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DIAGNOSTIC SENSITIVITY AND SPECIFICITY OF PRO- AND ANTIOXIDANT SYSTEM MARKERS IN MEN WITH ERECTILE DYSFUNCTION FOLLOWING COMBAT-RELATED INJURIES[1]

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Introduction. To study the predictive ability of using the quantitative value of the level of a single indicator to detect oxidative stress, ROC analysis was performed with the calculation of the optimal cut-off threshold selected according to the criterion of maximum balance between sensitivity and specificity.

The aim of this research is to analyze the diagnostic sensitivity and specificity of pro- and antioxidant system indicators in men injured as a result of combat operations.

Materials and methods. 116 samples of peripheral blood lymphocytes and serum from men, which were divided into two groups by age, were analysed for the sensitivity and specificity of pro- and antioxidant system indicators.

Results. The results of ROC analysis for indicators of the pro- and antioxidant system of lymphocytes and peripheral blood serum demonstrated their predictive ability for detecting oxidative stress. Malondialdehyde of blood lymphocytes for the age group 20–39 years, reduced serum glutathione and total glutathione of blood lymphocytes for all examined men were characterized by excellent discriminatory ability. Malondialdehyde of blood lymphocytes for the age group 20–39 years showed itself as a highly sensitive marker with a slightly lower specificity value. Reduced glutathione of blood serum of men of all age groups is characterized as a highly specific marker with a slightly lower sensitivity value. Total glutathione of blood lymphocytes is simultaneously characterized by high sensitivity and specificity for all examined men.

Conclusions. The concentration of malondialdehyde in blood lymphocytes and the levels of total and reduced glutathione in blood serum were the most significant predictors for the detection of oxidative stress based on the ROC analysis. These markers demonstrate high sensitivity and specificity as indicators of the pro- and antioxidant balance.

Keywords: erectile dysfunction, combat trauma, pro- and antioxidant system, ROC analysis.

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ДІАГНОСТИЧНА ЧУТЛИВІСТЬ ТА СПЕЦИФІЧНІСТЬ ПОКАЗНИКІВ ПРО-/АНТИОКСИДАНТНОЇ СИСТЕМИ У ЧОЛОВІКІВ З ЕРЕКТИЛЬНОЮ ДИСФУНКЦІЄЮ, ЯКІ ПОСТРАЖДАЛИ ВНАСЛІДОК БОЙОВИХ ДІЙ
Державне некомерційне підприємство «Львівський національний медичний університет імені Данила Галицького», Львів, Україна

Для виявлення оксидативного стресу у чоловіків, які отримали поранення внаслідок бойових дій, було проведено ROC-аналіз з розрахунком оптимального порогу, вибраного за критерієм максимального балансу між чутливістю та специфічністю показників про-/антиоксидантної системи: малонового діальдегіду, загальної антиоксидантної активності, концентрації відновленого та загального глутатіону. Встановлено, що концентрація малонового діальдегіду в лімфоцитах крові та рівні загального та відновленого глутатіону у сироватці крові є найбільш значущими предикторами для виявлення оксидативного стресу. Ці маркери демонструють високу чутливість та специфічність як показники про-/антиоксидантного балансу.

Ключові слова: еректильна дисфункція, бойова травма, про-/антиоксидантна система, ROC-аналіз.

[1] *The study presented in this article was conducted with the support of the National Research Fundation of Ukraine. Name of the project “Improvement of diagnosis and treatment of disorders of sexual and reproductive functions of men suffered as a result of hostilities” (registration number 2022.01/0151).*

Introduction

Research into the physical, reproductive, and mental health of men who have survived combat trauma is part of a broad field of research into the stress response, which manifests itself at all levels of the body's organization. Trauma is characterized by a wide range of body reactions to injurious factors, involving all body systems, which leads to pronounced changes in the prooxidant-antioxidant balance [1; 2].

Injuries sustained in modern wars are mostly multisystemic [3; 4]. Post-traumatic stress disorder, depressive states, and post-traumatic chronic pain may develop as a result of combat injuries [4].

There is growing evidence that posttraumatic stress disorder is associated with higher rates of erectile dysfunction, decreased sexual desire, and premature ejaculation [4]. Men who have served in combat experience impaired sexual health, including decreased libido, difficulty maintaining arousal, and the ability to achieve orgasm [3]. Combat-related injuries can also have significant consequences for male fertility, as they typically serve during their peak sexual activity years [4].

