



# Serum Cholinesterase Activity in Elderly Female Patients with Different Screening Cognitive Status and Frailty Assessment Scores

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## Abstract

**Introduction.** Frailty and mild cognitive impairment (MCI) are common geriatric syndromes. Peripheral serum cholinesterase (pChE) is a laboratory indicator that may reflect dysfunction of cholinergic processes in the central nervous system. Published data demonstrate the potential utility of pChE as a marker for a range of neurodegenerative disorders.

**Aim.** This study aimed to identify and investigate the relationship between serum pChE levels in patients and various screening scores of cognitive status, frailty, and metabolic parameters.

**Materials and methods.** The study included 50 women aged over 60 years. Screening clinical examinations were conducted, including Montreal Cognitive Assessment (MoCA), Mini-Mental State Examination (MMSE), Frontal Assessment Battery (FAB), Age Is Not a Hindrance questionnaire, and Charlson Comorbidity Index. A blood chemistry analysis was performed, including a kinetic colorimetric assay of serum pChE.

**Results.** The Age Is Not a Hindrance score and pChE activity exhibited a moderate inverse correlation with a Spearman coefficient ( $r_s$ ) of  $-0.31$ ; 95% confidence interval (CI)  $-0.5$  to  $-0.03$ ;  $p < 0.05$ . The MoCA scores and pChE levels also showed a moderate inverse correlation with  $r_s$  of  $-0.32$ ; 95% CI:  $-0.55$  to  $-0.05$ ,  $p < 0.05$ . A high risk of MCI is defined by a pChE activity threshold point of 9978 U/L, with a sensitivity of 47% and a specificity of 97%. The association between pChE activity and the prevalence of cognitive impairment remained significant even when different socio-demographic and metabolic parameters were included in the regression model, odds ratio (OR) 1.0005; 95% CI: 1.0001–1.009;  $p = 0.01$ .

**Conclusion.** Women over 60 years of age in an outpatient setting exhibited an inverse correlation between the Age Is Not a Hindrance questionnaire score and the pChE activity. A pChE activity of 9978 U/L or higher was associated with an elevated risk of concomitant mild cognitive impairment. However, it is important to consider the high probability of false negatives in this context. This association persisted across a variety of clinical and metabolic factors.

**Keywords:** frailty; cognitive impairment; cholinesterase; biomarker; diagnosis

**Ethics approval.** The study was conducted with the informed consent of patients. The study protocol was approved by the local Ethical Committee of the Ural State Medical University (protocol No 9, December 18, 2020).

**Source of funding.** The study was carried out within the framework of the grant received as a result of the young scientist contest “Scientific Height – 2023”, financed by the Institute of High Temperature Electrochemistry of the Ural Branch of the Russian Academy of Sciences.

**Conflict of interest.** The authors declare no apparent or potential conflicts of interest related to the publication of this article.

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**For citation:** Kodincev A.N., Volkova L.I. Serum cholinesterase activity in elderly female patients with different screening cognitive status and frailty assessment scores. *Annals of Clinical and Experimental Neurology*. 2024;18(4):27–37. (In Russ.)

DOI: <https://doi.org/10.17816/ACEN.1097>

Received 22.02.2024 / Accepted 29.07.2024 / Published 25.12.2024

# Активность сывороточной холинэстеразы у пожилых пациенток с различными скрининговыми показателями оценки когнитивного статуса и старческой астении

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## Аннотация

**Введение.** Старческая астения (СА) и умеренные когнитивные нарушения (УКН) являются распространёнными гериатрическими синдромами. Периферическая холинэстераза (ПХЭ) сыворотки крови является потенциальным лабораторным показателем, отражающим дисфункцию холинэргических процессов в центральной нервной системе. Опубликованы данные, свидетельствующие о возможности использования ПХЭ в качестве маркера различных нейродегенеративных заболеваний.

**Цель** – выявление и изучение взаимосвязи активности ПХЭ сыворотки крови у пациенток с различными скрининговыми показателями когнитивного статуса, СА и метаболических параметров.

**Материалы и методы.** В исследование были включены 50 женщин старше 60 лет. Проведено скрининговое клиническое обследование: Монреальская когнитивная шкала, Краткая шкала оценки когнитивного статуса, батарея тестов на лобную дисфункцию, опросник «Возраст не помеха», индекс коморбидности Чарлсона. Выполнено биохимическое обследование, включавшее определение ПХЭ сыворотки крови кинетическим колориметрическим методом.

