


The Analysis of Oxidative Stress Markers May Increase the Accuracy of the Differential Diagnosis of Alzheimer's Disease with and without Depression

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Introduction: The aim of work is to assess the usefulness of oxidative stress parameters in the differential diagnosis of dementia of the Alzheimer's type and dementia of the Alzheimer's type with coexisting depression.

Methods: The study involved three groups of people: patients with Alzheimer's disease (AD) (AD; N=27), patients with Alzheimer's disease and depression (D) (AD+D; N=30), and a control group that consisted of people without dementia and without depression (C; N=24). The assessment of cognitive functioning was carried out using among alia, Auditory Verbal Learning Test and Verbal Fluency Test. Furthermore, we determined the activity of superoxide dismutase (SOD-1) and superoxide anion radical.

Results: Multiple models with different combinations of independent variables showed that SOD together with Rey delayed recall were the best significant predictors of AD with the area under curve (AUC) of 0.893 ($p = 0.001$) and superoxide anion radical ($O_2^{\cdot-}$) together with verbal fluency – sharp objects were the best significant predictors of AD +D diagnosis with the AUC of 0.689 ($p = 0.034$).

Conclusion: This study confirmed the value of neuropsychological diagnosis and analysis of oxidative stress markers in the diagnosis of AD and major depressive disorder (MDD) in the course of AD. The combination of the use of biochemical markers and neuropsychological tests seems particularly important for differential diagnosis.

Keywords: Alzheimer's disease, depression, oxidative stress, superoxide dismutase, superoxide anion radical, verbal fluency, Auditory Verbal Learning Test

Introduction

Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder related to age characterized by several neuropathological events including amyloid and tau deposition, neuronal loss, and synaptic dysfunction.^{1,2} These changes lead to deficits in the area of cognitive and behavioral functioning.³ Alzheimer's disease is now considered the most common cause of dementia, accounting for about 70% of all dementias, not only to the high frequency of diagnosis of this disease but also to the relatively long survival of patients.⁴⁻⁷ The prevalence of this type of dementia increases with age.⁴⁻⁷ Depression is among the most common neuropsychiatric disorder affecting more than 50% of patients diagnosed with AD.^{8,9} The etiology of both conditions may be influenced by various factors: genetics, toxins (endogenous and exogenous), bacterial and viral infections.^{8,9} Growing evidence suggests that oxidative stress, the lack or limited ability of the body to inhibit

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uncontrolled free radical reactions, plays an extremely important role in the pathogenesis of both Alzheimer's disease and depression.^{10,11}

Free radicals are molecules containing at least one unpaired electron on the atomic orbital.¹² They are very reactive because they seek to pair electrons by abstracting them from or donating them to other molecules.¹² A significant proportion of free radicals (approximately 90%) generated in the body is formed by reactions occurring in the respiratory chain in the mitochondria of cells.¹² The remainder (about 10%) is produced by physiological reactions occurring in different cell structures. Free radicals are also produced by affecting the external factor cell such as ultraviolet and ionizing radiation, ultrasound, elevated temperature or tobacco smoke, as well as in the metabolism processes of various exogenous chemicals.¹²

The presence of two unpaired electrons in separate atomic orbitals makes oxygen, which is an essential element for life, showing its "other side" as it is susceptible to reactive oxygen species (ROS), which can modify and damage cells by reacting with their components.¹² Since ROS is one of the most active bactericidal compounds, the "aerobic explosion" has an antibacterial effect in the body, making it easier for macrophages to eliminate the pathogen. Phagocytic cells, such as granulocytes, monocytes and macrophages, use the phenomenon of "aerobic explosion" to eliminate pathogens.¹³ In addition, ROS contributes to the increased activation of T lymphocytes and the adhesion of leukocyte cells into the endothelial, which allows these cells to penetrate from the circulatory system to the site of the inflammatory reaction.¹³ Elevated intracellular ROS levels cause damage to lipids, proteins and DNA, leading to pathologies at the level of systems and organs.¹²

Beta-amyloid deposits in the brain in the intercellular space and the deposition of hyperphosphoric tau protein inside neurons have been associated with neuronal loss in the course of AD.¹⁴⁻¹⁷ In addition, other proteins with a pathological structure are also involved in the transformations leading to neuronal atrophy in neurodegenerative disorders: alpha-synuclein and nuclear TDP protein 43.¹⁸ The presence of proteins with a pathological structure results in the activation of glial cells in the brain (astroglial and microglial), triggering inflammatory processes, as free oxygen radicals, excitatory amino acids, interleukins, as well as nitric oxide are released.^{1,18} Additionally, these compounds contribute to the death of neurons and their connections.^{1,18} Furthermore, there is a loss of cholinergic

neurons in the forebrain, there are also changes in the synapses of the cerebral cortex and hippocampus.^{1,18}