Modern scientific understanding of erectile dysfunction (ED) indicates the predominant secondary nature of sexual disorders to the diseases that cause them. Among the many pathological conditions that precede or complicate ED, neurotic mental disorders, blood vessel diseases, metabolic disorders, and partial androgen deficiency are the most common, especially in combatants [5–9]. Injury is not only physical, but also psychological, and often prolonged. ED causes significant psychological distress for men, which subsequently affects the quality of life of their partners and families [1]. Although ED is not life-threatening, ED and physical health are closely linked [6–8]. For example, ED may be a predictor of cardiovascular disease, dementia, Parkinson's disease, and premature death from various causes [6–8]. Today, the incidence of ED is increasing among younger men [9].

The connection between ED and oxidative stress has piqued the curiosity of many researchers [1].

Revealing oxidative stress in the body is important already in the early, sometimes preclinical, stages of various diseases, when assessing the severity of the pathological process. In this regard, the use of the ROC analysis (Receiver Operating Characteristic) method is promising [10; 11], which allows assessing the diagnostic sensitivity and specificity of pro- and antioxidant status indicators.

Sensitivity is the probability of a positive result for a patient; it characterizes the ability of the sample to detect a pathological condition (disease). Specificity is the probability of a negative result in a healthy person; it characterizes the ability of the sample to detect the absence of the disease [12]. To establish the specificity and sensitivity of the indicators in determining the likely development of oxidative stress, the ROC analysis module was used. The basis of this analysis is the construction of the so-called ROC curve, which is constructed in the coordinates "sensitivity – 1 – specificity" and shows the dependence of the number of correctly classified positive examples on the number of incorrectly classified negative examples. ROC analysis allows you to objectively determine the diagnostic significance of an individual test based on the AUC values (area under the curve). AUC is an integral indicator of the predictive effectiveness of a marker, which determines the probability that the value of a marker feature, or biomarker, in a randomly selected patient is greater than in a randomly selected healthy person [11].

The aim of this research is to analyze the diagnostic sensitivity and specificity of pro- and antioxidant system indicators in men injured as a result of combat operations.

Materials and Methods

The work was based on the results of a study of the indicators of the pro- and antioxidant system of lymphocytes and blood serum in 68 individuals injured as a result of combat operations (shrapnel and bullet wounds, mostly polysystemic), divided into two groups by age, since ED is closely correlated with the age of men [1]. The first group included 42 men aged 20–39 years, and the second group consisted of 26 men aged 40–53 years. To isolate lymphocytes, peripheral blood was collected from patients after the completion of the main course of treatment for combat trauma and rehabilitation and clinical examination, before prescribing them a course of treatment. The control group consisted of 48 practically healthy men: 30 men aged 20–39 years and 18 men aged 40–53 years. The men reported no complaints related to sexual dysfunction, cardiological, neurological or endocrinological diseases.

ROC curves were calculated to determine the optimal cut-off values of biochemical parameters of peripheral blood lymphocytes and serum for predicting the development of oxidative stress in combat casualties. Calculations were performed for the following biochemical parameters – malondialdehyde (MDA) content, total antioxidant activity (TAA), reduced (GSH) and total (GSHt) glutathione concentrations. The area under the curve (AUC) was calculated using a receiver operating characteristic curve (ROC curve) to determine the predictive ability of pro- and antioxidant system indices of lymphocytes and serum of patients who suffered as a result of hostilities. To construct the ROC curve, the sensitivity of the test (true positives) is plotted on the Y axis against its false positive rate (1 − specificity) on the X axis for all possible cutoff points.

The sensitivity (Se) and specificity (Sp) of peripheral blood lymphocytes and serum indices were calculated for all possible cut-off points to find the optimal threshold value, which is the cut-off value. The point on the ROC curve, which corresponds to the maximum value of the Youden index, determines the threshold of the test at which the best ratio between the number of correctly identified patients (sensitivity) and the number of correctly identified healthy individuals (specificity) is achieved. The area under the ROC curve, at the same time, is an integral indicator that reflects the overall ability of the test to distinguish between two conditions regardless of the choice of a specific threshold [10]. In daily clinical practice, the cut-off value classifier of diagnostic tests, i.e. the normal level of the indicator, is used [11].

