*
[2]*Regional Medical Compliance Manager –Europe, Basel Area, Switzerland*

Corresponding author: Simona Opris, simonaopris11@yahoo.com

Abstract. Lipid profile and atherogenic index (AI) have been shown to be powerful predictors for metabolic disturbances including dyslipidemia, atherosclerosis, hypertension and cardiovascular diseases. Our study aimed to investigate the impact of obesity on lipoprotein profiles –i.e. lipid ratio AI and to calculate 10-year risk of cardiovascular mortali¬ty based on the HeartScore, at elderly patients. Patients (n=168) were divided into 3 groups: Group 1 (n=27) normal weight patients - as control group; Group 2 (n=77) overweight patients; Group 3 (n=64) obese patients. HeartScore are significantly higher ($p<0.0001$) in group 3 and 2 compared with control group 1 and also comparing group 3 to group 2. While comparing group 3 vs. control group 1 and vs. group 2 we noticed a significant increased in AI ($p<0.005$ respectively $p<0.001$). Between obesity class – I, II and III, HeartScore and AI shown a slight increase, but insignificant. Linear regression equation revealed at group 1 that HeartScore is positive significantly correlated with BMI ($r=0.54$, $p<0.001$). Also, at group 2, we observed a positive significant correlation between: HeartScore and BMI ($r=0.24$, $p<0.01$); AI and BMI ($r=0.34$, $p<0.0001$); and respectively HeartScore and AI ($r=0.59$, $p<0.0001$). Our study confirms that obesity affects serum lipoprotein profile-i.e. lipid ratio AI and that, in presence of obesity, it leads to higher cardiovascular risk. Lipid ratios remain useful tools for the diagnosis and prognosis of cardiovascular disease and by their associations with lipid parameters and their predictive values, these biomarkers could be helpful in the management of clinical treatments.
Key words: obesity, atherogenic index, HeartScore, aging

IMPACTUL OBEZITATII ASUPRA PROFILULUI LIPOPROTEINELOR SI RISCUL CARDIOVASCULAR LA PACIENTII VARSTNICI

Opriș *Simona* [1]**,** ***Constantin*** *Gianina* [1]**,** ***Grigorie*** *Mihaela* [1]**,** ***Halici*** *Florentina* [2]**,** ***Valuch*** *Anton* [1]

[1]*Institutul Național de Gerontologie și Geriatrie "Ana Aslan", București, Romania*
[2]*Regional Medical Compliance Manager –Europa, Zona Basel, Elvetia*

Autor corespondent: Simona Opris, simonaopris11@yahoo.com

Rezumat. Profilul lipidic si indicele aterogenic (AI) s-au dovedit a fi predictori puternici pentru tulburarile metabolice, inclusiv dislipidemie, ateroscleroza, hipertensiune si bolile cardiovasculare. Studiul nostru a urmarit sa investigheze impactul obezitatii asupra profilului lipoproteic -i.e. raportul lipidic AI si de a calcula riscul cardiovascular pe 10 ani, bazat pe HeartScore, la pacientii varstnici. Pacientii (n = 168) au fost impartiti in 3 grupe: Grupul 1 (n = 27) pacienti cu greutate normala - grupul de control; Grupul 2 (n = 77) pacienti supraponderali; Grupul 3 (n = 64) pacienti obezi. Valorile HeartScore sunt semnificativ mai mari ($p<0.0001$) la grupul 3 si 2 comparativ cu grupul de control 1 si, de asemenea, la grupul 3 comparativ cu grupul 2. In timp ce, comparand grupul 3 vs grupul 1 si vs grupul 2, am observat o crestere semnificativa AI ($p<0.005$, respectiv $p<0.001$). Intre clasele de obezitate - I, II si III, se observa o foarte usoara crestere, dar nesemnificativa, a HeartScore si AI. Ecuatia de regresie liniara a aratat la grupul 1, o corelatie semnificativ pozitiva intre HeartScore si BMI ($r = 0.54$, $p<0.001$). De asemenea, am observat la grupul 2, o corelatie pozitiva semnificativa intre: HeartScore si BMI ($r = 0.24$, $p<0.01$); AI si BMI ($r = 0.34$, $p<0.0001$); si, respectiv, HeartScore si AI (r =

0.59, p <0.0001). Studiul nostru confirma faptul ca obezitatea afecteaza profilul seric lipoproteic – i.e. AI si ca, in prezenta obezitatii, acesta duce la un risc mai mare cardiovascular. Raporturile lipidice raman instrumente utile pentru diagnosticul si prognosticul bolilor cardiovasculare si prin asocierea cu parametrii lipidici si valorile lor predictive, acesti biomarkeri ar putea fi utili in administrarea tratamentelor clinice.
Cuvinte cheie: obezitate, indice aterogenic, HeartScore, imbatranire

INTRODUCTION

Obesity, type 2 diabetes mellitus, dyslipidemia and hypertension remain major cardiovascular risk factors, whose prevalence and impact on overall cardiovascular risk are increasing.

Numerous studies have shown that the ratio of triglycerides/ HDL cholesterol (TG/HDL) is a strong predictor of heart attack. An abnormal report indicates an atherogenic lipid profile and a risk of developing myocardial infarction (1). Atherogenic index (AI), calculated as log(TG/HDL), reflects atherogenic potential of full lipoprotein fractions spectrum and has been described as a biomarker of plasma atherogenicity (2,3,4).

In the prevention of cardiovascular diseases, the European Society of Cardiology recommends the SCORE scale (5). To calculate the 10-year risk of cardiovascular death, the HeartScore calculator was used, which included age, systolic blood pressure, plasma total cholesterol level, and smoking habits. The relative risk of death was compared with the risks acceptable for the age of each person and the difference was calculated. The HeartScore risk estimation is based on the following risk factors: sex, age, smoking, systolic blood pressure, and total cholesterol. The threshold for high risk based on fatal cardiovascular events is defined as "higher than 5%" (6,7).

Our study aimed to investigate the impact of obesity on lipoprotein profiles –i.e. lipid ratio AI and to calculate 10-year risk of cardiovascular mortality based on the HeartScore, at elderly patients.

MATERIALS AND METHODS

Subjects

Elderly patients from NIGG "Ana Aslan" (>65 years, n=168) were divided into 3 groups:

- Group 1 (n=27) normal weight patients - as control group
- Group 2 (n=77) overweight patients
- Group 3 (n=64) obese patients
  - obesity class I (30-34.9 Kg/m^2) n=38
  - obesity class II (35-39.9 Kg/m^2) n=21
  - morbid obesity-class III (> 40 Kg/m^2) n=5

Anthropometric measurement were determined using standard protocols for each patients and included height, body mass, waist circumference, systolic and diastolic pressure.

Body mass index (BMI) was calculated and based on this, the patients were classified as: normal (BMI 18.5-24.9 Kg/ m^2), overweight (25-29.9 Kg/ m^2) and obese (>30 Kg/ m^2).

AI values are associated with:

- low risk 0.3 ÷ 0.1
- medium risk 0.1 ÷ 0.24
- high risk above 0.24

The HeartScore risk was divided into three subclasses according to the various algorithms:

- low risk (HeartScore < 2%),
- intermediate risk (HeartScore 2% but < 5%) and
- high risk (HeartScore>5%)

Statistical analysis

All values are presented as mean ± standard deviation. The results were statistically analyzed by using Student's "t" test, by Pearson's correlation coefficient and $p<0.05$ is considered to be statistically significant. The relationship between HeartScore, AI and BMI was assessed using a linear regression model.

RESULTS AND DISCUSSIONS

Abnormalities of blood lipids are related mainly to different dietary habits of people, lifestyle and heredity along with the other factors. Obese people seem to have an

adverse pattern of plasma lipoproteins. This could be due to increase in adipocyte mass and accompanying decrease in insulin sensitivity associated with obesity has multiple effects on lipid metabolism.

Kopelman et al. (8) reported alteration in lipid profile associated with obesity, elevated LDL concentrations as well as high concentrations of TG which rises the coronary heart disease risk.

Table I. Comparation of atherogenic index and HeartScore risk in relation to the BMI groups

	Group 1-Normal weight patients (n=27)	**Group 2- Overweight patients** (n=77)	**Group 3- Obese patients** (n=64)
Atherogenic index (AI)	$0.33 \pm 0.25^{**}$	0.37 ± 0.29^{tt}	0.49 ± 0.21
HeartScore	$1.88 \pm 1.28^{*t}$	$2.84 \pm 1.57^{*}$	4.29 ± 1.94
BMI (Kg/m^2)	$22.71 \pm 1.81^{*}$	$27.51 \pm 1.31^{*}$	$34.74 \pm 3.71^{*}$

Results are presented as means±D.S.; $p^{*}<0.0001$ vs. 3, $^{t}p<0.001$ vs. 2, $^{tt}p<0.001$ vs. .3, $^{**}p<0.005$ vs. 3

Between obesity class – I, II and III, HeartScore and AI shown a slight increase, but insignificant. This could be due to the fact that once lipid metabolism is altered (triggered by obesity), the risk of cardiovascular disease remains high, whatever are the classes of obesity.

Values of HeartScore are significantly higher ($p<0.0001$) in group 3 and 2 compared with control group 1 and also comparing group 3 to group 2 (Tab. I).

Comparation between group 2 and control group 1 showed no significant differences in AI values (Tab. I). While comparing group 3 vs. control group 1 and vs. group 2 we noticed a significant increased in AI ($p<0.005$ respectively $p<0.001$). In agreement to our study, Oladipo et al. (9) shown significant increase of AI values too.

At control group 1, HeartScore is positive significantly correlated ($r=0.54$, $p<0.001$) with BMI, according to linear regression equation (Fig.1).

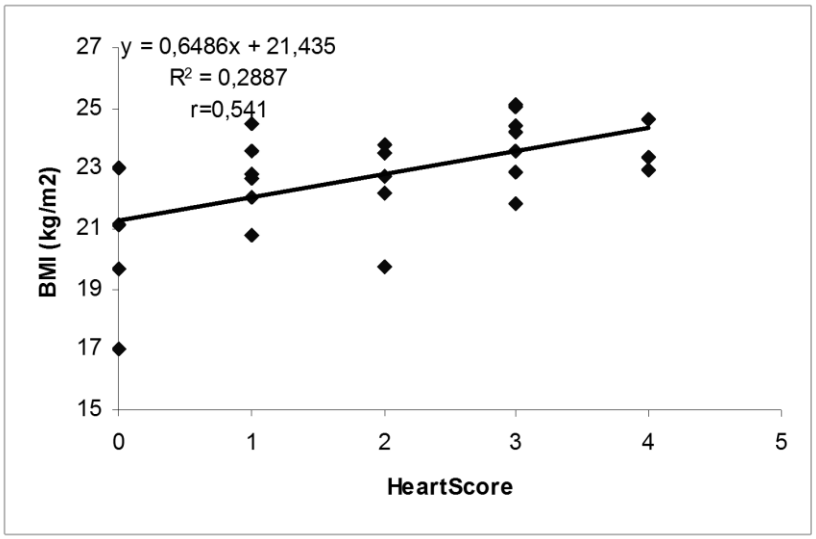

Fig.1. Correlation between HeartScore and BMI in normal weight group patients
Curve fitting was by linear regression; r = correlation coefficient

As shown in Fig.2, Fig.3 and respectively Fig.4, we observed at group 2, positive significant correlation between: HeartScore and BMI ($r=0.24$, $p<0.01$); AI and BMI ($r=0.34$, $p<0.0001$); and respectively HeartScore and AI ($r=0.59$, $p<0.0001$).

