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CLUSTER ANALYSIS OF HORMONAL CONSTELLATION AT WOMEN AND MEN WITH HARMONIOUS AND DISHARMONIOUS GENERAL ADAPTATION REACTIONS

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Abstracts

Background. It is known that the nervous, endocrine and immune systems interact closely with each other to form a three-pronged neuro-endocrine-immune complex. On of manifest of causal neuroendocrine-immune relationships is Leukocytogram of peripheral blood that differ by various constellation of Principal Adaptation Hormones. **Material and research methods.** We determined twice at ten women and ten men aged 33-76 years without clinical diagnose serum levels of Cortisol, Aldosterone, Triiodothyronine, Testosterone and Calcitonin (ELISA) as well as Popovych's Adaptation Index by Leukocytogram. **Results.** All case was distributed among four clusters. Cluster N1 contains 5 cases with drastically hypercortisolemia and moderately hypercalcitoninemia; 5 other cases characterized moderately hypercortisolemia and borderline level of Triiodothyronine, together reflected chronic stress. Majority of cases (Cluster N3) characterized upper borderline level of Cortisol and normal levels others hormones, while in 5 cases (Cluster N4) hormonal costellation is also normal but with lower borderline level of Cortisol. Estimation by Leukocytogram Popovych's Adaptation Index shown that at 25 cases take place Harmonious General Adaptation Reactions (GAR) as markers of health while at 15 cases detected Disharmonious GAR as markers of premorbidic stage. **Conclusion.** Constellations of principal Adaptation Hormones displays widely variability representing different gradations of health and premobide states. Calcitonin may be considered as one of principal Adaptation Hormones.

Keywords: Cortisol, Aldosterone, Triiodothyronine, Testosterone, Calcitonin, General Adaptation Reactions.

INTRODUCTION

The problem of the causal relationships between neuro-endocrine and immune systems has more than a century. The opening in 1898 the fact that the thymus increases in castrated rabbits, considered the birth of the hybrid medical discipline - immunoendocrinology [cit. by: 14]. EA Korneva [10] in 1993 put forward the hypothesis of the existence of a "indivisible immune-neuro-endocrine complex, which is involved in ensuring constancy of internal environment". IL Popovych [21] changing almost term for "neuroendocrine-immune complex", initiated research led him Truskavetsian Spa Scientific School of its role in the mechanism of therapeutic and prophylactic action of well-known bioactive water Naftussya. The results were published in numerous articles and several books [3-5,11-13,16-18,20,22,24,25,38,39], which presented a great interest in the scientific public and evaluated by experts as the backbone trend of the last decade Ukrainian SPA [26].

Although there are still some disagreements about specific aspects of neuro-immune causal relationships, brought a lot of evidence to support the existence of numerous interactions between the central nervous system, the peripheral nervous system (both sympathetic and parasympathetic divisions), endocrine and immune systems. Neurohormonal mechanisms include circulating hormones such as cortisol (in humans) or corticosterone (in rats) and catecholamines, in particular norepinephrine [2,9,15,30,31,36].

On a chart, offered EM Sternberg [30], CNS-mediated adjusting of immunity is carried out through system, regional and local ways. The peripheral nervous system provides the first line of defence in the place of inflammation through freeing of neuropeptides, that on the whole strengthens local inflammatory responses. Sympathetic (or adrenergic) and parasympathetic (or cholinergic) [32-35,37] nervous systems on the whole brake inflammation at regional level, through innervation of immune organs. Neuroendocrine responses control the inflammation at the system level hypothalamic-pituitary-adrenal (HPA) axis through anti-inflammatory effects of glucocorticoids, are released from the adrenal cortex, hypothalamic-pituitary-gonadal (HPG) axis by sex hormones, are released from ovaries and testicles, and the hypothalamic-pituitary-thyroid (HPT) axis due to thyroid hormones.

