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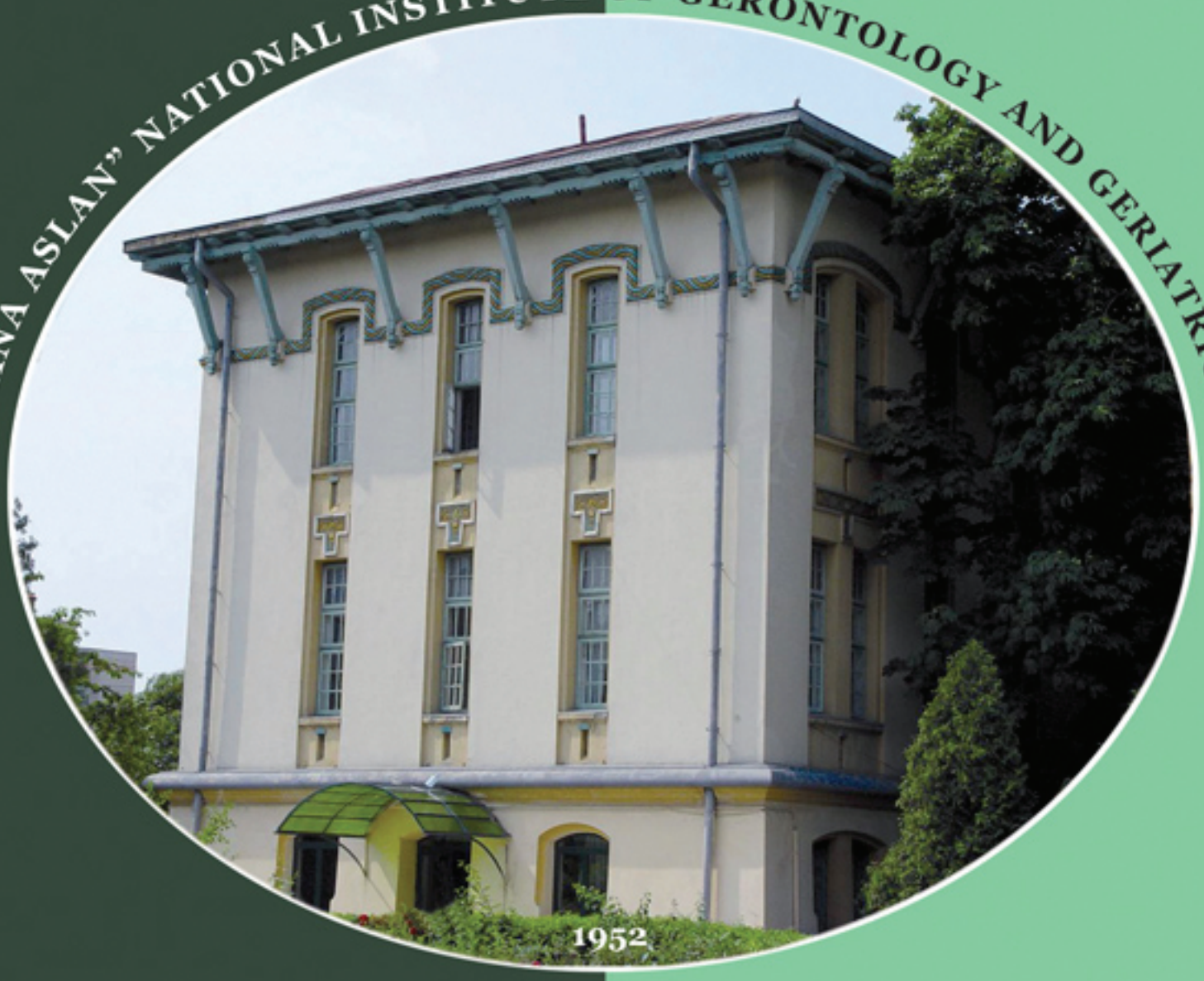

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STUDY OF ANTHROPOMETRIC PARAMETERS TO SUBJECTS OVER 80 YEARS

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Abstract. Old age is associated with increased prevalence of overweight and obesity, defined as BMI > 30 kg/m^2. BMI does not differentiate muscle mass from fat and other anthropometric parameters that define abdominal adiposity have been used to properly define the concept of obesity. Obesity is a risk factor and prediction for aging associated diseases. The aim of the study is to highlight changes in anthropometric parameters and determine the health risk for elderly subjects over 80 years of age. The study was conducted on 120 subjects distributed in three age groups: A-group 80-84 years; group B 85-89 years and group C 90 years +. Body weight, height, body mass index (BMI), waist circumference (WC), hip circumference (HC), waist-to-hip ratio (WHR), waist-to-height ratio (WHtR) and body adiposity index (BAI) were measured. Correlations of anthropometric parameters with the subject's age and between all anthropometric parameters were evaluated. The prevalence of health risk based on WC and the relationship between WC and BMI was assessed. There is a tendency to diminish the anthropometric parameters with the age of the subjects and a significant negative correlation of CT, RTI and BMI with age was revealed. Subjects of 90 years are normoponderal and overweight in equal proportions (38.89%) and only 11.11% with obesity. Significant changes in their anthropometric parameters with BMI increase were observed. Analyzing the prevalence of health risk, it has been observed that subjects aged 90 years and over have the highest prevalence of the "no increased risk" category and the lowest prevalence for "very high risk". Data show that anthropometric parameters are simple and useful tools for assessing health risk and targeting the therapeutic strategy of obesity, malnutrition and fragility in aging.
Key words: aging, anthropometric parameters, obesity, health risk factors

STUDIUL PARAMETRILOR ANTROPOMETRICI LA SUBIECŢI DE PESTE 80 ANI

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Rezumat. Vârsta înaintată este asociată cu creşterea prevalenţei supraponderei şi obezităţii, definită că BMI > 30 kg/m^2. BMI nu diferenţiază masă musculară de cea grasă şi alţi parametrii antropometrici care definesc adipozitatea abdominală au fost utilizaţi pentru definirea adecvată a conceptului de obezitate. Obezitatea este un factor de risc şi predicţie pentru bolile asociate procesului îmbătrânirii. Scopul studiului este să evidenţieze modificările parametrilor antropometrici şi să determine riscul pentru sănătate la subiecţii vârstnici de peste 80 ani. Studiul s-a realizat pe 120 subiecţi distribuiţi în trei grupe de vârstă: A-grupa 80-84 ani; grupa B-85-89 ani şi grupa C-90 ani +. S-au determinat greutatea corporală, înălţimea, indicele de masă corporală (BMI), circumferinţa taliei (CT), circumferinţa soldului (CS), raportul talie-sold (TSR), raportul talie-înălţime (RTI) şi indicele de adipozitate corporală (IAC). S-au evaluat corelaţiile parametrilor antropometrici cu vârstă subiecţilor şi între toţi parametrii antropometrici. S-a evaluat prevalenţa riscului pentru sănătate pe baza CT şi relaţiei dintre CT şi BMI. Există o tendinţa de diminuare a parametrilor antropometrici cu vârstă subiecţilor şi s-a

evidenţiat o corelaţie semnificativ negativă a CT, RTI şi BMI cu vârstă. Subiecţii de 90 ani sunt în proporţii egale normoponderali şi supraponderali (38,89%) şi numai 11,11% cu obezitate. S-au observat modificări semnificate ale parametrilor antropometrici acestora cu creşterea BMI. Analizând prevalenţa riscului pentru sănătate, s-a observat că subiecţii de 90 ani şi peste au cea mai mare prevalenţa a categoriei"nici un risc crescut" şi cea mai mică prevalenţa pentru "risc foarte mare". Datele arată că parametrii antropometrici sunt instrumente simple şi utile în evaluarea riscului pentru starea de sănătate şi orientarea strategiei terapeutice a obezităţii, malnutriţiei şi fragilităţii în îmbătrânire.
Cuvinte cheie: îmbătrânire, parametrii antropometrici, obezitate, factori de risc pentru sănătate

INTRODUCTION

Several factors have influence on life expectancy: heredity, lifestyle, exposure to environmental toxic chemicals, health states. Physical health is related to functional states of cardiovascular, digestive, bone and joints, muscle, respiratory, sensory (visual, hearing) systems, gait, balance, nutritional status, metabolic, hematological, immune, hormonal parameters, etc.

Anthropometric parameters such as body weight, height, body mass index (BMI), waist circumference (WC), hip circumference (HC), waist-hip ratio (WHR), waist-height ratio (WHtR), body fat index (BAI) define nutritional status, are risk factors and predict cardiovascular diseases, type 2 diabetes, metabolic syndrome, cognitive impairment, etc

Antropometric parameters change during the aging process [1]. Advancing age has been associated with increase of prevalence of obesity and overweight. Currently, obesity is defined as BMI > 30 kg/m^2. Obesity of elders has been associated with increases of morbidities such as infections, functional limitations, decrease of quality of life, gait decline and autonomy reduction. Aging- associated - changes in body composition as well as reduction in muscle mass (sarcopenia) associated with increase in fat mass (sarcopenic obesity), height decrease due to compression of vertebral bodies and cyphose, alter relationships between BMI and body fat. Uses of only body weight and BMI in order to define obesity might underestimate degree of adiposity in subjects with muscle mass loss. So, even though BMI does not change, older persons may present with an increase in visceral adipose tissue and fat areas of muscle tissue [2, 3, 4].

General obesity is a risk factor for various diseases associated or not with aging [5]: cardiovascular diseases (CVD), type 2 diabetes, functional disabilities, cognitive impairment [6, 7] but central obesity is more strongly associated with CVD risk than general obesity [8, 9].

Therefore, other parameters that define central/abdominal obesity as determined by waist circumference (WC) and visceral adiposity index (VAI), but also various associations of anthropometric indicators such as waist-to-hip ratio (WHR), waist-to-height ratio (WHtR) and body adiposity index (BAI) have been considered as contributing to adequately defining the concept of obesity and predict better than BMI the cardiovascular disease risk [10-15].

Paradoxically, a series of literature data show that in the elderly the mortality rate is lower in overweight and obese subjects [2, 16].

On the other hand, underweight defined as BMI<18.5 kg/m^2, malnutrition and frailty are important events associated with the aging process.

The purpose of this study is to highlight changes in anthropometric parameters: body weight, height, body weight index, waist circumference, hip circumference, waist-to-hip ratio, waist-yo-height ratio and body fat index, with which to determine the risk of illness for people aged over 80 years.

MATERIAL AND METHODS

Experimental groups: In the study, 120 patients, men and women, over 80 years of age, hospitalized in INGG were included. Subjects were informed about the

assessments to be made and agreed in writing to participate in this study. Subjects were divided into 3 groups according to age: A - age group 80-84 years, B - group 85-89 years old and C - age group 90 years and over 90 years.

Determinations: Measurement of anthropometric parameters such as body weight, height, body mass index (BMI), waist circumference (WC), hip circumference (HC), waist-hip ratio (WHR), waist-height ratio (WHtR), body fat index (BAI) was performed in subjects from the three age groups. The results were expressed as mean ± standard deviation. The comparison between the two results was achieved by Student's "t" test, and the values for $p < 0.05$ were considered significant. For the calculation of "t" and the correlation coefficient r (Pearson) the program excel - Windows 2007 was used. Correlation studies of the anthropometric parameters with the age of the subjects were made, as well as correlations between all the anthropometric parameters studied. The prevalence of the risk of impairment of health was determined based on waist circumference and body mass index in subjects in the three age groups.

RESULTS

The measurements showed that there was a tendency to reduce body weight, BMI, waist and hip circumference, waist-hip ratio and waist-height ratio, but no anthropometric parameter studied significantly changed in 85-89 year-old subjects and those over 90 years compared to subjects in the age group 80-84 years (Tab. I).

Tab. I Distribution of anthropometric parameters in elderly patients over 80 years of age

Age Parameters	**A** **80 – 84 years**	**B** **85 – 89 years**	**P**	**C** **90 + years**	**P**
Age (years)	82,02 ± 1,36	86,66 ± 1,24	**< 0,001 vs A**	92,36 ± 2,26	**< 0,001 vs A** **< 0,001 vs B**
Number Sex (W/M)	**36** (33W / 3M)	**57** (37W / 20M)	-	**19** (12W / 7M)	-
Weight (kg)	65,62 ± 13,03	66,85 ± 12,46	0,357 vs A	63,00 ± 12,20	0,799 vs A 0,265 vs B
Height (cm)	156 ± 8	160 ± 10	0,07 vs A	158 ± 10	0,522 vs A 0,374 vs B
BMI (kg/m^2)	26,72 ± 4,98	26,07 ± 4,45	0,975 vs A	25,17 ± 4,11	0,855 vs A 0,533 vs B
Waist (cm)	96,91 ± 14,39	94,15 ± 12,09	0,301 vs A	90,58 ± 8,88	0,104 vs A 0,321 vs B
Hip (cm)	105,95 ± 12,89	103,97 ± 11,84	0,276 vs A	100,76 ± 8,75	0,137 vs A 0,291 vs B
Waist-Hip Ratio	0,90 ± 0,04	0,90 ± 0,05	0,951 vs A	0,89 ± 0,05	0,628 vs A 0,643 vs B
Waist-Height Ratio	0,61 ± 0,08	0,59 ± 0,07	0,376 vs A	0,57 ± 0,06	0,154 vs A 0,420 vs B
BAI (body adiposity index) (%)	35,08 ± 5,74	33,82 ± 6,87	0,348 vs A	32,98 ± 5,89	0,267 vs A 0,641 vs B

However, the calculation of correlations of anthropometric parameters with the age of the patients revealed that the waist circumference, waist-height ratio and body adiposity index (BAI) correlated significantly negatively with the age of the investigated subjects (Tab. II).

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Tab. II Correlation of anthropometric parameters with the age of the subjects

Parameter	**r**	**R^2**	**T exp.**	**P**
Weight (kg)	- 0,1064	0,01132	1,12206	> 0,05
Height (cm)	0,05251	0,00276	0,5537	> 0,05
BMI (kg/m^2)	- 0,1498	0,02244	1,5958	> 0,05
Waist circumference (cm)	**- 0,2285**	0,05219	2,1967	**< 0,05**
Hip circumference (cm)	- 0,17649	0,03115	1,6928	> 0,05
Waist-Hip Ratio	- 0,12897	0,01663	1,2245	> 0,05
Waist-Height Ratio	**- 0,20227**	0,04091	1,9601	**0,05**
BAI (body adiposity index)	**- 0,11173**	0,01248	1,07169	**< 0,05**

Obesity, defined as BMI ≥ 30 kg/m^2 is a risk factor for some pathological conditions, and is often associated with aging, as well as malnutrition and fragility. For these reasons, we evaluated the prevalence of subjects in the age groups studied: 80-84 years, 85 = 89 years and 90 and over 90 years in all BMI categories (Fig.1).

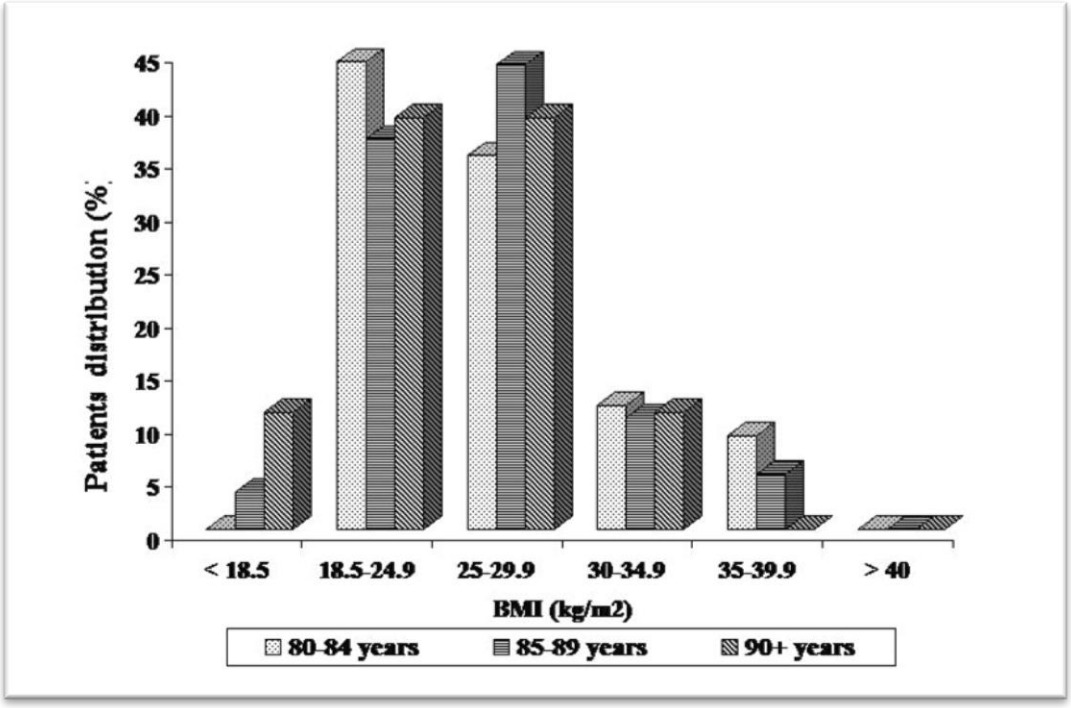

Fig. 1 Distribution of subjects by BMI in the three age groups (%)

Thus, only 3.51% of subjects aged 85-89 years and 11.11% of subjects over 90 years of age were underweight (BMI <18.5 kg/m^2 (Fig.1). 44,12% of subjects aged 80-84 years, 36,84% of those aged 85-89 years and 38,89% of subjects over 90 years of age are normoponderal (BMI = 18,5-24, 9 kg/m^2. Overweight (BMI: 25-29.9 kg/m^2 is 35.29% of subjects aged 80-84 years, 43.86% aged 85-89 years and 38.89% over 90 years of age . Fewer subjects have grade I obesity (BMI: 30-34.9 kg/m^2: 11.76% subjects aged 80-84 years, 10.53% subjects aged 85-89 years and 11.11 % of subjects over the age of 90. With obesity grade II (BMI: 35-39.9 kg/m^2 were 8.83%

of 80-84 year-old subjects and 5.26% of subjects aged 85-89 years. Note that no subject in the 80-84 age group is underweight, no subject over the age of 90 is not with obesity grade II, and no age group has morbid obesity (BMI> 40 kg/m^2) (Fig. 1). Synthesizing, in the age group 80-84 years, most subjects are normoponderal (44,12%) and overweight (35,29%) and 20,36% are obese; in the 85-89 age group most subjects are overweight (43.86%) and normoponderal (36.84%) and 15.79% are obese, and subjects over 90 years old are in equal proportion (38, 89%) normoponderal and overweight and only 11.11% with obesity.

As the aging process takes place changes in body composition in the sense of reducing muscle mass and increasing fat mass, but also a reduction in height that alters the relationship between BMI and body fat, we have further evaluated the relationship between BMI and the other anthropometric parameters (Tab. III) for all age groups.

I. Changes in anthropometric parameters according to the BMI category

In the *80-84 age group*, overweight patients showed a significant increase in waist circumference (p = 0.05) and hip circumference (p = 0.0256) versus normoponderals. Subjects with obesity gr. I have a significant reduction in height (p = 0.037) and a significant increase in waist-to-height ratio (p = 0.039) and body adiposity index (p = 0.028) compared to normoponderal. Subjects with obesity gr. I have a significant reduction in height (p = 0.037) and a significant increase in waist-to-height ratio (p = 0.039) and body adiposity index (p = 0.028) compared to normoponderal. Subjects with obesity gr. II have a significant increase in waist circumference compared to normoponderal (p = 0.037), overweight (p = 0.003) and obesity gr. I (p = 0.05); of the hip circumference compared to normoponderal (p <0.001), overweight (p <0.01) and obesity gr. I (p = 0.05); of the waist-to-height ratio compared to overweight (p <0.001) and obesity gr. I (p = 0.05) and body adiposity index (BAI) versus normoponderal (p = 0.005) and overweight (p = 0.008) (Tab. III).

In the *age range of 85-89 years***,** overweight subjects showed a significant increase in waist and hip circumference, waist-hip and waist-height ratios, and body adiposity index (BAI) versus underweight and normoponderal. Subjects with obesity gr.I showed a significant increase in waist circumference, hip circumference, and waist-to-height ratio compared to underweight and normoponderal, and a significant increase in BAI compared to underweight, normoponderal and overweight. In subjects with obesity gr. II significant reduction in height was noted compared to overweight subjects, signify-cant increase in waist circumference compared to normoponderal and overweight, hip circumference compared to underweight, normoponderal and overweight, waist-hip ratio compared to obesity gr. I, waist-height ratio compared to underweight, normoponderal, overweight and obesity gr. I., and BAI compared to underweight, normoponderal and overweight (Tab. III).

In the *age group of 90 years and over*, normoponderal subjects had a significant increase in hip circumference and BAI compared to underweight subjects. Overweight subjects had a significant increase in waist and hip circumference, waist-height ratio compared to underweight and normoponderal and a significant increase in BAI compared to underweight. Patients with grade I obesity had a significant increase in waist and hip circumference and waist-to-height ratio compared to normoponderal, and BAI versus underweight and normoponderal. Summarizing, in all studied age groups there were significant changes in the anthropometric parameters that accompany the increase of body mass index (BMI).