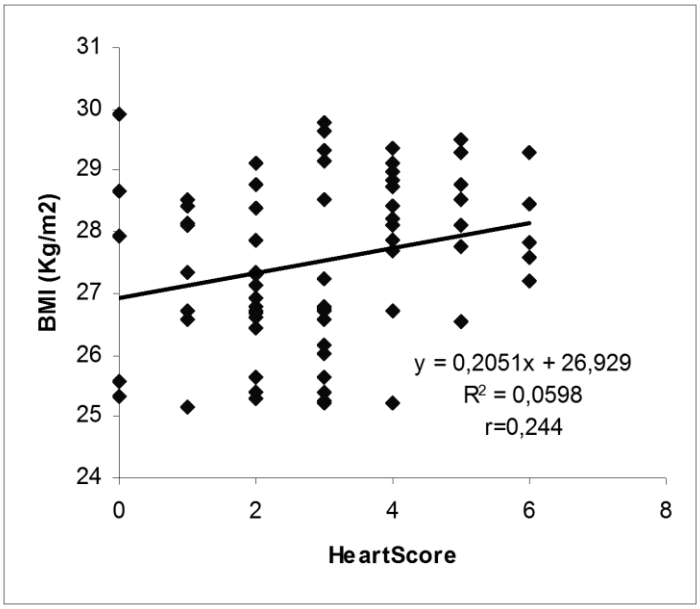

Fig.2. Correlation between HeartScore and BMI in overweight group patients
Curve fitting was by linear regression; r = correlation coefficient

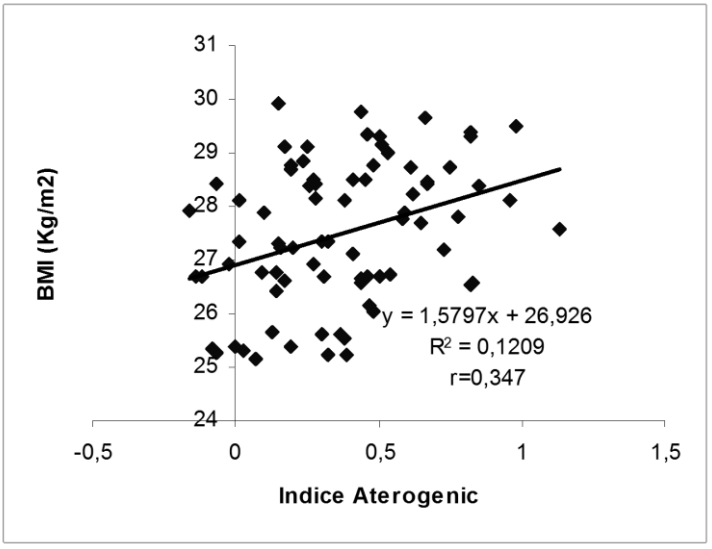

Fig.3. Correlation between AI and BMI in overweight group patients
Curve fitting was by linear regression; r = correlation coefficient

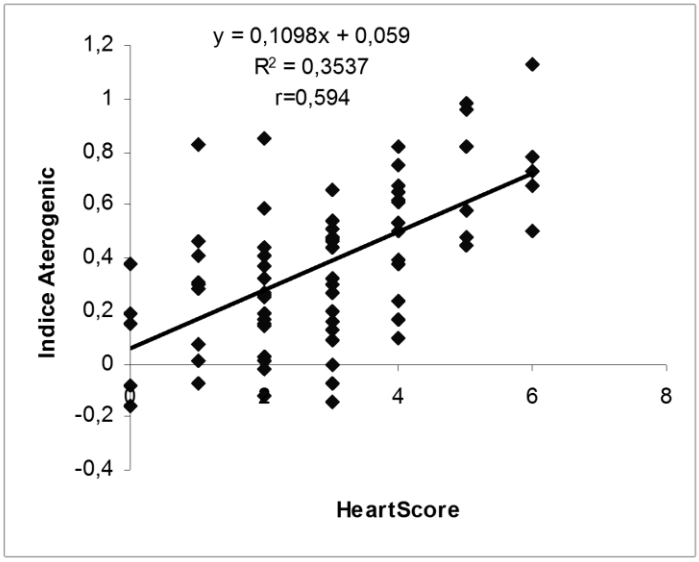

Fig.4. Correlation between HeartScore and AI in overweight group patients
Curve fitting was by linear regression; r = correlation coefficient

Other positive significant associations between HeartScore and AI (r=0.24, $p<0.05$), at group 3, were also observed (Fig.5).

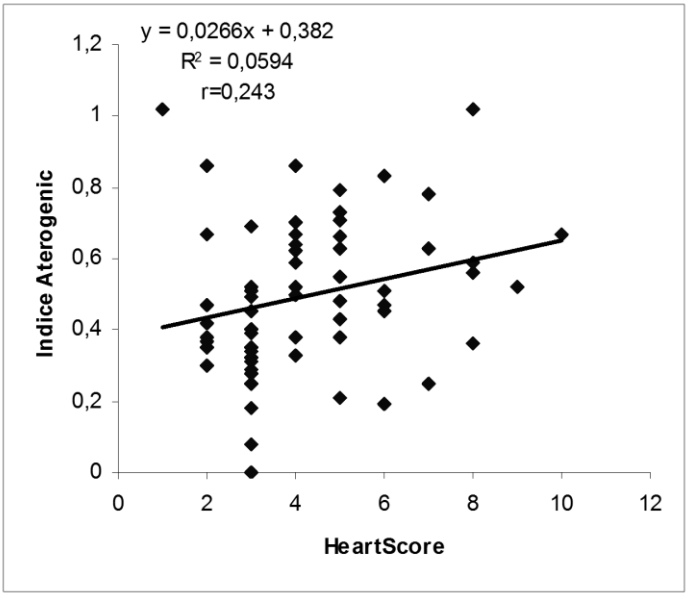

Fig.5. Correlation between HeartScore and AI in obese group patients
Curve fitting was by linear regression; r = correlation coefficient

The positive correlations observed between the lipids/ratio lipids and BMI were in corroboration with other studies (10,11,12,13,14) and reaffirmed the role of lipids in the pathophysiology of overweight and obesity as well as increasing accumulation of lipids with aging.

Torng et al. (15) reported obesity to be strongly associated with elevated levels of lipids and significant association between BMI and HDL, TG and LDL which was similar observed in our study. Studies even revealed the adverse effect of abnormal blood lipid and lipoprotein levels in the pathogenesis and progression of atherosclerosis and cardiovascular diseases, in obese patients.

CONCLUSIONS

Our study confirms that obesity affects serum lipoprotein profile-i.e. lipid ratio AI and that, in presence of obesity, it leads to higher cardiovascular risk. Management targeting lifestyle chages, such as low-caloric and low-fat diets and regular physical activity, may also avoid cardiovascular complications.

Lipid ratios remain useful tools for the diagnosis and prognosis of cardiovascular disease and by their associations with lipid parameters and their predictive values, these biomarkers could be helpful in the management of clinical treatments. Moreover, AI and HeartScore together can be useful in major cardiac events prevention.

REFERENCES

[1] Veerendra KA, Nutakki V, and Ramarao J. *Dose-dependent Impacts on the Diagnostic Efficacies of Atherogenic Lipids in Adult Indian Smokers*. Journal of Clinical and Diagnostic Research, 2011, 5, 7; 1352-1355.

[2] Raslova K, Dobiasova M, Hubacek JA, et al. *Association of metabolic and genetic factors with cholesterol esterification rate in HDL plasma and atherogenic index of plasma in a 40 years old Slovak population.* Physiol Res., 2011,60:785–795.

[3] Dobiasova M, Frohlich J. *The plasma parameterlog (TG/HDL-C) as an atherogenic index:correlation with lipoprotein particle size and esterification rate in apoB-lipoprotein-depleted plasma (FER(HDL)).* Clin Biochem,2001,34:583–588.

[4] Kanthe PS, Patil BS, Bagali S, et al. *Atherogenic index as a predictor of cardiovascular risk among women with different grades of obesity*. Inter J Coll Res Int Med&Public Health, 2012, 4, 10, 1767-1774.

[5] *European Guidelines on cardiovascular disease prevention in clinical practice* (version 2012) The Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice. Eur Heart J, 2012; 33: 1635–701.

[6] Krzysztof J, Jolanta G, Dorota L. *Prevalence of the coronary artery disease risk factors and 10-year risk of cardiovascular mortality based on HeartScore in people qualified for kidney donation.* Ann Transplant, 2013,18: 393-398.

[7] Diederichsen AC, Mahabadi AA, Gerke O, et al. *Increased discordance between HeartScore and coronary artery calcification score after introduction of the new ESC prevention guidelines*. Atherosclerosis, 2015, 239(1):143-9.

[8] Kopelman P. *Health risks associated with overweight and obesity*. Obesity Reviews, 2007,8, 1, 13-17.

[9] Oladipo A. *Plasma lipid profile, atherogenic and coronary risk indices in some redidents of Abeokuta in south-western Nigeria*. Biokemistri, 2008,20, 2, 85-91.

[10] Ikewuchi CJ. *Alteration of Plasma Lipid Profile and Atherogenic Indices of Cholesterol Loaded Rats by Tridax Procumbens Linn: Implications for the Management of Obesity and Cardiovascular Diseases*.Biokemistry,2009,21(2):95199.

[11] Ugwuja EI, Ogbonna NC, Nwibo AN, Onimawo IA. *Overweight and obesity, lipid profile and atherogenic indices among civil servants in Abakaliki, south eastern Nigeria*. Ann Med Health Sci Res, 2013,3, 1, 13-18.

[12] Santos APC, Vieira MS, Débora F. et al. *Atherogenic Indices and HDL Particle Size as Laboratory Parameters to Evaluate Cardiovascular Risk in the Presence of Dyslipidemia.* Journal of Biophysical Chemistry, 2014,5, 24-32.

[13] Khanam M, Khan AH, Rahman R, et al. *Comparison of Atherogenic Index of Plasma (AIP) Between Postmenopausal Women with Central Obesity and Postmenopausal Nonobese Women.* Chattagram Maa-O-Shishu Hospital Medical College Journal, 2014,13, 2, 47-50.

[14] Essiarab F, Hassan T, Lebrazi H et al. *Usefulness of lipid ratios and atherogenic index of plasma in obese Moroccan women with or without metabolic syndrome.* Ethnicity & Disease, 2014, 24,201-212.

[15] Torng PL. *Effect of menopause and obesity on lipid profiles in middle-aged Taiwanese women: the Chin-Shan Community Cardiovascular Cohort Study*. Atherosclerosis, 2000, 153, 413-421.

EPIGENETIC FACTORS IN CARDIOVASCULAR DISEASE. AGEING STUDIES. REVIEW

***Valuch** Anton[1], **Gherasim** Petru[1], **Opriș** Simona[1]*

[1] "Ana Aslan, National Institute of Gerontology and Geriatrics" Romania

Corresponding author: Anton Valuch, valuch_anton@yahoo.com

Abstract. Epigenetic is defined as changes in phenotype and gene expression (RNA, proteins) that occur without alterations of DNA sequence. Epigenetic changes involve alterations in DNA methylation patterns, posttranslational modification of histones, and chromatin remodelling. As a potential risk factor for cardiovascular diseases, hyperhomocysteinemia may initiate or motivate atherogenesis by modification of DNA methylation. This review focuses on correlation between some factors with epigenetic roles B9, B12 vitamins and homocysteine in human cardiovascular disease, based on some our data. B9 and B12 vitamins are inverse correlated with homocysteine ($r = -0.369$, $p < 0.05$, $n = 32$ and $r = -0.389$, $p < 0.05$, $n = 32$, respectively). This suggests that both vitamins have a benefic effect on vascular system through diminishing homocysteine and hypomethylation level. We observed an inverse correlation between age and serum folates ($r = - 0.373$, $p < 0.05$, $n = 29$), in cardiovascular diseases. We found that both vitamins correlates direct with HDLC (HDL cholesterol levels): B9 ($r = 0.484$, $p < 0.01$, $n = 29$) and B12 ($r = 0.673$, $p < 0.01$, $n = 16$). This suggests that both vitamins have a benefic effect on vascular system through HDLC mediation.
Key words: epigenetic, vitamin B9, vitamin B12, homocysteine, ageing

FACTORII EPIGENETICI ÎN BOALA CARDIOVASCULARĂ. STUDII DE ÎMBĂTRÂNIRE. ANALIZĂ

***Valuch** Anton[1], **Gherasim** Petru[1], **Opriș** Simona[1]*

[1]Institutul Național de Gerontologie și Geriatrie "Ana Aslan", București, Romania

Autor corespondent: Anton Valuch, valuch_anton@yahoo.com

Rezumat. Epigenetica se ocupă cu schimbările fenotipului şi ale expresiei genetice (ARN, proteine) ce se produc fără alterări ale secvenţei ADN. Schimbările epigenetice implică alterări în modelul de metilare al ADN, modificări posttranslaţionale ale histonelor şi remodelării acromatinei. Hiperhomocisteinemia, ca potenţial factor de risc in boala cardiovasculară, poate iniţia sau susţine aterogeneza prin modificarea metilării ADN. Lucrarea trece in revista corelaţiile dintre câţiva factori epigenetici: vitaminele B9, B12 şi homocisteină, în boala cardiovasculară, bazat si pe câteva din datele noastre. B9 şi B12 sunt invers corelate cu homocisteina ($r = -0.369$, $p < 0.05$, $n = 32$ and $r = -0.389$, $p < 0.05$, $n = 32$, respectiv). Aceasta sugerează că ambele vitamine au un efect benefic asupra sistemului vascular prin diminuarea homocisteinei şi a nivelului de hipometilare. Am observat ca exista o corelaţie inversă între vârstă şi folaţii serici ($r = - 0.373$, $p < 0.05$, $n = 29$), in boala cardiovasculară. Ambele vitamine se corelează direct cu nivelurile de HDL colesterol (HDLC): B9 ($r = 0.484$, $p < 0.01$, $n = 29$) şi B12 ($r = 0.673$, $p < 0.01$, $n = 16$), ceea ce sugerează că au un efect benefic asupra sistemului vascular şi prin medierea HDLC.
Cuvinte cheie: epigenetică, vitamina B9, vitamina B12, homocisteină, vârsta

INTRODUCTION

Epigenetics refers to external modifications to DNA that turn genes "on" or "off." These modifications do not change the DNA sequence. The term epigenetic has a generic meaning "extra, out genetics". A consensus definition of the concept of epigenetic trait as "stably heritable phenotype resulting

from changes in a chromosome without alterations in the DNA sequence" was formulated at a Cold Spring Harbor meeting in 2008. We used to think that a new embryo's epigenome was completely erased and rebuilt from scratch. But this isn't completely true. Some epigenetic tags remain in place as genetic information passes from generation to generation, a process called epigenetic inheritance.