Early we [17] have been analysed neuro-endocrine-immune relationships by chronic restraint stress at male rats. It is detected considerable (R=0,67) canonical correlation between autonomous regulation parameters and thymocytogram. Thymic canonical radical receives negative factor loading on the relative weight of the thymus gland and levels there macrophages, endothelial cells and Gassal corpuscules, while positive factor loading on radical give lymphoblasts and lymphocytes. The canonical correlation between vegetative parameters and spleenocytogram very strong (R=0,94). Splenic canonical radical receives negative factor loading of macrophages and reticulocytes and positive - of the mass of the spleen and the contents therein neutrophils, lymphocytes and eosinophils. Revealed a strong (R=0,79) canonical correlation between autonomous regulation parameters and immune parameters of blood. In this immune root is represented B-lymphocytes, plasmacytes, basophils, eosinophils, neutrophils, stub and segmented, completeness of neutrophil phagocytosis, phagocytic activity of monocytes, leukocytosis and general lymphocytosis. We found a close relationship (R=0,89) between the endocrine and immune parameters. Endocrine canonical radical right represented relative adrenal weight, thickness of fascicular, glomerular and reticular zones, excretion of 17-ketosteroids, plasma level of triiodothyronine and inverse represented plasma level of corticosterone. Immune radical receives positive factor loading on the relative weight of the thymus and spleen, content

in the last lymphoblasts and neutrophils, content in blood leukocytes, completeness and intensity of phagocytosis of neutrophils. Instead, the negative loadings on the immune radical given level of spleenic fibroblasts, macrophages, reticulocytes, thymic epithelial cells and Gassal corpuscules, level in blood of NK-lymphocytes, plasmacytes, basophils and monocytes.

According to conception of general adaptation reactions of organism (GARO), created by LKh Garkavi, EB Kvakina and MA Ukolova in 1977 [6] on the base of H Selye classic conception of stress [28,29] and developed then by them [7], and also by OM Radchenko [27], GARO of **training** and **quiet** and **heightened activation** of high levels of reactivity (HLR, **harmonious**) represent different gradations of **health,** but the same GARO of low levels of reactivity (LLR, **disharmonious**) and stress of HLR (**eustress**) characterize the **premorbide** states of organism, while nonspecific nosotropic basis of **illness** are stress of LLR (**distress**) and reaction of **superactivation** as second hypostase of chronic stress.

Early LG Barylyak et al. [1] conducted a comparative estimation based on HRV, endocrine and immune parameters of informativity of leukocytary index of adaptation by Garkavi and by Popovych. An examination of 20 healthy men found that index of adaptation by Popovych that takes into account the relative content in leukocytogram of lymphocytes and deviations of monocytes, eosinophils and stub neutrophils, moderately or significantly correlated with the HRV, endocrine and immune (phagocytose) parameters. Recently we [16] shown that factors favourable influence the adaptive capacity of the body, measured at Leukocytary Popovych's Adaptation Index, may be considered Sympathetic tone and activity of neural structures generating δ-Rhythm in Left Parietal locus while activity of neural structures generating β-Rhythm in Right Occipital locus as well as θ-Rhythm in Right Medial Frontal and Central loci affect unfavourable adaptation. Unfavourable factors also appeared Entropy of relative PSD all Rhythms in Anterior Frontal and Right Anterior Temporal loci.

The ultimate aim our study: estimation of causal relationships between Principal Adaptation Hormones and parameters of Immunity. In first communication we приводимо характеристику constellation of Principal Adaptation Hormones as well as Calcitonin at women and men with harmonious and disharmonious General Adaptation Reactions

MATERIAL AND RESEARCH METHODS

The study involved twentee volunteers – ten women and ten men aged 33-76 years without clinical diagnose. In the morning on an empty stomach determined content in serum of venous blood principal adaptation Hormones: Cortisol, Aldosterone, Testosterone, Triiodothyronine [6,7,20,27] as well as Calcitonin (by the ELISA with the use of analyzers “Tecan” and “RT-2100C” and corresponding sets of reagents from “Алкор Био”, XEMA Co., Ltd and DRG International Inc.). In portion of capillary blood counted up leukocytogram, on the basis of which determined Adaptation Index according IL Popovych scale [5,16,19]. After week all tests repeated.

Results processed by methods of correlation and cluster (k-mean) analyses, using the software package "Statistica 5.5".

RESULTS AND DISCUSSION

According Instruction for use sets of reagents for the determination of Testosterone in human serum or plasma (“Testosterone EIA”, XEMA Co., Ltd, RF), the following normal range(nM/L) is recommended: femals <0,15÷4,6; males 20-39 yrs 9,0÷38; 40-55 yrs 6,9÷21; >55 yrs 5,9÷18,1.

