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Interplay between antioxidant activity, health and disease

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ABSTRACT
The article discusses the relationship between oxidative stress (OS) and pathological conditions, the possibilities and benefits of estimating OS considering the integral antioxidant activity (AOA) as an OS criterion, and using a simple accessible hybrid potentiometric method (HPM) with a mediator system for AOA monitoring. The results of AOA of blood serum in healthy volunteers and patients with various diseases are presented. Preliminary reference values are found. The lower levels of AOA of blood serum in patients with different diseases in comparison with the control group are observed. The potential mechanisms of changes in the AOA levels and it's clinical significance are discussed from the position of biointerfaces interplay. With AOA equal or greater than 1.40 mmol-eq l^{-1} the person is healthy, the range from 0.95 to 1.40 mmol-eq l^{-1} indicates that the patient is at risk and needs to undergo a further medical examination. When AOA blood serum is below 0.95 mmol-eq l^{-1}, detailed diagnostics and relevant treatment are required. The findings allow suggesting that the approach determine antioxidant/oxidant activity of biological fluids holds considerable promise for monitoring OS; it opens up new opportunities in expanding the use of analytical chemistry in medicine.
Keywords: *Oxidative stress; Antioxidant activity; Antioxidants; Hybrid potentiometric method; Cardiovascular diseases; Cancer.*

1. INTRODUCTION

The relationship between oxidative stress (OS) and the state of human health has become more clear for the latest decades as the result of research in this field carried out in chemical, biological and medical laboratories of the world [1–3]. The term "oxidative stress" refers to the imbalance between the production of oxidants (hydrogen peroxide, organic hydroperoxides, nitric oxide, oxygen and nitrogen reactive species, etc.), which generate oxidation processes, and exhaust activity of the system of antioxidant protection of the living organism. The latter suppresses the generation of oxidants or scavenges them [4–6].

It is well known that the energy required for the life of aerobic organisms is formed in cells in the oxidation of certain substrates, primarily during oxidative phosphorylation [7]. In the mitochondria, the oxidation of glucose with oxygen to carbon dioxide and water, localized at the respiratory chain at the cristes of mitochondria results in the synthesis of adenosine triphosphate acid, with more than 90 % of the oxygen consumed reduced to water with the participation of the enzyme cytochrome oxidase [8]. A very small part of the oxygen is converted into partially reduced products – reactive oxygen species (ROS): superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\bullet}$); unlike oxygen, they are reactive, whereby in normal conditions and at physiological concentrations participate in the processes of growth, differentiation, development and cell death, as well as in several other processes of bioregulation as natural molecules, signaling, intracellular messengers and potential factors of intercellular communication [9]. Normally, excess of ROS is scavenged by antioxidant defense system in the mitochondria. In case of excessive formation of ROS, as well as their insufficient inactivation by the system of antioxidant protection of the body, there is a phenomenon of OS, which causes the development of oxidative destruction of deoxyribonucleic acid (DNA), proteins, lipids, carbohydrates, and damage of biomembranes, cells, tissues [10], i.e. expansion of aggressive chain chemical reactions of biooxidation out of the borders of the cell organells and membranes. The modern definition of OS was proposed in 1991 by H. Sies, who considered OS as "imbalance between pro-oxidants and antioxidants in favor of oxidizing substances, leading to possible damage" [11]. In recent years, special attention is paid to the need to include in the concept of OS violations of signal functions of ROS: OS is proposed to be considered as "an imbalance between oxidants and antioxidants in favor of oxidants, leading to a violation of redox signaling and control and/or molecular damage" [6, 12].

OS forms the basis for] developing of main pathological processes within a wide range of diseases (including cardiovascular, endocrine, respiratory, neurodegenerative diseases, cancer; infertility; anxiety and depressive disorders, etc.) [1, 2]. Involvement of OS mechanisms in pathogenesis of multiple diseases can be explained by universality and importance of redox reactions occurring in human cells both in normal and pathological conditions. The universality of OS makes its measure and monitoring as a possible integral indicator of health status an important task. One of the main problems associated with the study of OS is the existence of a large number of different methods for its assessing and the lack of a common standard for such measurements [13]. There are many approaches and attempts to measure the OS level and activity of oxidants by the assessment of the properties of separate oxidants and the products of oxidation reactions - the received data are often characterized by ambiguity and inconsistency, it makes published results incomparable and irrelevant for diagnostic purposes [13–15]. The degree of expression of chain/oxidation reactions, initiated by free radicals, due to their non-specificity and significant differences in the

response of antioxidant systems, cannot and should not be considered as an independent diagnostic feature, and as the only cause of OS [16]. The significant problem is the understanding of the location of OS reactions in the cells and cell membranes and their surfaces in concrete cases and diseases, the evaluation of mechanisms, limiting or increasing their development. The processes of oxygen and products of its metabolism diffusion through several biointerfaces (alveolar-blood, blood-organ histohaematic barriers, biomembranes (cells and mitochondria) are difficult to be measured. Many existing ways of OS detection use chemical methods to determine individual substances that are products of oxidation by free radicals, thus fixing the consequences caused by OS. There are many serious limitations for accurate measurement of oxidants level – after sampling the rapid changes in composition of complex biological matrices are usually obtained, there are multiple compounds with different chemical nature, and different antioxidant and oxidant properties, with difficult interaction in biological matrix, some of them can achieve synergy effect, many of them (reactive oxygen and nitrogen species) have short lifetime, etc.