Tab. III Distribution of anthropometric parameters by BMI and age groups

Age (years)	Anthropometric parameters	< 18,5 Under weight **A**	18,5 – 24,9 Normalweight **B**	25 – 29,9 Overweight **C**	30 – 34,9 Obesity gr. I **D**	35 – 39,9 Obesity gr. II **E**
80 – 84 N = 36	Weight (kg)	-	55,00 ± 8, 21	70,33 ± 6,84 **P < 0,001 vs B**	74,50 ± 1,91 **P = 0,0002 vs B**	88,00 ± 13,45 **P < 0,001 vs B** **P = 0,005 vs C**
	Height (cm)	-	155,73 ± 8,53	160,25 ± 6,62	152,00 ± 4,32 **P = 0,0370 vs C**	153,00 ± 8,54
	BMI (kg/m^2)	-	22,55 ± 1,76	27,37 ± 1,61 **P < 0,001 vs B**	32,30 ± 1,72 **P < 0,001 vs B** **P < 0,001 vsC**	37,40 ± 1,60 **P < 0,001 vs B** **P < 0,001 vs C** **P = 0,010 vs D**
	Waist circumference (cm)	-	89,63 ± 16,39	100,85 ± 4,05 **P = 0,0512 vs B**	100,00 ± 4,35	118,50 ± 9,19 **P=0,037 vs B** **P=0,003 vs C** **P=0,05 0 vs D**
	Hip circunference (cm)	-	99,00 ± 14,60	110,85 ± 4,18 **P = 0,0256 vs B**	108,33 ± 4,93	123,50 ± 6,36 **P < 0,001 vs B** **P = 0,010 vs C** **P = 0,055 vs D**
	Waist-Hip Ratio	-	0,89 ± 0,04	0,90 ± 0,03	0,92 ± 0,01	0,95 ± 0,02
	Waist-Height Ratio	-	0,56 ± 0,08	0,61 ± 0,02	0,65 ± 0,04 **P = 0,039 vs B** **P = 0,054 vs C**	0,75 ± 0,02 **P < 0,001 vs C** **P = 0,051 vs D**
	BAI (%)	-	32,02 ± 4,97	35,04 ± 3,61	39,91 ± 4,36 **P = 0,028 vs B**	44,78 ± 1,01 **P = 0,005 vs B** **P = 0,008 vs C**
85 -89 N = 57	Weight (kg)	42,00 ± 4,24	59,28 ± 9,52 **P = 0,021 vs A**	70,80 ± 9,50 **P < 0,001 vs A** **P < 0,001 vs B**	78,08 ± 8,45 **P = 0,011 vs A** **P = 0,001 vs B**	81,00 ± 9,00 **P = 0,011 vs A** **P = 0,001 vs B**
	Height (cm)	157,00 ± 2,82	160,90 ± 10,99	161,64 ± 9,32	156,83 ± 9,86	148,00 ± 9,00 **P = 0,023 vs C**
	BMI (kg/m^2)	17,01 ± 1,10	22,70 ± 1,97 **P < 0,001 vsA**	26,97 ± 1,42 **P < 0,001 vs A** **P < 0,001 vs B**	31,72 ± 1,71 **P < 0,001 vsA** **P < 0,001 vs B** **P < 0,001 vs C**	36,92 ± 9,16 **P < 0,001 vs A** **P < 0,001 vs B** **P < 0,001 vs C** **P = 0,002 vs D**
	Waist circumference (cm)	76,00 ± 14,14	86,10 ± 6,74	100,23 ± 7,57 **P < 0,001 vs A** **P < 0,001 vs B**	103,50 ± 9,57 **P = 0,043 vs A** **P < 0,001 vsB**	118,50 ± 4,94 **P < 0,001 vs B** **P = 0,004 vs C**
	Hip circumference (cm)	89,50 ± 7,77	96,89 ± 9,52	108,58 ± 7,04 **P = 0,002 vs A** **P < 0,001 vs B**	114,25 ± 11,78 **P = 0,049 vs A** **P = 0,004 vs B**	126,00 ± 2,82 **P = 0,024 vs A** **P < 0,001 vs B** **P = 0,003 vs C**
	Waist-Hip Ratio	0,84 ± 0,08	0,89 ± 0,04	0,92 ± 0,04 **P = 0,032 vs A**	0,90 ± 0,01	0,94 ± 0,02 **P = 0,037 vs D**
	Waist-Height Ratio	0,48 ± 0,08	0,53 ± 0,03	0,62 ± 0,04 **p<0,001 vs A** **p<0,001 vs B**	0,66 ±0,06 **P < 0,001 vs B**	0,77 ± 0,01 **P = 0,036 vs A** **P < 0,001 vs B** **P < 0,001 vs C** **P = 0,032 vs D**
	BAI (%)	27,45 ± 2,72	29,58 ± 4,73	35,68 ± 4,21 **P = 0,016 vs A** **P < 0,001 vs B**	41,40 ± 6,53 **P = 0,021 vs A** **P < 0,001 vs B** **P = 0,039 vs C**	49,37 ± 4,13 **P = 0,024 vs A** **P < 0,001 vs B** **P < 0,001 vs C**
90 + N = 18	Weight (kg)	43,50 ± 0,70	60,07 ± 6,84 **P < 0,001 vsA**	67.58 ± 10,32 **P = 0,016 vs A**	79,00 ± 1,41 **P < 0,001 vsA** **P = 0,007 vs B** **P = 0,026 vs C**	-
	Height (cm)	157,00 ± 7,07	159,28 ± 10,12	156,28 ± 11,78	158,50 ± 4,94	-
	BMI (kg/m^2)	17,63 ± 1,23	23,63 ± 0,92 **P < 0,001 vsA**	27,48 ± 1,44 **P < 0,001 vs A** **P < 0,001 vs B**	31,47 ± 1,40 **P = 0,008 vs A** **P < 0,001 vsB** **P = 0,011 vs C**	-
	Waist circumference (cm)	80,50 ± 10,60	85,85 ± 2,79	97,71 ± 4,15 ***** **P = 0,015 vs A** **P < 0,001 vs B**	104,50 ± 12,02 ¶ **P = 0,002 vs B**	-
	Hip circumference (cm)	88,00 ± 5,65	97,42 ± 3,25 **P = 0,015 vs A**	104,00 ± 3,87 **P = 0,002 vs A** **P = 0,004 vs B**	115,00 ± 12,72 **P = 0,006 vs B** **P = 0,053 vs C**	-
	Waist-Hip Ratio	0,92 ± 0,17	0,88 ± 0,03	0,91 ± 0,02	0,91 ± 0,003	-
	Waist-Height Ratio	0,50 ± 0,08	0,54 ± 0,03	0,61 ± 0,04 **P = 0,037 vs A** **P = 0,003 vs B**	0,65 ± 0,05 **P = 0,003 vs B**	-
	BAI (%)	26,07 ± 0,77	30,74 ± 4,50 **P = 0,035 vs A**	35,72 ± 5,16 **P = 0,040 vs A**	39,53 ± 3,67 **P = 0,036 vs A** **P = 0,041 vs B**	-

II. Changes in anthropometric parameters by age within the same BMI category

Subponderal subjects (BMI <18.5 kg/m^2) were only in the 85-89 and 90+ years of age groups (Tab. III). Normal subjects (BMI: 19.5-24.9 kg/m^2) of these age groups did not show significant changes in the anthropometric parameters studied compared to the underweight subjects (Tab. III). Overweight subjects (BMI: 25-29.9 kg/m^2) of 85-89 years did not show significant changes in anthropometric parameters compared to subjects 80-84 years. In 90+ years, there was a significant reduction in waist circumference compared to subjects 80-84 years ($p = 0.016$) and 85-89 years ($p = 0.033$). Subjects with grade I obesity (BMI: 30-34.9 kg/m^2) aged 85-89 did not show significant changes in anthropometric parameters compared to subjects 80-84 years. Subjects aged 90 years and over had only a significant increase in waist circumference compared to 80-84 year olds ($p = 0.007$) and those aged 85-89 years ($p = 0.05$). Subjects with grade II obesity (BMI: 35-39.9 kg/m^2) of 85-89 years did not show significant changes in anthropometric parameters compared to subjects aged 80-84 years. In the 90+ age group there is no subject with grade II obesity.

Summarizing the analysis of the anthropometric parameters variation according to the age of the subjects, within the same BMI category, we found that only waist circumference (abdominal obesity) underwent significant changes in subjects aged 90+, overweight and obese grade I, compared to subjects between 80-84 years and 85-89 years of age, from the same BMI categories.

Starting from significant changes in anthropometric parameters by BMI, we calculated the Pearson correlation coefficient and its significance in all subjects participating in the study in order to highlight the possible link between changes in all anthropometric parameters.

The results showed that BMI correlated significantly positively with body weight, waist and hip circumference, waist-hip and waist-height ratio and BAI (Tab. IV). The Body Adiposity Index (BAI) correlated significantly positively with body weight, BMI, waist and hip circumference, waist-height ratio and significantly negative with patient height.

Tab. IV Correlations between anthropometric parameters in subjects over 80 years of age

Parameters	BMI		BAI		Waist circumference		Waist-Hip Ratio		Waist-Height Ratio	
	r	p	r	p	r	p	r	p	r	p
Weight (kg)	0,7844	**<0,001**	0,3725	**<0,001**	0,7948	**<0,001**	0,3050	**<0,01**	0,5824	**<0,001**
Height (cm)	-0,1376	> 0,05	-0,4639	**<0,001**	0,2453	**< 0,05**	0,0499	> 0,05	-0,2112	**<0,05**
BMI (kg/m^2)	1		0,7436	**<0,001**	0,7360	**<0,001**	0,2974	**<0,01**	0,8035	**<0,001**
Waist circum-ference (cm)	0,7360	**<0,001**	0,6684	**<0,001**	1		0,4805	**<0,001**	0,8935	**<0,001**
Hip circumference (cm)	0,7043	**<0,001**	0,7148	**<0,001**	0,9177	**<0,001**	0,1028	> 0,05	0,7993	**<0,001**
Waist-Hip Ratio	0,2974	**<0,01**	0,0802	> 0,05	0,4805	**<0,001**	1		0,4601	**<0,001**
Waist-Height Ratio	0,8035	**<0,001**	0,8916	**<0,001**	0,8935	**<0,001**	0,4601	**<0,001**	1	
BAI (body adiposity index)	0,7436	**<0,001**	1		0,6684	**<0,001**	0,0801	> 0,05	0,8916	**<0,001**

The waist circumference significantly correlated with all analyzed anthropometric parameters. The waist-to-hip ratio significantly correlated with body weight, BMI, waist circumference, and waist-to-height ratio. The waist-height ratio correlated significantly positively with body weight, BMI, waist and hip circumference, waist-to-hip ratio and body adiposity index (BAI) and significantly negative with the height of the subjects.

The results of this study, as well as the literature, have shown that other anthropometric parameters other than BMI contribute to the definition of obesity. Thus waist circumference is a parameter that defines abdominal obesity and undergoes significant changes within the same BMI category in patients aged 90 years and over as compared to subjects 80-84 years and 85-89 years old (Tab. III). Abdominal adiposity (waist circumference) provides information on visceral and subcutaneous adiposity without differentiating them. Visceral adiposity is a risk factor for cardiovascular pathology, type 2 diabetes, atherosclerosis, etc. Under these conditions, waist circumference benchmarks have been established, based on which a risk scale for the health status of people, taking into account race (ethnicity) and sex [17]. Thus, low risk is seen for women with WC <80 cm and men with WC <94 cm; high risk shows women with WC 80-88 cm and men with WC 94-120 cm and very high risk are women with WC> 88 cm and men with WC. 102 cm [13].

Considering these criteria, the evaluation of the distribution of subjects in the 80-84 age group revealed that 13.04% of women were at low risk (WC <80 cm), 13.04% were at high risk (WC 80-88 cm) and 65.23% have a very high risk (WC> 88 cm), while 8.69% of men have a very high risk (WC> 102 cm) (Tab. V) to affect their health status.

Tab. V Distribution of subjects according to waist circumference and sex in different risk categories for health status (%)

Subjects age (years)	Low risk		High risk		Very hight risk	
	W < 80 cm	M < 94 cm	W: 80-88 cm	M: 94 -102 cm	W > 88 cm	M > 102 cm
80-84	13,04	0	13,04	0	65,23	8,69
85-89	9,09	11,36	22,73	9,09	38,64	9,09
90+	5,55	22,22	27,78	16,67	27,78	0

In the 85-89 age group, 9.09% of women have low risk (WC <80 cm), 22.73% are at high risk (WC 80-88 cm), and 38.64% very high risk (WC > 88 cm). In men 11.36% have low risk (WC <94 cm), 9.09% have a high risk (WC 94-102 cm) and 9.09% have a very high risk (WC> 102 cm).

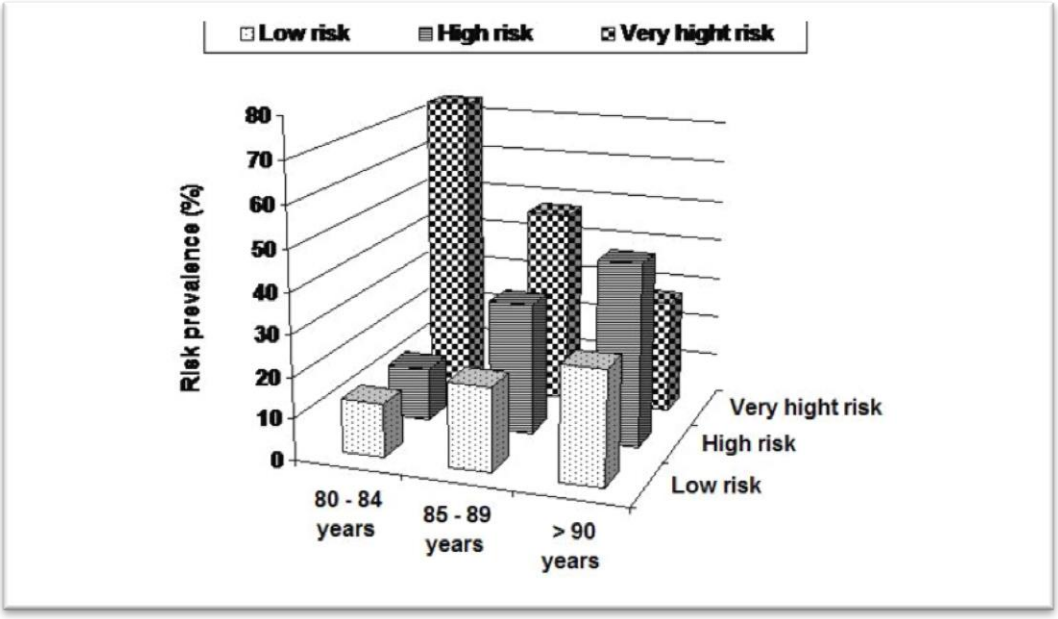

Fig. 2 Prevalence of health risk based on waist circumference

Of women aged 90 years and over 5.5% have low risk (WC <80 cm), 27.78% are at high risk (WC 80-88 cm) and 27.78% very high risk (WC> 88 cm). In the same age group, 22.22% of men have low risk (WC <94 cm), 16.67% have high risk (WC 94-102 cm) and 0% very high risk (WC>102 cm).

The analysis of the prevalence of health risk for waist circumference for women and men together revealed that subjects aged 80-84 have the highest prevalence (73.92%) of very high risk, while subjects of 90 years and over have the lowest prevalence (27.78%) of very high risk (Fig. 2). Furthermore, subjects aged 90 years and over have the highest prevalence (27.77%) of low risk for impairment of health (Fig. 2).

From the relationship of nutritional status based on BMI to waist circumference, there were several levels of risk to people's health: least risk, increased risk, high risk and very high risk [18].

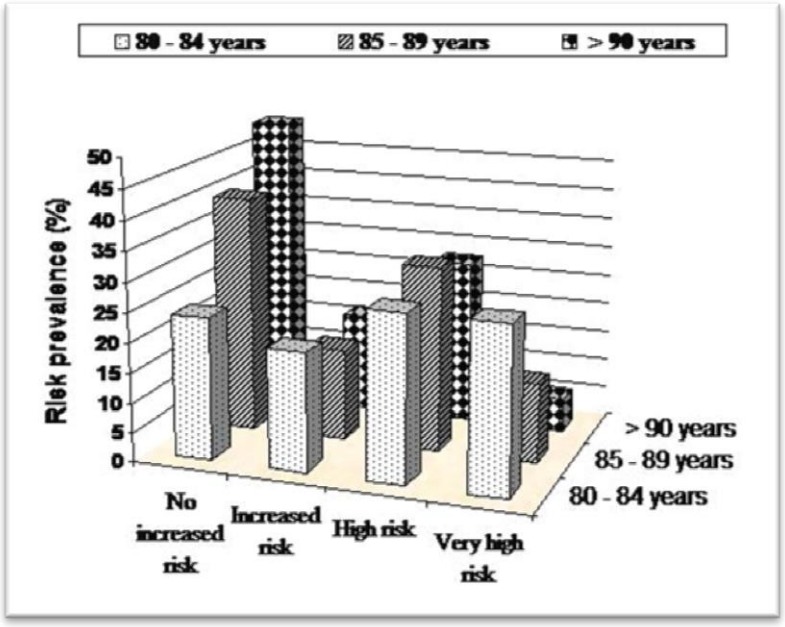

Fig. 3 Prevalence of Health Risk (%), based on waist circumference (WC) and Body Mass Index (BMI), in patients over 80 years

In the 80-84 year age group, women and men together, 24% show no increased risk, 20% increased risk, 28% high risk and 28% very high risk of harm to health. (Fig. 3). In the age range of 85-89 years, 40% of subjects are risk-free, 15.55% with increased risk, 31.12% with high risk and 13.33% with very high risk. In subjects aged 90 years and over, no increase risk is 50%, increases risk by 16.67%, high risk of 27.78% and very high risk of 5.55%. In conclusion, subjects aged 90 years and over have the highest prevalence of no increase risk and the lower prevalence of very high risk (Fig. 3).

The study of correlations between subject age and health risk based on waist circumference showed that the age-related relationship was positively correlated with low risk and high risk and significantly negative with very high risk (Tab. VI). The calculation of the correlation between subject age and health risk based on waist circumference and BMI showed that age correlated significantly positive with no increased risk, significantly negative with increased risk and very high risk, and not correlated with high risk category (Tab. VI).

Tab. VI Pearson's correlation between health risk and subjects age

Health risk	Risk category	Pearson's correlations	
		r	p
based on WC	Low risk	0,9994	< 0,001
	High risk	0,9936	< 0,001
	Very high risk	- 0,9969	< 0,001
based on WC and BMI	No increased risk	0,9912	< 0,001
	Increased risk	- 0,7193	< 0,01
	High risk	0,0059	> 0,05
	Very high risk	- 0,9846	< 0,001

DISCUSSIONS

Due to technological advances and scientific discoveries in the medical field, society is experiencing a steady and rapid increase in life expectancy in Western European countries, coupled with a major increase in population aging. And Romania is experiencing the aging phenomenon of the population. Increased life expectancy is accompanied by an increased risk of aging-related illnesses such as obesity, type 2 diabetes, atherosclerosis, cancer, and neurodegenerative diseases. These diseases represent enormous challenges, both for individuals and for society, in terms of quality of life and economic burden. Thus, aging societies have to urgently address public health issues and develop services for the elderly.

Maintaining independence, quality of life, high function, and health is crucial for the older population. So, obesity and the loss of muscle mass and muscle function (sarcopenia) are important health risk factors in old age leading to functional decline and mobility limitations [3].

Obesity is associated with increased incidence of cardiovascular disease, type 2 diabetes, dyslipidemias, metabolic syndrome, and cognitive impairment in the general population and the elderly population.

Obesity prevalence is increasing in the older population, and like sarcopenia, obesity (ie, a body mass index (BMI) >30 kg/m²) and severe obesity (ie, a BMI .35 kg/m²) have been consistently associated with several negative health outcomes, disabilities, falls, and mobility limitations. The effect of obesity on mortality by cardiovascular disease, however, is less relevant in older than in younger age groups, as obese older patients with cardiovascular disease have demonstrated

better survival rates compared with nonobese older patients (the so-called "obesity paradox" [19]. But even if mortality rates might be affected positively by obesity, the problem remains that its negative effects on function may lead to considerable disability during this extended lifetime.

Body mass index is the standard for classifying weight and is the most practical method to determine the extent of obesity. Obesity is commonly classified as BMI≥30 kg/m^2, whereas a BMI < 18.5 is classified as underweight, a BMI of 18.5–24.9 as normal weight and a BMI of 25–29.9 as overweight. This classification does not take into account sex or age. Classification of obesity by using BMI does neither differentiate between fat and fat free mass, nor between the distributions of body fat. Other parameters like waist circumference, waist to hip ratio, direct measurement of visceral body fat, or classifications which incorporate parameters of body composition, the relation between fat and fat free mass and the concept of sarcopenic obesity have been proposed to be more valid parameters for mortality risk assessment in the elderly.

A BMI in the obese range in the elderly increases the risk of frailty by 3.5 and 96% of community-dwelling subjects aged 65–80 are classified as frail. Not only increased BMI, especially sarcopenia and sarcopenic obesity are associated with frailty. Frailty in elderly subjects is associated with increased mortality. On the other hand, not only obesity, but also underweight, the other extreme on the BMI scale can be responsible for the development of frailty. This fact seems paradoxical; however, the way underweight and obesity lead to frailty follow different pathophysiological paths with only the same outcome, frailty. A good nutritional status can contribute to the prevention of frailty through the prevention of underweight and adiposity.