An additional method for evaluating a diagnostic test is the Likelihood Ratio (LR) [12]. A positive likelihood ratio (+LR) indicates how many times the probability of having the disease increases in a patient with a positive test result, while a negative likelihood ratio (-LR) indicates how many times the probability of having the disease decreases in a patient with a negative test result. ROC curves demonstrate the discriminatory power of a diagnostic test. The further the curve is in the upper left corner, the better the test. The area under the ROC curve AUC is a measure of the diagnostic power of the test. A test that does not distinguish between sick and healthy people will have an AUC of 0.5 or less, so considering a curve with such AUC values is inappropriate. An ideal test is characterized by an AUC of 1.0. The AUC value interval is divided by model quality into: 0.9–1 – excellent; 0.8–0.9 – very good; 0.7–0.8 – good; 0.6–0.7 – average; 0.5–0.6 – unsatisfactory [12].

All patients were fully informed about the medical procedures and consented to treatment and the use of their data. The study was performed in accordance with the Declaration of Helsinki for Human Research (WMA, 2013), and the study programme was approved by the Bioethics Committee of Danylo Halytsky Lviv National Medical University (protocol No. 7 of 26.06.2023).

Data analysis was performed using MedCalc® Statistical Software version 22.018 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2024). All P values were two-tailed, and $P < 0.05$ indicated that the difference was statistically significant.

Research results and their discussion

For qualitative analysis of the diagnostic significance of markers of the pro- and antioxidant system of lymphocytes and blood serum, ROC analysis was used. The results of the analysis of ROC curves of various indicators of the pro- and antioxidant system of lymphocytes and blood serum are summarized in Tables 1 and 2.

The ROC analysis for serum malondialdehyde (MDA) is presented in Figure 1. Serum MDA supports the diagnostic profile, showing an area under the ROC curve of 0.814 (95% CI: 0.705 – 0.896) for the age group 20–39 years and

Table 1

Results of ROC curve analysis of pro- and antioxidant system indicators of blood lymphocytes and blood serum for the age group of patients with erectile dysfunction aged 20–39 years, injured as a result of hostilities

Indicators	MDA (blood serum)	GSH (blood serum)	MDA (blood lympho-cytes)	TAA (blood lympho-cytes)	GSH (blood lympho-cytes)	GSHt (blood lymphocytes)
AUC	0.814	0.907	0.910	0.736	0.851	0.982
95% CI	0.705–0.896	0.815–0.963	0.819–0.965	0.619–0.833	0.747–0.924	0.918–0.999
P, significance level regarding AUC=0.5	<0.0001	<0.0001	<0.0001	0.0002	<0.0001	<0.0001
SE	0.0501	0.0348	0.0384	0.0625	0.0469	0.0128
cut-off	>35 µmol/mg protein	≤16 nmol/mg protein	>67 µmol/mg protein	≤3.3 µmol/l	≤26 µmol/l	≤42 µmol/l
Se, %	69.05	85.71	95.24	80.95	88.10	97.62
Sp, %	86.67	80.00	76.67	63.33	70.00	93.33
Youden Index, J	0.5571	0.6571	0.7190	0.4429	0.5810	0.9095
+LR	5.18	4.29	4.08	2.21	2.94	14.64
-LR	0.36	0.18	0.062	0.30	0.17	0.026

Note: AUC – area under the ROC curve; CI – confidence interval); SE – Standard Error; Se – Sensitivity; Sp – Specificity).

Table 2

Results of the analysis of ROC curves of the pro- and antioxidant system of lymphocytes and blood serum for the age group of patients with erectile dysfunction aged 40–53 years, injured as a result of hostilities

Indicators	MDA (blood serum)	GSH (blood serum)	MDA (blood lympho-cytes)	TAA (blood lympho-cytes)	GSH (blood lympho-cytes)	GSHt (blood lymphocytes)
AUC	0.833	0.957	0.884	0.799	0.857	0.927
95% CI	0.690–0.928	0.849–0.995	0.751–0.960	0.651–0.905	0.718–0.944	0.807–0.984
P, significance level regarding AUC=0.5	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Standard Error	0.0624	0.0268	0.0638	0.0703	0.0583	0.0527
cut-off	>36 µmol/mg protein	≤14 nmol/mg protein	>72 µmol/mg protein	≤2.4 µmol/l	≤21 µmol/l	≤41 µmol/l
Se, %	76.92	84.62	96.15	65.38	80.77	96.15
Sp, %	77.78	94.44	83.33	88.89	83.33	88.89
Youden Index, J	0.5470	0.7906	0.7949	0.5427	0.6410	0.8504
+LR	3.46	15.23	5.77	5.88	4.85	8.65
-LR	0.30	0.16	0.046	0.39	0.23	0.043