The development of radical oxidative processes in a living organism is limited by the action of antioxidant system aimed at retaining pro- and antioxidant balance. Thus, the study of antioxidant defense system seems to be the appropriate way for monitoring and evaluation of OS. A lot of works concerning the properties of separate antioxidants and their groups were published, but the determination of total content of antioxidants can be preferable because it is impossible to determine all different compounds exhibiting antioxidant properties, and possible synergism of the action of antioxidants and many other known and unknown factors modulating their antioxidant properties in vivo in each specific situation cannot be taken into account [17, 18]. The huge problem is that the term "antioxidant properties" has a different understanding of scientific publications; some of them do not have specific interpretations or use relative units to measure it, making the obtained results difficult or impossible to compare [19, 20].

One of the integral correct diagnostic indicators of the balance in the system of antioxidants/pro-oxidants as a whole is the antioxidant activity (AOA), by which we understand the total concentration of antioxidants in the biological sample [21]. AOA of the biological system is determined by a set of protein, enzyme and non-enzyme antioxidants, quantitatively determining the integral value of AOA [22].

Since the AOA reflects the properties of the system as a whole and is determined by a complex of compounds, it is advisable to use methods for determining AOA as an integral parameter.

A number of methods of AOA determination are described [3, 5, 14, 15, 22–26].

Some authors use stable radicals synthesized preliminary or during the analysis. Since the composition of used and natural radicals is different, it should be recognized that their interaction with antioxidants should also be different. The latter makes the interpretation of the results of the analysis not always correct.

With rare exceptions, different relative values are usually used as the parameter characterizing the OS, which makes the results and conclusions of such investigations incomparable. From our point of view, electrochemical methods [27, 28] are preferable because the methods are simple, as a rule, give the result in units of concentration. However, in this case, the latter quality is not always realized.

The hybrid potentiometric method (HPM), which is free from these drawbacks, was described in several earlier works [29, 30]. It was shown, those mentioned fundamental difficulty could be overcome by inserting the sample in a mediator system, containing the oxidized and reduced forms of the element and chemically react with antioxidants or oxidants. At the same time, a reversible signal is formed in this case due to the reaction of the mediator system components with the analyte. Thus, the use of the mediator system allows for measuring the equilibrium potential rather than the unstable stationary one.

Based on these considerations, we think, that HPM appears to be preferable for AOA determination, estimation of OS and use it in clinical investigations.

The important goal is to estimate the normal values of AOA in healthy people, deviations of AOA in patients with different diseases, and explain the possible cause of such deviations.

The aim of this work is to determine the boundaries of normal levels of AOA in healthy people, to compare these values with the AOA levels obtained in patients with arterial hypertension and cancer, and to assess the possibility of use of AOA values measured by a HPM in practice.

2. MATERIALS AND METHODS

2.1. Ethic statements.

The study was conducted in accordance with the rules of Good Clinical Practice and Declaration of Helsinki. All patients who were involved in the study have provided informed consent. The protocol of the study was approved by the Ethics Committee of “Medical Technologies” JSC (Project identification code 16-01-18 MT-AO).

2.2. Study groups.

305 people aged from 19 to 89 years were involved in the study. Three groups were formed: the group of 110 healthy volunteers; the group of 170 patients with cardiovascular diseases (CVD): arterial hypertension (HT) of stages I-III (in 34 patients the HT of III stage was complicated with stable coronary heart disease (CAD)) in accordance with the European Society of Cardiology and European Society of Hypertension of Cardiology Guidelines for the management of arterial hypertension, 2018; the group of 25 patients with diagnosed cancer (adenocarcinomas of different locations, verified by biopsy and histochemical test), 10 of them with generalized forms of cancer (metastases), 15 – with non-generalized forms. Patients of control group have no chronic diseases, their anthropometric data, results of objective examination, biochemical and clinical full blood-count tests, urine test, electrocardiogram data and spirometry test were within normal values. In patients with arterial hypertension, the values of the following parameters were additionally studied: total cholesterol, high and low density lipoproteins, uric acid, creatinine concentrations in serum; measurement of the pulse wave velocity,

measurement of the thickness of the intima-media complex of the common carotid arteries were also performed.