Body mass index is not only used to classify obesity but also to determine life expectancy and prevalence of obesity-related issues and comorbidities. The risk of developing a comorbid condition increases with increasing BMI [4]. Additional factors that increase disease risk in the overweight and class I and II obesity groups are large WC (>102 cm in men and >88 cm in women) and ethnicity. The rationale for measuring the WC in clinical practice is to identify metabolically obese and overweight patients whose BMI is normal and thus would not be considered for lifestyle intervention and treatment. The WC measurement has been highlighted as a key component in several recently released algorithms for overweight and obesity management.

Waist circumference is also a method often used to diagnose metabolic syndrome in overweight and obese patients. When a large WC is factored into BMI-associated disease risk, there is an increased disease risk in the overweight and class I obesity groups. The WC is less useful as an independent marker of medical risk when the BMI is greater than 39. Overall risk is independently associated with excess abdominal fat (WC > 102 cm in men and > 88 cm in women). The visceral deposition of adipose tissue is easily ascertained by measuring WC or the waist-to-hip ratio.

Waist circumference was shown to be a surrogate marker for intra-abdominal adiposity in a study conducted upon men and women. In this study, WC strongly correlated with intra-abdominal adiposity as measured using computed tomography or magnetic resonance imaging, which is considered the criterion standards for imaging adipose tissue. Waist circumference is also used in the screening of the metabolic syndrome and to establish the cardiovascular risk factors in the elderly [19]. The metabolic syndrome and its components which include excess abdominal fat, insulin-resistance, dyslipidemia, and high blood pressure are highly prevalent in older populations (NCEP, 2002). The prevalence of the metabolic syndrome increases with age and reaches a

peak in men aged 50–70 years and women aged 60– 80 years. In the adult population with age fasting plasma glucose and postprandial glucose increase by 1–2 mg/dl and 10–20 mg/dl, respectively, for each decade. Such as, the prevalence of type 2 diabetes mellitus, also increases with age and reaches a peak in women aged 85 years and older and in men aged 75–84 years [20].

Both anthropometric parameters, MBI and waist circumference were also used to assess disease risk. Thus, a stratification of the health risk was made depending on the BMI category and the waist circumference [4, 17].

In this context, the measurement of anthropometric parameters currently in the clinic, regardless of age, gender, race or pathology, allows the detection of risk factors for impairment of health and the adoption of an appropriate treatment strategy. By simple means, easy to achieve, one can predict the incidence of a disease or death.

Given the increase in life expectancy, the increase in the proportion of the elderly population globally, along with the increase in the prevalence of diseases associated with the aging process, with economic and social impact, our study has highlighted changes in anthropometric parameters: body weight, height, body weight index, hip circumference, waist-to-hip ratio, waist-yo-height ratio and body fat index, and determine the risk of illness for people aged over 80 years.

Our study revealed that no anthropometric parameter suffers significant changes in subjects over 90 years of age and those aged 85-89 years. However, the correlations studies of anthropometric parameters with of the patients age revealed that the waist circumference, waist-height ratio and body adiposity index (BAI) correlated significantly negatively with the age of the investigated subjects. Analyzing the prevalence of obesity in the three age groups, the study showed that in the age group 80-84 years, most subjects are normoponderal (44,12%) and overweight (35,29%) and 20,36% are obese; in the 85-89 age group most subjects are overweight (43.86%) and normoponderal (36.84%) and 15.79% are obese, and subjects over 90 years old are in equal proportion (38, 89%) normoponderal and overweight and only 11.11% with obesity.

The assessment of the relationship between BMI and other anthropometric parameters showed significant changes in WC, HC, WCR, WHtC, and BAI that accompany the increase of BMI in all studied age groups. The analysis of the anthropometric parameters variation according to the age of the subjects, within the same BMI category, showed that only waist circumference (abdominal obesity) underwent significant changes in subjects aged 90+, overweight and obese grade I, compared to subjects between 80-84 years and 85-89 years of age, from the same BMI categories.

Correlations between anthropometric parameters in subjects over 80 years of age highlight the possible link between changes in all anthropometric parameters.

To be highlighted as subjects over 90 years of age have a lower prevalence of high and very high risk and an increased prevalence of no increase risk and low risk for health based on BMI and WC.

Although, in general, obesity is a risk factor for CVD, type 2 diabetes, atherosclerosis, cognitive impairment, cancer, and the incidence increases with age, it is not a risk factor for mortality in the elderly, especially at those with serious pathologies. That is why we are discussing the appropriateness of indications of reducing BMI in elderly patients, targeting treatment to disease control and addressing a healthy lifestyle that includes movement/ exercise and proper nutrition.

CONCLUSIONS

Obesity is defined as BMI> 30 kg/m^2, but in the process of aging there are changes in body composition that in turn modify the

relationship between lean and fat mass. Thus, BMI does not adequately measure body adiposity and has a limited capacity to predict mortality. The concept of obesity has been complemented by the assessment of other anthropometric parameters such as WC, WH, WHR, WHtR, BAI, that provide information about fat mass and its distribution according to age and pathology.

WC, WHR and WHtR measurements allow the assessment of abdominal adiposity, which is a risk factor for cardiovascular disease, type 2 diabetes, cognitive impairment, etc.

It is known that the prevalence of overweight, obesity, abdominal obesity and adiposity is higher in the elderly, but it is not a risk factor for mortality ("obesity paradox").

Simple assessment of anthropometric parameters and their various associations was at the base of the health risk stratification and the mortality prediction.

Our study found that after the age of 80 there was a tendency to reduce the values of the anthropometric parameters studied, and the waist circumference, the waist-height ratio and the body adiposity index (BAI) correlated significantly negatively with the age of the investigated subjects. Subjects aged 90 years and over had the highest prevalence of no increase risk and the lowest prevalence of very high risk.

Measurement of anthropometric parameters allows the orientation and pursuit of a therapeutic strategy of obesity, abdominal obesity, underweight, malnutrition and fragility associated with the aging process.

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BIOLOGICAL MARKERS USED TO IDENTIFY AND EVALUATE FRAILTY IN ELDERLY PEOPLE

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Abstract. Use of biomarkers as feasible endpoints has been proposed for frailty identification, since they would provide a more accurate detection of frail subjects in early stages, when this syndrome can still be potentially reverted. It is possible that, with laboratory parameters, a functional alteration and its evolution can be detected. This should prevent the development of a manifest disease. Experts agreed that no single biomarker by itself is adequate for the assessment of frailty, suggesting a need for a combination of multiple biomarkers. Currently, there are no standardized tests or biomarkers that can be used to identify frail patients and for this reason, identification of biomarkers for frailty is a major consideration for future studies of this syndrome. Scientists working in the field claim that the special measured values which play a specific role in the context of frailty syndrome are there in relation with anemia, vitamins, hormones and inflammatory proteins/cytokines.
Key words: frailty, biomarkers, anemia, vitamins, hormones, inflammaging

MARKERI BIOLOGICI FOLOSIŢI PENTRU IDENTIFICAREA ŞI EVALUAREA FRAGILITĂŢII LA PERSOANELE VÂRSTNICE

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Rezumat. Pentru identificarea fragilităţii a fost propusă folosirea biomarkerilor ca şi obiective fezabile, dat fiind că aceştia ar putea asigura detecţia cu mai mare acurateţe a subiecţilor fragili încă din stadii timpurii, când acest sindrom este potenţial reversibil. Este posibil ca prin intermediul parametrilor de laborator să putem detecta o alterare funcţională şi evoluţia acesteia, ceea ce poate preveni dezvoltarea unei afecţiuni manifeste. Experţii din domeniu sunt de acord asupra faptului că nu există un singur biomarker adecvat pentru evaluarea fragilităţii, sugerând necesitatea combinării mai multor biomarkeri. În prezent nu există teste standardizate sau biomarkeri care să poată fi folosiţi pentru evaluarea pacienţilor fragili şi pentru acest motiv identificarea biomarkerilor fragilităţii este o provocare majoră pentru studiile viitoare asupra acestui sindrom. Oamenii de ştiinţă care lucrează în domeniu susţin că valorile măsurabile cu rol specific în contextul sindromului fragilităţii sunt în legătură cu anemia, unele vitamine, hormoni şi proteine inflamatorii sau citokine.
Cuvinte cheie: fragilitate, biomarkeri, anemie, vitamine, hormoni, "inflammaging"

INTRODUCTION

Frailty, being multi-factorial in etiology, is a multidimensional clinical age-related syndrome affecting multiple organs. Therefore, many biological, clinical and social factors are involved in the development of frailty [1] and all of these factors interact to produce conditions to develop disability and decrease quality of life. It is characterized by increased

vulnerability to stressors, decline of different physiological systems and cognitive abilities, all of these leading to co-morbidity and loss of independence with diminished ability to perform activities of daily living (ADL). For these reasons, it is associated with increased risk of falls and fractures, institutionalization, hospitalization and increased mortality (Fig.1).

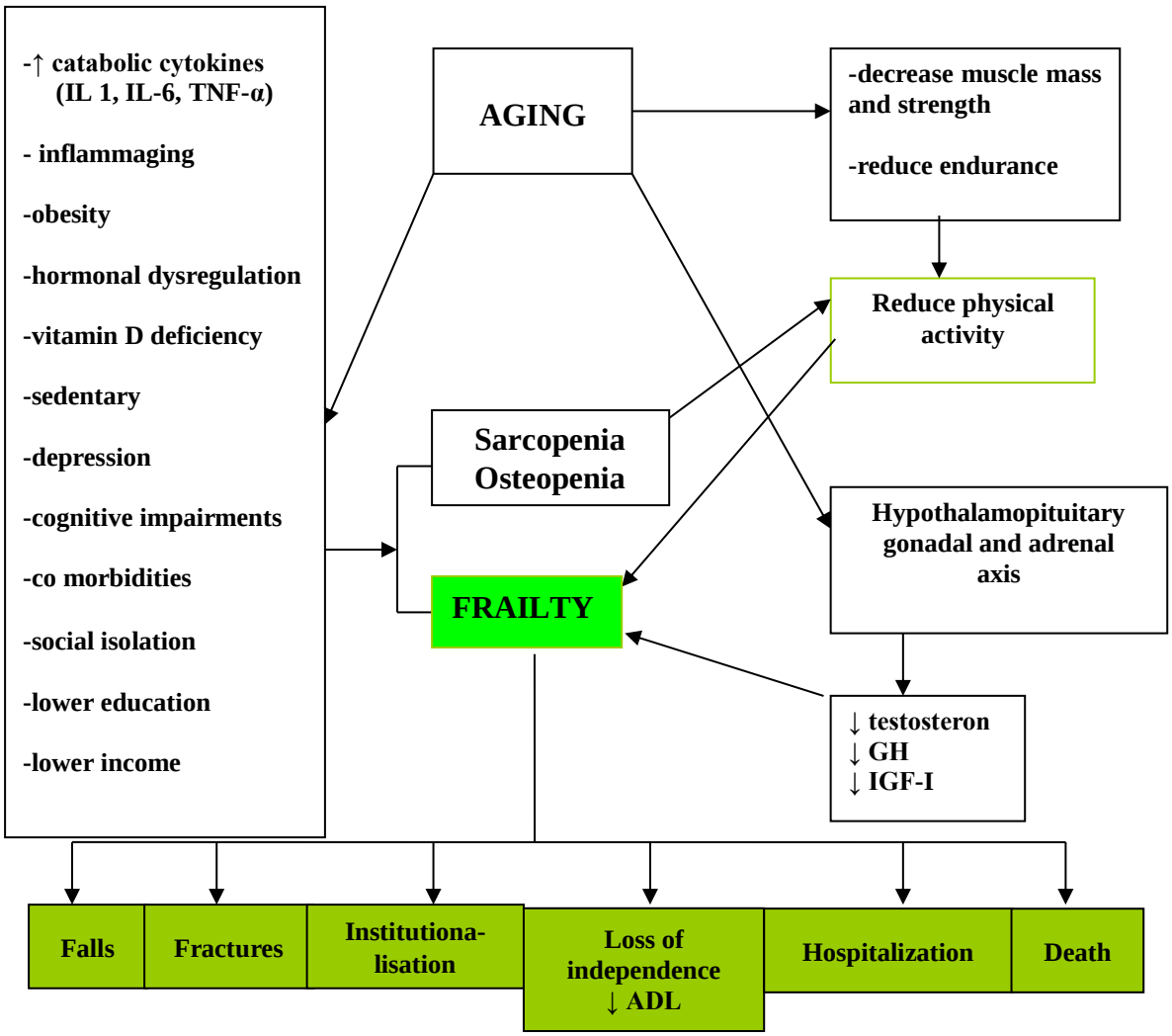

Fig. 1 Diagram representing etiopathogenesis and consequences of frailty

Use of biomarkers as feasible targets has been proposed for frailty identification [2], since they would provide a more accurate detection of frail subjects in early stages, when this syndrome still can be potentially reverted. In this way it is possible that, with laboratory parameters, a functional impairment and its evolution can be detected and this should prevent the development of a manifest disability.

Experts accepted that no single biomarker by itself was able for the assessment of this syndrome, suggesting that it is necessary for a combination of multiple biomarkers. So it was found agreement regarding the necessity to combine biomarkers but no agreement regarding which combination of biomarkers. Until now there are no standardized tests or biomarkers that can be used to identify frail patients and for this reason, identification of biomarkers for frailty is a major consideration for future studies of this syndrome. About this, Ferruci et al. [3] stated: "The factors that contribute to this cyclic metabolic pathway, currently defined as the frailty syndrome,

are still unclear and, therefore, there is still uncertainty on what circulating molecules should be considered as biomarkers of frailty".

The special measured values which play a specific role in the context of frailty syndrome are there in relation with: anemia, vitamins, hormones and inflammatory proteins/cytokines.

Anemia in the context of frailty syndrome

Anemia negatively impacts quality of life and is associated with poorer survival in older adults [4].

Among elderly people with anemia, one third have evidence of folic acid, iron, and/or vitamin B_{12} deficiency, another third have chronic inflammation and/or renal insufficiency, and the remaining third have unexplained anemia, when investigations can not suggest a specific cause (the term "anemia of unknown etiology" - AUE) [5]. One study [6] revealed that erythropoietin (EPO) levels are significantly lower and inappropriately in patients with AUE, suggesting that decreased EPO production may play a key role in the pathogenesis of AUE, being a distinct entity.

EPO is a hormone that plays an important role in the regulation of erythropoiesis, promoting the differentiation and proliferation of the colony-forming unit-erythroid (CFU-E) and other erythroid progenitors. A possible mechanism for the decrease in EPO levels in this elderly population is the presence of a subclinical pro-inflammatory state. Inflammatory cytokines such as IL-1 and TNF alpha are postulated to play a role in development of anemia of chronic disease through inhibition of EPO synthesis.

Iron deficiency is of particular significance, but the most important criterion to make difference between pathologies (chronic inflammation and iron deficiency) is the serum concentration of the ferritin (iron storage protein). This is lowered in anemia with iron deficiency and raised in anemia of chronic diseases.

Another cause of anemia is the deficiency of folic acid and/or vitamin B12, resulting in the impaired DNA metabolism. Therefore hyperchromic, macrocytic anemia develops, which is characterized by an increased mean erythrocyte volume (VEM) with a simultaneously high hemoglobin content of the erythrocytes.

Vitamins in the context of frailty syndrome

The importance of folic acid and B12 vitamin in the development of frailty has already been explained, but another vitamin which is important for the functionality of the elderly is D vitamin, with it's especially role in the regulation of calcium metabolism (stimulation of bone mineralization, absorption of calcium in the intestine, primary role in the maintenance of extra cellular fluid calcium concentration). The association between D vitamin deficiency and bone disease are well recognized, but have been increasing indications that the D vitamin system plays an important role in the metabolism of a wide variety of cells and systems, so as can be seen in Tab. I.

.

Tab. I D Vitamin deficiency and associated conditions

Cardiovascular	orthostatic hypotension, aortic dilatation
Respiratory	obstructive sleep apnea, bronchiolitis, bronchiectasis, asthma
Gastrointestinal	chronic hepatitis, liver cirrhosis, pancreatitis
Neurological	myasthenia gravis, multiple sclerosis
Musculoskeletal	osteoporosis,osteoarthritis, rheumatoid arthritis, muscle weakness, falls, fractures
Metabolic	diabetes mellitus, diabetic nephropathy, metabolic syndrome
Cancer	colorectal, lung ovarian, prostate, breast
Skin	systemic lupus erythematosus, eczema, psoriasis

D vitamin deficiency is associated with muscle weakness predominantly of the proximal muscle groups. This leads to slower walking speed, prolonged sit-to-stand time, lower quadriceps strength, and a higher rate of falls [7] which are a major problem in the elderly, leading to significant morbidity, increased mortality and substantial consumption of healthcare resources. Observational findings have been confirmed by interventional studies with daily dosing of D vitamin from 800 to 1000 IU per day associated with a 20– 30% reduction in falls rate and significant improvements in body sway [8].

Osteoporosis causes substantial morbidity and mortality in older people, but whether chronic insufficiency of D vitamin is a reversible determinant of osteoporosis and related risk of fractures is controversial. Observational studies indicate that low plasma levels of 25 OH vitamin are associated with higher risk of fractures and with vascular and non-vascular mortality [9-12], but it is unclear if these associations are causal. Randomized trials assessing the effects on fracture and other health outcomes have generally failed to demonstrate beneficial effects of D vitamin supplementation [13-16], but in a major study in subjects over 65 years old, Trivedi et al. [17] showed that the additional intake of D3 vitamin reduced the fracture rate with 33%.

Hormones in the context of frailty syndrome

The process of aging strongly affects entire endocrine system, because some target tissues become less sensitive to their mechanism of hormonal control or the amount of hormones produced may also change (Tab. II).

Four main groups of hormones are involved in frailty: thyroid hormones; growth hormone/insulin-like growth factor-1 (GH)/IGF-1 and insulin; sexual hormones (testosterone and estradiol); cortisol/dehydroepiandrosterone (DHEA).

Tab. II The association of variation of the hormonal profile in the process of aging with longevity and frailty

	Hormones	**Variations in aging**	**Longevity**	**Frailty**
1	DHEA	↓	Higher levels with predictive factor for long-term longevity	Predict the risk of developing frailty
2	Testosteron	↓	Associated with low longevity in men and women	Prevalence of frailty increases, whereas testosterone decreases, as men age.
3	Estrogens	↓	Long-term replacement therapy is associated with lower all-cause mortality in older women.	In contrast to testosterone, frailty seems to be associated with high estrogen levels in postmenopausal women (especially if are associated to inflammation)
4	GH	↓	Survival to extremely old age - associated with reduced GH and IGF-1 signaling	Associated +/-(possible utility of GH in the treatment of frailty and sarcopenia)
5	fT4/TSH	↑	Inverse correlation suggest a potential role of decreased thyroid function leading to longevity	fT4 within normal range associated with frailty in ♂

Thyroid gland is certainly impacted in elderly, the prevalence of thyroid disorders increasing with age. Subclinical disturbances of thyroid function are more frequent in the elderly and the symptoms are more subtle, often attributed to normal aging. The natural history of subclinical hypothyroidism depends on the presence

or absence of antithyroid peroxidase antibodies (TPOAb). Thus, a quite high rate of reversion of subclinical hypothyroidism to euthyroid status in adults aged at least 65 years with lower baseline TSH levels and TPOAb negativity was observed [18]. In turn, higher TSH level and TPOAb positivity were independently associated with lower chance of reversion to euthyroidism [18]. Moreover, TSH levels ≥ 10 mIU/l were independently associated with progression to overt hypothyroidism [18]. Similar findings, showing that higher baseline TSH levels are associated with progression from subclinical to overt hypothyroidism and that higher TSH level (>8 mIU/l) is a predictive value for development of overt hypothyroidism, were recently reported by Imaizumi et al. [19]. On the other hand, there is strong evidence that thyroid hypofunction may contribute to increased lifespan (see further in the text). Therefore, taking into account all mentioned observations, the replacement therapy with L-thyroxine is not uniformly recommended in elderly people with subclinical hypothyroidism.

DHEA is a steroid precursor of testosterone produced by the adrenal cortex and the biological role of it is not yet well defined. Observational studies have demonstrated that plasma levels of DHEA and DHEAS decline by 80% between 25 and 75 years, and this decrease is greater after 80 years [20], serum DHEAS being a predictive factor for long-term longevity. The pattern of steroid secretion from corticoadrenal gland shows a progressive significant reduction of DHEAS levels, being cortisol nearly unchanged, and consequently with a significant age related increase of cortisol/DHEAS ratio. Therefore, low DHEA levels and a high cortisol/DHEA ratio predict the risk of developing frailty.

Data from the InCHIANTI study [5] suggest that DHEAS levels are related to lower extremity muscle strength and have reported that frail people have lower levels of serum IGF-I and DHEAS and higher levels of IL-6 than non-frail, age-matched individuals.

The physiological pathways leading to frailty are complex, but there is evidence that testosterone may play an important role in aging men.