Resting on this repering points, we builded regressive model for calculation of normal averages for males from 20 to 69 years (Fig. 1).

Taking into account as extended norm range 80÷120 % from average, we sertify that in 65% cases at femals Testosterone level was normal, in 30% heightened (139÷209 %), and only in 5% (one case) reduced (70%), while at males Testosterone level was normal only in 25%, in 15% heightened (136÷169 %), but in 60% cases reduced (76÷42 %). Mean Testosterone level makes at Men 85±8% from average norm while at Women 121±9%.

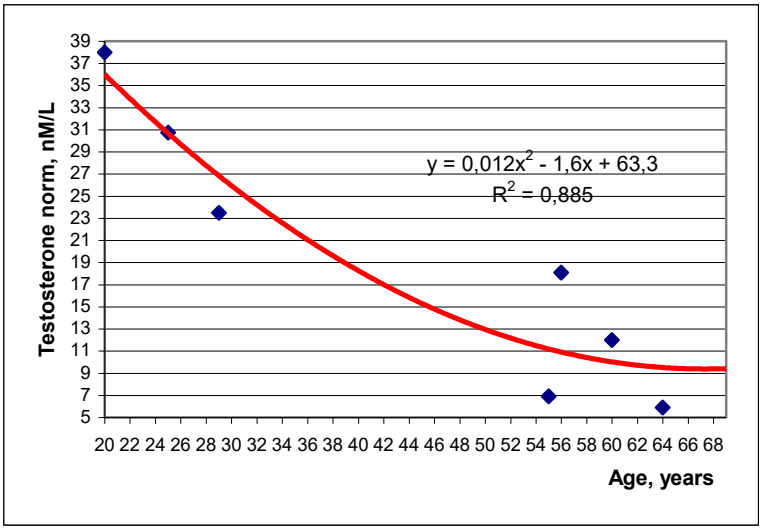

Fig. 1. Testosterone normal averages for males various age

According Instruction for use sets of reagents for the determination of Calcitonin (DRG® Calcitonin ELISA) the values obtained on the normal females ranged from 0,1 to 10,0 ng/L while on males from 0,2 to 27,7 ng/L. On these grounds we sertify that at femals Calcitonin level was normal (87÷111 %) only in 25% cases, but reduced (78÷26 %) in 40% and heightened (131÷202 %) in 35% cases, while at males Calcitonin level was normal (80÷98 %) only in 15% cases, but in 85% cases reduced (79÷11 %). Mean Calcitonin level makes at Men 52±6% from average norm, that reflects hypocalcitoninemia, while at Women makes 99±12%, that masks discalcitoninemia. Fig. 2 visualyses absence connection between Testosterone and Calcitonin levels.

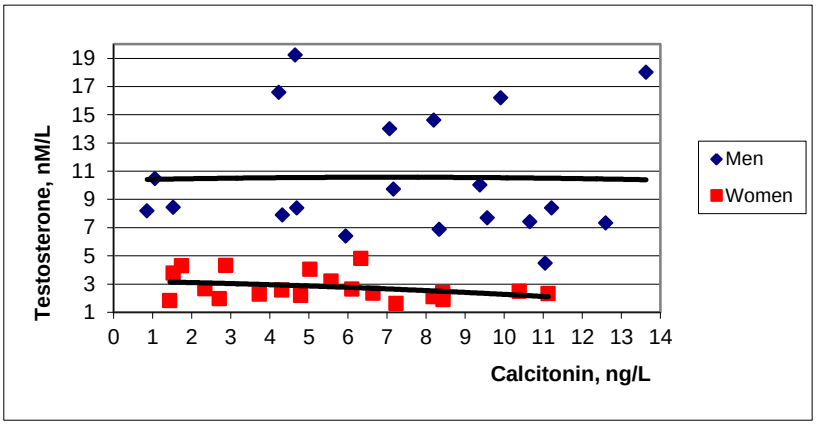

Fig. 2. Personal serum levels Calcitonin (axis X) and Testosterone (axis Y)

Normal range Cortisol level makes 150÷660 nM/L at both Men and Women. It was heightened at 85% Men and 80% Women, normal (80÷120 % from average 405 nM/L) only at 5% and 15% as well as reduced (77÷66 %) at 10% and 5% Men and Women correspondingly. Mean Cortisol level makes at Men 159±13% as well as at Women 180±18% from average norm.