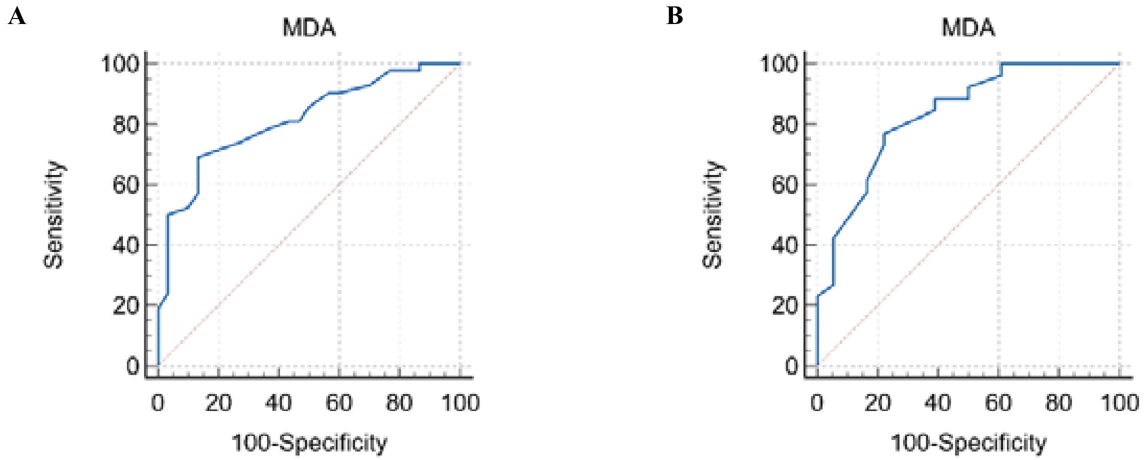

Fig. 1. ROC curve for serum malondialdehyde (MDA) as a marker of oxidative stress (A – age group 20–39 years, B – age group 40–53 years)

0.833 (95% CI: 0.690 – 0.928) for the age group 40–53 years. The diagnostic value of the test is very good for both age groups. The sensitivity values are excellent for both age groups. Thus, the MDA test for the age group 20–39 years is able to detect only 69.05% of men with high levels of oxidative stress, while 30.95% with pathology are determined as healthy, which is a false positive result. The detection of oxidative stress for men of the older age group (40–53 years) is 76.92%. The proportion of true negative cases that were correctly identified by the MDA test for the younger group is 86.67%, while the specificity result for the older group of subjects is 77.78%. The optimal cut-off threshold may indicate that the development of oxidative stress is observed in men if the concentration of MDA in the blood serum is >35 μmol/mg protein with a Youden index value of 0.5571 and >36 μmol/mg protein with a Youden index value of 0.5470, respectively, for each of the age groups and the specified values of specificity and sensitivity. An additional method of evaluating a diagnostic test is the likelihood ratio. A value of +LR for the MDA age group of 20–39 years indicates that the test confirms the presence of a pathological condition, while for the age group of 40–53 years it weakly confirms the presence of a pathological condition. A negative likelihood ratio conditionally excludes the presence of a pathological condition.

The results of the ROC analysis for serum reduced glutathione concentrations are presented in Figure 2. The optimal cutoff value for the age group 20–39 years is ≤16 nmol/mg protein with a sensitivity of 85.71% and a specificity of 80%, while the Youden index of 0.6571 indicates that the test has some diagnostic value, but is not ideal. The AUC is 0.907 (95% CI: 0.815 – 0.963, $P < 0.0001$), which is consistent with the excellent quality of the model. Similarly, to the previous age group the results for the age group 40–53 years are characterized by excellent discriminatory ability with AUC 0.957 (95% CI: 0.849–0.995, $P < 0.0001$) and an optimal cut-off point ≤ 14 nmol/mg protein with a sensitivity of 84.62% and a specificity of 94.44%. The Youden index of 0.7906 indicates that the test has a high diagnostic value. The high +LR value for the age group 40–53 years of the examined men means that a positive test result significantly increases the likelihood of developing oxidative stress.