Testosterone

- promotes erythropoesis
- maintains bone mineral density
- supports muscle function and growth
- anabolic actions
  - -stimulate appetite and food intake
  - -increase protein synthesis
  - -inhibition of adipocyte production
  - -stimulation of cells for muscle repair
  - -enhance amino acid reuse in muscle

Hypogonadal men typically present:

- decreased bone mineral density
- loss of energy
- muscle wasting

Several studies have explored the association between sex hormones and various components of the frailty syndrome. In 2005 Schaap and colleques [21] reported results of a cross-sectional analysis in older men. Total and free testosterone levels were positively associated with grip strength, whereas free testosterone levels were associated with better mobility and were less likely to fall during a follow-up period of 4 years. These findings suggest that testosterone therapy could potentially treat or prevent the development of frailty.

Estrogens may have a central role in the long life expectancy of women. A study

from 2012, Carcaillon et al. [22] found the potential association between estradiol (E2) levels and frailty among older postmenopausal women not taking hormonal therapy. The results demonstrated that E2 levels decreased significantly with age and educational level; whereas they increased with body mass index and high-sensitivity C-reactive protein (hs-CRP). Higher E2 levels were associated with the prevalence of frailty among postmenopausal women younger then 79 years, but not in the oldest group.
The synergism between higher E2 and hs-CRP levels suggests the existence of physiopathological mechanisms connecting inflammation and estrogen to frailty.

Estrogen levels correlates to:

- favorable impact on serum lipid profiles
- high risk for breast and ovarian cancer
- low risk for heart disease, stroke and all cause mortality
- protective effects on cerebral areas known to be involved in age-related cognitive functions and Alzheimer's disease.

Growth hormone (GH), produced in the anterior pituitary gland, is released into the circulation in a pulsatile manner and predominantly stimulates the liver to produce IGF-1. The GH /IGF-1 axis in aging, like deficiencies in sex steroid hormones, appears to be linked with changes in the hypothalamic-pituitary-adrenal axis, affecting muscle and bone physiology. GH levels in men and women fall significantly with age. Also, decline in IGF-1 often occurs with increasing age, and low IGF-1 levels are associated with frailty and an increased risk of death. In this same regard, insulin resistance is also associated with frailty, supporting the contribution of low-functioning insulin/IGF-1 signaling to a prolonged survival, but at the same time, to the presence of frailty.

Inflammation in the context of frailty syndrome

Chronic inflammation has been postulated like a key mechanism involved in frailty, acting either directly or indirectly through its negative influence on other physiological systems [23]. Thus, studies testing the hypothesis that frailty is associated with alterations in the concentration of immune activation markers, in different lymphocyte subpopulations, and pro-inflammatory molecules are becoming more in the last years [24].

Cross-sectional studies reported [25] that compared to robust participants; both frail and pre-frail ones had significantly higher levels of C-reactive protein. Frailty and pre-frailty were associated with higher serum levels of IL-6, and were also significantly associated with elevated number of white blood cells/mmc and fibrinogen levels compared to people who were robust.
The study of Marcos-Perez M et al. published in may 2018 [26] confirmed the involvement of chronic inflammation in frailty in later life; particularly strong associations were obtained in the regression analysis for IL-6 and for sTNF-RII. This last biomarker showed a high accuracy for predicting frailty. Although results from this study revealed limited strength associations between frailty and the lymphocyte subsets, data obtained for the different inflammatory mediators provide additional reinforcement to the widely established hypothesis that inflammaging is involved in the frailty status in older adults.
There is a complex physiological interaction between various anabolic hormones (GH, IGF-I and testosterone), inflammatory cytokines (IL-2, IL-6, TNF-α), biochemical and molecular pathways mediating catabolism of muscle protein, age related muscle loss and consequently to physical frailty.

CONCLUSIONS

I. Frailty becomes more prevalent with increasing age but is not an inevitable consequence of aging
II. Use of biomarkers as feasible endpoints has been proposed for frailty identification, since they would provide a more accurate detection of frail subjects in early stages, when this syndrome can still be potentially reverted
III. Experts agree that there is no single biomarker there is adequate to predict or identify frailty, but it is necessary a combination of multiple biomarkers
IV. Additional research work is needed to identify the specific combination of clinical and laboratory biomarkers that can be used for the diagnosis of frailty
V. It can be stated that laboratory diagnostic, in addition to clinical observation and anthropometric data, represent important components for the clarification of preventive approaches and the causality of the frailty

Conflicts of interest

The authors declare no conflicts of interest.

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PREVALENCE OF FRAILTY SYNDROME AMONG TYPE 2 DIABETES MELLITUS ELDERLY PATIENTS

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Abstract. Frailty defined by decreased adaptability to stress factors as a result of decreasing functional reserves is one issue discussed more and more often by those that care for the elderly and are concerned about their quality of life. Frailty leads to an increased vulnerability to the loss of physiological functions that becomes visible in stress conditions. The association of frailty with chronic disease, like diabetes mellitus leads to poor prognosis for the elderly patient. This study aims to establish a correlation between frailty syndrome and diabetes mellitus type 2 in the elderly patient. Also, the study aims to calculate the prevalence of frailty syndrome among elderly diabetics and also to evaluate the relation between the degree of diabetes control and the evolution of the frailty syndrome. The increased prevalence of this syndrome with the elderly patient with diabetes increases the need for medical interventions in order to prevent evolution to serious complications. The study documents the prevalence of frailty syndrome with 23.3% frail patients and an equal percentage of pre-frail patients of the total study group. The prevalence of frail elderly, among the elderly included in this study is 46.66%.
Key words: frailty, diabetes mellitus, elderly

PREVALENŢA SINDROMULUI DE FRAGILITATE LA PACIENŢII VÂRSTNICI CU DIABET ZAHARAT TIP 2

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Rezumat. Fragilitatea definită de scăderea capacităţii de adaptare la factori de stress că urmare a scăderii rezervelor funcţionale este una dintre problemele puse în discuţie, tot mai des, de cei care îngrijesc vârstnici şi sunt preocupaţi de calitatea vieţii acestora. Fragilitatea aduce o vulnerabilitate crescută la pierderea functilor fiziologice şi care devine vizibilă în condiţii de stress. Asocierea fragilităţii cu boli cronice, cum este şi diabetul zaharat, determina evoluţii cu prognostic grav în viaţă pacientului vârstnic. Acest studiu îşi propune stabilirea unei corelaţii între sindromul de fragilitate şi diabetul zaharat tip 2 la pacientul vârstnic. De asemenea, studiul urmăreşte calcularea prevalenţei sindromului de fragilitate în rândul pacientiilor vârstnici diabetici dar şi evaluarea relaţiei dintre gradul de control al diabetului şi evoluţia sindromului de fragilitate. Prevalenţa crescută a acestui sindrom la pacientul vârstnic, diabetic ridică nevoia de intervenţii, care să reducă evoluţie spre complicaţii grave. Studiul documentează o prevalenţa a sindromului de fragilitate de 23,3% fragili şi un procent egal de pre-fragili de vârstnici din totalul lotului de studiu. Prevalenţa vârstnicilor fragili, din totalul vârstnicilor incluşi în studiu este de 46,66%.
Cuvinte cheie: fragilitate, diabet zaharat, vârstnic

INTRODUCTION

The association of frailty with chronic disease, like diabetes mellitus leads to poor prognosis for the elderly patient [1, 2]. Demographic data mention an increase in the number of elderly people who, through multiple, chronic and progressive suffering evolving into complications, attract a

growing need for specialized care and multidisciplinary management [3, 4].

In close connection with the ageing phenomenon, the published literature is beginning to outline a new syndrome - the frailty syndrome, whose definition has stirred up some controversy. At present, it is considered to be a multidimensional geriatric syndrome [5], as a consequence of decreasing physiological reserves concomitant with a multisystem disorder and a limited capacity to maintain homeostasis [6]. Two frailty patterns have been accepted: the frailty phenotypes and Rockwood's frailty index. The frailty phenotypes proposed by Fried and colleagues in the CHS study, a cohort study of more than 5,300 subjects, are: a decrease in muscular grip strength, decreased walking speed, decreased physical activity level, fatigue, unintended weight loss [2,7]. Thus, the presence of frailty in the elderly patient increases the risk of a serious evolution such as falls, disabilities and mortality [8].

Frailty seems to be associated, as studies have shown, with serious illnesses and physiopathological changes at the level of the endocrine system and inflammatory response level, as well as with malnutrition or obesity [9].

This study aims to establish a correlation between the frailty syndrome and type 2 diabetes mellitus in elderly patients. The study also aims to track the prevalence of the frailty syndrome among elderly diabetic patients, as well as to assess the relationship between the degree of managing diabetes and the evolution of the frailty syndrome.

METHODS

Subjects

Participants in the study are patients admitted to "Ana Aslan" INGG between December 2015 and May 2016. Two patient groups were selected with an equal number of participants, broken down by age groups as follows: Lot 1 aged 50-64 and lot 2 aged>= 75 years. Exclusion criteria are type 1 diabetes, MMSE score less than 12 points.

Data collection

The data needed to achieve the study's objectives were collected from the observation records of the patients included in the study and by using the standardized Frail Scale questionnaire. Thus, the parameters collected from the observation records are: the duration of diabetes evolution, the maximum glycemia value, the value of the last fasting glycemia, the glycated hemoglobin (HbA1c), the body mass index (BMI), the presence of comorbidities (hypertension, heart failure, heart ischemic disease, myocardial infarction, stroke, renal failure nephropathy, chronic pulmonary disease, asthma, neoplasia, arthrosis, MMSE test, GDS depression scale evaluation, nutritional assessment using the Mini Nutritional Assessment (MNA) scale.

The Frail Scale Questionnaire is used in order to establish the frailty status. At least three positive responses to the five questions of the questionnaire qualify the patient as frail, one or two positive responses qualify the as pre-frail, and all negative responses qualify the subject as non-frail.

The inclusion criteria were those related to age groups, 50-64 years old and over, or equal to 75 years, as well as the diagnosis of type 2 diabetes (DM).

Statistical analysis

This is a case-control study where type 2 DM was considered the risk factor to which the patients were previously exposed.

The data obtained were centralized using the online Google Forms application and subsequently processed in a database using the Microsoft Excel 2010 spreadsheet program; the same program was used to draw the charts.

To compare the frequency of comorbidities in different age groups and sexes, I used Independent Samples T-test equations.

The correlation of the frailty score in lot 1 and in lot 2 with the duration of diabetes and the degree of diabetes management was made using the Pearson correlation equation.

RESULTS

The study followed a total of 120 subjects with an equal number of patients for each age group. In each age group, the gender distribution was also equal. In the adult group, most of the participants come from urban areas and have a medium or higher education level. In comparison, most elderly adults come from rural areas and have a predominant level of primary school education (Tab. I).

Tab. I Socio-cultural study groups' characteristics.

		Adults (50-64 years) N=60	Older adults (≥ 75 years) N=60
		%/Mean (SD)	%/Mean (SD)
Age		61,83 (2.46)	78,13 (3.02)
Education	Primary school	12,33	48,33
	High school	46,66	33,33
	University	41	18,33
Settlement	Urban	81,66	78,33
	Rural	18,34	21,66

The average developmental duration of diabetes is 12.43 years among adult men and 13.43 among adult women, while in the elderly it is higher, as expected, 15.9 years in elderly men and 17 years in elderly women. Comparing the mean value of the maximum blood glucose in lot 1, men versus women, there a no statistically significant differences ($p > 0.05$). However, comparing these values in lot 2, statistically significant differences ($p < 0.05$) are obtained. By comparing the mean value of the maximum blood glucose between the two lots, a small statistically significant difference is obtained (Tab. II).

The study also evaluated the presence of other geriatric syndromes, such as cognitive impairment, urinary incontinence, falls and malnutrition as shown in Tab. II. In this assessment, a clear incidence of syndromes among women in both age groups can be noticed.

Tab. II Patients' characteristics in adults and older adults groups

Characteristics			Adults (50-64 years)	Old adults (≥75 years)	p value
Mean duration of DM (years)	Male		12.43	15.9	
	Female		13.43	17	
Patient distribution according to the duration of DM evolution N (%)	<1 year	Male	1 (1.66)	0	
		Female	1 (1.66)	0	
	1-5 years	Male	10 (16.66)	8 (13.33)	
		Female	10 (16.66)	3 (5)	
	5-10 years	Male	11 (18.33)	8 (13.33)	
		Female	9 (15)	6 (10)	
	10-19 years	Male	7 (11.66)	11 (18.33)	
		Female	10 (16.66)	15 (25)	
	> 20 years	Male	1 (1.66)	3 (5)	
		Female	0 (0)	6 (10)	
Mean maximum blood sugar level (mg/dl)	Male		214.2	210.1	<0.05
	Female		234.64	285.67	
Last recorded mean blood sugar level (mg/dl)	Male		129.6	135.86	
	Female		140.26	149.73	

Patient distribution according to type of treatment for DM N (%)	Diet	Male	7 (11.66)	1 (1.66)	<0.05
		Female	5 (8.33)	5 (8.33)	
	OAD	Male	19 (31.66)	21 (35)	
		Female	22 (36.66)	18 (30)	
	Insulin	Male	4 (6.6)	8 (13.33)	
		Female	3 (5)	7 (11.66)	
Geriatric syndromes N (%)	Cognitive impairment*	Male	0	9 (15)	<0.05
		Female	0	11 (18.33)	
	Depression*	Male	1 (1.66)	11 (18.33)	
		Female	16 (26.66)	15 (21.6)	
	Urinary incontinence	Male	4 (6.66)	14 (23.33)	
		Female	10 (16.66)	12 (20)	
	Falls	Male	2 (3.33)	4 (6.66)	
		Female	3 (5)	5 (8.33)	
	Malnutrition*	Male	0	0	
		Female	0	0	

* cognitive impairment - MMSE cut off score of 24 [10]; depression – GDS cut off score of 6 [11]; Malnutrition – MNA cut off score of 11[12]

Fig. 1 highlights the greater distribution of comorbidities among elderly patients, as expected. Among the most frequent comorbidities is high blood pressure both in lot 1 (where it is found in 31.67% of men, compared to 40% of women) and in lot 2 (50% among man and 43.3% among women).

In lot 1, the prevalence of most comorbidities among subjects is higher in women than in men (Myocardial infarction, kidney diseases and heart failure are prevalent among adult women, 26.67%, 18.3% and 13.3% compared to men, with prevalence of 6.6%, 3.3% and 5%).

In the lot of elderly adults, heart failure, angina pectoris, asthma and COPD have higher prevalence among men, appearing in 25%, 20%, 16.66% and 6.66% of patients respectively compared to the women in the same group, affected in proportion of 11.66%, 16.66%, 8.33% and 3.33%, respectively.

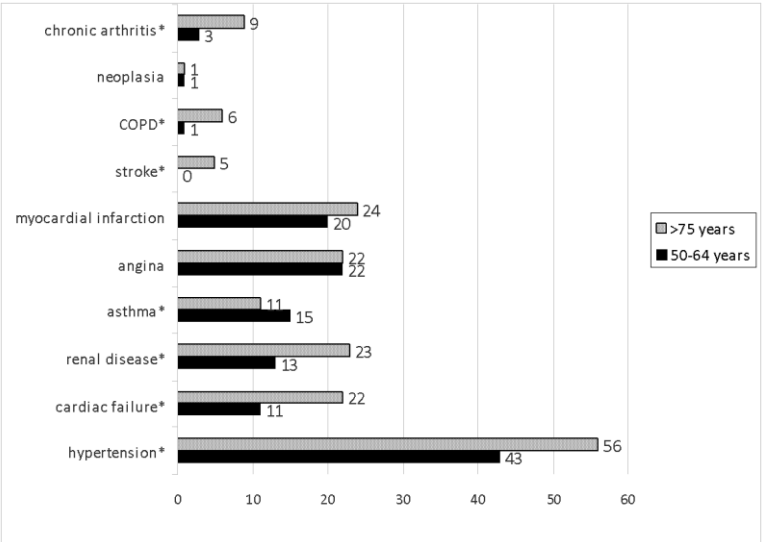

Fig. 1 Distribution of comorbidities in adult and older adult groups; * = T- Test $p < 0,05$ (CI 95%).

Of those aged 50-64, 25.83% are non-frail, 19.16% are pre-frail and only 5% are frail. Of those>/= 74 years, only 3.3% are non-frail, 23.3% are pre – frail, and 23.3% are frail. It can be noticed that in lot 1, that of the adults, non-frail and pre-frail patients predominate compared to group 2, that of the elderly, with predominantly pre - frail and frail patients. There is a high statistically significant difference between the two lots (p <0.01).

The distribution of the patients in lot 1 and lot 2, depending on the sex, and the result obtained after applying the Frail Scale questionnaire are shown in Tab. III. There is a statistically significant difference (p <0.01) in the proportion of frailty and pre-frailty among women compared to the men in the adult category. In the older adults category, there is a statistically significant (p <0.05) proportion of frailty among women compared to men.

Tab. III Patient distribution between the studied groups regarding frailty phenotypes

Clinical phenotypes of frailty	Adults (50-64 years)		Old adults (≥75 years)	
	Male	Female	Male	Female
Frail % (N)	0	10 (6)	18,3 (11)	28,3 (17)
Pre-Frail %(N)	11,66 (7)	28,3 (16)	26,66 (16)	20 (12)
Robust %(N)	36,66 (22)	15 (9)	5 (3)	1,66 (1)

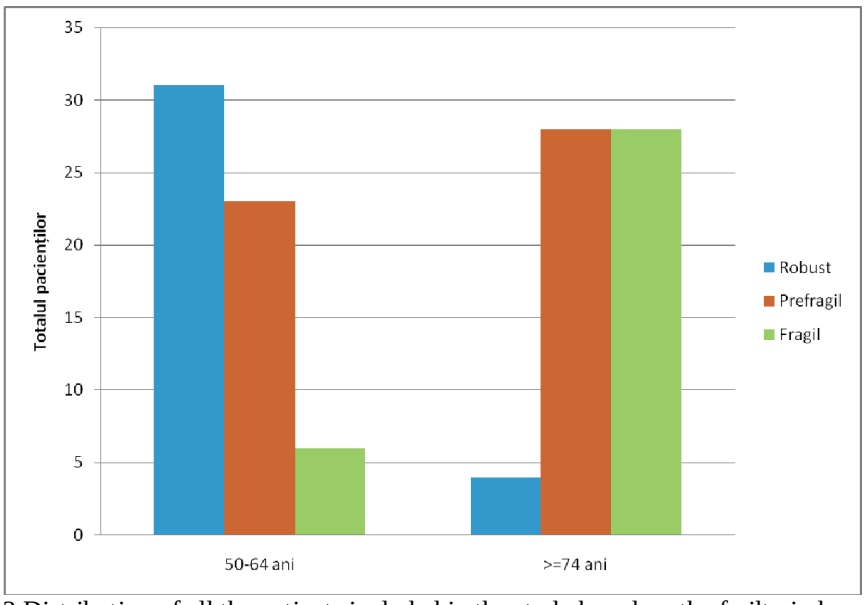

Fig. 2 Distribution of all the patients included in the study based on the frailty index score

A significant positive correlation was found between the frail score and the duration of diabetes and the level of glycemic control in groups I and II (Tab. IV).

Thus the duration of exposure to diabetes mellitus can be a risk factor for increasing the incidence of fragility syndrome. High and poorly controlled blood glucose levels increase the risk of developing co-morbidities and associating frailty syndrome with a higher degree of gravity (by the presence of a greater number of symptoms that it is defined by).

Tab. IV Correlation between Frail test scores and duration of exposure to risk factor (DM), glycemic values, glycosylated hemoglobin values

Variables	Frail scale	
	Adults (50-64 years)	Old adults (≥75 years)
Duration of diabetes (years) Pearson's correlation p	 0.816 <0.001	 0.865 <0.001
Fasting blood glucose (mg/dl) Pearson's correlation p	 0.851 <0.001	 0.918 <0.001
HbA1c (%) Pearson's correlation P	 0.858 <0,001	 0.897 <0,001

DISCUSSIONS

The results of this study demonstrate the impact that diabetes mellitus has on frailty syndrome among elderly patients compared to adults.

The frailty component is significantly higher among elderly and affects more often women in both age groups. The difference in incidence of frailty syndrome between men and women decreases with age.

There are no frail men in the adult group, compared with women that are represented by 10% in the same group. Therefore, we can conclude that women are more affected at a younger age by frailty then men.

The first positive symptom in the 'frail scale' evaluation reported by the adult women was the inability to climb 10 steps without difficulty, followed by walking. Among men, the main symptoms reported were fatigue, difficulty climbing 10 steps and involuntary weight loss, all representing 1.67% of symptoms.

Elderly women reported as the primary symptom fatigue (26.67%), followed by difficulty in climbing 10 steps (6.67%). Elderly men report in equal measure the presence of fatigue and difficulty in climbing 10 steps as the primary symptoms, accounting to 18.3%.

Multiple co-morbidities were encountered in 16.67% of elderly men, the majority of the group being represented by frail and pre-frail. Compared with the adult group, the frail elderly have a higher prevalence of co-morbidities, which were not present in the non-elderly group, such as stroke, cardiac failure, COPD. The symptoms encountered in the elderly frail women group were in decreasing order high blood pressure, angina pectoris, cardiac failure and arthritis.

We can conclude that the unfavorable evolution of the degree of frailty is correlated with a higher number of symptoms.