The exclusion criteria were the presence of chronic renal and hepatic failure, respiratory failure, severe heart failure, blood diseases, cerebral ischemic stroke or myocardial infarction within 6 months before the date of inclusion in the study, acute infectious diseases.

2.3. Blood collection and sample preparation.

Blood sampling of patients with cancer and arterial hypertension was performed at fasting in the early morning hours (7.00–8.30), in healthy volunteers – at fasting in the early morning (7.00–8.30) and morning (8.31–10.00) hours. Blood was taken by venipuncture at the bend of the elbow joint into polyethylene terephthalate vacuum tubes (Chengdu Puth Medical Plastics Packaging Co., Ltd., China) containing a coagulation activator (SiO_2). To obtain serum, the blood was centrifuged at a speed of 3500 rpm for 15 minutes using centrifuge SM–6M (SIA ELMI, Latvia).

2.4. Blood clinical analysis.

A full blood count blood test was performed at BC-6800 hematology analyzer (Shenzhen Mindray Bio-Medical Electronics Co., Ltd., China) and biochemical tests were performed at BA-400 biochemical analyzer (Biosystems S.A., Spain).

2.5. Determination of Blood Serum AOA.

HPM was used for analysis. Serum sample was introduced into the solution, containing 0.01 M $K_3[Fe(CN)_6]$, 0.0001 M $K_4[Fe(CN)_6]$, 0.0545 M Na_2HPO_4 and 0.0121 M KH_2PO_4 (pH 7.4). Potential shift (signal) observed as a result of signal generating reaction (1) between antioxidants containing in the sample and $K_3[Fe(CN)_6]$:

$$a \times [Fe(CN)_6]^{3-} + b \times AO = a \times [Fe(CN)_6]^{4-} + b \times AO_{Ox}, \quad (1)$$

where a and b are the stoichiometric coefficients, AO is the antioxidant(s), AO_{Ox} is the oxidized form of the antioxidant(s).

Measurements were carried out at the temperature 23 ± 3 °C using the potentiometric analyzer Antioxidant (Antioxidant Ltd., Russia). AOA was calculated with the use of equations (2) and (3):

$$AOA = \frac{C_{Ox} - aC_{Red}}{1+a} \times d, \quad (2)$$

$$a = \left(\frac{C_{Ox}}{C_{Red}}\right) \times 10^{(E_1 - E)nf/2.3RT} \quad (3)$$

where E is the initial potential of the mediator system, V; E_1 is the potential of the mediator system, established after the introduction of the sample, V; C_{Ox} – concentration of $K_3[Fe(CN)_6]$, M; C_{Red} is the concentration of $K_4[Fe(CN)_6]$, M; n is the number of electrons in the electrode reaction (here n = 1); F is the Faraday constant (96485.34 C/mol); R is the universal gas constant (8.314 J/(mol×K)); T is the absolute temperature, K; d is dilution of the sample in the electrochemical cell (for plasma and serum d ≤ 6).

Analysis of blood serum by HPM was performed using two-electrode cell comprising a platinum screen-printed electrode (Iva Ltd., Russia), which served as an indicator electrode and silver/silver chloride electrode Ag/AgCl, 3.5 M KCl type EVL-1M3.1 (JSC Gomel Plant of Measuring Devices, Belorussia) as a reference electrode. After operation in a biological matrix (blood serum), the platinum screen-printed electrode was regenerated by annealing at 750 °C for 1 hour [31].

2.5. Pulse wave velocity measurement.

Determination of the pulse wave velocity was carried out in patients with arterial hypertension in accordance with the commonly used classical method by means of simultaneous registration of sphygmograms of carotid, radial and femoral arteries on the device POLY-SPEKTR-8/E with the help of software Poly-Spectr.NET with the connected module Poly-Spectr-SRPV (Neurosoft Ltd., Russia). At the distance from the bifurcation of common carotid artery to the proximal part of femoral artery the pulse wave of elastic type (Se) vessels was measured and determined, and at the distance from the bifurcation of common carotid artery to the proximal part of radial artery the pulse wave velocity of muscular type (Sm) vessels was determined.

2.6. Intima-media thickness measurement.

Ultrasound examination of common carotid arteries intima-media complex was performed on at the ultrasound scanner Philips IU–22 (Philips N.V., Netherlands) by the linear probe with the working frequency of 5-12 MHz. The measurements were carried out with the accordance to a commonly used method, by the experienced physician, manually at three points of each common carotid artery posterior wall, in the zone located 1 cm proximal to the bifurcation of the common carotid artery. The intima-media thickness (IMT) was measured as the distance between the vessel lumen – intimal layer surface boundary and the media –adventitia layers boundary. The arithmetical mean of three dimensions was calculated, its value was taken as the value of IMT.