Depression of the elderly is characterized, inter alia, by anhedonia and the coexistence of various cognitive dysfunctions: in terms of executive function, memory, processing speed, attention and visual-spatial skills¹⁹ and it is associated with an increased risk of developing AD compared to healthy older adults.⁴⁻⁷ Pathomechanism of the development of major depressive disorder (MDD) is still associated with many unknowns, but it is believed to have a multi-factorial origin, involving dysfunction of many areas of the brain, such as the hippocampus, prefrontal cortex, nucleus and amygdala.²⁰ In addition, MDD pathophysiology is associated with an inflammatory process due to microglial activation, increased cytokine release and increased oxidative stress, astrocyte atrophy and changes in glutamatergic regulation, which can lead to local damage.²¹ The activated microglial initiates the fission of precursor forms of interleukin-1 β (IL-1 β) into its active form.^{22,23} Exacerbation of the inflammatory process can result in a significant increase in the production and expression of pro-inflammatory cytokines such as tumor necrosis factor- α (TNF- α) and IL-1 β , as well as reactive oxygen species (ROS) and nitric oxide, contributing to neurodegenerative processes associated with psychiatric disorders.^{22,23}

The relationship between depression and Alzheimer's disease is complex and not fully explained. Scientific research indicates that depression in the elderly may be one of the first signs of the dementia process. On the other hand, it is believed that brain damage caused by progressive Alzheimer's disease can contribute to the development of depression. Changes in the brain in the same structures can be the biological cause of both depression and dementia.²⁴

Due to the similar etiology and clinical picture of AD and MDD, it is necessary to look for comprehensive diagnostic methods that take into account both the biological and functional aspects of cognitive performance, especially at the prodromal stage. Another crucial aspect is the availability of the methods used and the relative ease of diagnosis. Due to the above mentioned AD structural and functional changes, neuropsychological tools to assess episodic memory and the ability to acquire new information are particularly useful.^{25,26} The development of a combination of high sensitivity and specificity markers is particularly urgent in the diagnosis of neurodegenerative dementia.²⁶

Therefore, for the purposes of this work, tools classified as in-depth neuropsychological diagnosis were selected. Furthermore, they were relatively simple and not very expensive to perform, and they were available to most clinical psychologists. Due to the emerging new evidence supporting the role of oxidative stress in the pathogenesis of MDD and AD, they were combined with biochemical markers: superoxide dismutase SOD and superoxide anion radical ($O_2^{\cdot-}$).

Materials and Methods

The research was conducted among patients of the Geriatric Clinics and Geriatric Outpatient Clinic in Bydgoszcz. Patient selection was based on the WHO definition of old age, which assumes that the onset of old age is at the age of 60.

The study involved three groups of participants: 27 patients with Alzheimer's disease (AD), 30 patients with Alzheimer's disease with coexisting major depressive disorder (AD+D), and a control group of 24 healthy participants without dementia and without depression.

The diagnosis of probable Alzheimer's disease (prodromal stage) was based on the current criteria of the International Statistical Classification of Diseases and Health Problems ICD-10. The cognitive impairments appeared gradually (more than 6 months earlier) and slowly increased. They significantly disrupted the daily functioning of patients (this was confirmed by the collected interviews from the subjects and in many cases from objective caregivers). The deficits not only affected memory processes but also decreased levels of other mental functions, in relation to the pre-disease status of the subjects. The subjects were classified into groups based on the profile of cognitive impairment characteristic of dementia of the Alzheimer's type and biochemical and CT tests. The following screening scales were used for the initial assessment of the severity of dementia: The Folstein's Mini Mental State Examination (MMSE) and the Clock Drawing Test (CDT). As a part of the routine diagnosis, subjective and physical examinations were carried out, as well as the following laboratory tests: blood count, general urine test, ionogram, creatinine, AspAT, ALAT, lipid profile, EST, CRP, total protein, vitamin B12 and folic acid levels, TSH. The results of computed tomography (CT) of the head were collected, or this examination was performed as a part of a planned diagnosis.

The following exclusion criteria were used: the occurrence of another cause of dementia (vascular brain disease,

HIV infection, Parkinson's disease, Huntington's disease, normotensive hydrocephalus, hypothyroidism, vitamin B12 or folic acid deficiency, hypercalcemia, alcohol or drug abuse).

The diagnosis of major depressive disorder was made on the basis of the criteria of the International Statistical Classification of Diseases and Health Problems ICD-10. The Geriatric Depression Scale (GDS) in its abbreviated version including 15 traits was used to study the severity of depressive symptoms. Qualified personnel collected blood from the basilic vein from patients who were included in the study. The clot and anticoagulant were taken within one blood draw during routine tests in the amount of about 7 mL. Blood was collected at 8 am to polyethylene tubes with lithium heparin and tubes without anticoagulant. The material was transported to the Department and Biochemistry Department of the Collegium Medicum in Bydgoszcz of Nicolaus Copernicus University in Torun. The tests were conducted on the same day, approximately 1 hour after the material was collected. Plasma was separated from the blood obtained from the morphotic elements and haemolysate was prepared from isolated red blood cells by the addition of 1:1, v/v bidestylated water).

The Bioethical Commission at Nicolaus Copernicus University in Torun Ludwik Rydygier Collegium Medicum in Bydgoszcz (KB 260/2007) agreed to carry out the research.