The results of this study highlight a poorer metabolic control in older diabetic patients compared to younger ages. Diabetic comorbidities were also more prevalent in older age.

The elderly group is significantly associated statistically with a longer exposure period to the risk factor – diabetes mellitus, but also to average blood sugar levels with higher HbA1c indicating poor glycemic control and by association a worse evolution toward complications.

CONCLUSIONS

The elderly group can also be significantly associated statistically with a higher number of events and symptoms that define the frailty syndrome. The prevalence of frailty syndrome is higher among elderly, compared with adults, and women are more affected by this syndrome than men, regardless of age.

We consider that the study's limitations are the small number of sample (n=120) and their selection from a single institution.

Conflicts of interest

The authors declare no conflicts of interest.

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GLYCOXIDATIVE STRESS IN AGING AND PATHOLOGY

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Abstract. The glycoxidative stress is involved in aging and age-related pathologies. The advanced glycation end products (AGEs) and the activation of AGEs – receptor for AGEs (RAGE) axis may lead to functional decline in aging and to the onset and development of age-related diseases: metabolic, cardiovascular and neurodegenerative diseases. This minireview presents the AGEs involvement in aging and pathology, their mechanisms of action as well as the potential therapeutic strategies to counteract their deleterious effects. Systemic AGEs levels, biomarker of glycoxidative stress, could become a marker of overall health status. Inhibition of AGEs generation, AGEs crosslink formation and AGEs-RAGE axis activation could represent useful approaches in development of alternative, complementary or novel therapies in healthy aging and active longevity.
Key words: advanced glycation end products, receptor for advanced glycation end products, aging

STRESUL GLICOXIDATIV ÎN ÎMBĂTRÂNIRE ŞI PATOLOGIE

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Rezumat. Stresul glicoxidativ este implicat în îmbătrânire şi patologiile dependenţe de vârstă. Produsii finali de glicare avansată (AGEs) şi activarea axei AGEs- receptorul pentru AGEs (RAGE) pot conduce la declinul funcţional în îmbătrânire şi la apariţia şi dezvoltarea bolilor dependenţe de vârstă: metabolice, cardiovasculare şi neurodegenerative. Acest minireview prezintă implicarea AGEs în îmbătrânire şi patologie, mecanismele lor de acţiune precum şi potenţialele strategii terapeutice de a contracara efectele lor dăunătoare. Nivelele sistemice ale AGEs, biomarker al stresului glicoxidativ, ar putea deveni un marker al statusului de sănătate total. Inhibarea generării AGEs, a formării legăturilor încrucişate ale AGEs şi a activării axei AGEs-RAGE ar putea reprezenta cai utile în dezvoltarea terapiilor alternative, complementare sau noi în îmbătrânirea sănătoasă şi longevitatea activă.
Cuvinte cheie: produşii finali de glicare avansată, receptorul produşilor finali de glicare avansată, îmbătrânire

INTRODUCTION

The glycoxidative stress plays a key role in aging and age-associated or age-related pathologies, acting together with oxidative and nitrosative stress. The major consequences of glycoxidative stress include damage to proteins, lipids and DNA, dysfunction of cellular homeostasis and accumulation of damaged molecules. The end products of glycoxidation processes, named advanced glycation end products (AGEs) are age-accumulated, being also involved in the onset and progression of various age-associated diseases, such as: metabolic, cardiovascular or neurodegenerative diseases [1-5].

It has been documented that AGEs may have negative effects on vessels by modifying collagen and other proteins from vascular wall, rising vascular rigidity, thus contributing to the onset and progression of cardiovascular diseases. Also, AGEs may modify lipids and proteins from lipoproteins, especially low density lipoproteins (LDL), enhancing LDL capture in subendothelial compartments [6].

Experimental and clinical studies have underlined the AGEs involvement in oxidative stress enhancing, endothelial dysfunction, cardiovascular and degenerative diseases and even in rising the cardiovascular mortality risk [7, 8].

The specific consequences of glycoxidative stress and AGEs accumulation are direct related to the signal transduction receptor specific for glycoxidation compounds named advanced glycation end products receptor (RAGE). These consequences involve the activation and upregulation of RAGE and RAGE-dependent cellular signal pathways, the sustaining and intensification of pro-inflammatory, pro-trombotic and atherosclerotic processes, the enhancement of oxidative, metabolic and nitrosative stress, cellular stress maintaining, cellular and tisular dysfunctions, damages and modifications specifically to human aging, especially to vascular aging [3, 4, 8].

The AGEs-RAGE interraction contributes to free radicals generation, pro-inflammatory cytokines release, modifications of extracellular matrix or hormons action. Numerous studies have underlined that the inhibition of AGEs generation, AGEs tissular accumulation and of glycoxidative stress may conduct to the elimination or amelioration of their damaging effects [6, 9].

By binding to the specific transmembranar receptor RAGE, the AGEs are involved in various pro-oxidant or pro-inflammatory pathways activated by the nuclear factor NF-kB. The AGEs-RAGE axis contributes to cellular signaling either on NF-kB pathway, or by activation of extracellular signal-regulated kinases (ERK), named ERK1 and ERK2 [3, 6, 9].

The inhibition of AGEs production and AGEs-RAGE axis activation has been shown to prevent the pathogenesis of age-associated diseases in human. The therapeutic advances in this field have resulted in several agents that may prevent the adverse effects of glycoxidative stress [6, 9].

This minireview presents the AGEs involvement in aging and pathology, their mechanisms of action as well as the potential therapeutic strategies to counteract their deleterious effects.

Clinical studies on glycoxidative stress, aging and age-related pathology

The accumulation of AGEs and their crosslinks with long-lived proteins during aging may contribute to the age-related decline of the functioning of cells, tissues and organs in normal aging. Also, AGEs may activate RAGE signaling pathways leading to enhancement of oxidative stress and inflammation conducting to endothelial and vascular dysfunctions. On the other hand the oxidative stress could enhance glycoxidative stress by rising AGEs production. The important studied

biomarkers of glycoxidative stress are represented by: N-ε-carboxy-ethyl-lysine (CEL), N-ε-carboxy-methyl-lysine (CML), imidazolone, methyl-glyoxal-lysine dimer (MOLD), glyoxal-lysine dimer (GOLD), pyrraline, glucosepane and pentosidine, or fluorescent AGEs [2, 3, 6, 9].

Recent clinical studies, presented in table I, have underlined the associations of AGEs with oxidative stress, inflammation, cardiovascular risk and even cardiovascular mortality, in healthy elderly [1, 2, 5, 7, 10, 11-13]. Moreover, AGEs levels were related to cognitive decline in elderly with and without age-associated diseases [14].

Many clinical studies (Tab. I) have also linked AGEs to cardiovascular disease (CVD), particularly in elderly patients with impaired glucose metabolism or metabolic syndrome [11, 12, 14-18]. Thus, AGEs levels were associated with metabolic, endothelial, oxidative stress or cardiovascular disease markers in elderly with IFG, T2DM and metabolic syndrome. These interrelations were also underlined in our recent researches (Tab. I). We pointed out significant positive correlations between systemic AGEs levels with oxidized low density lipoproteins (oxLDL), nitric oxide metabolic pathways products (NOx), insulin resistance (IR), interleukin-6 (IL-6), atherogenic and CVD risk markers in elderly with impaired glucose metabolism and metabolic syndrome [15, 16, 18].

The AGEs-RAGE axis activation leads to initiation of intracellular signaling, production of proinflammatory cytokines and free radicals. These processes may be limited by action of soluble RAGE (sRAGE), which counteracts the deleterious effects of glycoxidative stress by binding AGEs [3, 6, 9]. Our studies pointed out elevated levels of AGEs and AGEs/sRAGE ratio in elderly with T2DM and their relationships with metabolic, oxidative and cardiovascular markers (table I). Thus, our studies underlined that the AGEs/sRAGE ratio may be a more reliable marker of cumulative effects of AGEs, sRAGE and AGEs-RAGE axis [18]. All these studies underline the fact that AGEs levels, biomarker of glycoxidative stress could become a marker of overall health. Future researches are needed to well establish this fact.

Tab. I Clinical studies underlining the glycoxidative stress associations with oxidative, inflammatory and cardiovascular disease markers in healthy aging and age-associated diseases

Subjects	**Glycoxidative stress associations**	**References**
Healthy elderly	AGEs accumulate with age, AGEs ≈ OS and inflammatory markers (CRP)	Uribarri et al. 2005, 2007 [1,2]
Healthy adults and elderly	AGEs ≈ > OS, IR, inflammation in aging	Uribarri et al. 2007 [2] Meigs et al. 2007 [10]
Healthy elderly	CML ≈ CV mortality	Semba et al. 2009 [5]
Women with and without diabetes	↑ AGEs predict CV and coronary mortality	Kilhovd et al. 2005, 2007 [11]
Disabled older women	CML ≈ CV mortality	Semba et al. 2009a [12]
Older adults	CML ≈ increased risk of CV events; CML ≈ age, albuminuria, BP, IR	Kiser et al. 2014 [13]
Young, adults and elderly	Cardiac and systemic CML ≈ age, DM, CHD	Hu et al. 2014 [7]
Elderly with and without T2DM	↑ AGEs ≈ ↑ cognitive decline	Yaffe et al. 2011 [14]
Elderly with IFG and T2DM	↑ AGEs; AGEs ≈ FG, HbA1c, IR, oxLDL, NOx, CVD markers	Gradinaru et al. 2013 [15]

Elderly with metabolic syndrome	↑ AGEs; AGEs ≈ adiponectin	Gradinaru et al. 2016 [16]
Adults and elderly	AGEs ≈ endothelial dysfunction (FMD, NIV)	Kajikava et al. 2015 [17]
Elderly with T2DM	↑ AGEs; ↑ AGEs/sRAGE AGEs/sRAGE ≈ FG, Ai, IL-6, CVD markers	Borsa et al. 2017 [18]

≈, associated with
↑, higher values
AGEs, advanced glycation end products; Ai, atherogenic index; BP, blood pressure; CHD, coronary heart disease; CML, carboxy-methyl-lysine; CRP, C-reactive protein; CV, cardiovascular; CVD, cardiovascular disease; DM, diabetes mellitus; FG, fasting glucose; FMD, flow-mediated vasodilation, HbA1c, hemoglobin A1c; IL-6, interleukin -6; IR, insulin resistance; oxLDL, oxidized low density lipoproteins; NIV, nitroglycerine-induced vasodilation, NOx, nitric oxide metabolic pathways products; OS, oxidative stress; sRAGE, soluble receptor for advanced glycation end products

Molecular mechanisms of glycoxidative stress in aging and pathology

The glycoxidative stress activates important signalling pathways leading to enhance oxidative stress and inflammation. The cellular AGEs generation and accumulation are age-related and AGEs levels represent a reliable biomarker of "in vivo" aging. Two pathways are involved in AGEs contribution to aging and age-related disorders: AGEs receptor-independent pathway and AGEs receptor-dependent pathway. The AGEs receptor-independent pathway consists in accumulation of AGEs and AGEs crosslinks with long-live proteins, like is: haemoglobin, albumin and collagen; altering their structure, conformation, properties and reactivity. The AGEs receptor-dependent pathway activates signalling pathways for inducing oxidative stress and inflammation [2, 3, 8].

The AGEs-RAGE axis activation may increase ROS production and oxidative stress by two pathways: activation of mitochondrial electron transport chain and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. Also, the AGEs-RAGE axis activation and downstream signalling lead to induction of inflammatory mediators, such as: tumor necrosis factor-alpha (TNF-a), interleukin-1 (IL-1), interleukin-6 (IL-6), C-reactive protein (CRP); as well as activation of genes for inducible nitric oxide synthase (iNOS). The AGEs-RAGE axis contributes to cellular signaling either on NF-kB pathway, or by activation of extracellular signal-regulated kinases (ERK), named ERK1 and ERK2. AGEs- and RAGE-mediated ROS production and enhanced oxidative stress are involved in endothelial dysfunction, vascular stiffness, metabolic, cardiovascular and neurodegenerative diseases [3, 6, 9].

The RAGE-dependent signalling may be counteracted by advanced glycation receptor 1 (AGER 1) and soluble forms of RAGE, which bind AGEs, inactivate AGEs-RAGE axis, diminish cellular signalling related to oxidative stress, inflammation and RAGE-mediated pathogenesis [6].

Strategies targeting glycoxidative stress – related damages

Strategies for prevention and treatment of human AGEs-related disorders have been conducted on three directions:

1. inhibition of AGEs generation
2. degradation of AGEs and AGEs crosslinks with proteins
3. competitive inhibition of AGEs-RAGE axis activation

Experimental and clinical studies have underlined many agents that may counteract the damages induced by glycoxidative stress. These agents are classified as inhibitors of AGEs generation, breakers of AGEs crosslinks or antagonists of AGEs-RAGE signaling [4, 6, 9]. Some

representative agents, their actions and biological effects are presented in Tab. II.

The inhibitors of AGEs formation act either by targeting different inducers (ROS, metal ions) or intermediate products, especially reactive carbonyl species (RCS). In this category are included RCS quenchers, metal ion chelators, antioxidants or xenobiotics [6, 19-25].

The AGEs crosslings breakers have also the potential to effectively reduce AGEs products and act to lower the cardiovascular complication associated with aging and age-related diseases. Alagebrium and related compounds including: ALT-711, ALT-462, ALT-486, ALT-946 act as breakers of AGEs crosslinks with proteins [26-29].

RAGE activation and RAGE-mediated signal transduction are involved in aging and pathogenic mechanisms of age-related diseases. The AGEs-RAGE axis represents an important drug target and inhibition of axis activation exerts beneficial effects in various pathologies [6, 9].

The molecular strategies for inhibition the RAGE activation [30] consist in:

- inactivation of RAGE ligands by RAGE soluble forms
- inactivation of RAGE by antagonists or antibodies
- down-regulation of RAGE expression
- inhibition of RAGE signal transduction

Suppression of RAGE activation could be useful in preventing and slowing aging and age-related pathologies. Potential compounds for RAGE inhibition include:

- inhibitors of RAGE expression
- RAGE antagonists
- blockers of RAGE intracellular signaling
- enhancers of sRAGE and esRAGE production
- sRAGE supplementation

The antagonists of AGEs-RAGE signaling are small molecules that act by blocking RAGE receptor activation, RAGE dependent molecular processes and inhibition of RAGE ligand- triggered signal transduction. Recently, clinical trials studies have tested RAGE antagonists which may block the binding of ligand to extracellular domain of RAGE, such as azeliragone or compound FPS-ZM127. Also, soluble RAGE-type molecules are under study [31].

Recent studies have underlined the important role of exogenous-derived AGEs in rising glycoxidative stress. Thus, the restriction of dietary AGEs may lead to a fall in systemic AGEs and a decrease in biomarkers of oxidative stress, inflammation, endothelial dysfunction and insulin resistance in aging and age-related pathologies [6].

Tab. II Therapeutic agents for glycoxidative stress-related pathology

Compounds	**Actions**	**Effects**	**References**
AGEs inhibitors			
amonoguanidine	traps aldehyde group and reactive carbonyl intermediates	reduces nephropathy and retinopathy	Brownlee et al. 1994 [19]
piridoxamine	traps aldehyde group and reactive carbonyl intermediates, scavenges ROS, inhibits post-Amadori stages of AGEs formation	reduces diabetes and non-diabetes nephropathy and retinopathy	Degehhardt et al. 2002 [20] Stitt et al. 2002 [21] Voziyan et al. 2005 [25]
benfotiamine	reduces AGEs content in renal cells; reduces AGEs effects on endothelial functions	improves microalbuminuria; improves endothelial functions	Babaci-Jadidi et al. 2003 [23] Stirban et al. 2006 [6]

valsartan	reduces plasma and urinary pentosidine and plasma CML, scavenges ROS, chelation of transition metals	antihypertensive, antioxidant and anti- glycoxidation effects	Monacelli et al. 2006 [24]
polyphenols	chelation of transition metals, antioxidant activity	antioxidant effects, reduce albumin glycoxidation	Sadowska-Bartosz et al. 2014 [25]
AGEs breakers			
ALT-711 (dimethyl-3-phenyl-thiazolium chloride; N-phenocylthiazolium; N-phenocyl-4,5-dimethyl-thiazolium	breaks AGEs crosslinks via thiazolium structure	reduces cardiovascular complications in aging and diabetes; rises skin hydration; improves arterial compliance and cardiac function in elderly	Vasan et al. 2003 [26] Bakris et al. 2004 27] Monnier et al. 2006 [28] Little et al. 2005 [29]
Antagonists of AGEs-RAGE signalling			
Small molecules inhibitors of RAGE signalling	bind to ctRAGE; interfere to ctRAGE-DIAPH1 complex	inhibition of ctRAGE interaction with DIAPH1; inhibition of RAGE dependent molecular processes; inhibition of RAGE ligand- triggered signal transduction	Manigrasso et al. 2016 [31]

AGEs, advanced glycation end products; CML, carboxy-methyl-lysine; ROS, reactive oxygen species; RAGE, receptor for advanced glycation end products, ctRAGE, RAGE cytoplasmic tail; DIAPH1, mammalian diaphanous 1

CONCLUSIONS

Glycoxidative stress affecting all long-lived proteins and cell types leads to enhancement of oxidative stress, inflammation, endothelial and vascular dysfunctions.

Systemic AGEs levels, biomarker of glycoxidative stress, are associated with age and age-related diseases and could become a marker of overall health status.

Receptor-independent and RAGE-dependent mechanisms mediates the AGEs biological effects, especially ROS generation and stimulation of inflammatory pathways.

Inhibition of AGEs generation, AGEs crosslink formation and AGEs-RAGE axis activation could represent useful approaches in development of alternative, complementary or novel therapies in healthy aging and active longevity or age-related diseases.

Conflicts of interest

The authors declare no conflicts of interest.

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A PERSPECTIVE OF CARDIOVASCULAR EVENTS PREDICTION STRATEGIES

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Abstract. The incidence of cardiovascular events at elderly patients is very high. Therefore, it is necessary to detect the presence and severity of an acute heart condition as soon as possible. Prognostic evaluation is based on choosing the right risk markers, who must accomplish certain clinical features. Recent research has shown new emerging biomarkers, capable of providing significant information, which could be added to increase prediction ability. Thus, diagnostic and therapeutic strategies are highly needed. The latest studies have focused on finding a multimarker strategy which combines utility of several cardiac markers, employing different pathophysiological aspects. Present review aims to discuss new cardiac biomarkers and strategies that could facilitate not only risk stratification of short and long term adverse cardiovascular events appearance, but also a more accurate and proper diagnosis, prognostic and treatment.
Key words: cardiovascular diseases, biomarkers, multimarker strategy

STRESUL GLICOXIDATIV ÎN ÎMBĂTRÂNIRE ŞI PATOLOGIE

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Rezumat. La pacienţii vârstnici incidenţa evenimentelor cardiovasculare este foarte mare. Prin urmare este necesar să se detecteze cât mai curând posibil prezenţa şi severitatea unei afecţiuni cardiace acute. Analiza prognostică se bazează pe alegerea markerilor de risc adecvaţi, care trebuie să îndeplinească anumite caracteristici clinice. Cercetări recente au relevat noi biomarkeri capabili să asigure informaţii semnificative şi care ar putea fi adăugaţi pentru a spori capacitatea de predicţie. Astfel, sunt foarte necesare strategiile de diagnostic şi tratament. Ultimele studii s-au concentrat pe găsirea unei strategii multimarker, care să combine utilitatea mai multor markeri cardiaci, folosind diferite aspecte patofiziologice. Prezentul review urmăreşte să discute noi biomarkeri cardiaci şi strategii care ar putea facilita nu numai stratificarea riscului apariţiei evenimentelor cardiovasculare adverse pe termen scurt şi lung, ci şi un diagnostic, prognostic şi tratament mai precis şi adecvat.
Cuvinte cheie: boli cardiovasculare, biomarkeri, strategie multimarker

INTRODUCTION

Cardiac biomarkers are molecules linked with heart function and their detection could predict the presence and/or severity of cardiovascular diseases (CVD). The sooner they are detected an appropriate treatment can be initiated [1]. As we aged, the incidence of CVD is very high, so cardiac markers must accomplished certain clinical characteristics in order to be independent predictors. In screening and cardiovascular disease management there is an increasing interest in the use of new biomarkers [2].

To be good predictors, the new biomarkers must show a significant association with CVD events and bring new information into risk stratification when added to risk prediction models. Thus, new markers are

required, alone and/or in combination with other markers, to be used as prognostic indicators for future CVD events and in monitoring of treatments [1, 2].

POTENTIAL NEW BIOMARKERS - CLINICAL RELEVANCE

•Chitinase-3-like protein 1 (YKL-40), is a secreted glycoprotein by macrophages, chondrocytes, and some types of cancer cells. YKL-40 is regarded as an acute phase protein but in contrast to C-reactive protein (CRP), mainly produced by hepatocytes in response to high IL-6, YKL-40 is produced by macrophages and neutrophils in tissues with inflammation and by differentiated macrophages and activated neutrophils [3].

Studies suggest that serum YKL-40: could be a new biomarker of acute and chronic inflammation in patients with stable coronary artery disease; can reflect the overall burden of coronary atherosclerosis or may identify a high-risk phenotype of atherosclerosis; may be a useful marker for myocardial ischaemia, remodelling and probably prognostic.