The ROC analysis for MDA content in blood lymphocytes demonstrated excellent predictive quality of

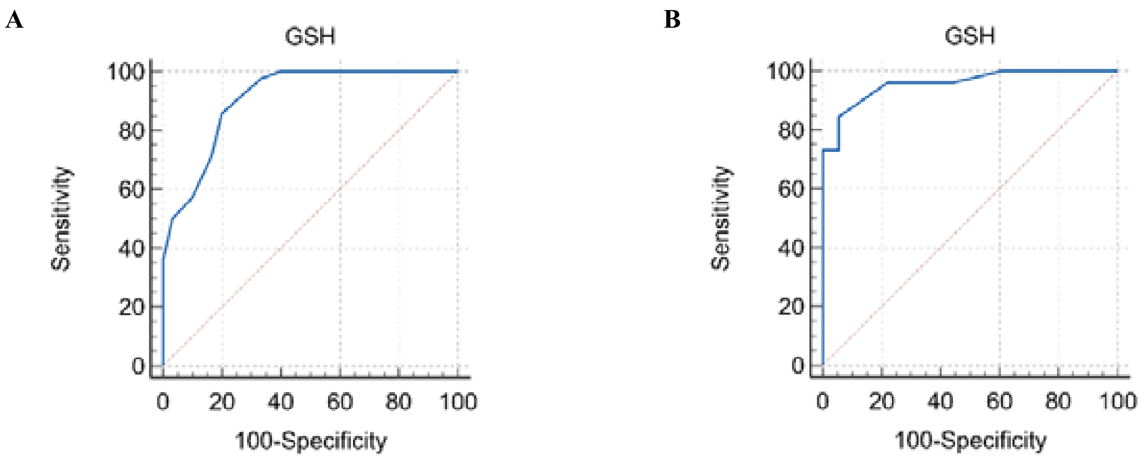

Fig. 2. ROC curve for the content of reduced glutathione (GSH) in blood serum as a marker of antioxidant status (A – age group 20–39 years, B – age group 40–53 years)

the test for men aged 20–39 years, as confirmed by the AUC value of 0.910 (95% CI: 0.819–0.965, $P < 0.0001$, Figure 3 A). For the age group 40–53 years, the area value is slightly lower at 0.884 (95% CI: 0.751–0.960, $P < 0.0001$), which corresponds to a very good predictive quality of the model (Figure 3 B). The sensitivity and specificity of MDA in blood lymphocytes for determining the development of oxidative stress were 95.24% and 76.67% for the first age group with an optimal threshold value of > 67 μmol/mg protein. The proportion of true positives was 96.15%, the proportion of negative results in the absence of oxidative stress was 83.33% for the second group with an optimal cutoff threshold of >72 μmol/mg protein, respectively. The high value of the Youden index indicates that the test for the content of MDA in blood lymphocytes is quite effective in distinguishing individuals with a pathological condition.

ROC curve analysis for total antioxidant activity (TAA) of blood lymphocytes determined AUC 0.736 (95% CI: 0.619–0.833, $P=0.0002$) for patients aged 20–39 years and AUC 0.799 (95% CI: 0.651–0.905, $P<0.0001$) in men aged 40–53 years (Figure 4). The value indicates a good overall diagnostic test performance. The optimal cut-off point for a given sensitivity of 80.95% and specificity of 63.33% is ≤3.3 μmol/L for the examined men with pathology of the younger age group. Whereas, for the older age group, the optimal cut-off point is lower and is ≤2.4 μmol/L with a given sensitivity of 65.38% and specificity of 88.89%. The probability of a positive test result, i.e., the detection of a pathophysiological condition, is higher for younger patients, while the probability of a negative test result in the absence of the disease is higher in older examined men. The positive likelihood ratio is higher in the 40–53 age group compared to the 20–39 age group, indicating the ratio of the probability of obtaining a positive result in a patient with the disease to the probability of obtaining a positive result in a healthy subject. Similarly, the negative likelihood ratio is higher in the 40–53 age group compared to the 20–39 age group, indicating the ratio of the probability of obtaining a negative result in a patient with the disease to the probability of obtaining a negative result in a healthy examined patient.

ROC analysis indicates that the content of reduced glutathione in blood lymphocytes for the age group 20–39 years very well distinguishes between men with