**Результаты.** Показатели опросника «Возраст не помеха» и активность ПХЭ обладают обратной умеренной корреляцией, коэффициент Спирмена ( $r_s$ ) =  $-0,31$ , 95% доверительный интервал (ДИ):  $-0,54$ – $(-0,03)$ ;  $p < 0,05$ . Показатели шкалы MoCA и активность ПХЭ также обладали умеренной обратной корреляцией:  $r_s = -0,32$ ; 95% ДИ  $-0,55$ – $(-0,05)$ ;  $p < 0,05$ . Пороговая точка активности ПХЭ 9978 ЕД/л позволяет с чувствительностью 47% и специфичностью 97% определить высокий риск умеренных когнитивных нарушений. Ассоциация между показателями ПХЭ и распространённостью когнитивных нарушений сохранялась при введении в регрессионную модель социально-демографических и метаболических параметров: отношение шансов 1,0005; 95% ДИ 1,0001–1,009;  $p = 0,01$ .

**Заключение.** У женщин старше 60 лет, наблюдающихся амбулаторно, выявлена обратная корреляция показателей опросника «Возраст не помеха» и активности ПХЭ. Уровень активности ПХЭ 9978 ЕД/л и выше ассоциировался с высоким риском сопутствующих умеренных когнитивных нарушений, при этом важно учитывать большую вероятность ложноотрицательных результатов. Данная ассоциация сохранялась в условиях воздействия различных клинических и метаболических факторов.

**Ключевые слова:** старческая астения; когнитивные нарушения; холинэстераза; биомаркер; диагностика

**Этическое утверждение.** Исследование проведено при добровольном информированном согласии пациентов. Протокол исследования одобрен локальным этическим комитетом Уральского государственного медицинского университета (протокол № 9 от 18.12.2020).

**Источник финансирования.** Исследование выполнено в рамках гранта, полученного по результатам конкурса молодых учёных «Научная высота – 2023», финансируемого Институтом высокотемпературной электрохимии УрО РАН.

**Конфликт интересов.** Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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**Для цитирования:** Кодинцев А.Н., Волкова Л.И. Активность сывороточной холинэстеразы у пожилых пациенток с различными скрининговыми показателями оценки когнитивного статуса и старческой астении. *Анналы клинической и экспериментальной неврологии*. 2024;18(4):27–37.

DOI: <https://doi.org/10.17816/ACEN.1097>

Поступила 22.02.2024 / Принята в печать 29.07.2024 / Опубликовано 25.12.2024

## Introduction

The increase in life expectancy is responsible for the growing proportion of elderly and senile individuals in Russia, which requires the development of personalized medical care for patients over 60 years of age. Aging is associated with the onset of geriatric symptoms, a multifactorial age-related clinical condition that increases the risk of adverse outcomes and functional impairment [1]. In this regard, physicians of various specialties are increasingly encountering clinical manifestations of frailty and cognitive impairment in their practice. Pre-frailty and frailty are more common in females than in males [2]. Data suggesting an association between frailty and cognitive impairment have been published. In particular, frailty is associated with an increased risk of cognitive impairment of various origin and vice versa [3, 4]. Furthermore, cognitive frailty (CF) is currently being recognized as a distinct nosological entity, combining features of frailty and cognitive impairment. The prevalence of CF is 6 to 16% in the population over the age of 60 years [5].

In this context, there is an increasing need for timely diagnosis and personalized management strategy for patients at high risk of developing frailty in combination with cognitive impairment. A relevant diagnostic focus is to search for potential biomarkers that can be used as a patient stratification system, since frailty and cognitive impairment are currently diagnosed primarily by clinical examination.

Laboratory and instrumental diagnosis of cognitive impairment in Alzheimer's disease (A/T/N ( $\beta$ -amyloid/tau protein/neurodegeneration) system) is expensive and inaccessible and involves the use of labor-intensive methods (positron-emission tomography, lumbar puncture, biopsy) [6]. This approach is not commonly employed in clinical practice, thus the pursuit of more accessible and comparable biomarkers in terms of accuracy continues [7].