Serum YKL-40, CRP, and natriuretic peptide-NT-proBNP were measured in 4265 patients with stable coronary artery disease included in the CLARICOR trial [4], and death was registered in a 6-years follow-up period. Study concluded that serum YKL-40 is a predictor of long-term mortality in patients with stable coronary artery disease, independent of common risk factors.

•Recently, copeptin, a 39 amino acid glycopeptide comprising the C-terminal portion of vasopressin, has been shown to be a stable and sensitive vasopressin-releasing marker analogous to insulin peptide C. Measurement of copeptin has proven useful in various diseases, including the diagnosis of insipid diabetes and monitoring of CVD [5]. Because it is not specific for a particular disease, it could be used as an adjunct to more specific biomarkers, increasing the accuracy of the diagnosis.

-Normal values in healthy volunteers range from 1.70-11.25 pmol/l.

-In advanced and acute heart failure, it varies between 20 and 45 pmol/l.

-In septic shock, haemorrhagic shock, ischemic stroke and acute myocardial infarction, increase to over 100 pmol/l.

-In insipid diabetes, hyponatremia and other conditions associated with decreased vasopressin, it decreases.

•Modified ischemia albumin (IMA) is a marker formed after the destruction of the N-terminal part of albumin under ischemic conditions. The IMA test should be interpreted in association with other tests for cardiac function such as troponin, myoglobin and EKG. IMA increases in 6h and remains elevated for 12 hours. IMA is useful in differentiating angina pectoris from myocardial ischaemia: if IMA is within normal limits and troponin and myoglobin are within normal limits and there are no changes in electrocardiogram, then chest pain is not due to heart attack [6]. The IMA test is important in the first few hours, so if the chest pain occurred a few hours ago, then the test is not useful because the IMA may have returned to normal levels.

•Placental growth factor (PLGF) is a multitasking cytokine capable of stimulating angiogenesis by direct or indirect mechanisms. Recently, an increasing number of clinical evidence suggests that PLGF alone or in combination with other biomarkers is a strong predictor of survival or cardiovascular events [7] in patients with stable and unstable coronary disease (PLGF alone and BNP alone have similar diagnostic precision for prediction of cardiovascular events, and the combined use of PLGF and BNP would have a higher diagnostic accuracy than each biomarker taken separately). However, some studies have not demonstrated that PLGF is independently associated with survival in patients with chronic heart failure or suspected acute myocardial infarction [7].

•Sphingolipids have recently been discovered to be not only plasma membrane components but also bioactive mediators that can induce different biological responses. Of these lipids, sphingomyelin (SM) and sphingosine-1-phosphate (Sph-1-P) are proposed to be involved in the pathogenesis of atherosclerosis and could become biomarkers useful for atherosclerotic disorders [8]. However, the measurement of Sph-1-P and SM levels has not been introduced into clinical practice because of the difficulty of accurate, rapid and convenient measurement of these sphingolipids. But it is true that the level of sphingolipids can accurately predict acute coronary syndrome and undoubtedly can act as future biomarkers to confirm atherosclerotic disorders as well as neurological disorders [8].

•Homocysteine is a factor involved in promoting thrombosis and inflammation, as well as vascular damage [9].

Numerous studies have shown that hyperhomocysteinemia is associated with:

- Increased cardiovascular disease - independent risk factor for both women and men;
- Increased venous thrombosis

A meta-analysis of 27 epidemiological studies indicated that a 5 μmol/l increase in homocysteinemia is associated with a risk of coronary disease similar to that induced by a 0.5 mmol/l increase in cholesterol [10]. Theories have been developed to justify the pathogenic role of homocysteine such as favouring platelet aggregation, development of endothelial lesions, alteration of fibrin affinity of lipids and lipoproteins, alterations in smooth muscle cell proliferation and increased production of reactive oxygen species, leading to increased stress oxidative. Normal levels should be less than 10 μmol/l.

•The A-plasma protein (PAPP-A) is a homodimer that is not covalently bound to the major eosinophilic basic protein who has proteolytic activity and is considered to play an important role in cardiovascular disease [11]. Recently, PAPP-A has been found to be a useful biomarker for cardiovascular dysfunction, inflammation and malnutrition in patients with chronic renal disease undergoing hemodialysis. High levels of PAPP-A are found in patients with unstable plaques, aggravated unstable angina and acute myocardial infarction. Very high plasma stability has given it clinical potential but currently there is no clinically standardized method of determination. Levels above 1.74 mIU/l are considered abnormal.

•Phospholipase A2 (Lp-PLA2) is an enzyme produced by inflammatory cells and is associated with atherogenic proteins. Nearly 80% is linked to LDL and 20% to HDL and VDRL. Lp-PLA2 is an inflammatory biomarker secreted by the atherosclerotic plaque and blood levels would predict the most recent cardiovascular events in patients with ischemic heart disease or heart failure. This association seems to be independent of traditional risk factors [12]. Lp-PLA2 is believed to play a dual role in atherosclerosis, with both proaterogenic and anti-inflammatory properties. It has also been demonstrated that levels of LpPLA2 are reduced by statins, suggesting that it could be a target for therapies, besides being a cardiovascular risk biomarker.

•Fatty acid binding protein (FABP) is a small cytosolic protein responsible for the transport and storage of fatty acids in the cell. Recently, H-FABP has been shown to be useful as an early marker of myocardial infarction and early detection of minor myocardial events such as unstable angina [13]. It is also a potential marker for cerebral lesions and stroke with high specificity and predictive positive value. Tomographic analysis has demonstrated the association between high levels of H-FABP and infarction, and preliminary studies have shown that FABP isoenzymes are more sensitive markers in brain injuries than currently used markers-S100 and NSE-neuronal specific enolase.

•ST2 is a member of the interleukin-1 receptor family, being released from myocytes under mechanical stress. Soluble concentrations of ST2 are associated with adverse cardiac events [14]. In addition to its potential as a biomarker for adverse cardiovascular events, ST2 is believed to play a causal role in chronic cardiovascular disease such as atherosclerosis and heart failure. Furthermore, the combination of NT-proBNP, ST2 and transthoracic echocardiography can be useful as a strong predictor of mortality in patients with dyspnea and for triage and risk stratification [15].

•Growth differentiation factor 15 (GDF-15) is a stress-sensitive cytokine that could be used as a biomarker of cardiac and vascular dysfunction. Elevated levels of GDF-15 have been found in patients at increased risk of cardiovascular disease, from stable coronary artery disease to acute coronary syndrome and heart failure. The association between GDF-15 and cardiovascular disease is independent of stable risk factors and biomarkers, including NT-proBNP and troponin. Prognostic information provided by GDF-15 in cardiovascular disease may be useful in identifying patients with acute coronary syndrome without ST segment elevation who benefit from an invasive strategy or monitoring response to treatment in heart failure [16].

•Recent studies provide important information on the clinical relevance of CD40L in patients with acute coronary syndrome [17]. If the troponin reflects the ability and tendency of the coronary thrombus to embolize with micronecrosis, then CD40L appears to reflect the prothrombotic inflammatory activity of the plaque with recurrent and platelet activation. Its increase indicates an increase in cardiac risk at 6 months following follow-up. Furthermore, in patients without myocardial necrosis, CD40L appears to identify a high risk subgroup, suggesting that its measurement could have additional benefits when combined with current myocardial infarction analyses [17]. As CD40L is elevated also in those with inflammatory diseases the question arises about the specificity of this marker.

•miRNA is involved in all biological processes, from cell differentiation to cell death and apoptosis [18]. Many types of miRNA can be detected in the blood, with remarkable stability, each with a specific pattern of expression. miRNA that regulates the cardiovascular system can be divided into 4 groups:

1. miRNA that regulates endothelial function and angiogenesis: miR126, miR17-92 cluster, miR130a, miR221, miR21
2. miR208a specific miRNA miRNA
3. miRNA for myocytes and striated muscles: miR1, miR133a, miR499
4. miRNA for smooth muscle: miR1443, miR145

miRNA is very promising, being a very specific marker and with high accuracy for heart failure [18]. Tian Jian et al. [19] conclude that studies strongly implicate certain miRNAs such as miR499 as useful biomarkers of a given heart disease.

MULTIMARKER APPROACH - PROGNOSTIC ABILITY

All of the above markers (and many others who have not been included in this review due to the large amount of references) could be capable of providing significant prognostic information [1, 2]. Of late years, studies are focused on a strategy that combines utility of all these cardiac markers. Because these are very different, each of them reflecting various pathological ways, a multimarker strategy needs to be considered [20-24]. Using this multimarker strategy, clinicians could stratificate the risk of long/short term adverse cardiovascular events appearance.

James et al. [20] showed in a substudy of GUSTO trial that a combination of NT-proBNP, troponin T and creatinine clearance, leads to a better risk stratification, than each of them separately.

Also, Sabatine et al. [21] in OPUS-TIMI 16 and TACTICS-TIMI 18, found too than simultaneously measurement of CRP, BNP and troponin brings important prognostic information.

Zhang et al. [22] examined the inflammatory plasma cytokines: CD40, cathepsin S, chemokine ligand 16, interleukin-10, PLGF, GDF-15, matrix metallopeptidase-9, 1 and CRP in 964 patients with mild to moderate lesions and evaluated their association with the risk of cardiovascular events occurring in a follow-up of 3 years. It was concluded that CD40L, cathepsin S, PLGF and GDF-15 were useful biomarkers for predicting cardiovascular disease.

Similarly, Schnabel et al. [23] investigated 12 biomarkers linked to inflammation, lipid metabolism, renal/cardiovascular function and remodelling: CRP, GDF-15, neopterin, apolipoprotein AI, B100, cystatin C, serum creatinine, copeptin, proadrenomedullin (MR-proADM), MR-proANP (mid-region-pro-natrial natriuretic peptide) and Nt-proBNP. The study concluded that Nt-proBNP, GDF-15, MR-proANP, cystatin C and MR-proADM are the strongest predictors of cardiovascular outcomes in patients with stable angina.

Conflicts of interest

The authors declare no conflicts of interest.

The Food and Drug Administration approved in 2010 a multimarker strategy for cardiac ischemia combining EKG, troponin I and IMA with a 95% sensitivity for acute coronary syndromes [24].

Therefore, these studies have shown that multimarker approach is much more useful to predict the evolution of cardiovascular disease than individual marker approach.

CONCLUSIONS

- The role of cardiac markers in diagnosis, risk stratification and treatment of patients with cardiovascular disease is central according to the new diagnostic and treatment guidelines;
- Further investigations will clarify which of these potential markers have clinical value;
- Longitudinal studies are required to confirm whether discriminatory markers maintain long-term prognostic ability;
- The multimarker strategy can help stratify the risk of adverse cardiovascular events occurring both in the short and long term.

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UNDERSTANDING RISK FACTORS AND PREVENTING CARDIOVASCULARY PATHOLOGY

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Abstract. Worldwide cardiovascular diseases are still the leading cause of death. Objectives: highlighting some cardiovascular risk factors and estimation of the cardiovascular risk for a lot of NIGG patients. Material and methods: The groups (study and control) consist of 60 and 33 subjects, respectively. Both are characterized by polypathology, but in the study lot, cardiovascular diseases are more numerous. The overall assessment was based on clinical data and medical-social tests for life style, physical functionality, nutritional status (BMI, MNA) and food preferences. Results: We enumerate some significant differences between the two groups regarding cardiovascular risk factors. In the study group we find • age factor-females over 65 years, and •family history of hypertension (un-modifiable determinants) which are higher than in the control group, with 22% and 19%, respectively. In the control group, modifiable determinants- the education level and the income, have weights higher with 10-12%, showing their favorable health effect. In the case of biological determinants, the percentages of risk factors are higher in the study group (e.g. hypertension and obesity, higher with 30 percents). But in the control group, the lifestyle determinants have more increased percents: there are three times more smokers and 5% of them consume more salty foods and more fats. The CV risk is estimated by the "Cardboard Risk Test" with four levels: very high, high, medium and low risk. In the study lot, the very high and high risks are each, more than 10 percent higher compared to their equivalents from the control group. Conclusions: the presence of CV risks fairly frequent in the control group highlights the need for active prevention measures among those considered "healthy".
Key words: prophylaxis, risk factor**,** lifestyle

CUNOAŞTEREA FACTORILOR DE RISC CARDIOVASCULAR ŞI PREVENŢIA PATOLOGIEI CARDIOVASCULARE

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Rezumat. În lume, bolile cardiovasculare (BCV) sunt prima cauză de deces. Obiective: evidenţierea unor factori de risc cardiovascular, pe un lot de pacienţi din INGG. Material şi metode: Subloturile (studiu şi control) sunt reprezentate de 60, respectiv 33 subiecţi. Ambele sunt caracterizate de polipatologie, dar în lotul de studiu, BCV sunt mai numeroase. Evaluarea globală s-a bazat pe date clinice şi teste medico-sociale pentru: stilul de viaţă, funcţionalitatea fizică, statusul nutriţional (BMI, MNA) şi preferinţele alimentare. Rezultate: Enumerăm câteva diferenţe semnificative între cele două subloturi, în privinţa factorilor de risc. În grupul de studiu găsim factorul •vârstă, la femeile peste 65 ani şi •istoricul familial de hipertensiune arterială (determinanţi nemodificabili), ambii având valori mai înalte decât în grupul de control, cu 22%, respectiv, 19%. În grupul de control, determinanţii modificabili: •nivelul educaţional şi •venitul, au ponderi mai mari cu 10-12%, sugerându-se efectul lor favorabil asupra sănătăţii. În cazul determinanţilor biologici, mărimile procentuale ale factorilor de risc sunt mai mari în grupul de studiu (e.g. hipertensiunea şi obezitatea, mai mari cu 30%). Dar în grupul de

control, determinanţii stilului de viaţă au ponderi mai înalte: există de trei ori mai mulţi •fumători şi 5% din grup consumă mai multă •sare şi mai multe •grăsimi. Riscul CV este estimat prin „Testul cartonaşelor", cu patru niveluri de risc: foarte înalt, înalt, mediu şi scăzut. În lotul de studiu, riscurile foarte înalt şi înalt, sunt fiecare cu 10 procente mai mari decât echivalenţele din lotul control. Concluzii: prezenţa riscurilor cardiovasculare, destul de frecvente în grupul de control, indică necesitatea unei prevenţii active, aplicată şi celor care se consideră „sănătoşi".
Cuvinte cheie: profilaxie, factor de risc, stil de viaţă

INTRODUCTION

In 2015, World Health Organization published a report which shows that cardiovascular disease (CVD) is still the leading cause of death in the world [1]. Over the past 30 years, there has been a significant decrease in CVD-related deaths in developed countries. More than half of the CV mortality decrease has been attributed to changes in risk factor levels in the population, primarily the reduction of cholesterol, blood pressure (BP) levels and smoking. This favourable trend is partly diminished by an increase in other risk factors, mainly obesity and type 2 diabetes. Aging of the population also increases CVD deaths [2].

In Europe, the high CV mortality rate is prevalent in the eastern countries, with a poor socio-economic situation. According to the latest American Heart Association statistics (2014), Romania ranks third in the world, after Ukraine and the Russian Federation, in terms of ischemic cardiopathy mortality and stroke [1]. And the World Bank's 2015 report shows that our country ranks 1st in Europe as mortality due to cardiovascular disease. (We have an average of 108.9 deaths per 100,000 inhabitants, compared with U.E.: 43.8 per 100,000 inhabitants). And in the mortality structure of our country, these diseases represent the first cause of death (57%) [3].

I. The concept of cardiovascular prophylaxis

In general, cardiovascular pathology, regardless of localization and etiopathogenic characteristics, is based on degenerative atherosclerotic changes. When talking about cardiovascular prophylaxis, the essential goal is to prevent or delay the onset of these lesions. Ideally, primary prophylaxis should prevent the development of cardiovascular disease. Starting from observational and interventional epidemiological studies, it was developed a unitary prophylactic concept on CV disease. The concept is based on the existence of determinant and favorable conditions in the occurrence of ischemic cardiopathy [4].

The risk is the likelihood that a person will be affected by certain pathology within a certain period of time. Individualization of the degree of risk is the first step of effective primary prophylaxis.

The risk factor is defined as the concept which includes •lifestyle elements, and also •physiological elements, •biochemical, and some •un-modifiable personal elements. These characteristics determine the significant increase in the frequency of a particular pathology. Typically, the same individual has several risk factors, each contributing to global risk.

The global risk is the result of the interaction of all risk factors but can be considerably higher than the sum of the effects of isolated risk factors. Currently, over 280 CV risk factors are known. Some are not directly involved in the pathogenesis of atherosclerosis, but cause specific lesions which subsequent are completed. For this reason, 6 risk factors (smoking, hypercholesterolemia, diabetes mellitus, age, gender, BP) were selected from all CV risk factors and they are known as major risk factors [4].

The absolute risk defines the likelihood of developing the condition (e.g. coronary ischemic disease - CID) over a certain period of time. Depending on the length of the period, the absolute risk can be: short-

term (maximum 10 years) or long-term (over 10 years).

Estimating cardiovascular risk requires a rapid assessment of the risk status in order to establish screening methods.

Quantification of cardiovascular risk consists in accurately assessing the risk status in order to establish therapeutic goals and assess the effectiveness of clinical management.

In 1994, the European Cardiology Society, the European Atherosclerosis Society and the European Society of Hypertension created the first European Guide, in order to outline CVD preventing means. Its purpose was to establish a consensus on the global risk assessment in the primary prevention of CID. The European Guide is revised every 4-5 years, the last publication being in 2016. Each meeting of expert societies brings new elements.

The 1998 version states that: "In CID, a multi-factorial etiology disease, it's important to •estimate the absolute risk for all healthy people, taking into account all risk factors".

The 2003 Guidelines extend the target of the prevention recommendations from coronary ischemic disease alone to all cardiovascular diseases. The Systematic Coronary Risk Assessment (SCORE) database is also modified for the CVD overall risk assessment. Also in 2003, • the concept of primary and secondary prevention is replaced by the recognition of an important fact: atherosclerosis is a continuous process, its onset being present even at younger ages.

In 2007, the new elements are:

• recommending to family doctors and cardiovascular nurses a greater involvement in CV prevention,

• emphasizing the importance of lifestyle counselling and

• reviewing CVD risk in young people [5].

The 2016 European Guidelines on CV prevention place greater emphasis on a population-based approach, on disease-specific interventions and on specific conditions of females, younger individuals and ethnic minorities.

In 2006, the European Heart Health Charter was launched, consisting in a public health statement supported by most EU member states. Among other recommendations, the Charter defines the profile of a healthy individual: "•non-smoker, •suitable physical activity- at least 30 minutes, five times a week, •healthy eating habits, •no overweight, •blood pressure below 140/90 mmHg, •serum cholesterol below 190 mg/dl, •normal glucose metabolism, •avoid excessive stress" [6].

Regarding prevention strategies, two distinct but complementary action approaches are delineated: the "population" strategy and the "high risk" individualized strategy - addressed to patients with known CVD or at high risk of developing CVD in the future [7]. Theoretically, most people could benefit through the high-risk strategy. But most of the deaths in a community come from the group of individuals with low levels of risk, because this group is more numerous than those of high-risk individuals. The latter, paradoxically, develop fewer events in absolute terms (the Rose paradox). For this reason, the strategy for high-risk individuals needs to be complemented by measures of the population strategy, i.e. measures to reduce risk factors and encourage a healthy lifestyle [1].

We need to know the importance of the specific risk factors to the population in our country, in order to control the alarming rise of cardiovascular disease. The objective is possible by conducting classical epidemiological studies to reflect the real profile of our population and a greater involvement of experts at national level.

SEPHAR studies (2005 and 2012) have shown a 40% prevalence of hypertension, 37% of obesity, 46% of dyslipidemia and 29% of smoking in the general adult population of Romania. Over 50% of newly diagnosed hypertensive did not

know their diagnosis at the time of screening, and of known hypertensive, only 39% were under treatment. The study highlighted an increased prevalence of cardiovascular risk factors in regions with low socio-economic levels. The cardiovascular risk estimated on the SCORE diagrams for the next 10 years was 3.5%, in the general population of Romania [1]. Another Romanian study is PREDATORR – The national study on the prevalence of diabetes, prediabetes, overweight, obesity, dyslipidemia, hyperuricemia and chronic kidney disease. Its results (published in 2014) show large figures of obesity (31.4%) and overweight (34, 6%) in line with the increase of type 2 diabetes, important risk factors for CVD [1].

II. Prevention through information, intervention and evaluation

1. Information

The guidelines of the European Cardiovascular Health Charter (2006) also regulate CV prophylaxis activities. Article 4 provides a classification of risk factors for informing the population and the medical world [6].

Risk factors associated with cardiovascular risk

A. General Determinants

• Unmodifiable: age, gender, genetic predisposition, ethnicity;

• Modifiable: income, education, living conditions, working conditions.

B. Biological determinants:

Hypertension, hyperglycemia, hyper-cholesterolaemia, overweight or obesity

C. Determinants of lifestyle

Smoking, unhealthy diet, alcohol abuse, physical inactivity

General Determinants: Unmodifiable risk factor "age"

In the USA, by the 1990s, the incidence of acute myocardial infarction in people under 45 was between 2% and 6%, of which 5-10% was women; after 1990 this incidence was between 4% and 10%. Also, in the last years in Romania, a significant increase in the number of diagnosed CVD cases was found in the population younger than 40 years, including even myocardial infarction [3].