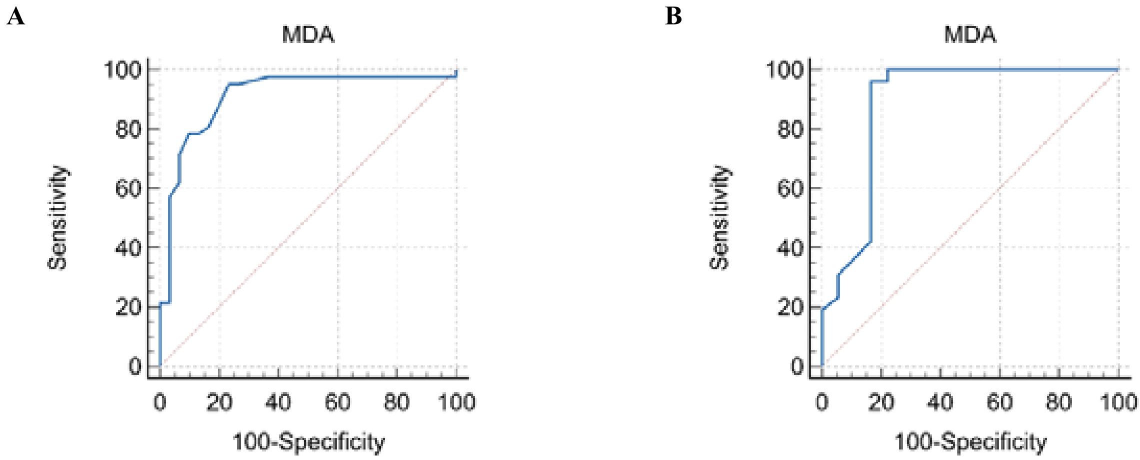

Fig. 3. ROC curve for malondialdehyde (MDA) in blood lymphocytes as a marker of oxidative stress (A – age group 20–39 years, B – age group 40–53 years)

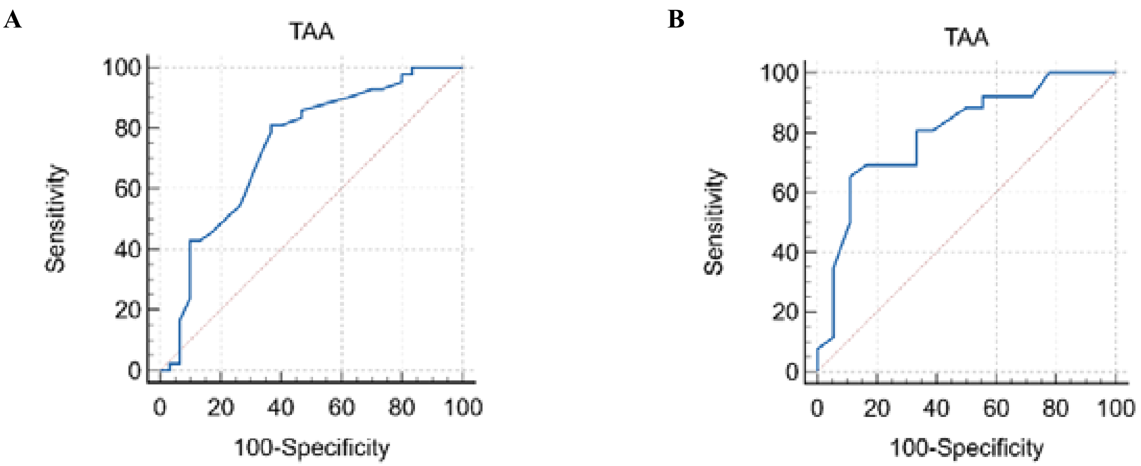

Fig. 4. ROC curve for total antioxidant activity (TAA) of blood lymphocytes as a marker of antioxidant status (A – age group 20–39 years, B – age group 40–53 years)

pathology and normal condition with 88.10% and 70% sensitivity and specificity, for which the optimal cut-off point is ≤26 μmol/L. Minor differences were determined in the ROC curve parameters for men with oxidative stress aged 40–53 years, namely sensitivity of 80.77% and specificity of 83.33%, which set the optimal cut-off threshold of ≤21 μmol/L. The AUC for GSH content in blood lymphocytes is: 0.851 (95% CI: 0.747–0.924, P<0.0001) and 0.857 (95% CI: 0.718–0.944, P<0.0001), respectively, which according to the expert scale for the values of the area under the ROC curve, the model is classified as very good (Figure 5). The Youden index for GSH content is 0.5810 and 0.6410.