Epigenetic inheritance is an unconventional finding. It goes against the idea that inheritance happens only through the DNA code that passes from parent to offspring. It means that a parent's experiences, in the form of epigenetic tags, can be passed down to future generations.

As unconventional as it may be, epigenetic inheritance is real. In fact, it explains some strange patterns of inheritance geneticists have been puzzling over for decades. At certain times during development (the timing varies among species), specialized cellular machinery scours the genome and erases its epigenetic tags in order to return the cells to a genetic "blank slate." Yet, for a small minority of genes, epigenetic tags make it through this process and pass unchanged from parent to offspring.

Epigenetic alterations affect all cells and tissues throughout life (1) (Fig. 1). Changes involve alterations in DNA methylation patterns, posttranslational modification of histones, and chromatin remodeling. Increased histone H4K16 acetylation, H4K20 trimethylation, or H3K4 trimethylation, as well as decreased H3K9 methylation or H3K27 trimethylation, constitute age-associated epigenetic marks. The multiple enzymatic systems assuring the generation and maintenance of epigenetic patterns include DNA methyltransferases, histone acetylases, deacetylases, methylases, and demethylases, as well as protein complexes implicated in chromatin remodeling.

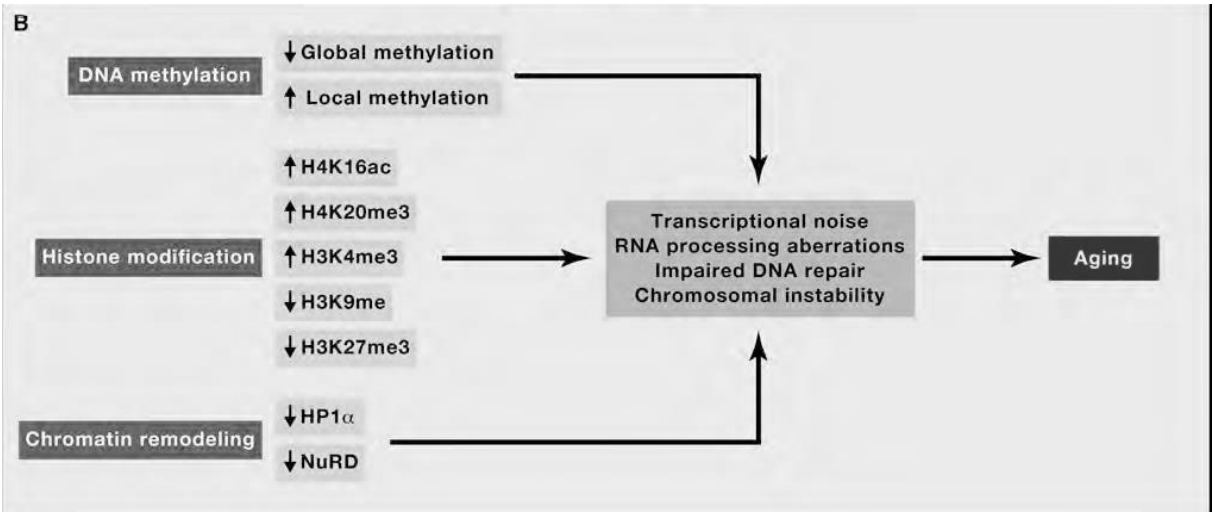

Fig. 1. Epigenetic alterations. Alterations in the methylation of DNA or acetylation and methylation of histones, as well as of other chromatin-associated proteins, can induce epigenetic changes that contribute to the aging process

DNA Methylation

Gene or DNA methylation in humans consist in the addition of a methyl group from S-adenosylmethionine and occurs at cytosine-phosphoguanine sites, near the gene promoter and has a major impact on gene expression. Methylation of mammalian genome suffers significant changes during early development, related to the rapid differentiation of many tissues and organs. After finishing differentiates the methylation pattern may show tissue specificity and will stabilize. DNA hypomethylation is one of the major DNA methylation states and indicates in general a decrease from the “normal” methylation

level. The first experimental of the age-dependent loss of genomic methylation was provided by Berdishev (3) and Vanyushin (4), who found that the content of 5-methylcytosine (5meC) in DNA isolated from the various organs of humpback salmon was decreased during ontogenesis. These findings were confirmed in later studies that documented age-dependent DNA hypomethylation in many mammalian tissues. The maximal amount of 5meC was observed in DNA isolated from tissues of embryos and newborn animals and gradually decreased upon aging. Further evidence confirming the loss of DNA methylation during the aging process was obtained in in vitro studies that demonstrated that a marked decrease in the 5meC content in DNA is associated with a number of cell divisions in normal diploid mouse, hamster, and human cells, in contrast to the immortal cell lines. Based on these findings, it has been proposed by Holliday that changes in DNA methylation patterns may have significance in the aging process. Since that time, a number of in vivo and in vitro findings have established that normal aging mammalian cells (mice, rats, and humans) show a progressive loss of 5meC content in DNA (5).

The mechanism of DNA hypomethylation is still unclear, and there is very likely no universal mechanism that describes demethylation of DNA alone. However, it is well established that upon aging, several factors including the activity and expression of DNA methyltransferases, the status of one-carbon metabolism, and the integrity of the genome may trigger and contribute to the loss of genomic methylation. Aging DNA hypomethylathion correlates also with the development of age-related pathologies such as cancer, atherosclerosis, neurodegeneration, and autoimmune disease.

Histone Modifications

Histones are basic proteins with a large proportion of positively charged amino acids, mainly arginine and lysine, and they can be posttranslationally modified through methylation, acetylation, phosphorylation, ubiquitination, and ADP-ribosylation. Most of these modifications take place on their "tail" domains (6). The histone tails, which protrude from the surface of the chromatin polymer and are protease sensitive, compromise 25–30% of the mass of individual histones, thus provides an exposed surface for potential interactions with other proteins. Modifications, performed by histone acetyltransferases (HATs), histone deacetylases (HDACs), histone methyltransferases (HMTs), and histone kinases (HKs), offer a mechanism through which upstream signaling pathways can converge on common targets to regulate gene expression. Acetylation of histones neutralizes their positively charged, lysine-rich amino-terminal tails, loosening the histone–DNA contacts, thus making DNA more accessible at these specific sites for transcription (7).

Histone methylation is a marker of aging, especially in invertebrates (8,9). Inhibition of histone demethylases (for H3K27) in worms may extend lifespan by targeting components of key longevity routes such as the insulin/IGF-1 signaling pathway (10). It is not clear whether manipulations of histone-modifying enzymes can influence aging through purely epigenetic mechanisms, by impinging on DNA repair and genome stability, or through transcriptional alterations affecting metabolic or signaling pathways outside of the nucleus.

Members of the sirtuin family of NAD-dependent protein deacetylases and ADP ribosyltransferases have been studied extensively as potential anti-aging factors. Several of sirtuin paralogs can ameliorate various aspects of aging in mice (11,12). In particular, transgenic over expression of mammalian SIRT1, which is the closest homolog to invertebrate Sir2, improves aspects of health during aging but does not increase longevity. The mechanisms involved in the beneficial effects of SIRT1 are complex and interconnected, including

improved genomic stability and enhanced metabolic efficiency (13).

Chromatin Remodeling

DNA- and histone-modifying enzymes act simultaneously with key chromosomal proteins, such as the heterochromatin protein 1a (HP1a), and chromatin remodeling factors, such as Polycomb group proteins or the NuRD complex, whose levels are diminished in both normally and pathologically aged cells (14). Alterations in these epigenetic factors determine changes in chromatin architecture, such as global heterochromatin loss and redistribution, which constitute characteristic features of aging (15). The causal relevance of these chromatin alterations in aging is supported by the finding that flies with loss-of-function mutations in HP1a have a shortened lifespan, whereas over expression of this heterochromatin protein extends longevity in flies and delays the muscular deterioration characteristic of old age (16).
Mammalian telomeric repeats are also enriched for these chromatin modifications, indicating that chromosome ends are assembled into heterochromatin domains. Subtelomeric regions also show features of constitutive heterochromatin, including H3K9 and H4K20 trimethylation, HP1a binding, and DNA hypermethylation. Thus, epigenetic alterations can directly impinge on the regulation of telomere length, one of the hallmarks of aging.

Transcriptional Alterations

Aging is associated with an increase in transcriptional noise and an aberrant production and maturation of many mRNAs (17). Microarray- based comparisons of young and old tissues from several species have identified age-related transcriptional changes in genes encoding key components of inflammatory, mitochondrial, and lysosomal degradation pathways. These aging-associated transcriptional signatures also affect noncoding RNAs, including a class of miRNAs (gero-miRs) that is associated with the aging process and influences lifespan by targeting components of longevity networks or by regulating stem cell behavior (18,19)

Reversion of Epigenetic Changes

Unlike DNA mutations, epigenetic alterations are—at least theoretically reversible, hence offering opportunities for the design of novel anti-aging treatments (20,21). Restoration of physiological H4 acetylation through administration of histone deacetylase inhibitors avoids the manifestation of age-associated memory impairment in mice, indicating that reversion of epigenetic changes may have neuroprotective effects. Inhibitors of histone acetyltransferases also ameliorate the premature aging phenotypes of progeroid mice and extend their lifespan. Conceptually similar to histone acetyltransferase inhibitors, histone deacetylase activators may conceivably promote longevity. Resveratrol has been extensively studied in relation to aging, and among its multiple mechanisms of action are the upregulation of SIRT1 activity, as well as other effects associated with energetic deficits.

One-Carbon Metabolism, folate, methionine cycles.

Important epigenetic compunds are implicated in folate, methionine cycles. The term folate is derived from the Latin word folium which means leaf. Folate refers to all pteroylglutamates possessing vitamin B9 activity. ‘Folic acid’ and ‘folate’ are the preferred synonyms for pteroylglutamic acid (PteGlu) and pteroylglutamate, respectively. Folate describes a family of related molecules that are capable of one-carbon transfer (DHF, THF, 5, 10-MTHF, 5-MTHF). Folic acid is oxidized form of B9 and is a synthetic form. Other hydrogenated forms are natural ones and can be synthesized by plants in mitochondria and by microorganisms, but must be ingested with food by animals, including humans.

Deficiencies in exogenous sources of methyl; vitamins B9, B12, methionine, serine, choline exert pathogenic effects in humans. It has been shown that halving the methionine intake will double the average amount of re-methylation cycles gone through per homocysteine molecule. The folates found in food consist of a mixture of reduced folate polyglutamates. These are forms the body can utilize easily. It is found in green vegetables, yeast, egg yolk, liver, and kidney. The reduced forms of the vitamin, particularly the unsubstituted dihydro and tetrahydro forms, are unstable chemically. There is a decrease in folate during harvesting, storage, processing, and preparation. Half or even three-quarters of initial folate activity may be lost during these processes. Thus, natural folates rapidly lose activity in foods over periods of days or weeks and this is one reasons garden fresh food is healthier than food that has been stored. This is in contrast to the stability of the synthetic form of this vitamin, folic acid that manufacturers use. Human can metabolize synthetic form via reduction reactions catalyzed by DHFR. Result DHF in a slow and incomplete reaction. Result also an increased concentration of folic acid for a long period that increased incidence of some adverse reaction. Folic acid must be used in measured concentration. Natural hydrogenated forms of folate are better tolerated. Folate is used for a good embryonic development but also to improve cognitive function in aging or reducing risk of vascular disease.

One-carbon metabolism (Fig. 2), a network of cellular methylation reactions is essential for nucleotide biosynthesis and nucleotide and protein methylation.