Cognitive Performance

The assessment of cognitive functioning was carried out, *inter alia*, with the help of the following neuropsychological tests.

Auditory Verbal Learning Test (AVLT) is characterized by a high diagnostic value in the diagnosis of Alzheimer's disease. It evaluates the process of acquiring new information. The subjects memorized 15 words presented verbally in five consecutive attempts. After each presentation of the words by the researcher, the subjects mentioned all the memorized words in the sample (specifying the learning curve). Then, after a 20-minute break, they pointed memorized words from the list presented five times earlier (indicating the durability of the memory trace). The final task of the test was to recognize words learned in five attempts out of 30 different words on the list. The 2010 European Federation of Neurological Societies guidelines for the diagnosis and treatment of Alzheimer's disease, in a psychological study, pay particular attention to the

inclusion of post-deferral reminders and post-hint reminders in Alzheimer's dementia diagnosis.²⁷

Verbal fluency test (semantic fluency – animals, phonemic fluency – words beginning with the letter k (without proper names), fluency of subcategories – sharp objects with which one can cut oneself). The respondent's task was to say as many words as possible according to the given criterion within one minute. It is a tool that mainly evaluates language functions (verbal production, ability to update names), as well as semantic memory and executive functions. Despite the lack of Polish test standards for verbal fluency assessment, the standard in the phonetic category is 12–16 words per minute, in the semantic category 18–20 words per minute, while in the subcategory of the fluency category 6–8 words per minute.

Biochemical Analyses

The Misra and Fridovich method was used to determine the activity of superoxide dismutase (SOD-1) in erythrocytes. SOD-1 activity is expressed in U/gHb. An amount of enzyme which inhibits adrenaline oxidation in 50% was taken as a unit of enzymatic activity (U).²⁸ The amount of superoxide anion radical ($O_2^{\cdot-}$) in whole blood was determined using the Bellavite et al. method, based on the reduction of cytochrome C in the presence of superoxide dismutase and 0.1 mL of whole blood. The extinction read at a light length of 550 nm from the supernatant to the air gave the value of the generation of superoxide anion radical. The stimulated value was obtained after stimulation of the sample with zymosan. The results were expressed in nmol/min/cell.²⁹

Statistical Analysis

Statistical analysis of the results was carried out using STATISTICA v. 13.1 computer program from StatSoft (Cracow, Poland). When two categorical variables were analyzed, the differences in groups were studied using the Kruskal-Wallis Chi-squared test. Single-factor logistic regression analyses were performed with age, gender, SOD ($O_2^{\cdot-}$), MMSE score, CDT score, verbal fluency test results and RAVLT results as independent variables, and clinical diagnosis as a dependent variable (AD or AD +D). These independent variables were also included in the multidimensional logistic regression analysis to evaluate their prognosis based on clinical diagnosis. Multiple models with different combinations of independent variables have been tested. The clinical diagnosis ROC curves were created in relation to psychological and biochemical

markers to assess optimal cut-off values. Youden Index has been calculated to find optimal thresholds. DeLong method and a two-stage nonparametric test that uses a shifted area under the ROC curve (sAUC), along with AUCs were performed in order to detect differences between ROC curves for different models.

Results

Table 1 contains the group characteristics.

The first univariate analysis showed that all examined parameters except for gender ($p = 0.1178$), years of education ($p = 0.0511$) and superoxide activity ($p = 0.0610$) were significant predictors of AD diagnosis (Table 2).

The univariate analysis showed that all parameters except for age, gender, years of education, AVLT percent forgetting, AVLT percent false negative errors and SOD were significant predictors of AD + D diagnosis (Table 3).

Multiple models with different combinations of independent variables were tested in the multivariate analysis for AD diagnosis prediction. In the multivariate analysis of the selected prediction model, it was found that SOD together with the raw score of AVLT delayed recall were the best significant predictors of AD disease with the AUC of 0.893 ($p = 0.0012$). The adjusted odds ratios (aOR) are presented in Table 4. For the AVLT – delayed recall aOR was 0.448 (95% CI: 0.300–0.668, $p = 0.0002$), which was similar to OR in univariate model (0.448, 95% CI: 0.300–0.668, see Table 2). For the result of SOD activity aOR was 0.983 (95% CI: 0.973–0.974, $p = 0.0034$), which is also quite similar to OR in univariate model for this parameter (0.986, 95% CI: 0.980–0.993, see Table 2). These results indicate that both lower scores in AVLT – delayed recall and lower SOD levels are predictive for AD.