2.7. Statistical Analysis.

Statistical analysis was performed with an acceptable level of significance α = 0.05. The Kolmogorov-Smirnov criterion, which was calculated in the IBM PSPP program with a General public license, was used to assess the compliance of the distribution of the AOA values of blood serum with the normal distribution. Statistical processing of the measurement results was carried out in Microsoft Excel 2010. The results are presented as $X \pm \Delta X$, where X is the average value, ΔX is the standard deviation. The statistical relationship between the parameters was estimated using the Pearson linear correlation coefficient, and the statistical significance of the differences between the parameters was estimated based on the Student's t-test. For the qualitative characteristics of the correlation relationships of the parameters, the Cheddok scale was used. text.

3. RESULTS

3.1. Evaluation of the normal distribution of AOA serum values.

The Kolmogorov-Smirnov test revealed no significant differences between the empirical distribution of AOA values of blood serum and the normal distribution: $K\text{-}S_{exp.}$ = 0.05 less than $K\text{-}S_{teor.}$ = 0.08 and AS = 0.431 is more than p = 0.05 for n = 305 (Fig. 1).

3.2. Study of healthy volunteers.

In the group of 110 healthy volunteers, 38.0 ± 13.2 years old, the average level of serum AOA was 1.22 ± 0.28 mmol-eq l^{-1}. Table 1 presents the results of the analysis of blood serum AOA for men

and women separately, and the levels of AOA in blood serum, which was obtained in the different time of fasting period duration. The data in Table 1 show that the AOA of the serum did not differ significantly between men and women in group of healthy volunteers. The average AOA value of the blood serum of healthy volunteers, whose blood was taken from 7.00 till 08.30 am, is significantly (t-test) higher than the level of AOA of those volunteers, whose blood sample was taken later than 08.30 am (from 8.31 till 10.00), after the more prolonged fasting period. These results demonstrate the strict need to comply with the time of blood sampling for AOA analysis (the minimal duration of fasting period in morning before blood sampling). According to those results, volunteers whose blood sampling was performed at period from 07.00 till 08.30 were selected as the control group.

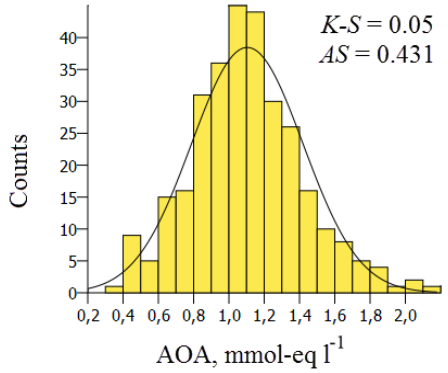

Figure 1. Histogram of frequency distribution of serum AOA values of healthy volunteers and patients involved in the study (total $n = 305$). The solid line shows the theoretical normal distribution curve. K-S: value of statistics Kolmogorov-Smirnov; AS: asymptotic significance.

Table 1. AOA of blood serum of healthy volunteers ($n = 110$).

Subgroup	AOA, mmol-eq l^{-1}	t-test
Men ($n = 42$)	1.21 ± 0.26	non-significantly difference
Women ($n = 68$)	1.22 ± 0.30	
Time of blood sampling:		
07.00–08.30 am ($n = 36$)	1.42 ± 0.29	significantly difference
8.31–10.00 am ($n = 74$)	1.12 ± 0.23	

3.3. Study of patients with cardiovascular diseases.

In the group of 170 patients 56.5 ± 16.7 years old with arterial hypertension, the average level of serum AOA was 1.09 ± 0.29 mmol-eq l^{-1}. The results of AOA measurements in comparison with other tests of patients with arterial hypertension are presented in the Table 2, where the patients are divided into subgroups according to the stage of HT (by the European Society of Cardiology Guidelines) and presence of CAD. In general, differences in the AOA values of blood serum in subgroups of patients with different stages of HT had a non-significantly (t-test) character. Only the serum AOA level in subgroup of patients with HT 1 stage was significantly higher than the serum AOA level in patients of other subgroups -HT stage 2, HT stage 3 and HT stage 3 complicated with CAD (t-test).

3.4. Study of patients with cancer.

In the group of 25 patients 57.4 ± 13.7 years old with cancer (verified adenocarcinomas of different locations), the average serum AOA level was 0.70 ± 0.21 mmol-eq l^{-1}. The results of AOA study patients with cancer are presented in Table 3, where the subgroups of patients with generalized and non-generalized forms of adenocarcinomas were formed. Serum AOA level of patients with generalized forms of cancer was slightly lower than this value observed in patients with non-generalized forms, however, this difference was not statistically significant (t-test).

Table 3. AOA of blood serum of patients with cancer ($n = 25$).