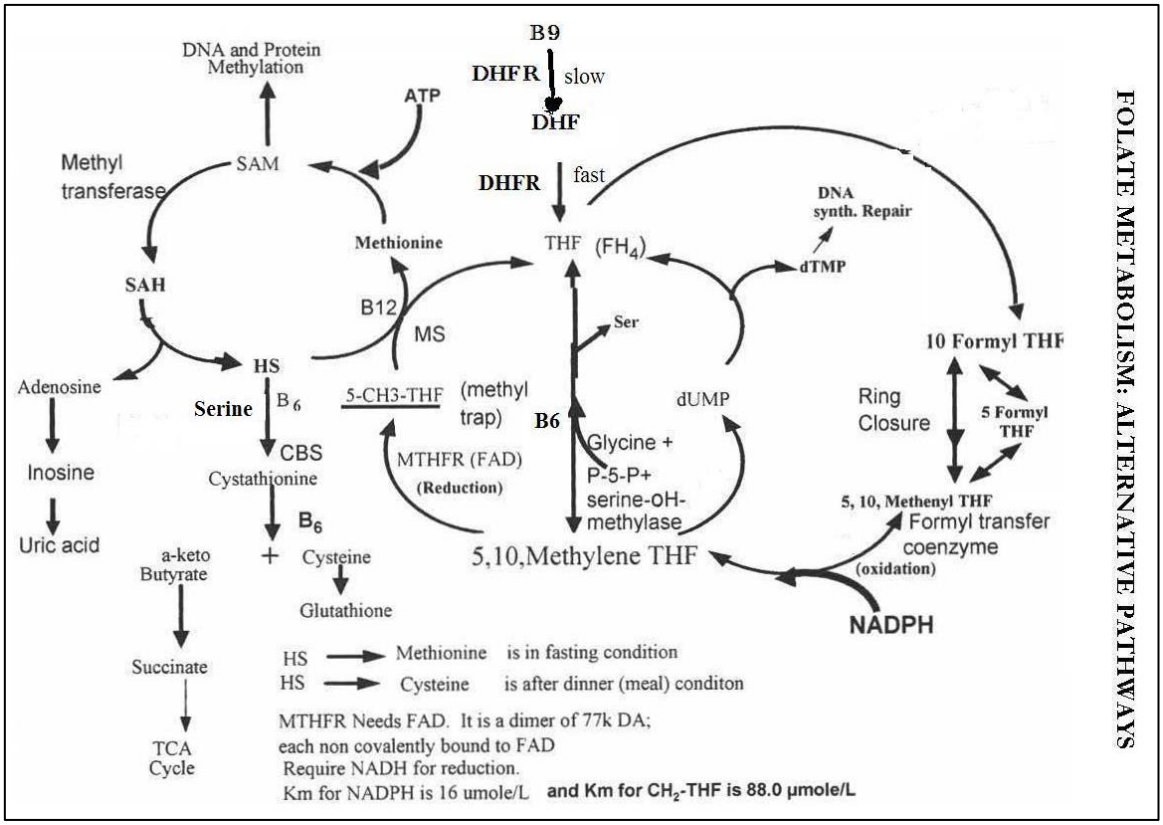

Fig. 2. One-Carbon Metabolism in folate, SAM cycles. This schematic shows the process by which folate/folic acid is used for DNA methylation. CBS, Cystathionine ß Synthase DHF, dihydrofolate; DHFR, dihydrofolate reductase; dTMP, deoxythymidine monophosphate; dUMP, deoxyuridine monophosphate; HS, homocysteine; MS, methionine synthase; MTHFR, methylenetetrahydrofolate reductase; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; THF, tetrahydrofolate; TS, thymidylate synthase.

The process of methylation requires two cycles of events; SAM cycle (also called one-carbon cycle or methionine cycle) and the folate cycle. The folate cycle is essential for regulation of the SAM cycle and for keeping methionine availability in producing SAM. The enzyme methionine synthase is an essential part of these cycles. 5 methyl -THF promotes the re-methylation of HS to methionine. HS is cytotoxic , increases oxidative stress, alters lipid metabolism and promotes excessive blood clotting. The effect does not seem to be directly because HS is an aminoacid with a reduced thiol group as cysteine or GSH. This one being synthesized from HS and serine through transsulfuration pathway. Three important way try to explain HS toxicity; 1. HS activates NMDAR receptors (N-methyl-D-aspartate receptor), non-specific cation channel that can allow the passage of Ca^{2+} into the cell and apoptosis. 2. "Hcy-thiolactone hypothesis 3. Damaging effects on the HDL-associated enzyme paraoxonase1, or PON1, which is required to protect LDL from oxidation?

The methyl groups that are needed for all cellular biological methylation reactions, including DNA methylation, are acquired from SAM, the primary universal donor of methyl groups in mammals derived from methionine in the one-carbon metabolic pathway. This indispensably connects the status of epigenetic modifications – DNA methylation status to the functioning level of the one-carbon metabolic pathway (Fig. 2). SAM can bind certain RNA structures called riboswitches that control transcription and/or translation (22). Deregulation of SAM through vitamin shortage is implicated in longevity and aging. B107. 95% of all SAM is used for methylation of a wide variety of molecules and 3–5% for the generation of decarboxylated SAM (dcSAM). In humans, 85% of all of these methylation reactions and 50% of all methionine metabolism takes place in a single organ, the liver. A small proportion of SAM is used by perhaps as many as 1000 different proteins for formation of 5-deoxyadenosyl radicals that perform vital reactions in the cell (23).

Demethylation of SAM yields SAH which through hydrolyze formes HS. HS is converted to cysteine by transsulfuration pathway (cysteine is a precursor for the antioxidant GSH). HS may be remethylated to methionine in methionine cycle, being substrate for methionine synthase (MS) with methylated tetrahydrofolate (MTHF) as methyl donor (B12 intermediar acceptor de metil si donor) and the cycle is complete after fusion of methionine and the adenosine part of ATP by methionine adenosyltransferase (MAT, also called S-adenosylmethionine synthetase).

SAM is the major regulator of folate-dependent homocysteine remethylation. SAM intrinsically mediates this shunting of homocysteine to maintain a stable re-methylation cycle. SAM has been shown to activate CBS while simultaneously inhibiting MTHFR, an enzyme responsible for 5 MTHF syntheses. Thus, a high level of methionine (after dinner) with its concurrent raised level of SAM will promote the transsulfuration pathway and suppress re-methylation, lowering the methionine and homocysteine back to the basal level. A low level of methionine with its concurrent low level of SAM will suppress the transsulfuration pathway and promote re-methylation, growing the methionine and homocysteine back to the basal level. It is also recognized that SAH functions as a potent product inhibitor of SAM-dependent methyltransferases. Decreasing the SAM: SAH ratio reduces the activity of the SAM dependent methyltransferase enzyme. For this reason, continual hydrolysis of SAH to homocysteine is essential to maintain normal DNA methylation (24).

B9 and B12 vitamins can not be synthesized in humans. All mammalian cells require these two vitamins to recycle

methionine and homocysteine (with methyl availability for biomethylation by SAM), for converting deoxyuridine to thymidine, pyrimidine and purine synthesis. In folate deficiency, the dependent reactions can be slowed and cell growth may be inhibited by insufficient intake of substrate or toxic intermediates accumulated. In mammalian cells, the synthesis of methionine from homocysteine asks the folate as a methyl donor and vitamin B12 as an intermediate methyl acceptor and donor. In case of folate or vitamin B12 deficiency, homocysteine or its byproducts can accumulate in plasma and other tissues with pathological effect.

We studied some of epigenetic factors implicated in these methylathion reactions like B9, B12 and homocysteine in correlation with age in cardiovascular disease. Our data are review from 3 papers and synthesized in Table I.

We observed an inverse correlation between age (68.4 ± 8.0) years and serum folates ($r = -0.373$, $p < 0.05$, $n = 29$), in cardiovascular disease (25). Decreasing of serum folate in aging means decrease of SAM with the danger of hypomethylation of promoters of oncogenes and activation of silent transposons, resulting in chromosome instability and cancer, but also a decrease in folate-derived factors with concomitant incorporation of uracil in DNA instead of thymine, as well as futile cycles of DNA repair and chromosome breaks.

Vitamins B9 and B12 are epigenetic factors, implicated in DNA methylathion, through SAM intermediation. But low levels of these vitamins are not correlated with age only on this pathway. We found that both vitamins correlates direct with high-density lipoprotein cholesterol (HDLC) levels. Our data shows a direct and significant correlation between serum B12 vitamin and HDLC ($r = 0.673$, $p < 0.01$, $n = 16$). Serum folates are also direct correlated with HDLC ($r = 0.484$, $p < 0.01$, $n = 29$). This suggests that B12 vitamin and serum folates have a benefic effect on vascular system through through HDLC mediation (26).

The involvement of DNA hypomethylation in the context of atherosclerosis was based on evidence that elevated plasma homocysteine is a risk factor for atherosclerosis and the fact that homocysteine and SAH efficiently inhibit DNA methyltransferases, causing hypomethylation of DNA (27).

B9 and B12 vitamins are inverse correlated with homocysteine ($r = -0.369$, $p < 0.05$, $n = 32$ and $r = -0.389$, $p < 0.05$, $n = 32$, respectively). This suggests that B12 vitamin and serum folates have a benefic effect on vascular system through diminishing homocysteine and hypomethylation level (28).

.

Table I. Pearson correlations between some epigenetic factors and age in human cardiac disease.

	B9	B12	HS	HDLC	AGE
B9	r = 1		r = -0.369 p < 0.05 n = 32	r = 0.484 p < 0.01 n = 29	r = - 0.373 p < 0.05 n = 29
B12		r = 1	r = -0.389 p < 0.05 n = 32	r = 0.673 p < 0.01 n = 16	
HS	r = -0.369 p < 0.05 n = 32	r = -0.389 p < 0.05 n = 32	r = 1		
HDLC	r = 0.484 p < 0.01 n = 29	r = 0.673 p < 0.01 n = 16		1 r = 1	
AGE	r = - 0.373 p < 0.05 n = 29				r = 1

r - Pearson correlation

CONCLUSIONS

There are multiple lines of evidence suggesting that aging is accompanied by epigenetic changes and that epigenetic perturbations can provoke aging process. SIRT6 exemplifies an epigenetically relevant enzyme whose loss of function reduces longevity and whose gain of function extends longevity (29,30).
Understanding and manipulating the epigenome holds promise for improving age-related pathologies and extending healthy lifespan.
B9 and B12 vitamins are benefic for cardiovascular system by methylathion mechanism and also by another mechanism associated with direct correlation with HDLC level.
Ageing in human cardiovascular disease is accompanied by B9 vitamin decrease, with producing of a hypomethylathion state, what may be a risk factor in in the pathogenesis of this disease.

REFERENCES

[1] Talens, R.P., Christensen, K., Putter, H., et al. *Epigenetic variation during the adult lifespan: cross-sectional and longitudinal data on monozygotic twin pairs*. Aging Cell, 2012,11, 694–703.
[2] Han, S., and Brunet, A. *Histone methylation makes its mark on longevity*. Trends Cell Biol., 2012,22, 42–49.
[3] Berdishev, G.D., Korotaev, G.K., Bojarskikh, G.V., et al. *Nucleotide composition of DNA and RNA from somatic tissues of humpback salmon and its changes during spawning.* Biochemistry (Mosc.), 1967,32:988–993.
[4] Vanyushin, B.F., Korotaev, G.K., Mazin, A.L., et al. *Investigation of some characteristics of the primary and secondary structure of DNA from the liver of spawning humpback salmon.* Biochemistry (Mosc.), 1969,34:191–198.
[5] Richardson, B.C. *Role of DNA methylation in the regulation of cell function: autoimmunity, aging and cancer*. J. Nutr., 2002,132:2401S–2405S.
[6] Bannister, A. J., and Kouzarides, T. *Reversing histone methylation*. Nature, 2005,436: 1103–1106.
[7] Roth, S. Y., Denu, J. M., and Allis, C. D. *Histone acetyltransferases*. Annu Rev Biochem, 2001,70:81–120.
[8] Greer, E.L., Maures, T.J., Hauswirth, A.G., et al. *Members of the H3K4 trimethylation complex regulate lifespan in a germline-dependent manner in C. elegans.* Nature, 2010,466, 383–387.
[9] Siebold, A.P., Banerjee, R., Tie, F., et al. *Polycomb Repressive Complex 2 and Trithorax modulate Drosophila longevity and stress resistance*. Proc. Natl. Acad. Sci. USA ,2010,107, 169–174.
[10] Jin, C., Li, J., Green, C.D., Yu, X., et al. *Histone demethylase UTX-1 regulates C. elegans life span by targeting the insulin/IGF-1 signaling pathway*. Cell Metab., 2011,14, 161–172.
[11] Houtkooper, R.H., Pirinen, E., and Auwerx, J. *Sirtuins as regulators of metabolism and healthspan.* Nat. Rev. Mol. Cell Biol. ,2012,13, 225–238.
[12] Sebastian, C., Satterstrom, F.K., Haigis, M.C., et al. *From sirtuin biology to human diseases: an update.* J. Biol. Chem, 2012,287, 42444–42452
[13] Nogueiras, R., Habegger, K.M., Chaudhary, N., et al. *Sirtuin 1 and sirtuin 3: physiological modulators of metabolism.* Physiol. Rev., 2012,92, 1479–1514.
[14] Pollina, E.A., and Brunet, A. *Epigenetic regulation of aging stem cells*. Oncogene, 2011,30, 3105–3126.
[15] Tsurumi, A., and Li, W.X. *Global heterochromatin loss: a unifying theory of aging?* Epigenetics, 2012,7, 680–688.
[16] Larson, K., Yan, S.J., Tsurumi, A., et al.*Heterochromatin formation promotes longevity and represses ribosomal RNA synthesis.* PLoS Genet. ,2012,8, e1002473.
[17] Harries, L.W., Hernandez, D., Henley, W., et al. *Human aging is characterized by focused changes in gene expression and deregulation of alternative splicing*. Aging Cell, 2011,10, 868–878.
[18] Boulias, K., and Horvitz, H.R. *The C. elegans microRNA mir-71 acts in neurons to promote germline-mediated longevity through regulation of DAF- 16/FOXO*. Cell Metab,2012,15, 439–450.
[19] Toledano, H., D'Alterio, C., Czech, B., et al. *The let-7-Imp axis regulates ageing of the Drosophila testis stem-cell niche.* Nature, 2012,485, 605–610
[20] Freije, J.M., and Lopez-Otın, C. *Reprogramming aging and progeria*. Curr. Opin. Cell Biol., 2012,24, 757–764.