The AUC values for GSHt content in blood lymphocytes of men aged 20–39 years are 0.982 (95% CI: 0.918–0.999, P<0.0001) and 0.927 (95% CI: 0.807–0.984, P<0.0001) for the age group 40–53 years, which allows classifying diagnostic tests with excellent diagnostic value (Figure 6). The Youden indices, which serve as an indicator of the quality of the tests, for total glutathione are 0.9095 and 0.8504 for the two age groups, respectively, and indicate that the test is able to distinguish between healthy and sick people. The optimal cut-off threshold for each screening test, which has the best combination of sensitivity and specificity, is located on the "knee" of the graph. For the content of GSHt lymphocytes in blood, the optimal cut-off point for the younger age group of men is ≤42 μmol/L with maximum sensitivity values of 97.62% and specificity of 93.33%. The optimal cut-off point value is ≤41 μmol/L with sensitivity of 96.15% and specificity of 88.89%. The value of +LR equal to 14.64 indicates that a positive result of the GSHt test in blood lymphocytes is a sign of the disease, and -LR = 0.026 indicates that a negative test result completely excludes pathology.

The antioxidant status of the cell is an important factor in the pathogenesis of a number of diseases. Most pathological conditions and diseases are characterized by increased free radical processes and a decrease in antioxidant capacity. This fully applies to combatants with combat trauma [1; 2]. Injuries, shrapnel and bullet wounds, as well as nervous disorders can cause oxidative stress. The last one occurs as a result of an imbalance between the generation of reactive oxygen species (ROS) and the activity of antioxidant defense [1; 13–16].

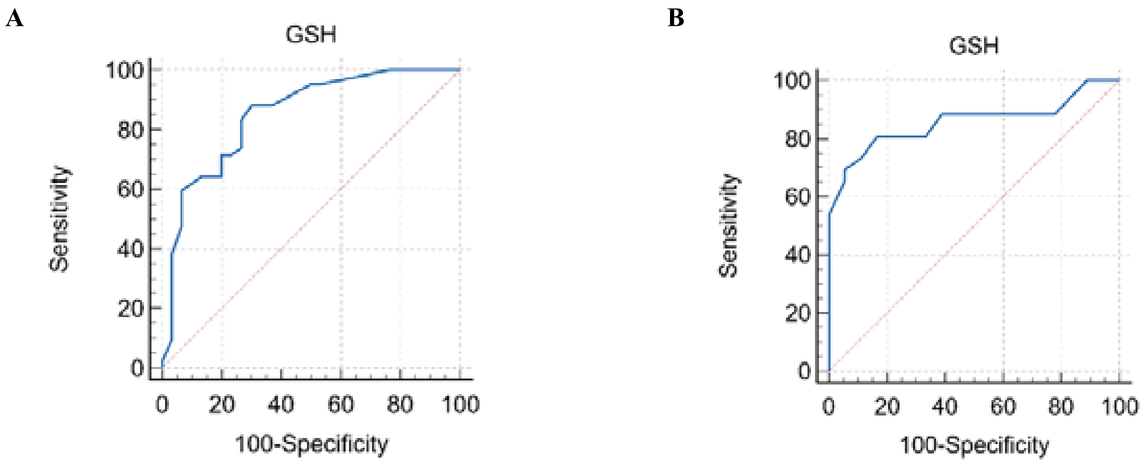

Fig. 5. ROC curve for reduced glutathione (GSH) of blood lymphocytes as a marker of antioxidant status (A – age group 20–39 years, B – age group 40–53 years)

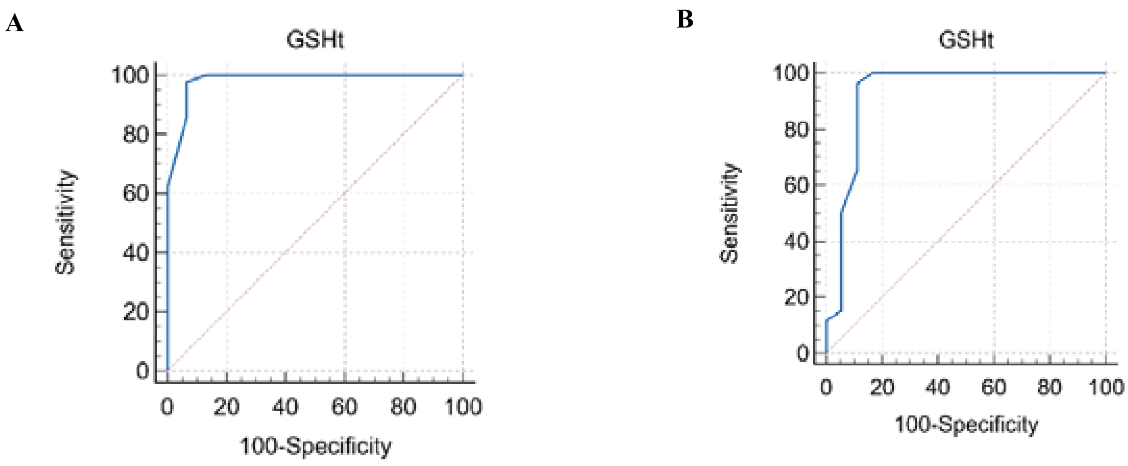

Fig. 6. ROC curve for total glutathione (GSHt) of blood lymphocytes (A – age group 20–39 years, B – age group 40–53 years)