According to studies, the causes of early onset of CVD are primarily smoking and family history of CVD, these also being the most common risk factors for young people with myocardial infarction. Additionally, dyslipidemia, abdominal obesity, hypertension and diabetes (the latter two most common in the elderly) must also be considered.

WHO data places young Romanians in the top places in the EU with regard to smoking: 50% of young people aged 15-16 years have started to smoke. And of these children, 23% of them claim they smoked the first cigarette at 13 or even earlier [3].

General Determinants: Unmodifiable risk factor "gender"

Ten or fifteen years ago, ischemic heart disease usually was occurring in women ten years later than in men. This gap was due to the protective role of female hormones until the age of menopause.

The proportion of women victims of infarction before 50 years increased from 3.7% in 1995 to 11.2% in 2005 (WHO data). Professor Bertrand, a cardiologist at CHU Lille, former chair of the European Cardiology Society, says that "the combination of contraceptives with smoking increases the cardiovascular risk by 20 times." These two risk factors add to the effects of obesity and stress [8].

In conclusion, the two CV risk factors, age and gender, considered causative risk factors, are unmodifiable, but do not act isolated. They are associated with: smoking, obesity, dyslipidemia and stress. For the latter two, there is a multitude of prophylactic intervention measures.

2. Intervention

Article 3 from Heart Charter states: "In cardiovascular disease, which is multifactorially determined, it is essential

that all risk factors to be treated both individually and socially throughout the community." WHO stresses that cardiovascular disease treatment is often limited to addressing an isolated risk factor rather than cardiovascular risk as a whole. However, most infarctions and strokes can be prevented if the treatment is targeted at all cardiovascular risk factors.
Article 2: The Charter recommends lifestyle-oriented interventions for considerably reducing the number of cardiovascular diseases. WHO estimates that: reduction in • blood pressure, • obesity, • smoking and • cholesterol would decrease the incidence of cardiovascular disease to over half.

3. Cardiovascular risk assessment
The Romanian Heart Foundation launched in May 2011 the Campaign: "SOS Cardio - Attention, cardiovascular risk!" The Campaign aims to inform the population about cardiovascular risk factors, but also to assess individual risks before and after the therapeutic and lifestyle interventions [9]. "Cardboard Testing" is a tool recommended for assessing risk factors and cardiovascular risk. A simple and accessible tool, it consists of studying ten images, representing ten risk factors: age, heredity, male gender, smoking, high cholesterol, obesity, sedentary, stress, high blood pressure, diabetes. The computer test records the responses and then calculates the overall cardiovascular risk of the respondent. For people who do not have internet access, there is a green phone line: 0-800-070-777, where a healthcare provider offers information about risk factors and helps the person who calls to perform the test.
The test was also used in our cardiovascular risk assessment.

Objectives
- highlighting population-specific cardiovascular risk factors;
- estimation of the global cardiovascular risk of the subjects in the two groups: study and control.

MATERIAL AND METHOD
The study group, consist mostly in patients from NIGG, but also from the Medical Clinic of the Coltea Hospital. It has 60 subjects (17 men and 43 women) with increased percents of cardiovascular disease, especially coronary heart disease. The control group has 33 subjects (7 men and 26 women) with reduced percent of coronary ischemic disease. Chronic polypathology is still increased, with higher weights of rheumatism, digestive diseases and respiratory diseases.
The overall health assessment was based on clinical data, diagnostics and paraclinical data, but also on tests included in a medical-social survey that assess:
• Physical functionality- adl (Index Barthel), iadl Lawton and Brody,
• The lifestyle assessment (smoking, alchohol consumption, stress),
• Food preferences,
• Nutritional status (BMI, MNA) [10],
• Cardiovascular risk assessment Cardboard Testing, from the Champagne ”SOS Cardio” of the Romanian Heart Foundation.

RESULTS AND DISCUSSIONS
I. The weights of the chronic diseases in the two lots
• There are percentage differences between chronic pathology from the two samples. So, in the study group some diseases are significantly more frequent compared to the control group, with the following percents: 25% - hypertension, 50% - coronary artery disease, 10% -diabetes melitus, 17.7%- obesity and with 10% - neurological diseases.
• Compared to the study group, in the control group, there are higher percentages for other conditions: rheumatic, digestive, respiratory diseases and also for neurotic syndromes.

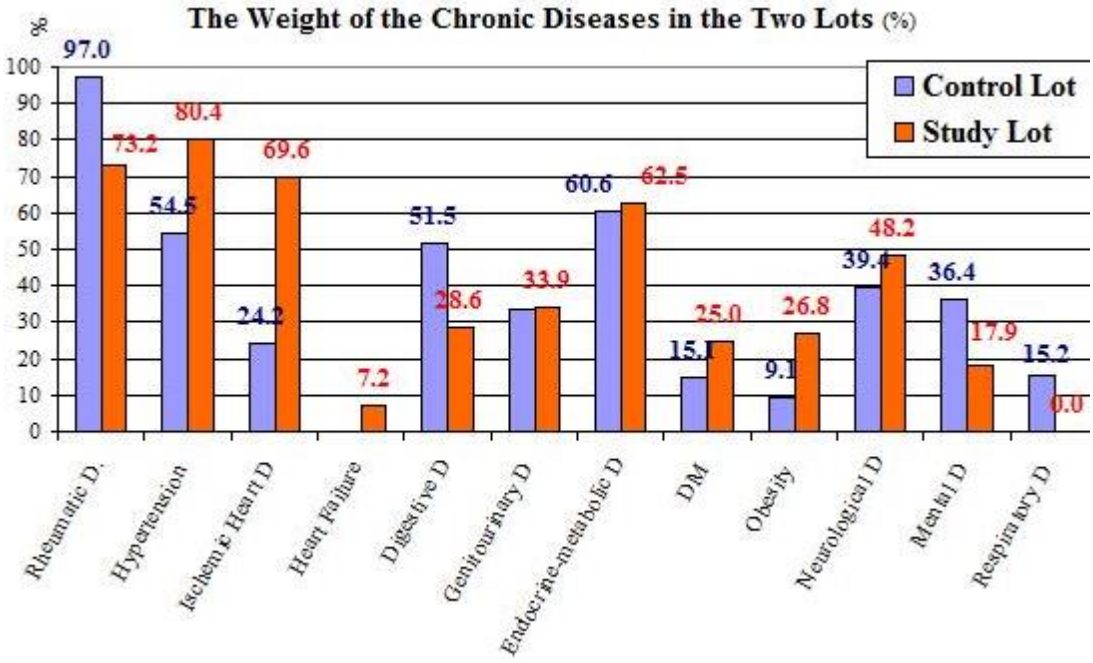

Fig.1The weights of chronic diseases in the study lot and the control lot (%)

II. Cardiovascular risk factors

The analysis data from our study emphasize cardiovascular risk factors. They are grouped after the classification indicated by the Cardiovascular Health Charter, as following:

(A1) General unmodifiable determinants

Tab. I Age and gender as risk factors

Study group (n=60)		Average age	**Control** group (n=33)		Average age
Males >55 years	= 90%	65.9 years	Males >55 years	100 %	68.9 years
Females >65 years	= 64.1%	68.5 years	Females >65 years	42.3%	63.5 years

Between genders, male gender is considered a risk factor. Also, as regarding the ages, males over 55 years old and females over 65 years are considered at risk of CVD.

Tab. II Heredo-collateral antecedents

Heredo-collateral antecedents of:	Study sample	Control sample
Hypertension	70.2%	51.5
Ischemic Heart Diseases	45.6%	**48.5**

Heredo-collateral antecedents are similar in severity for the control group as for the study group, as regarding coronary ischemic diseases. The proportion of antecedents of hypertension in the control group is less with a third as compared to the study group.

(A2) General modifiable determinants

Tab. III Education level in the two samples (%)

Education level	Study sample (%)	Control sample (%)
Primary courses (2-4 classes)	**17.7**	ns
Only gymnasium classes	**36**	36.4
Also high school	46.3	54.6

Tab.IV The income in the two samples (%)

The size of the income (pension mainly)	Study sample (%)	Control sample (%)
0 -349 lei	**10.9**	3,0 (ns)
350 – 999 lei	**56.4**	54,5
1000 – 4400 lei	32.7	42,4

Tab. III and IV, illustrating the education level and the income, show their favourable health effect: the control group, healthier regarding CVD, have the share of high school graduates by 8.3% higher than the study group; and those with larger pensions (1000-4400 lei) from the control group exceeds by 10% the share of those with similar income from the study sample.

B. Biological determinants

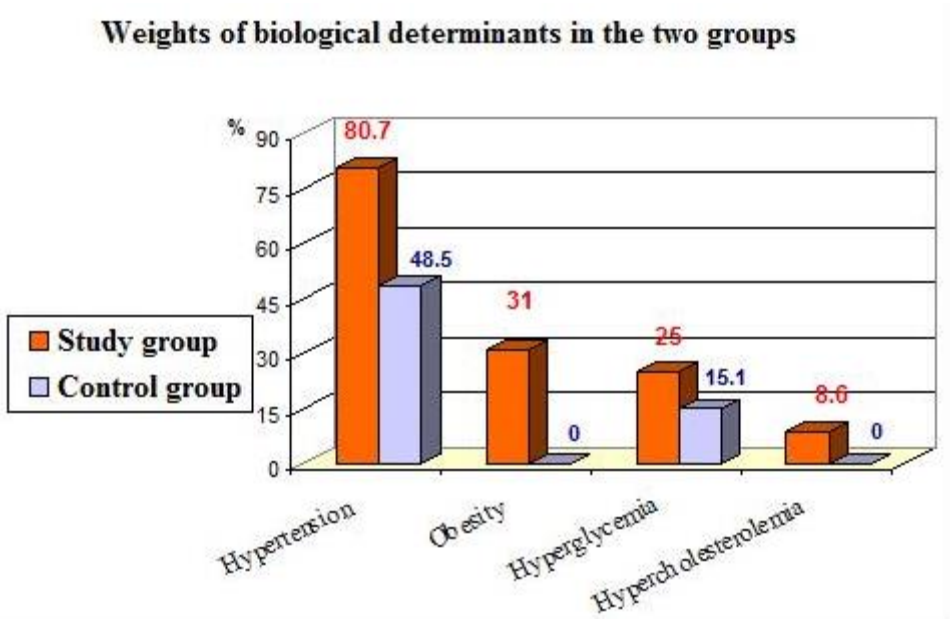

Fig. 2 Weights of biological determinants

The presence of CVD biological determinants is obviously higher in the study group than in the control group, with significant percentage differences: blood pressure occurs with 32.2% more frequent in the study group and hyperglycemia with 10 percentages higher. And obesity and hypercholesterolemia exist only in the study group.

C. Life style determinants

Tab. V Weights of unhealthy life habits (%)

Groups	Study Group	Control group
Smoking		
Smokers in the moment	8.6	21.2
Ex-smokers	17.2	12.1
Alcohol consumption		
Frequency: 4 or more times/week	8.8	9.1
Quantity: 2 or more units / day	12.3	ns
Unhealthy diets		
Excessively salty foods	31.5	36.4
Foods rich in cholesterol	36.1	39.4

By examining the figures from Tab. V, we can say that life style is just as damaging to health in both samples, or even more damaging, in the control sample. We can say this especially about smoking: in the control group there are almost 3 times more smokers than in the study sample. With regard to nutrition, 5% of the control group consumes more salty foods and 3.3% more fats, rich in cholesterol. Percentages of daily alcohol consumption are small, but almost equal in both groups. In fact, the situation described shows some compliance with the hygienic-dietary regimen prescribed to those with CVD: they left smoking and consumed somewhat less fat and salt.

Tab. VI illustrates the frequency of physical activities in the two groups. Those in the study group are less active, probably due to CV disease. In general, few subjects do gymnastics, 30 minutes 5 times a week (as recommended). Such gymnastics is surely performed by those who have garden and work in it. (The difference between the control and study group is high: 25.5%). Also, housekeeping and cooking are substantially reduced due to CV disease between the two groups (by 27.8% and 21% respectively).

Tab. VI Weights of exercise and physical activity (%)

Groups	Study group	Control Group
Physical exercise (sanogenetic scope)	37.5	42.4
Gardening	32.1	57.6
Walking	37.5	33.3
Housekeeping	66.1	93.9
Shopping	67.9	81.8
Doing laundry	69.6	87.9
Cooking	69.6	90.9

When defining the healthy individual, the Heart Health Charter also includes: avoiding excessive stress. In our studied groups we mention only the percentages: a) for the good mental state - 12.5% (the study group) and 21.21% (the control group); and for the b) satisfactory condition- 14.7% in the study group and 45.45% for the control group. In other words, the proportion of those with good mental health has close values in the two lots, whereas the satisfactory condition is more than 3 times higher in the control group than in the study one. The rest of the subjects, in both lots, have stress states of varying intensity (in the control group, percentages cannot be calculated due to the small number of people).

III. Assessment by calculation of cardiovascular risk

For the purpose of brief assessment individual CV risk assessment, the "Cardboard Risk Test" from the Campaign: "SOS Cardio" of the Romanian Heart Foundation was used.

▪ The 10 variables were mentioned in the study, separately in the two samples, for comparison.

Tab. VII The CV risk factors studied in our lots, also were included in "The Cardboard Testing"

General unmodifiable determinants	Biological determinants**:**	Life style determinants:
(1) age	(4) high cholesterol	(8) smoking
(2) male gender	(5) obesity	(9) sedentariness
(3) heredity	(6) blood hypertension	(10) stress
	(7) diabetes mellitus	

The test performs an estimation of the overall risk level. It allows for a repeated evaluation, before and after lifestyle changes and clinical and drug management. In our study lot, subjects go to the doctor preventively, not just for emergencies. The share of those concerned in prevention is higher in the study group (73.2%), compared to those in the control group (60.6%). In fact, among subjects with more severe heart disease (in the study group) this behaviour is the expression of compliance with recommended therapies. In other words, they are very worried about the severity of their health and respect a healthier lifestyle.

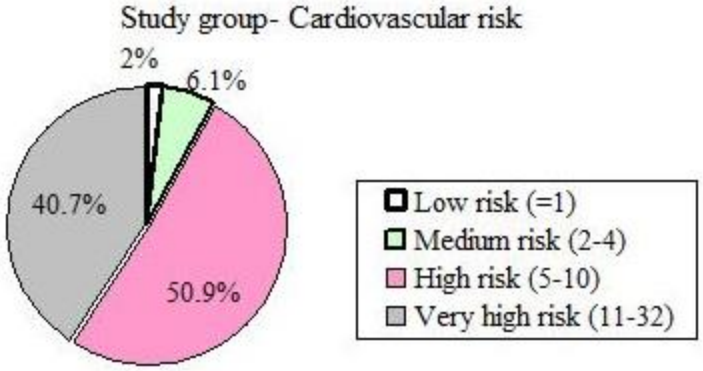

Fig. 3 Cardiovascular Risk Assessment – Study group

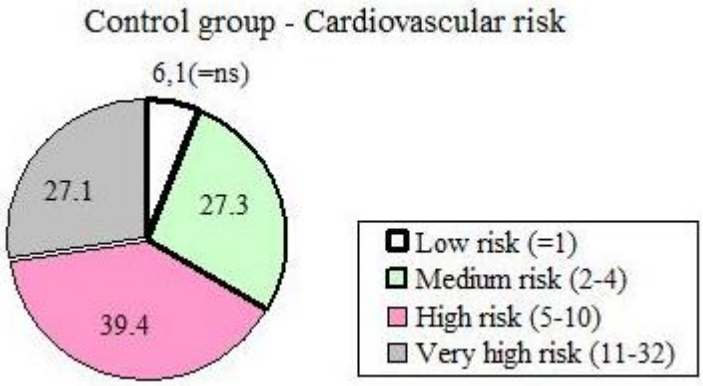

Fig. 4 Cardiovascular Risk Assessment – Control group

Tab. VIII Comparison between the CV risks in the two groups:

CV risk	Scores	Study group (%)	Control group (%)
Low (1)	1	2	6.1(ns)
Medium (2-4)	2-4	6.1	27.3
High (5-10)	5-10	**50.9**	39.4
Very high	11-32	**40.7**	27.1

By comparing the two graphs, we observe a higher CV risk in the study group (high and very high risks gathered means 91.7% of subjects). On the other hand, in the control group there are lower weights for high CV risk (66.5%).

Also, we see in the study group small weights of low and medium risks (summed up = 8.1%) compared to the higher values for these risks gathered, in the control sample (33.4%).

CONCLUSIONS

The study reveals a large share of CV risk factors both in the study and in the control group. More specifically, in the case of biological determinants, the weight of these factors is higher in the study group; but in the control group, the lifestyle determinants have a higher weight compared to those from the study lot.

The results of the CV risk estimated according to the test scores indicate four levels: very high, high, medium and low risk. In the study lot, the very high and high risks are each more than 10 percent higher than their equivalents from the control group.

However, the presence of fairly frequent CV risks in the control group highlights the need for active prevention measures among those considered "healthy". Without such type of preventive interventions, these subjects will develop coronary heart disease in the years to come.

"The benefits of a successful cardiovascular prophylactic activity: •awareness of cardiovascular risk factors; •visiting a doctor not only in emergency situations, but also for preventive purposes; •manifestation of preventive behaviour at all ages; •effective CV risk control can extend life expectancy by at least 10 years; •applying preventive measures would have the greatest impact and the costs, both the financial ones and the cost of suffering, could be much lower in society" [9].

Conflicts of interest

The authors declare no conflicts of interest.

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CLINICAL LABORATORY RESULTS EXPRESSED AS RATIOS

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Abstract. It is noticeable that there are many recent studies supporting the use of ratios instead of values of a stand-alone parameter. Elevated neutrophils per lymphocytes ratio (NLR), as a marker of the systemic immune response, has indicated poor prognosis in various cancer types, cancer treatment and cardiovascular diseases. For the late mentioned, NLR has prognostic values as regards short- and long-term mortality in patients with acute coronary syndromes, stable coronary artery disease, heart failure, coronary artery bypass and peripheral arterial disease treated with antiplatelet drugs. Neutrophilia and elevated NLR were associated with decreases in suppressive function of CD4 and CD25 regulatory T cells in acute phases of coronary syndromes. On other occasions, validated, well known ratios like the De Ritis ratio AST per ALT have been modified in order to develop scores with better performances. Among fibrosis risk scores, the AST: platelet ratio index APRI {AST/ULN) x 100}/platelet count (109/L) has been considered more reliable than the AST:ALT ratio. Nevertheless, APRI is used exclusively for selected populations with hepatitis virus C and extending its use to other diagnoses can lead to errors of diagnosis. The De Ritis ratio is now considered questionable as its calculation is requested for patients with transaminases already abnormal. As investigations by now used arbitrary threshold values for the neutrophils to lymphocytes ratio threshold values, future researches are needed to define its cutoffs.
Key words: neutrophils per lymphocytes ratio, AST per platelets ratio index, cardiovascular, cancer

REZULTATELE CLINICE DE LABORATOR EXPRIMATE CA RAPOARTE

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Rezumat. Este lesne de observat că numeroase studii recente sunt în sprijinul utilizării unor rapoarte între două valori numerice, în locul valorilor pentru un singur parametru. Raportul neutrofile per limfocite (NLR) crescut, ca marker al răspunsului inflamator sistemic, indică prognosticul (negativ) în diverse tipuri de cancer, tratamentul cancerului şi boala cardiovasculară. În patologia cardiovasculară, NLR are valori prognostice privind mortalitatea pe termen scurt şi lung în boala coronariană acută, boala coronariană stabilă, insuficienţa cardiacă, bypass aorto-coronarian şi boala arterială periferică sub tratament cu anti-agregante. În boala coronariană acută, neutrofilia şi NLR crescut au fost asociate cu scăderea numărului limfocitelor T-reglatorii, subseturile CD4 şi CD25. Alteori, rapoarte numerice validate şi foarte cunoscute, cum este raportul De Ritis AST:ALT, au fost modificate cu scopul de a introduce scoruri mai performante. Dintre scorurile care indică fibroza ficatului, raportul index AST per plachete APRI{AST/ULN) x 100}/număr trombocite (109/L) este mai sigur comparativ cu raportul de Ritis. APRI este folosit însă exclusiv pentru populaţii selectate cu hepatită virus C, iar extinderea utilizării lui pentru alte diagnostice poate conduce la erori de diagnosticare. În prezent raportul de Ritis este considerat discutabil deoarece calculul este solicitat pentru pacienţi când valorile transaminazelor sunt deja

anormale. Deoarece investigațiile de până acum au utilizat valori prag arbitrare pentru raportul neutrofile per limfocite, noi cercetări sunt necesare pentru a defini valorile limită ale raportului.
Cuvinte cheie: raport neutrofile per limfocite, raportul index AST per număr trombocite, cardiovascular, cancer

INTRODUCTION

It is noticeable that there are by now many clinical researches supporting the use of ratios instead of data of stand-alone parameters. Also, long-time known ratios like the validated De Ritis ratio (AST/ALT) have been modified in order to develop scores with better performances. Future investigations will clarify whether or not to rely on and use these ratios to replace measurements of some parameters of the clinical laboratory.