[21] Rando, T.A., and Chang, H.Y. *Aging, rejuvenation, and epigenetic reprogramming: resetting the aging clock.* Cell, 2012,148, 46–57.
[22] Corbino KA, Barrick JE, Lim J et al. *Evidence for a second class of S-adenosylmethionine riboswitches and other regulatory RNA motifs in alpha-proteobacteria.* Genome Biol, 2005; 6:R70.
[23] Buckel, W., B.T. Golding. *Radical enzymes in anaerobes*. Annu. Rev. Microbiol. 2006, 60, 27–49.
[24] James SJ, Melnyk S, Pogribna M et al. *Elevation in S-adenosylhomocysteine and DNA hypomethylation: potential epigenetic mechanism for homocysteine-related pathology.* J Nutr,2002; 132:2361S–6.
[25] Valuch A, Lupeanu E, Gherasim P, et al. *B9 and B12 vitamins in human cardiovascular aging.* International Academy on Nutrition and Aging. Hyatt Regency Tamaya Resort & Spa Santa Ana Pueblo New Mexico – USA, 2010,July 26-27.
[26] Valuch A, Lupeanu E, Gherasim P, et al. *Vitamins induced methylation in human cardiovascular aging.* Congresul Internaţional de Geriatrie şi Gerontologie "Comunităţile Longevive şi Fragilitatea Vârstnicilor" « Sociétés De Longue Vie Et Fragilité Des Aînés », 2010, Palatul Parlamentului – Bucureşti – 17 Iunie–18 Iunie.
[27] Yi P, Melnyk S, Pogribna, M, et al. *Increase in plasma homocysteine associated with parallel increases in plasma S-adenosylhomocysteine and lymphocyte DNA hypomethylation.* J. Biol. Chem, 2000,275: 29318–29323.
[28] Valuch A, Andrei V, Opris S. *Risk factors in human cardiovascular aging* (AF0108). The 20th IAGG World Congress of Gerontology and Geriatrics. Digital aging: A New Horizon for Health Care and Active Ageing, 2013,June 23-27, Coex, Seoul, Korea.
[29] Kanfi, Y., Naiman, S., Amir, G., et al. *The sirtuin SIRT6 regulates lifespan in male mice.* Nature, 2012,483, 218–221.
[30] Mostoslavsky, R., Chua, K.F., Lombard, D.B., et al. *Genomic instability and aging-like phenotype in the absence of mammalian SIRT6*. Cell, 2006,124, 315–329.

THE ASSESSMENT OF THE SPEED OF PROCESSING USING MMSE-2 EV AND FLANKER AC AGED PERSONS

***Stan** Polixenia*

[1] *"Ana Aslan, National Institute of Gerontology and Geriatrics", Bucharest, Romania*

Corresponding author: Stan Polixenia, xeryn5@yahoo.com

Abstract. Psycho-cognitive aspect in elderly, stongly influences their healts status, physical and mental health. Not the age is important, becouse the understanding and speed of information proccesing depends on the cognitive abilities of brain plasticity, rapid transmission and decoding information. Lately, in clinic there has been assessing global health. Some of the tests used are MMSE Test and Clock Test to assess cognition and GDS (Geriatric Despression Scale - short form) and BDI-II (Beck Depression Inventory – Second Edition) for the assessment of depression. Currently there is a new form MMSE test - MMSE-2 EV (evaluation of minimal cognitive status second edition).These test takes account in evaluation of tuition and subjects age. It has a section that measures the speed and perceptual-motor learning ability. A new test that measures processing speed is AC Flanker test. We intend in these research to establish a correlation between the processing speed of the MMSE-2 EV and AC Flanker sample. Using a batch aged 40-60 years who come from both rural and urban areas.The correlation made, it was taken account the state of depressed mood, ability to concentrate, anxiety, age group, enviroment, storage capacity of a story.
Key words: elder, cognitiv, conmcentration, speed processing, memorizing

EVALUAREA VITEZEI DE PROCESARE UTILIZÂND MMSE-2 EV ȘI FLANKER AC LA VÂRSTNICI

***Stan** Polixenia*

[1] *"Ana Aslan, National Institute of Gerontology and Geriatrics", Bucharest, Romania*

Corresponding author: Stan Polixenia, xeryn5@yahoo.com

Rezumat. Epigenetica se ocupă cu schimbările fenotipului şi ale expresiei genetice (ARN, proteine) ce se produc fără alterări ale secvenţei ADN. Schimbările epigenetice implică alterări în modelul de metilare al ADN, modificări posttranslaţionale ale histonelor şi remodelării acromatinei. Hiperhomocisteinemia, ca potenţial factor de risc in boala cardiovasculară, poate iniţia sau susţine aterogeneza prin modificarea metilării ADN. Lucrarea trece in revista corelaţiile dintre câţiva factori epigenetici: vitaminele B9, B12 şi homocisteină, în boala cardiovasculară, bazat si pe câteva din datele noastre. B9 şi B12 sunt invers corelate cu homocisteina ($r = -0.369$, $p < 0.05$, $n = 32$ and $r = -0.389$, $p < 0.05$, $n = 32$, respectiv). Aceasta sugerează că ambele vitamine au un efect benefic asupra sistemului vascular prin diminuarea homocisteinei şi a nivelului de hipometilare. Am observat ca exista o corelaţie inversă între vârstă şi folaţii serici ($r = - 0.373$, $p < 0.05$, $n = 29$), in boala cardiovasculară. Ambele vitamine se corelează direct cu nivelurile de HDL colesterol (HDLC): B9 ($r = 0.484$, $p < 0.01$, $n = 29$) şi B12 ($r = 0.673$, $p < 0.01$, $n = 16$), ceea ce sugerează că au un efect benefic asupra sistemului vascular şi prin medierea HDLC.
Cuvinte cheie: epigenetică, vitamina B9, vitamina B12, homocisteină, vârsta

INTRODUCTION

Problematic psycho-cognitive at the elderly strongly influences their health status, physical and mental health.

In clinic, lately, it has been the global assessment of their state of health. It was used even in the records of medical observation MMSE test and test the clock

for cognitive evaluation and GDS, short form, to evaluate the depressive status.
The researches reveals that attention deficit may influence significantly cognitive performance and behaviour. Specially in mild cognitive disorders.Some researchers demonstrates that attention defficit may make the differecence between healthy elders and those with cognitive disorders. These can be a point of start to evaluate and diagnose [1].
Cognitive decloine is associated with age, capacity to fix new informations, speed processing and work memory [2].
Cognitive performance is associated with executive control eficiency [3]. It is normal that elderly from time to time experience mild anxiety, depression or being mentally „low". It can be related to growing old, to day to day difficulties, to addapt to new circumstances etc but it should not affect on long term performances, day to day functions to a normal and healthy elder.
Last year it has purchased a assessment test cognitive more complex, who tries to correct the shortcomings of the test MMSE previously used (with 30 items manufacturer).
The new test, MMSE-2 EV (Evaluation of the minimal cognitive status edition-2a) takes account the assessment of the level of the schooling and the age of subjects. In addition, to the variant used in the present, it has: memorizing a story and the evaluation of processing speed [4].
In this test, the processing speed measure the speed of the perceptual-motor function and learning by (formation of pairs by symbols). The persons examined form pairs in the symbols and digits into a time limit of 30 seconds. The signs are different between them. The group of numbers is from 1 to 9, there is no action identical on adjacent rows, are not found in rapid succession on the same numeric or fluent.
Test Flanker AC, measure the processing speed and concentrated attention. In the group of 5 arrows it is switch-on to be identified as different arrow in the string and should be noted her direction : the left or the right hand. The arrows has each time financials in the love in the sequence. When all the arrows have the same direction, it skips that row. Browse itemilor shall be completed within a period of 3 minutes [5].
In these research it is intendent to find a correlation between the processing speed of MMSE-2 EV and test Flanker AC.

MATERIAL AND METHOD

STUDY GROUP: 124 subjects, mostly women (in the squad there are only 2 men) aged 40-89 years, where the average age is 66.7 years.

TESTS:
• MMSE 2EV
• Flanker AC
• Tests of depression: BDI, GDS (short form), depression screening scale of 9 items
• Test anxiety: Anxiety scale of 9 items
• Test the clock

Data were processed in SPSS, the study of the interaction between variables, using Pearson correlation analysis is mainly
For a better undestanding, we used methode of graphic illustration created in Excel.

RESULTS AND DISCUSSION:

The representation of age groups

Subjects were divided into the following age groups every 10 years: 40-49 years, 50-59 years, 60-69 years, 70-79 years, 80-89 years.

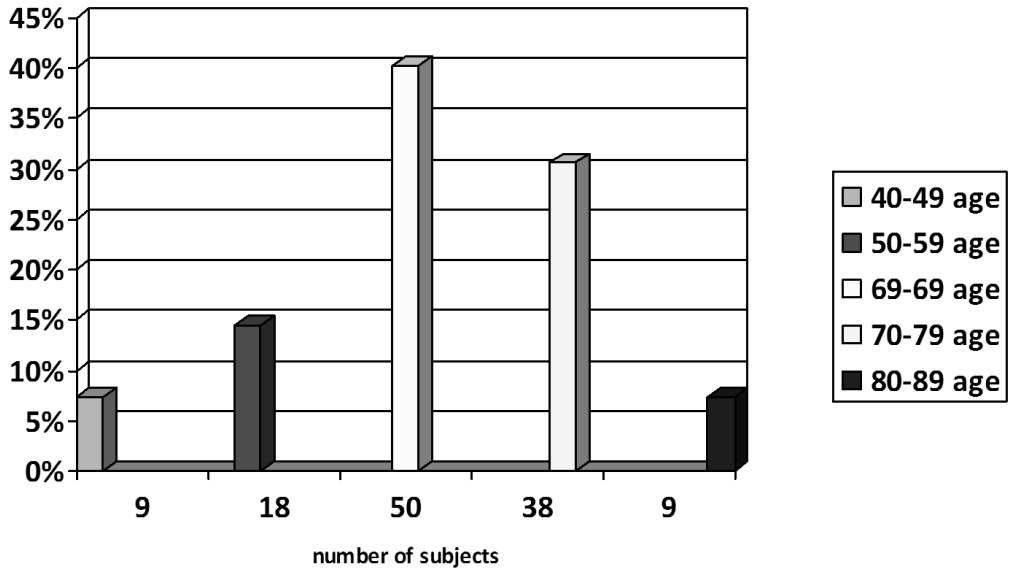

Fig. 1. The representation of age groups in batch (n = 124)

Representing training school

School grades 0-16.

The number of classes are divided into 0-4 grades, 5-8 grades 9-12 classes 13-15 classes and 16 classes. In this group, most were those with 9-12 grades, high school equivalent.