It is detected moderately negatively correlation between Cortisol and Testosterone levels at Men but not at Women (Fig. 3).

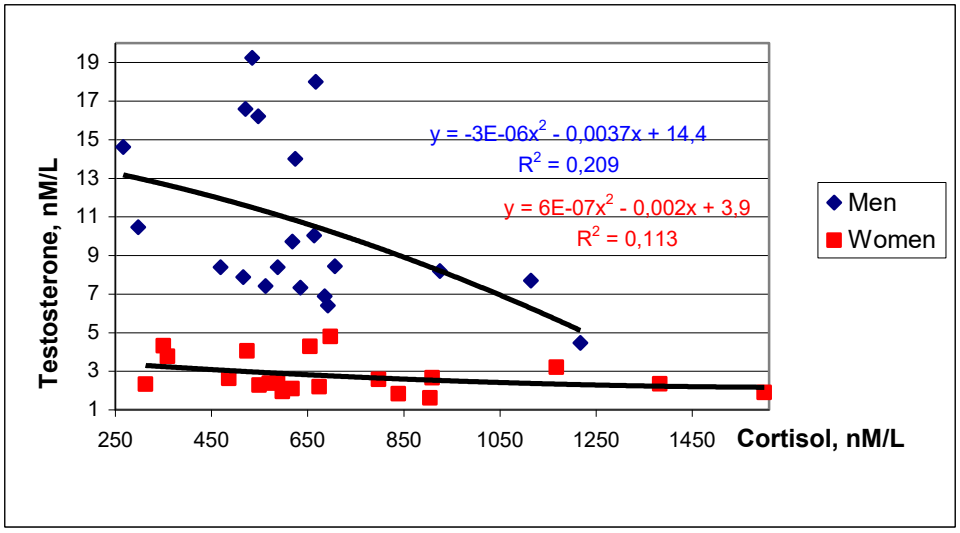

Fig. 3. Personal serum levels Cortisol (axis X) and Testosterone (axis Y)

According Instruction for use sets of reagents for the determination of Triiodothyronine in human serum or plasma ("T3 EIA", XEMA Co., Ltd, RF), is recommended the normal range 1,2÷3,2 nM/L for both female and male. In range 80÷120 % from 2,2 nM/L find oneself 35% cases, as much again in 35% cases Triiodothyronine makes 79÷40 % (68±3%) from average as well as in 30% cases 124÷242 % (157±12%) from average.

All cases Aldosterone levels was detected in range 218÷282 pM/L that coinsides with recommended normal range (190÷286 pM/L).

In the second stage of analysis, the information field of hormones was formed four clusters as homogeneous groups (Table 1).

Table 1. Summary Cluster Analysis of Hormones levels

Variables	Cluster N1 (n=5)	Cluster N2 (n=5)	Cluster N3 (n=25)	Cluster N4 (n=5)
Cortisol, nM/L	1297±88	875±24	600±14	317±17
Calcitonin, ng/L	8,3±1,0	3,2±1,1	6,9±0,7	5,0±2,0
Triiodothyronine, nM/L	2,15±0,27	2,99±0,65	2,20±0,17	1,71±0,22
Testosterone, nM/L	3,92±1,04	3,37±1,22	7,76±1,07	7,10±2,34
Aldosterone, pM/L	244±8	240±3	244±3	239±7
Portio of Norm	**Portio of Norm=Variable/Mean Norm**			
Cortisol (405 nM/L)	3,62±0,62	2,16±0,06	1,48±0,03	0,78±0,04
Calcitonin (5,5 & 13,95 ng/L)	1,54±0,15	0,90±0,28	1,11±0,13	0,92±0,35
Triiodothyronine (2,20 nM/L)	1,02±0,13	1,42±0,31	1,05±0,08	0,81±0,11
Testosterone (2,3; 25,2 nM/L)	0,87±0,16	0,89±0,10	1,04±0,09	1,25±0,22
Aldosterone (238 pM/L)	1,03±0,03	1,01±0,03	1,03±0,01	1,00±0,03
Z-score	**Z-score=(Portio of Norm - 1)/Coefficient of Variation**			
Cortisol (C_V=0,315)	8,32±0,87	3,69±0,19	1,53±0,11	-0,69±0,13
Calcitonin (C_V=0,492)	1,10±0,31	-0,21±0,56	0,23±0,26	-0,17±0,72
Triiodothyronine (C_V=0,222)	0,11±0,60	1,98±1,44	0,22±0,37	-0,87±0,50
Testosterone (C_V=0,253)	-0,63±0,50	-0,33±0,27	-0,07±0,28	0,29±0,45
Aldosterone (C_V=0,100)	0,28±0,33	0,11±0,27	0,27±0,14	0,04±0,30