Of these ratios that of neutrophil to lymphocyte (NLR) emerged as a marker of poor prognosis in cancer and cardiovascular diseases. "More recently, NLR was considered to be a reliable predictor of poor outcomes in clinical hepatic entities". For liver failure from acute and chronic hepatitis B the ratio served as a predictor of short-term mortality. NLR was as well underlined as a simple and easily accessible variable for evaluating therapeutic response in patients with chronic hepatitis C. Nevertheless, the utility of NLR in autoimmune liver disease is still being investigated [1].

PROGNOSTIC VALUE. SENSITIVITY AND SPECIFICITY

I. NEUTROPHILS PER LYMPHOCYTES RATIO - NLR

C-reactive protein (CRP), neutrophils to lymphocyte ratio NLR and the Glasgow Prognostic Score GPS combining CRP and albumin were all reported as indicators of poor prognosis especially in cancer. Tumor-induced systemic inflammatory responses change neutrophils, lymphocytes and platelets levels in peripheral venous blood and contribute to cancer progression and metastasis. Therefore, quantifications of some hematological parameters have been analyzed in various malignant tumors [2-4]. Also, NLR has been used in oncology for monitoring effects of treatments and then its use extended to cardiology. Additionally, increased concentrations of pro-inflammatory cytokines eventually causing cellular DNA damage were reported together with elevated NLR [5,6].

Neutrophils to lymphocyte ratio have been mostly analyzed in studies which investigated pancreatic cancer. According to Gao et al. [2], cancer-associated inflammation is characterized by key molecular features such as inflammatory cytokines, growth factors and proteinases "that are favorable for proliferation, invasion and metastasis of pancreatic cancer cells". Zhou et al. [7] as well as Inoue et al. [8] revealed NLR and CRP prognostic values in large pancreatic cancer cohorts. Low NLR was a favorable predictor of overall survival and disease-free survival DFS in patients with pancreatic cancer [7]. The NLR associations found out in patients with pancreatic cancer were with CA-199 marker, tumor size and cancer stage. Another study on associations of NLR and transition phenotypes of circulating tumor cells CTC showed that the ratio associated with CTC and pointed out that in patients lacking circulating tumor cells, lymphocyte counts and the neutrophil-to-lymphocyte ratio (NLR) were significantly different from those in patients testing positive for CTC subpopulation ($P<0.001$) and circulating tumor microemboli CTMs [9].

It was presumed that markers of the systemic inflammatory response (NLR, CRP) suggest poor prognosis also in cardiovascular diseases. In this sense, Bennites et al. [10] showed that NLR is a better "predictor of mortality than absolute neutrophil and lymphocyte counts in patients with acute decompensated heart failure". A high NLR might be a risk marker for advanced heart failure more than the altered leukocyte composition is.

Several studies documented on lymphocytopenia as frequently predicting poor prognosis for patients with heart failure [11-13]. A study conducted at cellular level [14], using blood samples from patients with heart failure showed that sympathetic activation and oxidative stress/pro-inflammatory statuses activated programmed lymphocyte death, thus causing lymphocytopenia in these patients [14]. As shown by authors, cutoff values indicating mild lymphocytopenia were between more than 1500 and less 2000 cells per microliter and those pointing at severe lymphocytopenia were less than 1500 cells per microliter. In patients who had only heart failure but not lymphocytopenia, lymphocytes were higher than 2000 cells per microliter [14]. Battin et al. [13] pointed out for hospitalized patients with heart failure and protein-losing enteropathy that lymphocytopenia was associated with loss of albumin and concomitant loss of lymphocytes.

Bhat et al. [15] listed as well, cardiovascular diseases for which NLR has prognostic values that indicate poor outcome. The ratio was reported "as an independent predictor of outcome in stable coronary artery disease and a predictor of short- and long-term mortality in patients with acute coronary syndromes, in patients with advanced heart failure and as well a prognostic marker for outcome from coronary artery bypass grafting. In addition, NLR pointed to increased risk of ventricular arrhythmias during percutaneous coronary intervention (PCI).

Spark et al. [16] demonstrated in his study that an elevated NLR predicted high mortality in patients aged 70 years old with peripheral arterial disease and critical limb ischemia who were on antiplatelet therapy. Because of an extremely high mortality rate of 43% in these patients during the 8.7 months-follow-up, additional parameters for risk stratification were necessary. Of these NLR proved clinically relevant at a cutoff value of 5.25, with an overall accuracy in the dataset of 66.4%. Decreases in both number and suppressive function of CD4 and CD25 regulatory T cells, neutrophilia and elevated NLR, were found out in acute phases of coronary syndromes and suggested presence of "circulating leukocyte-platelet aggregates that facilitate vascular plugging and infarct extension" [16].

An important finding was also that not only in cancer research could NLR aid to stratify patients but also in cardiovascular studies. Based on investigations regarding NLR utility in predicting long-term mortality in patients with non–ST-segment elevation myocardial infarction (NSTEMI), Azab et al. [6] showed that patients in the highest NLR tertile (NLR higher than 4.7) had a higher 4- year mortality rate (29.8% vs 8.4%) compared to those in the lowest tertile (NLR less than3). They underscored with regard to all above studies that several of these managed to categorize patients according to NLR intervals (tertiles, quartiles, quintiles), while other studies managed to establish NLR cutoff values [5].

Among limitations of these researches, Lin et al. [1] mentioned underlying mechanisms of elevated NLR that should be explored as the ratio is "a combined parameter of inflammation and host immune surveillance". Therefore, expanded neutrophils eventually give rise to a supporting milieu that enhances tumor growth and invasion but this inflammatory microenvironment is related to alterations in peripheral blood cells including lymphocytes. According to Lin et al. [1] several studies on primary biliary cirrhosis pointed to 10 to 100-fold increases in frequency of auto-reactive intrahepatic differentiation of CD4+ or CD8+ T cells, in comparison to lymphocytes in peripheral blood. Also, contradictory results of lymphocyte counts could be due to the absolute numbers of individual cell types, which change under infection, medication and stress. Azab et al. [5] added another limitation, which is lack of

standardization due to different time-points for collecting and analyzing blood samples.

II. AST: PLATELET RATIO INDEX - APRI

Liver fibrosis is another example illustrating advantages of using ratios as scoring to identify patients who need further investigations of liver disease. Among fibrosis risk scores, the AST: platelet ratio index APRI has been considered more reliable than the AST:ALT ratio as there has been confusion about clear indications of the later [17]. For example, alcohol use raises AST and implicitly the ratio AST: ALT but the latter ALT indicates more advanced liver fibrosis in patients with hepatitis C infection than liver fibrosis of those who abuse alcohol. Therefore, specifications on sensitivity and specificity of the AST:ALT ratio in cases different as regards their primary pathology would be needed. Also, Botros et al. [18] pointed out that a cutoff value for this ratio is questionable since the ratio is calculated for patients with transaminases already abnormal.

Cheung et al. [19] utilized APRI {AST/ULN) x 100}/platelet count (109/L) as a variable predictive of development of hepato-cellular carcinoma HCC in 144 patients with primary biliary cirrhosis. "The overall 5-, 10- and 15-year cumulative incidences of HCC were 2.3% (95% CI: 0% - 4.8%), 8.4% (95% CI: 1.8 - 14.5%) and 21.6% (6.8% - 34.1%), respectively". The study pointed out that APRI higher than 0.54 at one year after treatment (HR=3.94), older age (HR =1.07) and cirrhosis (HR = 4.38) were independent prognostic factors for development of hepato-cellular carcinoma. [19].

Joshita et al. [20] specified that APRI at values higher than 0.54 for patients with primary biliary cirrhosis (n=272) predicted disease progression to hepatic failure defined as decompensated liver cirrhosis in patients with jaundice, ascites, gastrointestinal bleeding, hepato-renal syndrome and encephalopathy. "The 5-, 10-, and 20-year cumulative incidences of hepatic failure were 3.5%, 5.8%, and 12.7%, respectively". Also according to this research, APRI had a high prognostic accuracy (AUROC 0.789, 95% confidence interval: 0.742–0.835) [20].

However, APRI is validated in referral populations with hepatitis C virus and thus, extending it to other conditions or populations may be misleading [21]. Drees et al. [22] lately demonstrated that stand-alone AST was as effective as FIB-4 index and APRI at predicting fibrosis of hepatitis viruses B and C. Also, authors specified that "cutoffs developed for AST, FIB-4 index, and APRI all had specificities of 79.2% to 80.3% for ruling-in severe fibrosis and enabled triage of one third of the study-population". Another advantage of using these indicators was that there was no need to wait for liver biopsies results before starting treatment. Nevertheless, at present there is no single serum fibrosis marker sufficiently sensitive to rule-out significant fibrosis [22].

CONCLUSION

As investigations by now used arbitrary threshold values for the neutrophils to lymphocytes ratio, future researches are needed to define its cutoffs.

Conflicts of interest

The authors declare no conflicts of interest.

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EMOTIONAL CUES COULD IMPROVE PROSPECTIVE MEMORY IN SUBJECTS WITH NEUROCOGNITIVE DISORDERS

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Abstract. There are a number of ways in which memory can fail us, forgetting to perform a previously formed intention at the appropriate occasion depends on prospective memory (PM). This type of memory involves binding in memory a cue to an action (during encoding), as well as successfully recognizing it and acting upon it when it is later encountered in ones environment (at retrieval). Studies have shown that the nature of the emotional cues (neutral, positive or negative) can either aid us in completing prospective memory tasks, or on the contrary impede us in doing so. The main purpose of the current article is to review some of the finings in the literature with regard to these effects, in both healthy and clinical populations (subjects with neurocognitive disorders).
Key words: prospective memory, emotional cues, subjects with neurocognitive disorders

STIMULII EMOŢIONALI AR PUTEA ÎMBUNĂTĂŢI MEMORIA PROSPECTIVĂ LA SUBIECŢII CU TULBURĂRI NEUROCOGNITIVE

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Rezumat. Există multiple modalităţi în care memoria poate eşua. A uită o anumită acţiune pe care ne-am planificat să o executăm într-un anumit moment viitor, reprezintă un eşec al memoriei prospective (PM). Acest tip de memorie implică asocierea mentală a unui anumit stimul cu o anumită acţiune (în procesul de codificare a informaţiei), identificarea stimulului atunci când apare în mediul extern şi efectuarea acţiunii asociate acestuia (în procesul de recuperare a informaţiei). Numeroase studii au arătat că natură stimulului (neutru, pozitiv sau negativ) poate avea fie un efect stimulator fie unul inhibitor asuopra memoriei prospective. Prezentul studiu îşi propune să revizuiască studiile care au investigat influenţă stimulilor emoţionali asupra memoriei prospective, în populaţii sănătoase şi în populaţii clinice (subiecţi cu tulburări neurocognitive).
Cuvinte cheie: memorie prospectivă, stimuli emoţionali, subiecţi cu tulburări neurocognitive

"He was still too young to know that the hearts memory eliminates the bad and magnifies the good, and that thanks to that artifice we manage to endure the burden of the past."

Gabriel Garcia Marquez, Love in the time of cholera

INTRODUCTION

The events in our lives that are the most memorable are usually those that are highly emotionally arousing. So in that sense, the minds memory tends to agree with the hearts. However, with regard to magnifying positive events while eliminating negative ones, it seems that the brain is less willing to allow us to forget burdens of the past no matter our age.

Because it seems that both young and old individuals' memory is enhanced by emotional cues (both positive and negative).

This idea, that emotional material attracts more attention or can better preserve encoded material has been well documented [1, 2]. Most studies agree that arousing events, whether positive or negative exhibit a time dependant advantage in memory compared to neutral events. However there is less of an agreement when it comes to positive memories compared to negative ones. Though some studies have shown that positive material is superiorly remembered to both negative and neutral material [3]. As well as specific instances where we "actively" forget or repress bad memories. However, the concept of repression, that forgetting protects the heart, or as Freud put it, the psyche from threatening thoughts or memories, has a very controversial history and is beyond the purpose of the present review.

The advantage of emotions on memory have been investigated for different memory systems, however the present review will mainly focus on prospective memory (PM). PM plays a central role in individual's ability to plan and execute daily activities and can be very important in maintaining social activities, health, independence and safety. The ability to fulfill certain tasks in the future is dependent on PM, this is essential in ensuring that people carry out their intentions at the appropriate occasion. PM is the ability to remember future intentions, as well as performing that intended action at the appropriate time, while involved with ongoing activities [4, 5]. To successfully complete a PM action, individuals have to remember its content (retrospective memory component) as well as to perform it in the future (prospective memory component). The PM action has to be carried out either at a set time (time-based PM) or when encountering a specific cue (event-based PM) [6].

There are two ways in which PM can fail: by forgetting to execute a task termed - omission error (e.g. taking a pill) or by mistakenly repeating the completed task - repetition error (e.g. taking the same pill twice).

Cue manipulation in PM paradigms has mainly been done in terms of cue familiarity [4, 7] or focality [8]. Interes-tingly, it has also been observed that PM cues with emotional valence are better remembered than PM cues with non-emotional content. The distinctiveness of an emotional cue may reduce the need for controlled monitoring of the cue as its detection is facilitated, and this may result in better PM performance [9, 10]. This view is supported by feedback theory of emotion [11] which proposes two ways in which cognition is influenced by emotion. Through conscious moods and through more automatically means of appraisal which arise when stimuli are encountered.

Research suggests that PM may be influenced by emotional stimuli through encoding or retrieval processes or, in synergy through both [12]. Deriving from the more general literature of cognition and emotion it's been proposed that emotion is likely to improve encoding processes by enhancing attention and visual processing [13, 14, 15]. It could also enhance the retrieval process by increasing the likelihood of detecting cues, because it's been shown that emotional stimuli attract more attention as compared to neutral stimuli [16, 17]. This finding holds both for consciously attended stimuli, in visual search tasks [18, 19, 20] and involuntarily, with subliminally presented stimuli [13, 16, 21]. This finding is in accordance with McDaniel and Einstein's [4] hypothesis that PM retrieval is supported by both automatic spontaneous retrieval and conscious monitoring processes.

The current paper aimed to review and synthesize some of the literature that has investigated the influence of emotion on PM and refer to some of the cognitive mechanisms that have been proposed to

support these effects by providing examples from clinical populations.

Research on emotional cues and PM in healthy populations

May et al. [22] explored the potential benefits of emotional cues in improving PM performance for both young and old individuals. They showed that both groups had higher PM execution rates (fewer omission errors) for the emotional arousing compared to neutral cues. With regard to the second type of failing in PM (repetition errors) they showed in a second experiment, that older adults had significantly less repetition errors with emotional than with neutral cues. They concluded that emotional cues can be used effectively to both initiate action and reduce repetition errors.

However, these findings are not always consistent in the PM literature. In a study by Altgassen et al. [23] PM performance was better for both positive and negative compared to neutral cues, but this result was only significant for older participants. A similar finding was reported by Schnitzspahn et al. [24] where older but not younger adults showed heightened PM with emotional cues relative to neutral cues. However, Rendell et al. [3] found better PM only for positive and not neutral and negative targets for older and younger adults and Altgassen et al. [25] reported the same pattern with young adults when compared to a depressed group. It seems that at least in some circumstances emotional cues can be more distinctive, and even more so for older compared to younger individuals.

A more conclusive understanding of these effects is presented in a recent synthesis conducted by Holster, Wood and Armitage [12]. They examined 67 effect sizes from 17 articles and found that overall, PM was enhanced when positively valences rather than neutral cues were presented ($d = 0.32$). Contrastingly, the synthesis revealed that cues that had a negative valence only improved PM performance when presented both during encoding and at retrieval ($d = 0.40$), and impair performance when presented only at encoding ($d = -0.25$). The main finding however is that cues with a positive valence do improve individual's performance on PM task [12].

Research on emotional cues and PM in clinical populations

Several studies proposed that this effect of emotion on PM performance is due to the nature of the structures that support it. Proposedly these item-emotion bindings are dependant on the amygdala, which enhances memory consolidation for this kind of stimuli [26] resulting in both enhanced long-term [27] and short-term memory [27]. Accordingly this type of binding is less rapidly forgotten that item-context bindings, supported by the hippocampus. While the hippocampus has a very unique physiology, it exhibits a high rate of neurogenesis and cell death that makes it more prone to forgetting. The amygdala is somewhat spared from forgetting because it's exposed to less cell death and less interference from new cells [28]. Another advantage may be that the amygdala is simply more resistant to interference because of fewer competing experiences (neutral events are more frequent compared to highly emotional ones).

A limited number of cases, with selective amygdala lesions (very rare) provide evidence toward this hypothesis. Cahill et al. [29] tested participants delayed recognition (one week) for a set of slides accompanied by a story that included neutral materials and negative arousing materials. They found no advantage for the patient group (bilateral amygdala damage) in recognizing the negative slides, though they performed well on the neutral ones. This lack of a memory advantage for negative stimuli has been observed with both verbal and visual stimuli when tested both for recall and recognition [30].

Research examining patients with lesion to multiple MLT regions, such as the

hippocampus, amygdala and surrounding perirhinal cortex, have also found a reduced effect of emotion on memory [31]. This however still seemed be due to damage to the amygdala rather than the other regions. Namely, when investigating recognition memory for negative versus neutral pictures, both patients with selective hippocampus damage and controls exhibited an emotional memory advantage in recognition tests, though no such advantage was seen for immediate test [32]. When examining patients with damage to MLT that do not include amygdala, an emotional advantage is found, even when controlling for overall memory performance [33]. Amygdala pathology was associated with reduced recollection for emotional but not neutral words in patients with temporal lobe epilepsy, while in hippocampus pathology reduced recollection was seen for both types of words.

To the best of our knowledge, only a small number of studies have investigated emotional enhancement in PM on clinical populations. A study by Mioni et al. [34] addressed the question of emotionally related improvement in PM performance in Parkinson Disease (PD) patients. They found that PM actions with emotional valence were better performed compared to PM actions with neutral valence, particularly with a greater enhancement of stimuli with positive emotional valence. However, the PD patients in their study had a lower performance than the control group, independently of the emotional valence of the PM cue. Consistent, with former studies assessing older adults [3] and other clinical populations [35] positive PM tasks were more likely to be performed than negative and neutral tasks.

Rendell and colleagues [35] tested multiple sclerosis patients and matched controls with Virtual Week, controls outperformed patients on both event based and time based tasks. However, a positive enhancement for event-based tasks was found for both groups.

Altgassen and colleagues [25] have tested whether or not emotional cues could be potentially advantageous for event-based PM with patients suffering from depression. They found that healthy controls outperformed individuals with depression across all PM conditions (neutral, positive and negative targets), and also found a positively effect in the control group only. While for depressed patients, performance was superior on neutral as compared to both positive and negative cues. Suggesting that, in the case of this clinical population, emotional valence does not yield any benefit on PM performance [25].

As far as we know, no studies have investigated emotionally valence material with PM in an Alzheimer's disease (AD) population. This may be due to the controversy around whether or not AD patients benefit from emotional enhancements. It is believed that this enhancement relies on limbic regions. However, while the neural substrate supporting this effect has not been thoroughly investigated, these may include the frontal lobe, as well as limbic circuits.

Kensinger et al. [36] found that the presence of AD reduces all emotional enhancement effects. Their results showed that both young and old adults, but not AD patients, have better memory for emotionally valance material. They also found that both elders and AD patients show no benefit when items are embedded in a emotional context as opposed to a neutral context, while younger adults do [36].

Nonetheless, while a hallmark of Alzheimer's disease (AD) is memory impairment, there is speculation that recall may be enhanced when an emotional component is associated with an event [37]. For example, Fleming et al. [37] showed better recall for emotionally valences material, as compared to neutral material, on a sample of AD patients. All subjects were tested with three word lists on three trials. The words were either of positive,

negative, or neutral valences and matched for concreteness, emotionality, and pleasantness. Controls outperformed AD patients, more importantly however; the pattern of recall for the emotions was different. Control groups recalled all emotions equally, whereas AD patients recalled significantly more negative words than positive or neutral words. These findings of improved immediate memory for emotional material in AD lend support to the notion that mnemonic functions are differentially affected in the disease [37].

The benefit of using emotional material to improve memory of Alzheimer's patients has also been assessed in memory and cognitive stimulation programs. Sandman [38] included 11 dyads of subjects with AD in a study using primarily a dyadic approach; however no control group was used. In this study, an attempt was made to stimulate memory by provoking emotional memories to produce "flashbulb" memories. (The author did not precisely define what are emotional or "flashbulb" memories—that is, the kind of emotions that should have been triggered by the memories and the required degree of emotional intensity). The patients and caregivers had to pay attention to specific information presented in films or pictures. The pictures contained information about the other subjects in the program. The training was carried out over 4 weeks. The author did not provide information about the duration and weekly frequency of the sessions. Memory was formally assessed at baseline or follow-up with a neuropsychological evaluation. Only the amount of information retained was recorded after the training. This study demonstrated that the emotional memories were best remembered: the patients retained six items in the high arousal condition, compared with approximately two items in the low arousal condition. There was no follow-up evaluation.

CONCLUSION

Thus far, for those populations that have been investigated, the evidence from the literature seems to collect in favor of positively valence material when attempting to enhance PM performance. However, while the extant studies are suggestive, additional data is needed for a more complete understanding of the interplay between PM and emotion processing, especially in clinical populations [39]. Finally, investigating possible emotional enhancement in PM performance in these populations could have important implications for rehabilitation programs, which would significantly enhance patient quality of life as well as reduce career burden [39].

Conflicts of interest

The authors declare no conflicts of interest.

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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.

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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.

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