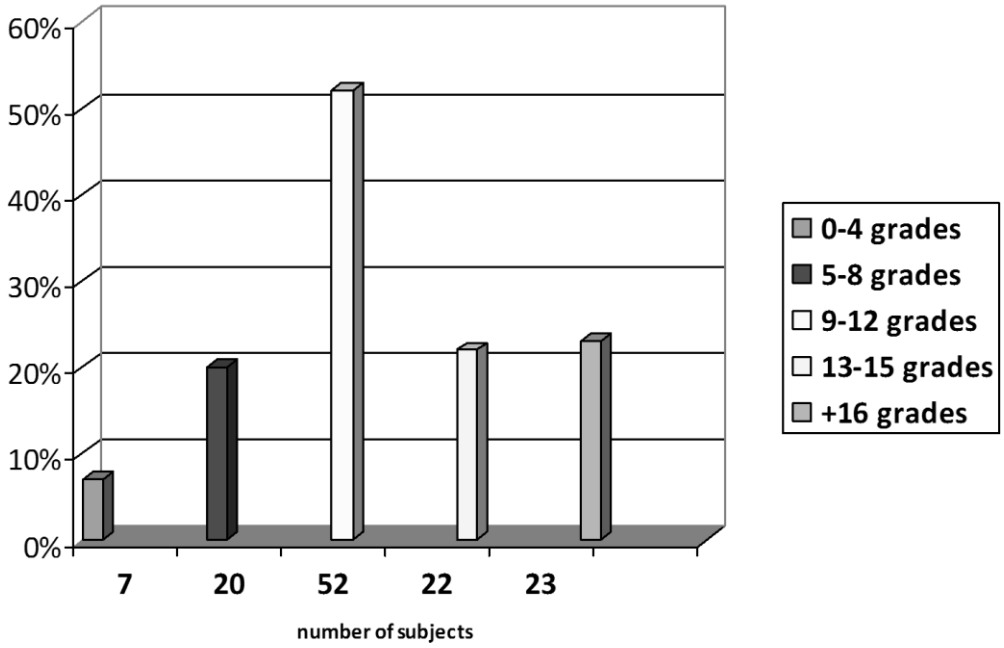

Fig. 2. Representation of weight training school in batch (n = 124)

Representing enviromental .

Coming both from urban and rural areas. Mostly those from urban areas and represent 88.70%.

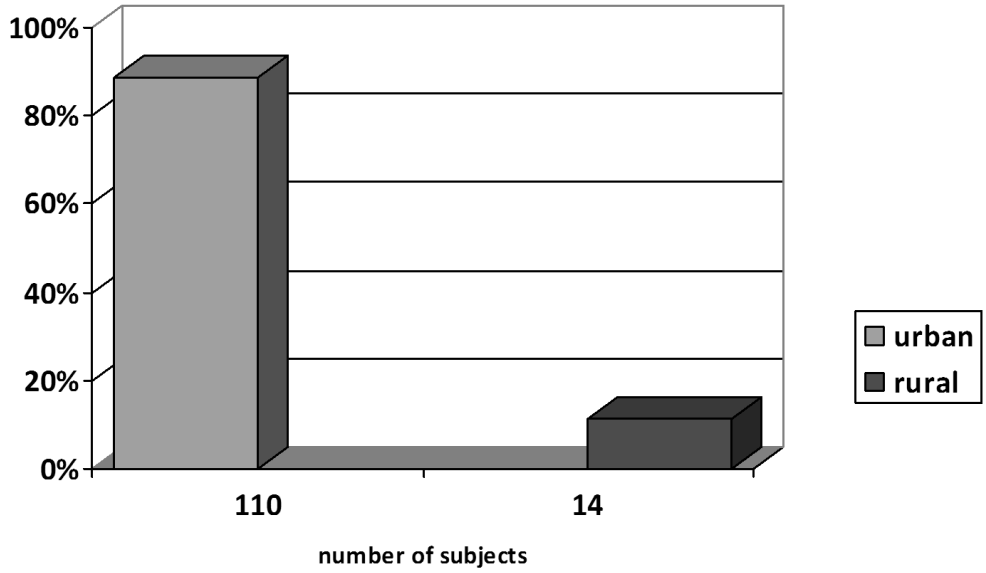

Fig 3. Enviromental

Chart 3 represents the distribution of married, widowed and separated. Thus, we see that most are married (55%), those widowed is 36% and the lowest rate of 9% is represented by those divorced or never married.

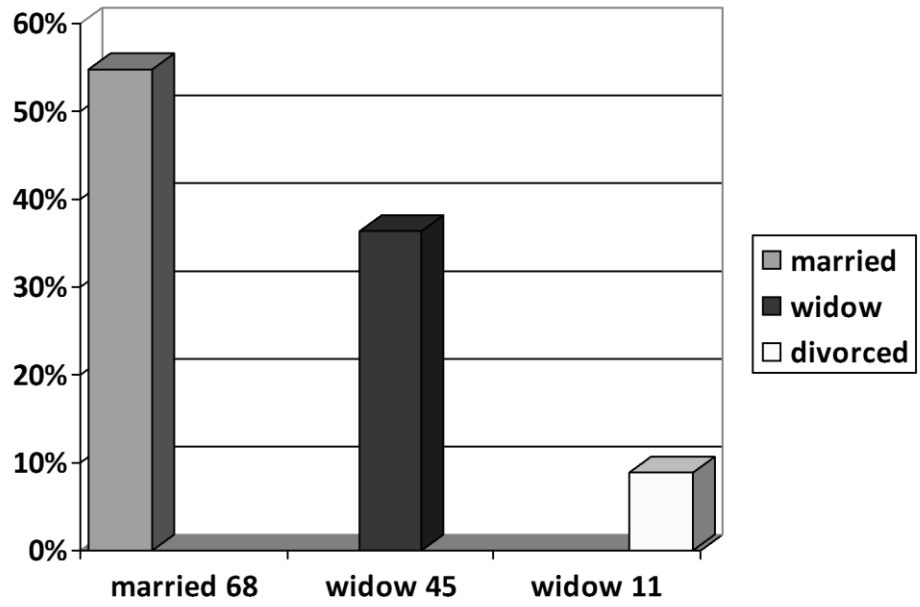

Fig. 4. Marital Status

The processing speed MMSE-2 EV and Flanker on age groups

Contact Pearson corelation between the two processing speeds of MMSE-2 EV and Flanker AC is significant and high intensity where r = 550 and p= 0.000.

Relations between the 2 types of processing speed (Flanker AC and the MMS EV) , calculated separately on each age group, are significant in terms of the age groups 60-69 years, 70-79 years but are not significant in the groups 40-49 years, 59 , years, 89 years. Possible due to the low number of patients. In group 70-79 years, the connection is the greater intensity indicating a correlation more powerful.

Table I. Processing speed of MMSE-2 EV and Flanker for the age groups 60-69 years and 70-79 years

		V. Procesing MMSE EV
60-69 age	speed F A.C Pearson Correlation Sig. (2-tailed) N (nr subiencti)	0,294** 0,038 50
70-79 age	Speed F A.C Pearson Correlation Sig. (2-tailed) N (nr subiecti)	0,409** 0,011 38

**Correlation is significant at the 0,05 level (2-tailed)

The processing speed MMSE-2EV and Flanker AC and training

At the global level, the correlation between the speed the processing of each test is significant with training, having coefficients strong.

In the case of test Flanker AC r = 0.450 with p= 0.000

In the case of test MMSE EV r = 0.454 with p = 0.000

Table II. The processing speed MMSE-2EV and Flanker AC and training

	V. Procesing MMSE EV	V F AC
Nr Clase Pearson Correlation	0,454**	0,450**
Sig. (2-tailed)	0,000	0,000
N (nr subiecti)	124	124

**Correlation is significant at the 0,01 level (2-tailed)

The processing speed MMSE-2EV and Flanker AC and the environment

Urban and rural areas significantly correlate with the processing speed. 88,7 % comes from urban environment.

Table III. Processing speed of MMSE-2EV and Flanker AC and urban and rural areas

	Speed ProcessMMSE-EV
Speed. FAC Pearson Correlation	0,525
Sig. (2-tailed)	0,000
N (nr subiecti)	110

Urban p=0.01

	Speed process MMSE-EV
Speed FAC Pearson Correlation	0,626
Sig. (2-tailed)	0,017
N (nr subiecti)	14

Rural p= 0,005

The correlation rates of MMSE-2 EV and Flanker AC with MMMSE -2 EV scores

In the group of those with changed values in minus the MMSE-2 EV, the correlation between the 2 speeds is significant . The correlation is more intense in the group of those with normal framed the results.

However, there are a percentage of people who have obtained a general lower value to MMM-2 EV, still having a good score in processing speed.

Table IV. Gear correlation of MMSE-2 EV AND FLANKER AC with MMSE scores-2 EV

MMSE Values-2 EV were under the standard deviation and p= 0,01

speed FAC Pearson Correlation	0,436**
Sig. (2-tailed)	0,001
N (nr subiecti)	52

**Correlation is significant at the 0,01 level (2-tailed)

MMSE Values-2 EV were under the standard deviation and p= 0,01

	V Processing MMSE-2 EV
speed FAC Pearson Correlation	0,641**
Sig. (2-tailed)	0,000
N (nr subiecti)	72

**Correlation is significant at the 0,01 level (2-tailed)

The correlation between depression and the processing speed of MMSE-2EV and Flanker AC

On the entire lot (N=124), the correlation between depression and the processing speed is significant for all three tests of depression (BDI-II GDS , and depression screening scale). It is an inverse relationship and significant. The higher the level of depression is higher with so many mistakes occur or speed is lower. In some cases, when depression is higher could result in difficult understanding the instructions and it takes several attempts to fail to understand the mechanism of filling samples.

Table V.The correlation between depression and the processing speed of MMSE-2 EV and Flanker AC"

	V. Processing MMSE EV	V. Processing Flanker AC
Scor screening depression scale	-0,186* 0,039 123	-0,124 0,172 123
Scor BDI	-0,256** 0,004 124	-0,211* 0,019 124
Scor GDS	-0,230* 0,010 124	-0,195* 0,030 124

*Correlation is significant at the 0,05 level (2-tailed)

**Correlation is significant at the 0,01 level (2-tailed)

The correlation with anxiety

In this study, the processing speed does not correlate with anxiety scale measured anxiety with 9 items.

The correlation between the degree of concentration, processing speed and scales of depression

The level of concentration of the BDI (Item 19) does not correlate with the processing speed of Flanker AC and MMSE-2 EV. But correlates with depression BDI-II, GDS, depression screening scale.

Table VI.The correlation between the degree of concentration (Item 19 of BDI-II) and scales of depression

		BDI (item 19)
Depresie total Scala screening	Pearson Correlation	0,545
	Sig. (2-tailed)	0,000
	N (nr subiecti)	123
Depresie scor Scala screening	Pearson Correlation	0,446
	Sig. (2-tailed)	0,000
	N (nr subiecti)	123
BDI total	Pearson Correlation	0,620
	Sig. (2-tailed)	0,000
	N (nr subiecti)	124
BDI scor	Pearson Correlation	0,497
	Sig. (2-tailed)	0,000
	N (nr subiecti)	124
GDS total	Pearson Correlation	0,541
	Sig. (2-tailed)	0,000
	N (nr subiecti)	124
GDS scor	Pearson Correlation	0,488
	Sig. (2-tailed)	0,000
	N (nr subiecti)	124

The processing speed Flanker AC and MMSE-2 EV and the stored story

Speed the processing Flanker AC and MMSE-2 EV correlates with the number of words/ideas reproduced correctly from the stord story MMSE-2 EV . The higher the speed is, they can reproduce more ideas/words. There is a significant direct correlation Link between the ability to store and good speed processing can be concentration.

Table VII. The processing speed Flanker AC and MMSE-2 EV and the stored story

		V procesare MMSE EV	V. procesare Flanker AC
Text din MMSE EV	Pearson Correlation	0,359**	0,317**
	Sig. (2-tailed)	0,000	0,000
	N (nr subiecti)	124	124

**Correlation is significant at the 0,01 level (2-tailed)

CONCLUSIONS

Because of the low number by *age* group, correlations were revealed only for age groups 60-69 years (group of 50 subjects) and 70-79 years (group of 38 subjects). In total there were 124 subjects

Values obtained in the test assessing processing speed and the MMSE-2 EV in AC Flanker test correlates between them. At the higher values MMSE-2 EV test are better, the greater the accuracy of the test Flanker. Globally, the correlation between the processing speed of each test is significant and school preparation, with strong coefficients. As schooling is better, and increase accuracy and processing speed.

If processing speed correlate with the final score of the sample MMSE-2 EV value appears stronger in people who scores within the limits mean and standard deviation.

Analyzing the total value of MMSE test-2EV with processing speed EV-2 MMSE test and test processing speed AC Flanker, it was found that there is a strong correlation between results. In the group with changed values minus the MMSE-2 EV, the correlation between the two speeds is significant. Correlation is more intense in the group classified results as being normal. However there is a percentage of people who have obtained a general value lower MMSE-2 EV, but still a good score in processing speed. The state of depressed

mood correlates with low speed processing.

The state of depressed mood correlates with low speed processing. It is an inverse correlation and significant. Higher the level of depression is there are more mistakes or the speed is lower.