An important factor in the pathogenesis of a number of diseases is the ratio of the antioxidant system to prooxidant indicators, which is called the antioxidant status of the cell. It is known that the pro- and antioxidant systems are in a state of dynamic equilibrium, and a violation of this balance causes oxidative stress. Most pathological conditions and diseases are characterized by increased free radical processes and a decrease in antioxidant capacity [13–16]. The content of malonaldehyde, which is the end product of lipid peroxidation, is considered a marker of oxidative stress, reflecting the degree of lipid damage due to the action of ROS [15; 16]. The most intensive free radical processes occur in male pathozoospermia, in particular leukocytospermia. This established fact is explained by the fact that leukocytes can generate ROS in high concentrations [16].

Given that GSH not only protects cells from free radicals, but also generally determines the redox characteristics in the intracellular environment, a decrease in the content of GSH indicates the suppression of antioxidant capacity. This leads to an increase in the formation of toxic ROS, depletion of the cellular pool of bioantioxidants and increased lipid peroxidation. The above can cause disruption of cell structure and function. The antioxidant properties of GSH are due to its direct interaction with toxic ROS and the functioning of glutathione-dependent enzymes, such as glutathione peroxidase and glutathione-S-transferase, which use GSH as a substrate. Therefore, depletion of its pool leads to inhibition of the activity of glutathione-dependent enzymes – glutathione peroxidase and glutathione-S-transferase. A decrease in the activity of enzymes of the glutathione system indicates the depletion of the compensatory mechanisms of the cell.

It is known that the formation of GSH occurs not only as a result of its de novo biosynthesis, but also with the participation of glutathione reductase – an enzyme that ensures the reduction of GSSG to GSH in the presence of NADPH as a source of reducing equivalents. The consequence of the decrease in GSH may be the accumulation of free radicals, which increases with the subsequent formation of the OH radical and damage to high-molecular structures, in particular DNA [17]. It is known that glutathione reductase uses NADPH as a substrate, the content of which decreases under conditions of oxidative stress.

Oxidative stress plays a key role in the development of ED. In response to cholinergic stimulation of the sympathetic nerve, nitric oxide (NO) is produced by the endothelial cells of the corpus cavernosum, which leads to an increase in the synthesis of cyclic GMP (cGMP), which in turn relaxes smooth muscle and facilitates erection [16]. Therefore, any disruption of this mechanism may contribute to ED. Under normal circumstances, various antioxidant enzymes and antioxidants maintain an optimal concentration of reactive oxygen species (ROS), thus maintaining redox balance. However, when ROS production exceeds normal levels or when antioxidant availability is reduced, oxidative stress occurs [13]. Excessive ROS impair NO production [18] and lead to both neurogenic and vasculogenic dysfunction, ultimately leading to ED [19]. Furthermore, the interaction between NO and excessive ROS can generate peroxynitrite, which inactivates superoxide dismutase (SOD) and establishes a reinforced positive feedback loop [14]. Despite the promising effects of phosphodiesterase-5 (PDE5) inhibitors in the symptomatic treatment of ED by inhibiting the hydrolysis of cGMP to GMP, these drugs are expensive and may cause unexpected side effects. In addition, these treatments are unable to address the progression of cardiovascular and neurological complications, which are common manifestations of systemic oxidative stress [20]. Thus, restoring the systemic pro- and antioxidant balance may be an important approach for the treatment and prevention of ED.

Conclusions

ROC analysis allowed us to establish the parameters that have the greatest predictive ability for the indicators of the pro- and antioxidant system of lymphocytes and blood serum for men sustaining combat-related injuries (shrapnel and bullet wounds), which can be used as additional criteria for detecting and confirming the development of oxidative stress. According to ROC analysis, the highest predictive ability for detecting oxidative stress was the content of malondialdehyde in blood lymphocytes, the content of total and reduced glutathione in blood serum, which are highly sensitive and highly specific markers of the pro- and antioxidant system.

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