Specifically, the level of peripheral serum cholinesterase (pChE) is an available indicator whose level changes may serve as a predictor of the development and progression of neurodegenerative processes associated with cognitive impairment [8]. PChE is an  $\alpha$ -glycoprotein synthesized by the liver and existing in two main forms: acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). In blood, the ratio of BChE to AChE is 412.5 : 1, but AChE is the more active enzyme [9]. In clinical practice, the assessment of BChE and pChE levels is used to diagnose organophosphate poisoning [10].

Recently, more and more data have emerged indicating a decrease in pChE activity in frailty, but a number of scientific papers show different changes in the levels of AChE and BChE in Alzheimer's disease and other neurodegenerative processes, making it difficult to formulate unified diagnostic algorithms [11–13]. Despite the clear correlation of pChE

activity with frailty and cognitive function scores, the validity and clinical significance of this parameter have not been studied, including in the presence of concomitant metabolic disorders. There is a lack of data on threshold enzyme levels that can be used as predictive values. Therefore, investigating the diagnostic role of pChE may be a promising area of personalized medicine. This parameter may be potentially employed not only as a laboratory marker of cognitive impairment and frailty, but also as a laboratory indicator of the efficacy of drug therapy for these conditions.

The **aim** of the study was to identify and investigate the relationship between serum pChE activity in elderly patients and various screening scores of cognitive status, frailty, and metabolic parameters.

## Materials and Methods

A total of 50 women over 60 years of age on the record of the outpatient clinic of the Institute of High Temperature Electrochemistry, Ural Branch of the Russian Academy of Sciences (Ekaterinburg) were randomly selected to participate in a single-time cross-sectional study. A comprehensive clinical examination was conducted to form the main referral sample, with inclusion and non-inclusion criteria applied. The Study Protocol and the Informed Consent Form (ICF) were approved by the Local Ethics Committee of the Ural State Medical University (meeting minutes No. 9 dated 18 December, 2020).

### *Study design: single-time cross-sectional study.*

Inclusion criteria:

- female;
- age > 60 years;
- signed ICF.
- Non-inclusion criteria:
- severe decompensated somatic, neurological, or psychiatric conditions;
- inability to perform neuropsychological testing due to the severity of somatic condition and mental disorders (dementia and/or depression according to neuropsychological testing: Geriatric Depression Scale-15 score 5 or higher, life history data);
- treatment with parasympathomimetics, muscarinic receptor antagonists, AChE inhibitors;
- chronic hepatitis, severe and decompensated liver disease.

A validated Age Is Not a Hindrance questionnaire was used to evaluate the frailty severity. The questionnaire scores were evaluated as follows: 0 – no signs of frailty; 1–2 – signs of pre-frailty; 3 and more – signs of frailty. For a more reliable assessment, scores of 0–2 were considered as low risk of frailty, while scores of 3 or more were considered as high risk of frailty [14]. The Age Is Not a Hindrance questionnaire scores demonstrate greater sensitivity for detecting an elevated risk of frailty compared to the thresholds recommended

in the Clinical Guidelines on frailty [15, 16]. O.N. Tkacheva et al. showed that the sensitivity was 87% for threshold score  $\geq 3$  and 46.7% for the threshold score  $\geq 5$  in relation to the frailty index. Compared to the frailty phenotype model, the sensitivity was 93% for the threshold score  $\geq 3$  and 46.4% for the threshold score  $\geq 5$ . Thus, the threshold score point  $\geq 3$  on the Age Is Not a Hindrance questionnaire is more valuable as a screening tool for frailty [16].

Three validated scales were used to assess cognitive status: Mini-Mental State Examination (MMSE), Montreal Cognitive Assessment (MoCA), and Frontal Assessment Battery (FAB). Mild cognitive impairment was diagnosed at MoCA score  $< 26$  and MMSE score  $> 24$ . The FAB results were interpreted in conjunction with the MMSE and MoCA scores.

The Charlson Comorbidity Index was used to assess the long-term prognosis of patients. Comorbidity (hypertension, type 2 diabetes mellitus (T2DM), postmenopausal osteoporosis, nonalcoholic fatty liver disease) data were established from medical history (outpatient record data).

### Measuring peripheral cholinesterase activity

Venous blood was drawn in the treatment room to measure pChE levels. The pChE level was estimated by means of a kinetic colorimetric assay (Cobas 6000, Roche Diagnostics) at NPF HELIX LLC. This assay is based on the method published by E. Schmidt et al. [17].