Becouse of fatigue and disinterest sometimes appear difficulties to keep attention focused especially while browsing items to sample Flanker AC. In some cases, when depression is higher could result in difficult understanding the instructions and it takes several attempts to fail to understand the mechanism of filling samples.

Although correlation is existing processing speed of depression scales, the correlation with the ability's concentration of item 19 (a subjective assessment of concentration) of the BDI-II test is not significant.

There appears to be significant correlation with anxiety scale measured anxiety with 9 items.

Processing speed Flanker AC and MMSE-2 EV correlates with the number of words / ideas reproduced correctly in MMSE-2 EV ability to reproduce as many ideas / words of the MMSE-2 EV to item memory of a story, grows as they manage to go through as much of the sample processing. It is a direct significant correlation. Link between the ability to store and good speed processing can be concentration.

REFERENCES

[1] http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0054035

[2] Hedden, T., Gabrieli,J.D. *Shared and selective neural correlates of inhibition, facilitation, and shifting processec during executive control*. NeurolImage, 2010

[3] Sarah Diana Bauermeister. *Lifestyle activities, menthal health and cognitive function in adults aged 50-90 years.* Thesis submitted for the degree of Doctor of Philosophy 2012.

[4] Manual de utilizare MMSE -2 – *Mini Mental State Examination* – adaptata in Romania de C. E. Munteanu, D Iliescu , R. Livinti, tiparit la Sinapsis Publishing Projects, Bucuresti 2013.

[5] F. A. Sava , L. Patrut. *Manual de utilizare Probele Flanker AC, Flanker AD si PON*. Ed. ArtPress, Timisoara, 2010.

NARRATIVE THERAPY – A WAY OF THERAPEUTICAL INTERVENTION

***Bălan** Claudia*

[1] *"Ana Aslan, National Institute of Gerontology and Geriatrics", Bucharest, Romania*

Corresponding author: Balan Claudia, bluegipt@yahoo.com

Abstract. Narrative therapy is centered on the idea that life is like a story which links past with present and the future. The story offers meaning and unity, specifies the personal niche of the human being on the world and reinforced the sense and continuity during the life events. The life story is a common product between a person and the environment in which he leaves. Stories in a narrative therapy context are made up of events, linked by a theme, occurring over time and according to a plot. A story emerges as certain events are privileged and selected out over others events as more important or true and becomes like a burden, positive or negative one. The themes lines that could appear are the mirror of the world dualism which bears the name of intimacy (love) and strength (war). Depression disorder impairs the structure of the psychic by inducing a lack of balance in a mind and soul of the individual and in his own personal story, is like a terrorist who hijacks a plane. Using narrative therapy like a strategy of counseling and intervention could restore the balance and re-meaning the personal history and by that recreate a meaning.
Key words: narrative therapy, the life story, the sense of meaning, depression disorder, lack of balance.

TERAPIA NARATIVĂ – UN MOD DE INTERVENȚIE TERAPEUTICĂ

***Bălan** Claudia*

[1] *"Ana Aslan, National Institute of Gerontology and Geriatrics", Bucharest, Romania*

Corresponding author: Balan Claudia, bluegipt@yahoo.com

Rezumat. Terapia narativa pleaca de la metafora perceperii vietii ca o poveste care leaga trecutul, prezentul si viitorul. Povestea ofera unitate si sens, specifica nisa personalizata a individului in lume si sensul continuitatii si neschimbarii de-alungul situatiilor de viata. Povestea vietii este un produs comun al persoanei si mediului. Povestea in terapia narativa este construita din evenimente critice si ofera sens prin linia tematica pe care o urmareste. Linia tematica poate fi privita ca un lait-motiv care tot apare in cursul povestirii. Liniile tematice care pot aparea si care sunt expresia dualismului lumii poarta numele de intimitate (iubire) si putere (conflict). Tulburarea de depresie induce o dezorganizare si un dezechilibru profund in psihicul si in istoria persoanala a individului este ca un terorist care deturneaza un avion. Aplicarea unui model de interventie terapeutica ca terapia narativa poate restabili si resemnifica istoria personala si induce o armonie in identitate.
Cuvinte cheie: povestea vietii, terapia narativa, sensul vietii, tulburare depresiva, dezechilibru

INTRODUCTION

These days the problem of identity is frightening because of lack of unity and purpose. Unity and purpose are the results of many and complex interactions between the person and the society. If identity is vital then in one way or another person touches the world and leaves a mark on it.

An unexamined life leads to neurosis and, in fact, a bad fate. In order to discover ourselves we need to separate us from the past or, at least, to develop new perspectives from which we can look at our past (1). We may conclude that the only stable element of our lives is change, which involves freedom; the freedom to be anything we want to be.

The theoretical objective of this case is to find out if a narrative approach is suitable for psychological treatment of reactive depression. Personal history represents a first class of resources upon which people can build a personal identity. You can't achieve unity and purpose while leaving the past behind. Depression disorder appears as a deep rift in the structure and dynamics of the person and everything that defined the person until then suffers an imbalance and enters dissonance. We're talking of a split identity and we're trying to find out to what extent a narrative therapeutic model represents an efficient way of intervention by connecting the past to the present and future.

METHODOLOGY: Case study

1. Identification
Male, 55 years old
Managing Director in his own company, currently retired, leaving the task of managing the business to his associate.
Married, one child.
2. Reason for admission
Tremor in the chest
Feeling weary
Agitation associated with anxiety
Tachycardia
Mixed insomnia
3. History of present illness
At the age of 50 the patient suffered an acute myocardial heart attack, without having any somatic diseases until then. Currently, he has two cardiovascular stents mounted and follows a specific medication.
History shows that he suffered multiple panic attacks from the moment of the heart attack until now and he was diagnosed with depressive anxiety disorder (not treated with drugs).
He was a heavy consumer of alcohol, tobacco and fats. He is currently abstinent.
4. Personal medical history.
Without significant history: common colds
5. Medical-therapeutic approach
Cardiology exam
Psychiatric examination: anxious-depressive disorder diagnosis (with treatment)

Psychological examination

- *Presentation:* proper hygiene, proper clothing, strained face, tense posture, anxious attitude.
- *Talk:* unperturbed ideational and verbal pacing, speech centered on somatic aspects.
- *Emotional expression:* the patient unaware and does not communicate his emotions, his speech was focused on recent somatic history and suffered symptoms to which he obsessively returns. The patient's mood is anxiety-anticipation.
- *Thinking and perception:* prevalent and recurrent thoughts focused on somatic condition, the investigations to be performed; cognitive distortions with a role of impairing judgment on cooperation regarding treatment; without pathological disorders at the perception level
- *Sensorium:* assessment of cognitive functions through the battery of specific tests indicates the presence of stable cognitive functions. These tests are MMSE-2: EV (2), the Wechsler test – memory scale (3), the Clock test (4), the Stroop test (5).

RESULTS AND DISCUSSIONS

The first meeting with the patient went slowly, the subject exhibiting resistance to his personal history and providing incomplete and superficial answers to questions on his personal life. I learned that he is married and has an adult son with whom he is not very satisfied.
His speech focused solely on somatic issues that generate the sensation of discomfort and captures his full attention and concern:
" In the evening, when I go to sleep I feel that my heart is pounding. If I watch TV I feel the heart beating hard and I feel the same thing during the day. I feel weak all the time as if I cannot do anything and I'm afraid it may be something serious"

I learned that the patient had ceded management of his part of the business to his associate, and his day to day life was limited to the perimeter of the house and he was very rarely engaged in the business aspects. His social life was also restricted to family members: his wife and unmarried son.

The analysis performed as a result of the session outlines, as a first presumptive diagnosis, anxiety disorder developed after the heart attack he suffered five years ago.

I note the patient's resistance towards personal-intimate aspects of his life and the reduced ability to manage the supported somatic distress. The patient feels moderately invalidated socially and professionally.

Thus, as a first therapeutic intervention strategy, the need for developing and strengthening the therapeutic alliance is developed.

The second and third sessions were focused on validating the patient's feelings and needs for catharsis and focusing the discussion on the somatic condition. The speech focused in an exacerbated way on the symptoms he faced and on how the heart attack was triggered and supported.

In parallel, the patient is sent by the geriatrician for clinical investigations in order to get an X-ray of his current health condition.

The therapeutic approach is steered towards emotional support and encouragement to express the fears and associated thoughts. The patient expresses a generalized anxiety caused by the existence of dysfunctional negative thoughts:

"I have no strength, I feel emaciated, I'm afraid my blood pressure will rise"

Next, the patient is still resistant to questions about the personal aspects of his life and shows a belief that he suffers from something somatic, not taking into account, or even rejecting, the psychological aspects of his condition.

Following the results of the conducted investigations showing that today's physiological condition is stable and that he should continue with the treatment prescribed by the cardiologist; the idea that the patient has a psychosomatic disorder is emerging.

From this moment, the therapeutic intervention will focus on attracting the patient in the therapeutic approach to discover the psychological underpinning of his condition as well as on the exploitation of the resources and the development of skills needed to contain and face the symptoms and states he bears.

In the next sessions, by developing the therapeutic alliance and explaining the neuro-psycho-physiologic functioning mechanisms, the patient recounts events of his personal life with an emphasis on the impact these have had on him.

Personal history of the patient shows a life lived to the full from a qualitative and quantitative standpoint:

"I drank, played in casinos, I had an intense professional life and, just as intense, a personal side. I didn't spend much time at home; Miss, I thought I was immortal!!!! Then I had the heart attack ..."

From this arose the hypothesis of a masked depressive disorder (6) with the role of influencing the patient's thought and affection: ideas and feelings of frustration and objection, negativism, weakening the control capacity and stress tolerance, dysphoric attitude and negative expectations towards the environment. There are profound adaptation difficulties to another existential context.

At this point the patient is proposed to follow a lengthy psychotherapy that focuses on two directions:

- A first phase that includes an in-depth analysis of strategies for containment of his states and a deep understanding of the mechanisms involved by following the model of cognitive therapies
- In the second phase, a narrative psychotherapy with the role of understanding of how life events leave a mark on our health and re-signifying them (7).

Narrative therapy is developed around the concept of scaffolding (8), a term that designates the process by which the individual's internal resources and resorts are brought to the surface in order to restore the affected identity structure.
In this case, the process consisted of two phases: "the life story" and interview phase.
"The life story" refers to the process by which the patient narrated, from the beginning until the present day, his life as an autobiography with focus on events important for him. This process allowed the identification of multiple resources, the re-signification of past events but also awareness on the meaning on which he has built his life. Meaning that now, in light of his new somatic condition, he lost.
As a result of the the interview phase, together with the patient, we identified that the motivation he used to build his life was the motivation to gain power.
The motivation to gain power designates the people that function in life as an agent or organizer. It shows a tendency towards control and domination, and their purpose is to change, transform, leaving impressions on the world and those around them (1).
In this case, the patient has identified this trait which now he feels is useless and powerless. The motivation towards transformation is there, but he perceived the context he was in as hostile and limiting. The feeling of loss was even more acute under the influence of his family which was locking his freedom and need to act even more.

CONCLUSIONS

The patient has built his existence around the motivation of power. His professional, family and social identities wore this mark: successful businessman, rich social life, an apparently united family.
The somatic distress he suffered caused an identity crisis by loss of his sense of power and domination. The motivation to gain power is still there but the context is not the same.
The deepest problem he faced was that of acceptance and adaptation to the new context of his life.
As a result of the therapy the patient began to exert a tendency of control and domination on the current context: he partially resumed his professional activity; he partially resumed his social relationships: he started playing tennis both as a social game as well as a physical activity.
His psychosomatic symptoms decreased, sleep improved. The patient does the mandatory health checks. The feeling of control over her life gave him the strength to accept, assume and control his somatic condition.

REFERENCES

[1] McAdams D.P., Janis L.A., Lynne E.S. *The handbook of narrative and psychotherapy: Practice, theory, and research*. Thousand Oaks, CA, US: Sage Publications, Inc, xi, 2004, 159-173, 404 pp.
[2] Folstein MF, Folstein SE, White T, Messer MA, Munteanu CE, Iliescu D, Livinti R. *MMSE-2: Mini-Mental State Examination.* Ed. a 2-a: manual de utilizare a testului. O.S. Romania, 2013, Bucuresti. www4.parinc.com/WebUploads/.../MMSE-2.pp
[3] *Wechsler test – Memory scale.* 2009 https://ptc.bps.org.uk/test/wechsler-memory-scale
[4] *The clock – drawing test.* 1998 http://ageing.oxfordjournals.org/content/27/3/399.full.pdf
[5] *Stroop test.* 2014 http://www.snre.umich.edu/eplab/demos/st0/stroopdesc.html
[6] A.P.A, *DSM-5.* Traducere & co-editie cu American Psychiatric Association**:** Diagnostic and Statistical Manual of Mental Disorders: DSM-5. Editura Medicala Callisto, 2016, Bucuresti, pg. 1000.
[7] Carey M. *Narrative therapy and trauma. Narrative Approaches to Brain Injury.* 2013, books.google.com
[8] Morgan A, Carey M, Russell S, Markey C. and Mann S. *How Stories Shape Us* [online]. International Journal of Narrative Therapy & Community Work, Vol. 2008, No. 1, 42-45. <http://search.informit.com.au/documentSummary;dn=024229530439815;res=IELHEA>.