The univariate analysis for predictors of AD+D is presented in Table 3 and showed that, although SOD activity was not significant here ($p = 0.0983$), the activity of $O_2^{\cdot-}$ was negatively associated with AD+D (OR = 0.991, 95% CI: 0.985–0.998, $p = 0.0140$). Also, MMSE and CDT scores were negative predictors of AD+D (OR 0.851, $p = 0.0001$, and OR = 0.766, $p = 0.0031$, respectively). Out of AVLT indices sum of immediately recalled words, learning and delayed recall were negatively associated with AD+D (OR 0.910, $p = 0.008$; OR = 0.720, $p = 0.0152$ and OR = 0.632, $p = 0.002$ respectively), whereas false-positive errors and the sum of all errors were positive predictors of AD +D (OR = 1.14, $p = 0.0046$ and OR = 1.16, $p = 0.0016$, respectively). Also, patients from AD+D group performed worse in all verbal fluency task,

Table 1 Group Characteristics

	AD		AD + D		C	
	N = 27		N = 30		N = 24	
	N	%	N	%	N	%
Sex						
Females	14	52%	19	63%	16	67%
Males	13	48%	11	37%	24	33%
Disease stage						
Mild dementia	13	48%	14	47%	N/A	
Moderate dementia	14	52%	16	53%	N/A	
	Me	Min-Max	Me	Min-Max	Me	Min-Max
Age (years)	79.0	60.0–92.0	82.0	68.0–88.8	75.3	65.0–91.0
Females	77.5	66.0–86.0	80.0	68.0–85.0	72.5	65.0–68.0
Males	80.0	60.0–92.0	80.0	75.0–88.0	79.5	68.0–91.0
Education (years)	11.0	7.0–16.0	10.5	7.0–17.0	12.0	7.0–17.0
MMSE score	23.0	11.0–26.0	18.0	8.0–26.0	29.0	27.0–30.0
CDT score	2.5	0.0–7.0	2.0	0.0–7.0	4.0	1.0–5.0
GDS score	3.0	1.0–7.0	8.0	2.0–14.0	7.0	3.0–7.0
AVLT scores:						
SUM (TRIALS 1–5)	23.0	3.0–39.0	23.0	8.0–33.0	39.0	20–56.0
LEARNING	2.0	0.0–10.0	2.0	0.0–5.0	5.0	1.0–8.0
FORGETTING	3.0	0.0–8.0	3.5	0.0–7.0	2.0	0.0–14.0
DELAYED RECALL	2.5	0.0–9.0	1.0	0.0–6.0	8.0	0.0–12.0
FALSE POSITIVE ERRORS	4.0	0.0–15.0	4.5	0.0–15.0	0.0	0.0–4.0
FALSE NEGATIVE ERRORS	2.0	0.0–7.0	2.0	0.0–11.0	0.0	0.0–5.0
ALL ERRORS	6.0	0.0–15.0	8.5	0.0–15.0	1.0	0.0–5.0
VERBAL FLUENCY scores:						
PHONEMIC (K LETTER)	6.0	0.0–14.0	7.0	2.0–12.0	11.5	4.0–18.0
SEMANTIC-ANIMALS	12.0	3.0–23.0	11.0	4.0–22.0	18.0	11.0–26.0
SEMANTIC-SHARP OBJECTS	5.0	0.0–10.0	3.0	0.0–8.0	7.0	4.0–11.0

Abbreviations: AD, patients with Alzheimer's disease; AD+D, patients with Alzheimer's disease with comorbid depression; C, control group; MMSE, The Folstein's Mini Mental State Examination; CDT, Clock Drawing Test; GDS, The Geriatric Depression Scale; AVLT, Rey Auditory Verbal Learning Test.

including phonemic verbal fluency task (OR = 0.863 for AD+D diagnosis, $p = 0.0262$), and both semantic tasks (OR = 0.894, $p = 0.0183$ for animals and OR = 0.667, $p = 0.0012$ for sharp objects).

Multiple models with different combinations of independent variables were tested in the multivariate analysis for AD+D diagnosis prediction. In the multivariate analysis of the selected prediction model, it was found that $O_2^{\cdot-}$ together with verbal fluency – sharp objects were the best significant predictors of AD +D diagnosis with the AUC of 0.689 ($p = 0.0312$). Adjusted odds ratios (aOR) are presented in Table 4. For the verbal fluency task – sharp

objects and $O_2^{\cdot-}$ aOR's was even a lower than OR's: 0.612, $p = 0.0008$ and 0.990, $p = 0.0065$, respectively. These results indicate that both lower scores in verbal fluency task – sharp objects together with SOD levels are most predictive for AD+D.

Furthermore, the comparison between receiver operating curves (ROC) with DeLong method showed that discrimination is superior when combining neuropsychological and oxidative stress markers than when using either of them alone either for AD (Figure 1) or for AD+D (Figure 2, Table 5). For AD diagnosis, the multivariate model increased all discrimination characteristics when compared to SOD (p