Subgroup	AOA, mmol-eq l^{-1}	t-test
Generalized forms of cancer ($n = 10$)	0.64 ± 0.15	non-significantly difference
Non-generalized forms of cancer ($n = 15$)	0.73 ± 0.24	

3.5. Correlation analysis.

Correlation analysis for a group of healthy volunteers has revealed only the weak correlation between serum AOA and other blood counts (biochemical and full blood count tests). In group of patients with HT the positive moderate correlation ($r = 0.48$) of serum AOA with serum uric acid level, and negative moderate ($r = –0.45$) correlation of serum AOA with the pulse wave velocity in the vessels of muscle type (Sm) was established. In the group of patients with cancer the positive noticeable ($r = 0.64$) correlation for serum AOA with the level of uric acid was obtained. The results of the correlative analysis for uric acid are presented in Fig. 2.

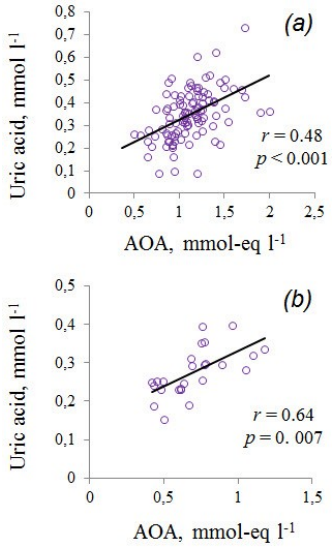

Figure 2. Pearson's correlation coefficient (r) between AOA and uric acid in the serum of patients with CVD (***a***) and patients with cancer (***b***).

Absolute values of AOA and uric acid concentration in serum for patient with CVD and cancer are shown in Fig. 3, from which it can be seen that these parameters are changed in different directions, which contradicts the results of the correlation analysis (Fig. 2).

3.6. Determination of preliminary reference values of serum AOA.

Fig. 4a shows the frequencies of AOA serum values distribution in three groups: control, patients with and patients with cancer. Analysis of these distributions allows us to conclude that no more than 10 % of patients with CVD fall into the group with AOA > 1.40 mmol-eq l^{-1}, and no more than 10 % of patients with cancer fall into the group with AOA > 0.95 mmol-eq l^{-1}. Based on this, we have identified three ranges of AOA for the purpose of assessing the state between healthy and ill patients

(Fig. 4b). Three areas of AOA values are presented for groups: (i) of healthy people (AOA ≥ 1.40 mmol-eq l^{-1}), (ii) patients who are at risk (AOA from 0.95 to 1.40 mmol-eq l^{-1}) and (iii) seriously ill patients (AOA < 0.95 mmol-eq l^{-1}). In the last second and particularly in the third groups detailed diagnostics and relevant treatment are required.

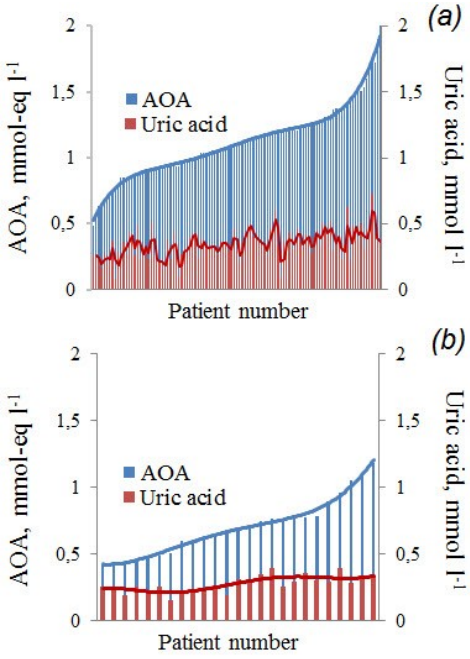

Figure 3. The values of the AOA and the concentration of uric acid in the serum of 170 patients with CVD (***a***) and 25 patients with cancer (***b***).

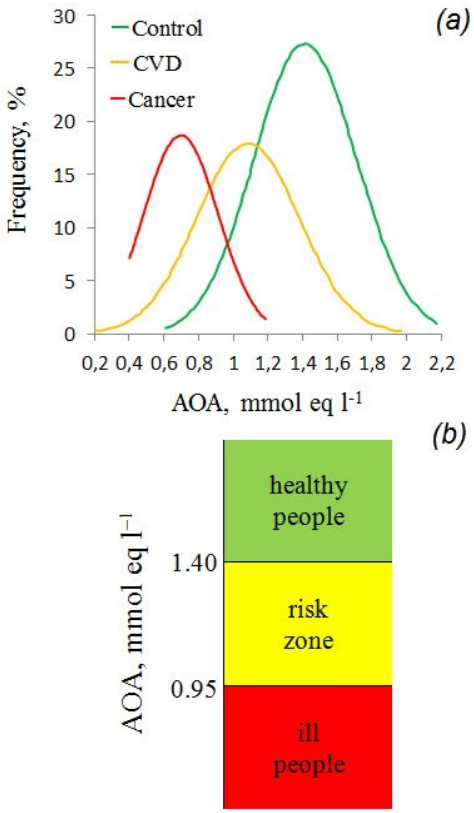

Figure 4. The frequency distribution of serum AOA values in control group (n = 36), group of patients with CVD (n = 170) and group of patients with cancer (n = 25) (***a***); diagram of serum AOA values for assessing the state "healthy – ill" people (***b***).