Table 1. Patient demographic and clinical profile

Group	<i>n</i>	Mean age, years	Body mass index	Hypertension, <i>n</i>	Type 2 diabetes mellitus, <i>n</i>	Higher education, <i>n</i>	Postmenopausal osteoporosis, <i>n</i>
Low frailty risk, no MCI	24	70.8 $\pm$ 3.3	27.7 $\pm$ 4.8	19	4	15	10
Low frailty risk, MCI	13	69.5 $\pm$ 4.2	27.3 $\pm$ 3.6	10	3	7	6
High frailty risk, no MCI	8	72.5 $\pm$ 3.5	26.5 $\pm$ 4.5	7	0	3	1
High frailty risk, MCI	5	69.6 $\pm$ 5.9	31.5 $\pm$ 6.0	4	1	2	1
Total	50			40	8	27	18

Table 2. Median serum cholinesterase levels in patient groups

Group	<i>n</i>	Serum cholinesterase, U/l	
		median	min-max
Low frailty risk, no MCI	24	8195	6962–9467
Low frailty risk, MCI	13	9603*	9061–10 952
High frailty risk, no MCI	8	8137	7772–9339
High frailty risk, MCI	5	8685*	7206–8714

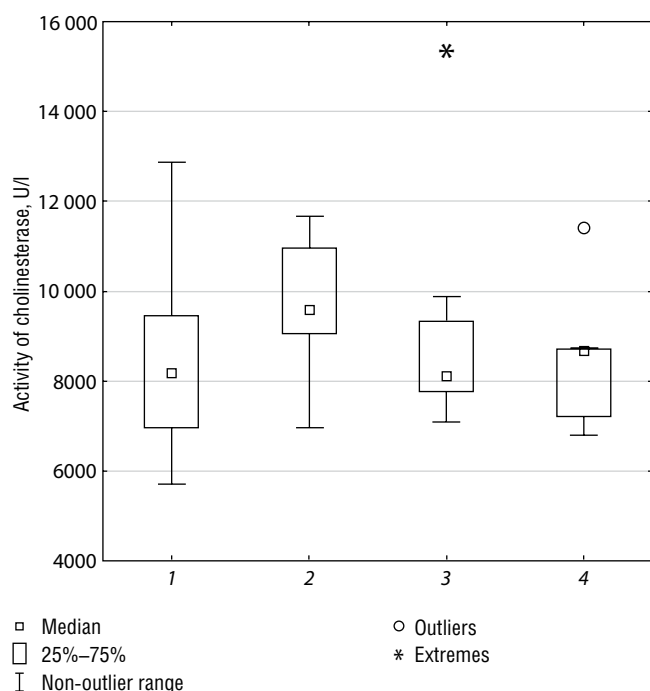
Note. \**p* < 0.05 as compared to groups without MCI.

### Statistical analysis

Statistica v. 10 (StatSoft Inc.), MedCalc, OpenEpi (<http://www.openepi.com>) were used for data processing. The selection of the criterion and test for statistical analysis was based on the evaluation of the normality of the distribution of each parameter conducted using Kolmogorov–Smirnov and Shapiro–Wilk tests. In the event that the data exhibited a normal distribution, the mean and standard deviation values were used for description, with the analysis conducted using parametric methods. In other instances, the median, lower and upper quartiles were employed for descriptive purposes, and nonparametric tests were utilized for analysis. The qualitative data were compared using the  $\chi^2$  criterion and Fisher's exact test (when the  $\chi^2$  criterion was not applicable) due to the independence of the samples. A ROC analysis with area under curve (AUC) estimation, univariate and multivariate logistic regression, and multiple linear regression were employed in the study. The threshold for statistical significance is *p* < 0.05. In the event of negative results, the probability of a type II error was estimated, and the study power was calculated.

### Results

A total of 50 women aged over 60 years (mean age 70.2  $\pm$  4.2 years) were included in the study. Patients were categorized into 4 groups according to their frailty and cognitive function scores (Table 1). The majority of patients (37–74%)



**Fig. 1. Median blood cholinesterase levels in patient groups.**  
1 – low frailty risk, no MCI; 2 – low frailty risk, MCI; 3 – high frailty risk, no MCI; 4 – high frailty risk, MCI.

exhibited low frailty risk, with a higher prevalence of those without MCI ( $n = 24$ ).