Table 2 The Univariate Model for Alzheimer's Disease Diagnosis

Effect	Wald	OR	-95% CI	95% CI	p
AGE	4.855	1.087	1.009	1.171	0.0276
Sex (female)	2.446	2.314	0.809	6.624	0.1178
Years of education	3.806	0.870	0.757	1.001	0.0511
Disease stage (moderate dementia)	4.217	1.421	1.314	1.764	0.0322
SOD	18.647	0.986	0.980	0.993	< 0.0001
O ₂ ^{•-}	3.509	0.994	0.989	1.000	0.0610
MMSE score	9.930	0.140	0.041	0.476	0.0016
CDT score	18.344	0.478	0.341	0.670	0.0000
AVLT scores:					
SUM (TRIALS 1–5)	18.425	0.740	0.644	0.849	0.0000
LEARNING	15.954	0.504	0.361	0.706	0.0001
FORGETTING	4.400	1.378	1.021	1.859	0.0359
DELAYED RECALL	24.403	0.463	0.341	0.628	< 0.0001
FALSE POSITIVE ERRORS	9.509	1.945	1.274	2.970	0.0020
FALSE NEGATIVE ERRORS	6.519	1.539	1.105	2.144	0.0107
ALL ERRORS	12.833	1.700	1.272	2.274	0.0003
VERBAL FLUENCY scores:					
PHONEMIC (K LETTER)	17.879	0.701	0.594	0.826	< 0.0001
SEMANTIC-ANIMALS	18.186	0.725	0.626	0.841	< 0.0001
SEMANTIC-SHARP OBJECTS	18.933	0.444	0.308	0.640	< 0.0001

Abbreviations: AD, Alzheimer's disease; SOD, superoxide dismutase; O₂^{•-}, superoxide anion radical; MMSE, The Folstein's Mini Mental State Examination; CDT, Clock Drawing Test; GDS, The Geriatric Depression Scale; AVLT, Rey Auditory Verbal Learning Test; OR, odds ratio; CI, confidence interval.

= 0.007) and AVLT DELAY RECALL (p = 0.015; Table 5). As for AD+D diagnosis, the multivariate model also improved both sensitivity to 0.811 (p = 0.018 when compared to O₂^{•-} and p = 0.028 when compared to verbal fluency – sharp objects alone); however, the specificity remained still relatively as low as 0.589 (Table 5).

To overcome the limitation of no non-AD depressed patients, and confirm the results, we performed also an analysis for all AD patients without control group (with non-depressed AD participants serving as controls). Results of this analysis are similar to the results of multivariate model for AD+D diagnosis, with area under ROC of 0.784 (SE=0.06, 95% CI 0.655–0.882, p = 0.0311), and presented in Table 6.

Discussion

With regard to AD diagnostics, it was observed that the greatest diagnostic value was the combination of SOD and RAVLT activity level studies, in particular, the delayed recall.^{27,30}

SOD superoxide dismutase belongs to the group of enzymes of the first line of defense against excess free radicals.³¹ It converts superoxide anion radical into

hydrogen peroxide and oxygen molecule.³¹ It neutralizes quickly and efficiently any molecule that can develop into a free radical or any free radical, with the ability to induce the production of other radicals.³¹ One of the first studies involving oxidative stress in AD, conducted by Gsell et al., provided evidence of H₂O₂ accumulation in the brain with Alzheimer's disease.³² These results were consistent in many post-mortem areas of the brain, from mild to strongly affected AD regions.³³ A few years later, Thome et al. reported a decrease in mitochondrial SOD 2 levels and unchanged serum SOD1 levels in AD patients, compared to the control group selected according to the age and gender.³³ Chang et al, in his well-crafted literature review, demonstrated, on the basis of a total of 80 cases, that AD is indeed associated with a significant increase in SOD levels.³⁴ In addition, differences in SOD levels may reflect different stages of the disease: SOD levels appear to be elevated in the early stages of AD, but there is an exhaustion in the later stages of the disease progression.³⁴ It therefore appears that SOD activity is a promising marker for enriching neuropsychological analysis of AD patients and, consequently, increasing sensitivity and diagnostic relevance.

Table 3 The Univariate Model for Alzheimer's Disease with Comorbid Depression (AD+D) Diagnosis

Effect	Wald	OR	-95% CI	95% CI	p
AGE	2.519	1.059	0.987	1.137	0.1125
Sex (female)	0.057	0.895	0.358	2.236	0.8119
Years of education	0.839	0.940	0.824	1.073	0.3596
Disease stage (moderate dementia)	1.121	2.441	0.414	5.312	0.1233
SOD	2.733	0.998	0.995	1.000	0.0983
O ₂ ⁻	6.037	0.991	0.985	0.998	0.0140
MMSE score	15.195	0.851	0.785	0.923	0.0001
CDT score	8.760	0.766	0.642	0.914	0.0031
AVLT scores:					
SUM (TRIALS 1-5)	11.189	0.910	0.862	0.962	0.0008
LEARNING	5.894	0.720	0.552	0.939	0.0152
FORGETTING	3.366	1.249	0.985	1.583	0.0665
DELAYED RECALL	13.507	0.632	0.495	0.807	0.0002
FALSE POSITIVE ERRORS	8.041	1.144	1.042	1.255	0.0046
FALSE NEGATIVE ERRORS	2.915	1.195	0.974	1.467	0.0877
ALL ERRORS	9.976	1.160	1.058	1.273	0.0016
VERBAL FLUENCY scores:					
PHONEMIC (K LETTER)	4.942	0.863	0.758	0.983	0.0262
SEMANTIC-ANIMALS	5.567	0.894	0.814	0.981	0.0183
SEMANTIC-SHARP OBJECTS	10.562	0.667	0.522	0.851	0.0012

Abbreviations: AD+D, AD – Alzheimer's disease with comorbid depression; SOD, superoxide dismutase; O₂⁻, superoxide anion radical; MMSE, The Folstein's Mini Mental State Examination; CDT, Clock Drawing Test; GDS, The Geriatric Depression Scale; AVLT, Rey Auditory Verbal Learning Test; OR, odds ratio; CI, confidence interval.