INSTRUCTIONS FOR AUTHORS

MANUSCRIPTS. Manuscripts must be written in English and must contain genuine material. They are accepted for consideration only if no parts of the manuscript (including tables, figures photos or legends) have been or will be published or submitted elsewhere. The journal discourages the submission of more than one article dealing with related aspects of the same study. All manuscripts are submitted as attachment Word Doc, with all figures, references, figure legends and tables in the same document, by e-mail to **rjgerontog@gmail.com**. Times New Roman font 12 and 1.0 spacing throughout will be used. Acceptable formats for pictures, photos, and figures are JPG or TIF.

Submission of papers will be confirmed to authors by e-mail.

TITLES AND AUTHORS' NAMES. The manuscript will contain a page with a concise and descriptive title (12 words recommended), Times New Roman font 12, upper case, bold. This page will include also the following information: the name (bold) and first name of the authors, without their professional and/or scientific degrees, including their affiliations, Times New Roman font 12, italic. Affiliations should be specified by numbers and not symbols (e.g. [1]"Ana Aslan" National Institute of Gerontology and Geriatrics, Bucharest, Romania, [2]"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania). If all authors were main authors and so, had equal contributions to the paper, then the authors should be listed in a subtitle, in alphabetical order, followed by their contact e-mails. The corresponding author and his contact e-mail must be written just after the institutional affiliations.

ABSTRACT. Abstracts in English and Romanian, each of them of no more than 250 words, Times New Roman font 12, will be inserted after the title and authors.

KEY WORDS. Three to five key words should be added in the bottom of each abstract.

HEADINGS. Manuscripts containing results of original studies should be of a maximum of 2500 words and must include the following sections: Introduction, in which the objectives and assumptions should be clearly stated; Materials and Methods; Results; Discussions; Conclusions. Tables, figures and figure legends should be included in the "Results"section.

REFERENCES. REFERENCES. References will contain maximum 30 titles of recent publications (in the past 10 years). Old publications are accepted only if they have special importance in the respective field. References must be numbered consecutively as they are cited. List only the first three authors followed by *et al*. The following are sample references:

For journal articles: Shapiro A.M.J., Lakey J.R.T., Ryan E.A., et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen, N. Engl. J. Med., 2000, vol. 343, 4: 230-238.

For journal articles in electronic format: Niki E. Role of vitaminE as a lipid-soluble peroxyl radical scavenger: in vitro and in vivo evidence, Free Radical Biology and Medicine, 2014, 66: 3–12. http://dx.doi.org/10.1016/j.freeradbiomed.2013.03.02223557727.

For books: Goadsby P. J. Pathophysiology of headache. In: S. D. Silberstein, R.B. Lipton and D. J. Dalessio (Eds.), Wolff's headache and other head pain 7th ed. 2001, Oxford, England: Oxford University Press (pp. 57-72).

For conference paper: Brown S. & Caste V. *Integrated obstacle detection framework.* Paper presented at the IEEE Intelligent Vehicles Symposium, May 2004, Detroit, MI.

For conference Paper from the Internet: Balakrishnan R. *Why aren't we using 3d user interfaces, and will we ever?* Paper presented at the IEEE Symposium on 3D User Interfaces March 25-26, 2006. doi:10.1109/VR.2006.148

ILLUSTRATIONS. Figures should be professionally designed. The title will be under the figure, Times New Roman font 10, and Arabic numerals will be used for numbering. Photo files must be submitted in JPG or TIF format with 300 dpi resolution.

If photographs of patients are used, either written permission of the patient is necessary or patients should not be identifiable in photographs.

TABLES. The title of every table should be written above, with Roman numerals for numbering, Times New Roman font 10. Place explanatory matters in footnotes. Do not repeat results of the tables as illustrations.

ACKNOWLEDGEMENTS. Acknowledgments of a maximum of 30 words should be included before "References". Authors can express gratitude for support in conducting research projects.

ABBREVIATIONS. The full term for which an abbreviation (acronym) stands should precede its first use in the text.

DRUG NAMES. Generic names should generally be used. When proprietary brands are used in research, include the brand name in parentheses in the "Materials and Methods" section.

UNITS OF MEASUREMENT. Measurements of length, height, weight and volume should be reported in metric units (meter, kilogram, or liter or their decimal multiples). Temperatures should be given in degrees Celsius. Blood pressures should be given in mm Hg. All hematological and clinical chemistry measurements should be reported in the metric system in terms of the International System of Units.

PERMISSIONS. The authors take full responsibility for original materials and copyright trespassing.

EVALUATION OF MANUSCRIPTS FOR PUBLICATION. Manuscripts are examined by the editorial staff and are usually sent to outside reviewers. Authors can suggest the names of possible reviewers, but we reserve the right of final selection. Decisions about potentially acceptable manuscripts may take longer.

The Romanian Journal of Gerontology and Geriatrics is published by the Romanian Society of Gerontology and Geriatrics, which is affiliated to the International Association of Gerontology and Geriatrics. More information about subscriptions to the journal or membership of this society may be provided at: Fax: 40 21 223 1480; Phone: 40 21 223 71 88; E-mail: rjgerontog@gmail.com; www.ana-aslan.ro or www.rjgg.ro.

INSTRUCȚIUNI PENTRU AUTORI

MANUSCRISELE. Manuscrisele trebuie redactate în limba engleză și este necesar să conțină material original. Ele sunt acceptate ca propuneri de articole în vederea publicării în această revistă, doar dacă nicio parte din articol (inclusiv tabele, figuri, fotografii sau legendă) nu a fost si nu va fi publicată sau trimisă spre publicare in alte reviste. Revista nu agreează trimiterea spre publicare a mai multor articole care se referă la aspecte conexe ale aceleiași problematici. Toate articolele se trimit în format electronic, Word Doc, cu toate figurile, tabelele, legendele și referințele bibliografice incluse în acelasi document, prin e-mail ca fișier atașat, pe adresa **rjgerontog@gmail.com**. Se vor folosi caractere Times New Roman 12 și spațierea la un rând a textului. JPG și TIF sunt formatele de fișier acceptate pentru figuri și fotografii.

Autorilor li se va confirma prin e-mail primirea lucrării spre publicare.

TITLUL ARTICOLULUI ȘI NUMELE AUTORILOR. Manuscrisul va conține o pagină cu un titlu concis și descriptiv al lucrării (recomandabil maxim 12 cuvinte), format Times New Roman 12, majuscule, bold. Această pagină va include și următoarele informații: numele (bold) și prenumele autorilor, fără titlu profesional și/sau stiintific precum și afilierea fiecărui autor, format Times New Roman 12 italic. Afilierile autorilor vor fi specificate cu numere si nu cu simboluri (de exemplu, [1]"Ana Aslan" National Institute of Gerontology and Geriatrics, Bucharest, Romania, [2]"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania). In situația în care toți autorii au avut contribuții egale la realizarea manuscrisului, ei vor fi menționați într-un subtitlu în ordine alfabetică împreună cu adresele lor de e-mail. Numele autorului corespondent și adresa sa de e-mail vor fi menționate imediat după enumerarea afilierilor instituționale ale autorilor.

REZUMAT. După titlu și autori se va insera rezumatul în limba engleză, precum și versiunea în limba română, fiecare cu un număr maxim de 250 de cuvinte, format Times New Roman 12.

CUVINTELE CHEIE. În partea de jos a fiecărei versiuni a rezumatului se vor include 3 până la 5 cuvinte cheie.

SECȚIUNILE. Manuscrisele care prezintă rezultatele unor studii originale trebuie să conțină maxim 2500 de cuvinte și să fie organizate în următoarele secțiuni: Introducere, în care vor fi specificate clar obiectivele și ipotezele studiului; Materiale și Metode; Rezultate; Discuții; Concluzii. In secțiunea "Rezultate" vor fi incluse tabelele, graficele și figurile împreună cu titlurile și legendele lor.

BIBLIOGRAFIA. Bibliografia va cuprinde maxim 30 de titluri reprezentând articole publicate recent (ultimii 10 ani). Se acceptă articole mai vechi dacă prezintă importanță deosebită în domeniul respectiv. Titlurile bibliografice se vor ordona în funcție de apariția în text. La fiecare lucrare vor fi menționați doar primii trei autori urmați de *et al.* Citarea articolelor se face după următorul model:

Pentru articole din revistele științifice: Shapiro A.M.J., Lakey J.R.T., Ryan E.A., et al. *Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen*. N. Engl. J. Med., 2000, vol. 343, 4: 230-238.
Pentru articole în format electronic*:* Niki E. *Role of vitaminE as a lipid-soluble peroxyl radical scavenger: in vitro and in vivo evidence*, Free Radical Biology and Medicine, 2014, 66: 3–12. http://dx.doi.org/10.1016/j.freeradbiomed. 2013.03.02223557727.
Pentru capitol sau subcapitole din monografii sau tratate*:* Goadsby P. J. *Pathophysiology of headache*. In: S. D. Silberstein, R.B. Lipton and D. J. Dalessio (Eds.), Wolff's headache and other head pain, 7th ed. 2001, Oxford, England: Oxford University Press, pp. 57-72.
Pentru articole prezentate la conferințe*:* Brown S. & Caste V. *Integrated obstacle detection framework*. Paper presented at the IEEE Intelligent Vehicles Symposium, May 2004, Detroit, MI.
Pentru articole prezentate la conferințe care apar online: Balakrishnan R. *Why aren't we using 3d user interfaces, and will we ever?* Paper presented at the IEEE Symposium on 3D User Interfaces March 25-26, 2006. doi:10.1109/VR.2006.148

FIGURILE. Figurile vor fi realizate profesional. Titlul va fi scris sub figura, format Times New Roman 10, iar pentru numerotare se vor utiliza cifre arabe. Fișierele cu imagini trebuie să fie în format JPG sau TIF iar rezoluția trebuie să fie de 300 dpi.
Dacă sunt incluse imagini ale unor pacienți, este necesar consimțământul scris al pacientului pentru difuzare publică sau pacientul trebuie să fie neidentificabil.

TABELELE. Titlul fiecărui tabel va fi scris deasupra, iar pentru numerotare se vor folosi cifre romane, format Times New Roman 10. Notele explicative vor fi în partea de jos a tabelului. Nu se accepta repetarea rezultatelor din tabel prin grafice.

EXPRIMAREA MULȚUMIRILOR. Vor fi menționate înaintea bibliografiei, utilizand maximum 30 cuvinte. Se pot exprima mulțumiri pentru sprijinul acordat în desfășurarea proiectelor de cercetare.

ABREVIERILE. La prima utilizare in text, abrevierea (acronimul) trebuie sa fie precedat de expresia integrală.

DENUMIREA MEDICAMENTULUI. Se utilizează numele generic al medicamentului. Atunci când marca de proprietate a fost utilizată în articolul de cercetare, aceasta se scrie în paranteze și apare în secțiunea "Materiale și Metode" a manuscrisului.

UNITĂȚILE DE MĂSURĂ. Inălțimea, greutatea, volumul, lungimea vor fi exprimate în unități de măsură din sistemul internațional (centimetru, kilogram, litru, unități decimale ale litrului, metrului). Temperaturile vor fi specificate în grade Celsius. Presiunea arterială va fi precizată în mmHg. Rezultatele analizelor laboratorului clinic vor fi exprimate în unitățile de măsură din sistemul internațional SIU.

PERMISIUNILE. Originalitatea conținutului materialelor trimise, respectiv încălcarea dreptului de autor (copyright) este doar responsabilitatea autorilor.

EVALUAREA MANUSCRISELOR SPRE PUBLICARE. Manuscrisele sunt examinate de către membrii redacției revistei și trimise de regulă unor referenți externi. Autorii articolelor pot sugera numele unor potențiali referenți, însă decizia asupra acceptării articolului poate să dureze un interval mai mare de timp.

Revista Română de Gerontologie și Geriatrie este o publicație a Societății Române de Gerontologie și Geriatrie, care este afiliată la Asociația Internațională de Gerontologie și Geriatrie. Mai multe informații despre revistă și statutul de membru al acestei societăți pot fi obținute de la: Fax 40 21 223 1480; Tel 40 21 223 71 88; E-mail: rjgerontog@gmail.com; www.ana-aslan.ro sau www.rjgg.ro.