According recommendation by IL Popovych [20] variables expressed as current level as well as portio of average norm (Fig. 4 above) and Z-score (Fig. 4 below, Fig. 5). Cluster N1 contains 5 cases with drastically hypercortisolemia and moderately hypercalcitoninemia; 5 other cases characterized moderately hypercortisolemia and borderline level of Triiodothyronine, together reflected chronic stress. Majority of cases (Cluster N3) characterized upper borderline level of Cortisol and normal levels othets hormones, while in 5 cases hormonal costellation is also normal but with lower borderline level of Cortisol.

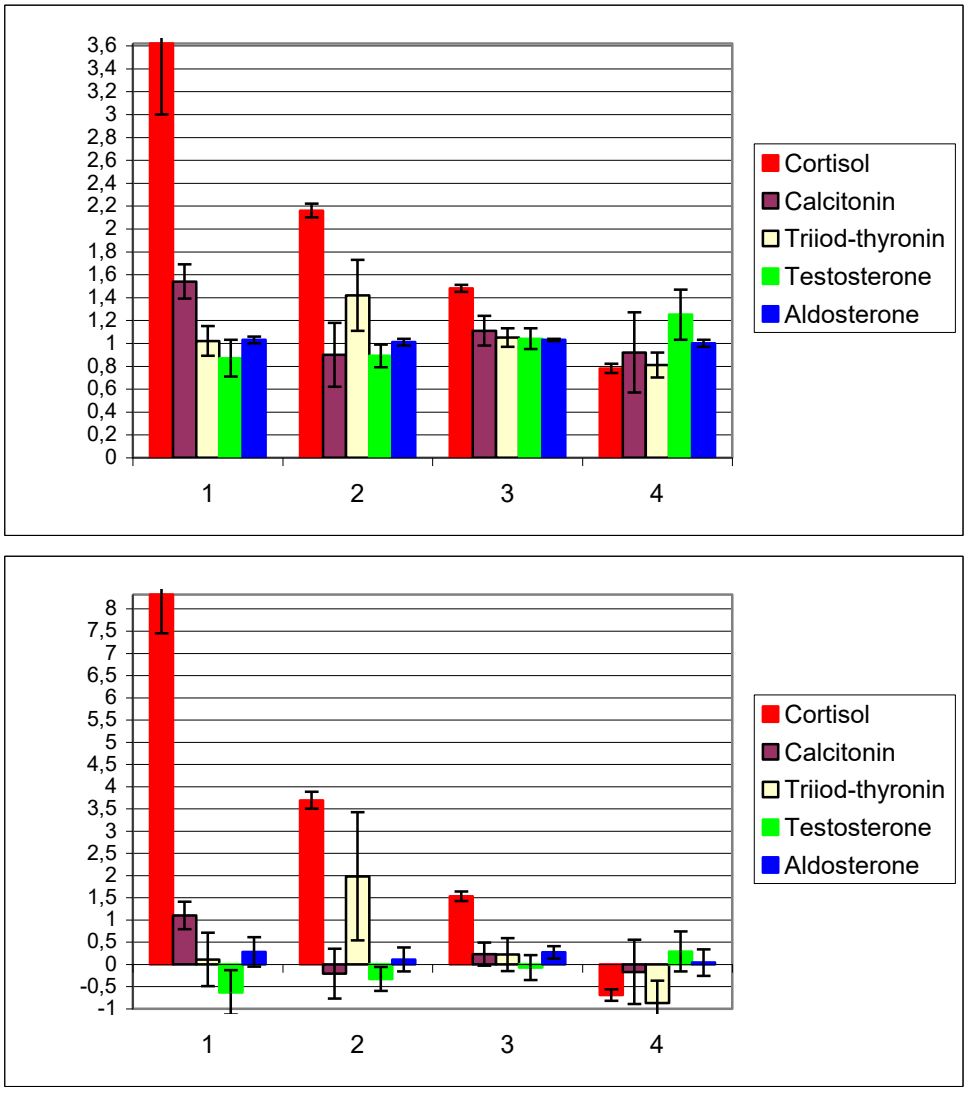

Fig. 4. Hormonal constellation at various clusters, expressed as portio of average norm (above) and Z-score (below)