The neuropsychological profile at the initial stage of Alzheimer's disease is an increasingly well-described important predictor of further development of the disease.^{35,36} The early stages of AD are characterized by episodic memory deficits, which are caused by medial lobe atrophy and loss of neurons in the primary cholinergic system of the forebrain.^{35,36} The effect of this disorder is the disruption of the neural network, which is crucial for episodic memory function.^{35,36} Thus, the clinical feature of AD is a deficit in the ability to learn and remember new information. Patients with AD show a general episodic memory deficit: they do not benefit from the guidance or structure of the test; their ability to recognize is just as flawed as free play after deferral. These patients have a deficit in acquiring new information.³⁷

The Rey Auditory Verbal Learning Test (AVLT) test is used to assess the process of acquiring new information and episodic auditory memory.²⁷ Previous studies have shown that the deterioration in AVLT results well reflects the underlying pathology caused by Alzheimer's disease (AD), making the indicators obtained in this test a good marker for early detection of AD among people reporting memory problems.²⁷

A study estimating AVLT results based on gray matter density showed that the best predictors of the results obtained in the test were medial temporal lobe structures and amygdala to evaluate immediate responses. Whilst angular gyrus, hippocampus and amygdala were the most accurate to assess AVLT forgetting percentage. In addition, the conversion of MCI to AD within 3 years could be predicted from observed or estimated AVLT results with

Table 4 Multivariate Models for Alzheimer's Disease without Depression (AD) and Alzheimer's Disease with Comorbid Depression (AD+D) Diagnosis

AD	Effect	Wald	aOR	-95% CI	95% CI	p
AD	Free	10.618	307.254	6.004	1618.120	0.0001
	AVLT delayed recall	15.484	0.448	0.300	0.668	0.0002
	SOD	9.387	0.983	0.973	0.994	0.0034
AD +D	Effect	Wald	aOR	-95% CI	95% CI	p
AD +D	Free	9.800	17.656	2.925	106.559	0.0017
	VERBAL FLUENCY: SHARP OBJECTS	11.100	0.612	0.458	0.817	0.0008
	O ₂ ⁻	7.385	0.990	0.982	0.997	0.0065

Abbreviations: SOD, superoxide dismutase; O₂⁻, superoxide anion radical; AVLT, Rey Auditory Verbal Learning Test; AD, Alzheimer's Disease without depression; AD+D, Alzheimer's disease with comorbid depression; aOR, adjusted odds ratio; CI, confidence interval.

accuracy comparable to MRI-based biomarkers.²⁷ Recent research suggests that there are both involvement of the widespread cortical network and the importance of its interactive roles in the memory process. In addition to the temporal lobe, prefrontal and parietal areas are associated with episodic memory.³⁸⁻⁴⁰ The structures involved in the memory formation process are the hippocampus and the structure surrounding the olfactory sulcus.³⁸⁻⁴⁰ It is in these areas that the degenerative process takes place at the early stages of Alzheimer's disease. It is manifested as a deterioration in memory trace consolidation represented by the result in the attempt to freely reproduce after a 20-minute deferral in AVLT.³⁸⁻⁴⁰ Many researchers have previously confirmed that those were independent predictors of the progression of Alzheimer's disease.⁴¹⁻⁴⁶

Both AD and AD+D groups were characterized by a lack of memory trace of deferred auditory hearing memory of any form and a significant decrease in memory skills.

With regard to AD+D diagnosis, it was observed that the greatest diagnostic value was the combination of superoxide anion radical and verbal fluency, in particular, the acute subject subcategory.

The results of our own work therefore indicate that in AD with coexisting MDD, the key roles are played not only by the body's defense capabilities (represented by SOD) but also oxidative stress itself (represented by an anion radical). This therefore leads to the hypothesis that stress with unbalanced defense forces is associated with the development of MDD in the AD course.

Indeed, the research carried out in the recent years highlights the potential role of oxidative stress in the pathogenesis of the clinical picture of depression as one of the factors relevant to the development of this disease. Increased levels of reactive oxygen species (ROS) and