Table 2. The results of serum AOA, biochemical tests, full blood count test, pulse wave velocity and IMT in patients with arterial hypertension (n = 170).

Parameter	HT stage 1 (n = 49)	HT stage 2 (n = 63)	HT stage 3 (n = 24)	HT stage 3 complicated with CAD (n = 34)
AOA, mmol-eq l^{-1}	1.12 ± 0.23	1.01 ± 0.29	1.07 ± 0.33	1.25 ± 0.31
Glucose, mmol l^{-1}	5.5 ± 2.0	5.7 ± 1.8	5.9 ± 1.2	5.4 ± 0.7
HGB, g l^{-1}	148.5 ± 13.7	146.1 ± 14.3	140.2 ± 19.3	144.0 ± 13.7
Total cholesterol, mmol l^{-1}	4.8 ± 1.1	5.7 ± 1.3	5.4 ± 1.4	5.2 ± 1.4
LDL, mmol l^{-1}	2.6 ± 1.4	3.5 ± 1.0	3.8 ± 1.3	3.5 ± 1.5
HDL, mmol l^{-1}	1.1 ± 0.4	1.5 ± 0.6	1.7 ± 0.8	1.1 ± 0.3
WBC count, 10^9 l^{-1}	5.5 ± 1.3	6.8 ± 2.1	7.2 ± 1.6	7.3 ± 2.1
RBC count, 10^{12} l^{-1}	4.7 ± 0.5	4.9 ± 0.5	4.8 ± 0.5	4.8 ± 0.4
HCT, %	42.7 ± 2.3	42.4 ± 3.9	41.5 ± 4.0	42.1 ± 3.7
Uric acid, μmol l^{-1}	343.5 ± 103.8	319.8 ± 108.1	353.8 ± 193.7	378.7 ± 99.0
Creatinine, μmol l^{-1}	90.3 ± 18.0	86.0 ± 15.0	91.7 ± 12.9	96.2 ± 26.2
Sm, m s^{-1}	10.3 ± 8.8	16.5 ± 12.8	21.8 ± 10.8	11.7 ± 4.5
Se, m s^{-1}	13.2 ± 7.8	11.4 ± 7.2	20.9 ± 6.7	18.1 ± 12.1
Sm/Se	0.8 ± 0.5	1.9 ± 1.7	1.0 ± 0.3	0.7 ± 0.2
IMT L, mm	0.07 ± 0.02	0.07 ± 0.02	0.09 ± 0.02	0.11 ± 0.02
IMT R, mm	0.07 ± 0.02	0.07 ± 0.02	0.09 ± 0.02	0.10 ± 0.02

AOA: antioxidant activity; CAD: coronary artery disease (ischemic heart disease); CVD: cardiovascular diseases; HCT: hematocrit (packed cell volume); HDL: high density lipoproteins; HGB: hemoglobin; HT: arterial hypertension; IMT: intima-media thickness; IMT L and IMT R: intima-media thickness of left (L) and right (R) common carotid artery; LDL: low density lipoproteins; RBC: red blood cells count; Se: pulse wave velocity through the vessels of elastic type; Sm: pulse wave velocity through the vessels of muscle type; WBC: white blood cells count.

4. DISCUSION

The dependence of the AOA value of the healthy volunteers on the time of blood collection, of course, has fundamental importance for the correct determination of the normal values of this indicator, and for the better understanding of the mechanisms, changing the level of AOA. In our opinion, the lower AOA rates in volunteers whose blood was taken for analysis at a later time can be explained from the standpoint of the stress theory of Hans Selye. The stress factor in this case is short nutrient deprivation. It is known that the enhance in the intensity of redox reactions occurs when the body's demand in energy is increased, and the body reacts by the generating of energy during the excitation phase of the stress. In aerobic organisms, it is the energy of redox reactions. The generation of energy is accompanied by the formation of more ROS, which is normally compensated by the elevated antioxidants synthesis and/or its supplement with food. In conditions of starvation, their reserves become depleted and OS appears. This is confirmed by the data of V. Luschak [32], who demonstrated the parabolic nature of changes in antioxidants concentrations in dynamics at different stress phases. The big problem is to understand, what phase of stress we measure in

plasma, what part of oxidants appear here from the cells after intracellular OS, what happens at the border between cell and extracellular environment, and what is the dependence between AOA of plasma and quantity and properties of oxidants, synthesed in cell and left out of its biomembrane? This requires in-depth research and cannot be performed as part of this work.