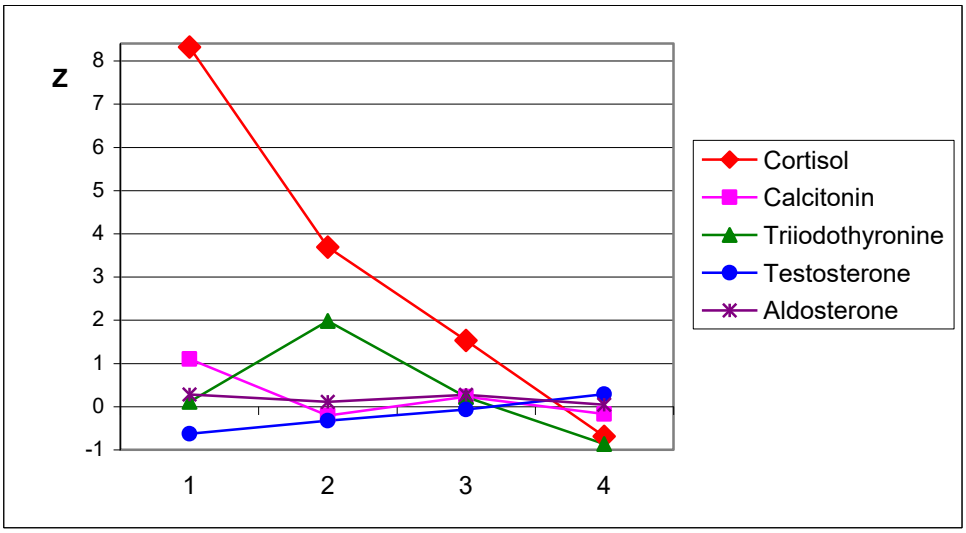

Fig. 5. Hormonal profiles various clusters, expressed as Z-scores

Estimation Popovych's Adaptation Index (PAI) shown (Table 2) that at 25 cases take place **Harmonious** GAR's as markers of health while at 15 cases detected **Disharmonious** GAR's as markers of premorbidic stage. However do not detect cases neither Stress-reaction nor reaction Superactivation, id est morbidic, that accorded with absence among volonteers clinical diagnose.

Table 2. Distribution cases among General Adaptation Reactions Scale

Leukocyto-gram Lymphocy-tes level, %	**G**eneral **A**daptation **R**eaction of **O**rganism	Eosinophiles and Stub Neutrophiles: 1÷6 %; Monocytes: 4÷7 %; Leukocytes: 4÷8 G/L		Eosinophiles and Stub Neutrophiles: <1; >6; Monocytes: <4; >7; Leukocytes: <4; >8 G/L	
		PAI	**n**	**PAI**	**n**
<21	Stress	**1,22**	**0**	**0,02**	**0**
21÷27	Training	**1,46**	**15**	**0,74**	**5**
28÷33	Quiet Activation	**1,95**	**8**	**0,98**	**8**
34÷43,5	Heightened Activation	**1,70**	**2**	**0,50**	**2**
≥44	Superactivation			**0,26**	**0**

Thus constellations of principal Adaptation Hormones displays widely variability representing different gradations of health and premobide states. Calcitonin is known as key regulator of Immunity at least cutaneous [8]. This proposition as well as our data gives grounds to conclusion that Calcitonin may be considered as one of principal Adaptation Hormones.

ACCORDANCE TO ETHICS STANDARDS

This study was approved by the local ethical committee of Truskavets' Scientists Assotiation. Tests in patients are conducted in accordance with positions of Helsinki Declaration 1975, revised and complemented in 2002, and directive of National Committee on ethics of scientific researches. During realization of tests from all participants the informed consent is got and used all measures for providing of anonymity of participants. For all authors (YL Hrytsak, LG Barylyak, W Zukow, IL Popovych) any conflict of interests is absent.

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