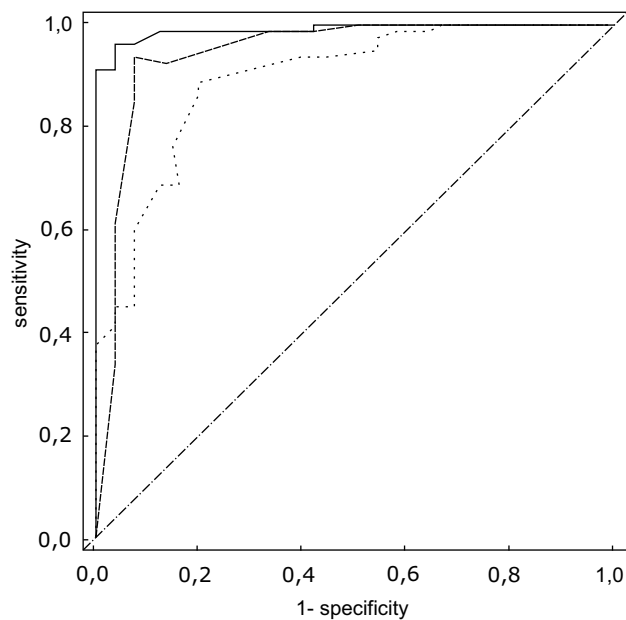
reactive nitrogen species (RNS) were observed in depression, as well as altered antioxidant concentrations ie GSH in depressed patients.⁴⁷

Patients with depression had lower antioxidant values such as vitamin E, zinc and serum coenzyme Q10, as well as lower antioxidant enzymes.⁴⁸⁻⁵⁰ Oxidative stress can also affect the pathogenesis of depression by interacting with neurogenesis and neuroplasticity processes, inflammatory processes in the brain and monoamine reuptake.

The effectiveness of depression treatment can consist of inhibiting the activity of pro-inflammatory cytokines and oxidative stress, tilting the balance in favor of antioxidant mechanisms.^{51,52}

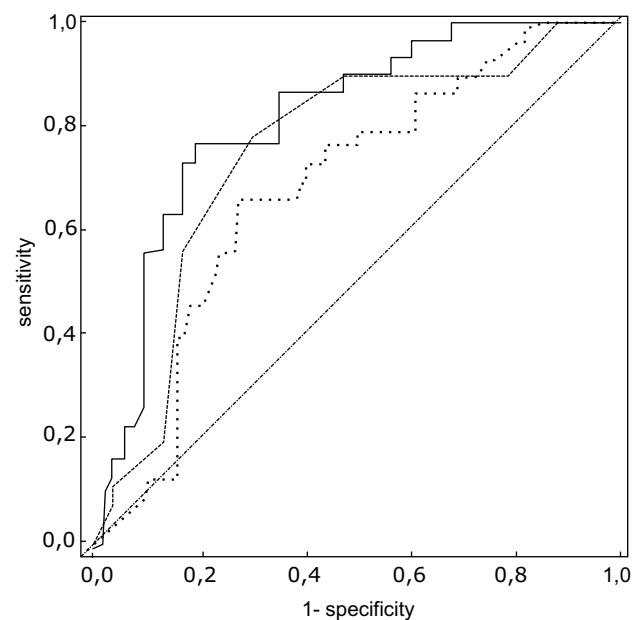
In the neuropsychological picture, people in the AD and AD+D groups revealed a decrease in the ability to create and speak words fluently according to the recommended criterion, as well as the ability to update vocabulary and systematically search semantic memory resources. In AD +D and AD groups, the sum of the words spoken in each category was significantly lower compared to the control group.

The Verbal Fluency Test is a tool that evaluates primarily language functions (verbal production, ability to update names), as well as semantic memory and executive functions.⁵³ Verbal fluency refers to the ability to generate as many words as possible in a limited time, without repetition, and according to phonology (each word begins with a given letter) or a semantic rule (each word belongs to a given semantic category).⁵³ Verbal knowledge requires not only access to and search for specific words in lexical memory but also response monitoring to avoid repetition and suppression of words irrelevant to an exercise, in order to stick to task rules.⁵³⁻⁵⁶ These tasks include language processing and elements of the executive



..... superoxide dismutase (SOD) activity
 ----- Rey's Auditory Verbal Learning Test - delayed recall score
 — multivariate model
 ---- reference line

Figure 1 The comparison of receiver operating curves (ROC) predicting diagnosis of Alzheimer Disease for activity of superoxide dismutase, the result of the subscale of Auditory Verbal Learning Test – delayed recall and multivariate model.



..... superoxide anion radical
 ----- verbal fluency task: sharp objects score
 — multivariate model
 ---- reference line

Figure 2 The comparison of receiver operating curves (ROC) predicting diagnosis of Alzheimer's Disease with coexisting depression for superoxide anion radical and scores in verbal fluency subscale: sharp objects and multivariate model.

function model.^{35,53,55,56} The standard evaluation of results is quantitative and includes the number of words according to the given criterion and errors – out-of-category responses and repetitions.^{35,53,55,56}

Previous studies of verbal fluency in depressed patients confirmed the observations made in our study: patients with depression produced fewer words in the semantic category than the control group, in the absence of a change in the tasks involving phonemic fluency.⁵³ It is worth to note that subcategory of sharp objects, which seemed in our analysis, to be predictive for depression in AD, reflects mainly the ability of abstractive thinking and executive functions.