Thus, the correct AOA levels can be obtained under the compliance of the correct time period for blood collection – in the early morning after waking up. Coming within a few hours of fasting first phase of stress (excitation phase) leads to an increase in the severity of OS and understated levels of AOA, probably due to depletion of stocks of antioxidants.

Certainly, more researches are needed on healthy patients with the modeling of dependence of AOA on the duration of the fasting period, the comparison of these data with the subsequent reaction of AOA for a meal.

The decrease in AOA levels in patients with cardiovascular pathology compared to the control group is a reflection of the OS, which plays an important role in CVD pathogenesis.

In many modern works, OS is considered as an important "pathogenetic link" in the pathology of the cardiovascular system in connection with the strengthening under its influence of nitric oxide (NO) and vascular endothelial disorders, which plays a key role in many diseases of the cardiovascular system, including atherosclerosis (atherogenesis), hypertension, coronary artery disease, thrombosis, heart failure and stroke [33–35].

In physiological conditions, the ROS does not have a significant impact on the production of nitric oxide [36]. In the presence of cardiovascular risk factors, the production of superoxide and other ROS leads to rapid inactivation of the nitric oxide, and its effects decrease, and the formation of high concentrations of the potent pro-oxidant peroxynitrite, which has a toxic effect on the endothelial cells [37] and leads to its dysfunction. OS is deeply involved in the pathogenesis of hypertension. These effects are mediated by the inactivation of nitric oxide by ROS in the vasculature and kidney, as well as H_2O_2–induced vascular remodeling [38].

In the study [39], a direct correlation between the average blood pressure and the concentration of products of reactions with thiobarbituric acid (TBARS), an important marker of oxidative degradation of lipids was demonstrated. It is noted that OS plays a key role in the different processes and mechanisms related to arterial hypertension, such as vascular remodeling, inflammation, endothelial dysfunction, the development of atherosclerotic plaques, the formation of aortic aneurysms, as well as vasoconstriction, endocrine dysregulation, and the activity of nervous and emotional factors that stimulate hypertension [40–44].

In our opinion, the decrease in the value of AOA in patients with hypertension reflects the presence of OS, damaging primarily the system of vascular regulation- the central link in the pathogenesis of hypertension, which is confirmed by our data on the existence of an inverse correlation between the value of AOA and the pulse wave velocity through the vessels of muscle type, i.e., showing the relationship of reducing the level of AOA with increasing stiffness of the vascular wall. It is important to know that such correlation was not obtained with the thickness of the intima-media complex of carotid arteries, as well as with the levels of total cholesterol and high-and low-density lipoproteins. It indicates, rather, that structural changes in vascular smooth muscles possibly have the greater influence on the level of AOA and on the severity of OS than concomitant lipid metabolism disorders. Probably it reflects the damaging effect of OS out of cell, in extracellular space, during the processes of vascular remodeling. This hypothesis, of course, needs to be tested and is likely to determine the key mechanisms of the impact of the OS on the cardiovascular system, its possible differences and regularities of their development in hypertension and atherosclerosis.

Uric acid is the final product of purine metabolism and holds a special place among water-soluble antioxidants [45] since it accounts for 35 to 65 % of the activity of antioxidant protection of lipoproteins from free radical oxidation. The antioxidant properties of uric acid are related to its ability to inactivate strong (strong transient) oxidants by means of an electron subsidy. This property is usually enhanced by the protonated state of the molecule of uric acid and the intermediate state of the free radical [46]. The antioxidant action of the uric acid is chelation of iron and copper ions that initiate free radical processes; it interacts with the free radicals ($O_2^{\bullet-}$, $OH^{\bullet}$), and peroxynitrite ($ONOO^-$); enhances the antioxidant effects of α-tocopherol and ascorbic acid, etc.

With the development of pathological conditions, the content of uric acid as a result of the response of the body increases significantly, and begin to show its pro-oxidant properties. In the Framingham study noted that elevated serum uric acid levels were associated with an increased risk of coronary heart disease and accelerated atherosclerosis in men aged 30–59 years [47]. Uric acid is able to stimulate the proliferation of smooth muscle cells of blood vessels, reduces the production of nitric oxide in endothelial cells. The question of correlation of concentration of uric acid with the total AOA of blood plasma is insufficiently studied, there is a contradiction between the expressed antioxidant properties of uric acid, and increase in the risk of development of CVD at its high concentration [48, 49]. Increasing the concentration of uric acid is considered as an adaptive response to the increasing OS in heart failure, which allows preserving the endothelial function through increasing the activity of extracellular superoxide dismutase [45]. In general, the AOA index did not show a direct dependence on the uric acid content, which can be a consequence of its pro-oxidant properties.

This confirms our postulate about the need for an integrated assessment of the OS.

A very important finding, which possibly can be considered as the diagnostic tool (the future studies are needed), is a significant reduction of AOA of blood serum of patients with malignant neoplasms in compare to the control group.

The malignant neoplasms growth in humans is associated with complex processes at the cellular and molecular levels, which are caused by various endogenous and exogenous causes, and the OS mechanisms play an obvious role in their implementation. Thus, ROS can induce mutagenesis, which can cause malignant transformation of the cells, using a variety of possible mechanisms (breaking of DNA chains, formation of DNA-adducts, activation of oncogenes, inactivation of tumor suppressor genes, DNA modification, etc.). Interaction of ROS with tumor suppressor genes and proto-oncogenes indicates their role in the occurrence of different types of human cancer. OS can stimulate carcinogenesis

also through epigenetic mechanisms that affect proliferation cell differentiation and apoptosis, oxidant changes in protein structure and functions, that, in particular can lead to transform normal cells into malignant [50–52]. Some ROS (for example, malonic dialdehyde), have a high cytotoxic and inhibitory action on protective enzymes, also act as the tumor promoters or co-carcinogenes, which was shown in some types of malignant tumors (breast cancer) [53]. Despite the large number of works, studying OS and antioxidants role in cancer, at the present time it does not allow to come to general conclusions about their role in cancer growth. Despite the malignant tumors metabolism has common patterns; the assertion that the sharp decrease in AOA can be a possible sign of tumor growth must be approached very carefully despite the first promising results of this study. The special well-designed clinical trials are needed to support this hypothesis.

Special research is needed for understanding the mechanism of those processes and their role in pathogenesis of neoplasms.

5. CONCLUSIONS, PROBLEMS, TRENDS

It is an accepted idea this time, that OS is an essential part of pathological processes in a living organism. The measure of its manifestation is the reducing of the AOA of biological fluids and tissues. As the origin and effect of OS are determined by the ox-red chemical reactions, the use of such kind of the reaction as signal generating one is important in the used analysis method. That is why HPM that includes correctly chosen mediator system is preferable to be used in laboratory clinic analysis for AOA determination.

The paper presents the results of own measurements of AOA of blood serum in healthy volunteers and patients with various diseases. The lower levels of AOA of blood serum in patients with CVD and malignancy in compare with the healthy volunteers are observed. Preliminary reference values of AOA blood serum are established. Thus, three areas of AOA values are observed in the groups:

- healthy people, AOA ≥ 1.40 mmol-eq l^{-1};
- who are at risk, 0.95 mmol-eq l^{-1} ≤ AOA < 1.40 mmol-eq l^{-1};
- ill people, AOA < 0.95 mmol-eq l^{-1}.

In the process of research, a very interesting fact was noted: the decrease in the AOA during fasting, which indicates the appearance of the OS and the need to observe a temporary regime during the analysis. These findings can be used in mass screening in medicine.

It is extremely important, that low AOA can be considered as a potential "red flag" because it was obtained in patients with malignant tumors – the detection of low AOA values in patients during screening can provide the physician with additional information suggesting a diagnostic search for malignancy.

The findings allow suggesting that the determination of AOA of biological fluids holds considerable promise for monitoring OS of the whole organism and its systems, it opens up new opportunities in expanding the use of clinical laboratory analysis in the evaluation of health state.

Most biochemical reactions in a living organism are heterogeneous, occurring on biointerfaces whose total surface is enormous. Whereas the implementation of the biochemical chain of ROS formation begins and mostly occurs in mitochondria as a heterogeneous process, and the reactions of ROS and RNS with antioxidants are hetero - or homogeneous depending on the nature of the antioxidants (hydro - or lipophilic) and place of their course. Taking into account the last circumstance, as well as:

- common redox interactions-reactions of oxidants with antioxidants,
- the relationship of these reactions with OS and the latter with AOA,
- a significant role of OS in pathologies,
- sufficient ease of monitoring OS by the magnitude of AOA,

it can be assumed that an in-depth and comprehensive study of AOA patients with various diseases will provide at least indirect information about the role of biointerface in the pathogenesis of various diseases.

Despite the apparent clarity of the above representations, it is necessary to point out the complexity and multifactorial nature of the above reactions. On closer examination, it is necessary to consider how pronounced is OS within cells, and how many products and what kind of them are released into the bloodstream (extracellular space) and activate the security system. This depends on the state and functioning of the barrier biological membranes, and the response of the antioxidant defense system to the release of oxidants, and the patterns of damage to the biomembranes, ie. from a complex of interactions of oxidants and antioxidants in different environments and at the interface of these environments, the resulting integral indicator is the AOA of biological environments (blood plasma, seminal fluid, saliva, skin, etc.) The clear understanding of processes of interaction of oxidants, leading to the emergence of OS, and antioxidants, determining the activity of the antioxidant defense system, in cells, intercellular environment and membranes and at the interface require serious research. The development of OS and AOA monitoring methods can help to optimize the treatment process of some diseases, for example, to find the right time and place of administration of antioxidants in cancer.

6. REFERENCES

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