The reduced ability to switch semantic subcategories was associated with a reduced ability to change mental attitudes, which supports the hypothesis that verbal fluency disorder reflects general executive problems in depression.⁵³ The evidence from neuroimaging studies indicates the neurobiological basis of this compound, primarily the need for simultaneous involvement of the hippocampus and frontal lobe structures. Sejunaite et al found

that executive function, which also acts as a mediator between neuropsychological domains and daily functioning, is the main area of cognitive impairment in depression.^{57–60} Impaired executive functions cause memory deficiencies in patients with depressive disorders and affect memory processes during coding, learning and search phases.⁶¹ The deficit in executive function in patients with depressive disorder was combined with functional disorders in the frontal lobes and impaired hippocampal function.⁶¹ The study of memory impairment focused mainly on omissions, ie false negative results. Memory omissions are due to the failure to recall or recognize information to which individuals have been exposed in everyday life or in laboratory conditions. Semantic fluency can also be particularly interesting in characterizing cognitive difficulties in MDD. The ability to perform well in verbal fluency requires integrating semantic search strategies and word generation.⁶¹ This may indicate that performance degradation may be due to a less integrated semantic network, leading to

Table 5 Comparison of Univariate and Multivariate Areas Under Curve (AUC) and Predictive Values for Alzheimer's Disease without Depression (AD) and Alzheimer's Disease with Comorbid Depression (AD+D)

Diagnosis	Model	AUC	Sensitivity	Specificity	PPV	NPV	LR(+)	p*
AD	Univariate: SOD	0.892	0.877	0.808	0.909	0.750	4.561	0.007
	Univariate: AVLT DELAY RECALL	0.942	0.930	0.923	0.964	0.857	12.088	0.015
	Multivariate model	0.998	0.965	0.962	0.982	0.926	25.088	—
AD+D	Univariate O ₂ ^{-•}	0.677	0.717	0.283	0.792	0.283	0.301	0.018
	Univariate: verbal fluency, sharp objects	0.751	0.698	0.302	0.841	0.302	0.277	0.028
	Multivariate model	0.816	0.811	0.589	0.860	0.589	0.405	—

Note: *p-value for comparison with multivariate model.

Abbreviations: AD, Alzheimer's Disease without depression; AD+D, Alzheimer's disease with comorbid depression; SOD, superoxide dismutase; O₂^{-•}, superoxide anion radical; AVLT, Rey Auditory Verbal Learning Test; AUC, area under curve; PPV, positive predictive value; NPV, negative predictive value; LR, likelihood ratio.

Table 6 Multivariate Model for Alzheimer Disease with Comorbid Depression (AD+D) Diagnosis with Alzheimer's Disease without Depression (AD) Group Serving as a Control Group Only (N = 57 Participants)

AD +D	Effect	Wald	aOR	-95% CI	95% CI	p
	Free	2.921	5.243	1.151	14.442	0.0012
	VERBAL FLUENCY: SHARP OBJECTS	3.214	0.664	0.243	0.765	0.0231
	O ₂ ^{-•}	7.271	0.978	0.963	0.994	0.0071

Abbreviations: AD+D, Alzheimer's disease with comorbid depression; O₂^{-•}, superoxide anion radical; aOR, adjusted odds ratio; CI, confidence interval.

unorganized search processes, making it difficult to search for words in the same category.

The results obtained in this work indicate that semantic fluency tests may help in the differential diagnosis of patients with depression in patients with early-stage dementia of the Alzheimer's type. This observation can be very useful clinically, due to the simplicity and low cost and low time effort, and thus causing a small burden on the patient, which is of particular importance in view of the ease of fatigue of this group of patients.

This study contains some limitations that can serve as a guidance for planning and implementing future research in this field. They concern, in particular, the methodological aspects of the analyses carried out.

Restrictive inclusion criteria have contributed not only to reducing the minimum test numbers needed to achieve adequate statistical strength of the tests but also to significant limitations in inference. It is also unclear how other disease units may affect the activity of antioxidant enzymes, which limits the possibility of reference of test results to people who do not have a disease with free radical etiology. In addition, the absence of people with depression but without dementia in the study group leads to difficulties in assessing the strength of the effect of depression itself on the obtained results. Another limitation is that other neurodegenerative disorders were not included. Thus, we cannot

evaluate whether this oxidative stress biomarkers are specific of AD or might be present in other neurodegenerative disorders.

Furthermore, we did not use any AD biomarkers such as tau levels in CSF or FDG-PET results. In fact, all these markers are helpful in scientific research, but usually not available in routine diagnostics.

These limitations continue to inspire further research into the issues raised in this work.

Conclusions

This study confirms the value of neuropsychological diagnosis and analysis of oxidative stress markers in the diagnosis of AD and MDD in the course of AD. The combination of the use of biochemical markers and neuropsychological tests seems particularly important for differential diagnosis. For AD diagnostics, the greatest diagnostic value was observed in a combination of SOD and RAVLT, in particular, delayed recall. For MDD diagnosis in the course of AD, the greatest diagnostic value was observed in the combination of the examination of the anion radical concentration and a verbal fluency test.

Data Sharing Statement

The data presented in this study are available on request from the corresponding author.

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Institutional Review Board Statement

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board Bioethical Commission at Nicolaus Copernicus University in Torun Ludwik Rydygiel Collegium Medicum in Bydgoszcz (KB 260/2007).

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflict of interest.

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