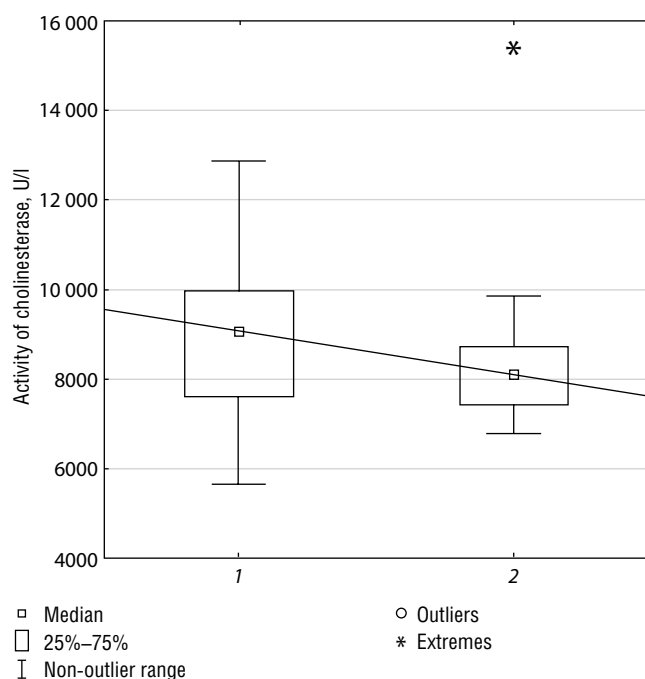
The patients in the groups did not differ significantly by age and BMI (two-way ANOVA;  $p > 0.05$ ), prevalence of hypertension, T2DM, postmenopausal osteoporosis, and presence of higher education ( $\chi^2$  criterion  $> 0.05$ ). Therefore, the obtained samples were comparable in terms of the main socio-demographic and certain clinical characteristics.

A Kruskal–Wallis test, followed by subgroup analysis, revealed that, although there was no statistically significant difference in pChE activity ( $H = 5.6$ ;  $p = 0.13$ ), higher pChE levels were observed in the patient groups with MCI (irrespective of the frailty risk) (Table 2).

Therefore, it can be assumed that the level of pChE is more strongly correlated with cognitive impairment than with the high frailty risk (Figure 1). To clarify this relationship, it was necessary to examine pChE levels, frailty scores, and cognitive status.

#### Relationship of cholinesterase levels to frailty assessment

When the correlation was evaluated, an inverse relationship was found between the pChE level and the Age Is Not a Hindrance questionnaire scores, Spearman's rank correlation coefficient ( $r_s$ ) =  $-0.31$ , 95% CI:  $-0.541$ – $(-0.0334)$ ;  $p < 0.05$ . However, pChE activity was not significantly different between the low and high frailty risk groups (9095 U/L (7613–9978)



**Fig. 2. Median cholinesterase levels in low (1) and high (2) frailty risk groups.**

and 8137 U/L (7457–8756), respectively), Mann–Whitney test significance level (pMW)  $> 0.05$  (Figure 2). Thus, changes in pChE levels were not significantly associated with the high frailty risk, despite the inverse correlation of the Age Is Not a Hindrance questionnaire with pChE level.

The constructed logistic regression model did not yield a significant association between elevated pChE levels and the increased frailty risk, odds ratio (OR) =  $-1$ ; 95% CI 0.99–1.0003. Moreover, the absence of a significant relationship ( $p = 0.09$ ) precluded the construction of a viable linear regression model evidenced by the exceedingly low value of the coefficient of determination ( $R^2 < 0.3$ ), not normally distributed residuals (Shapiro–Wilk test = 0.0075). To minimize the likelihood of false-negative results, the power of the study was calculated and found to be less than 80%, suggesting a high probability of a type II error. Therefore, although a correlation was identified between pChE activity and the Age Is Not a Hindrance questionnaire scores, further investigation is required in a larger sample in order to assess a reliable relationship between these parameters.

#### Relationship of cholinesterase levels to cognitive status assessment

The relationship between pChE level and MoCA score was assessed, revealing a moderate inverse correlation:  $r_s = -0.32$  (95% CI  $-0.55$ – $(-0.05)$ );  $p < 0.05$ . The Mann–Whitney test revealed a statistically significant difference in pChE levels between the two patient groups: 8173 U/L (7110–9256) without MCI and 9603 U/L (8267–11418) with MCI, pMW = 0.008.

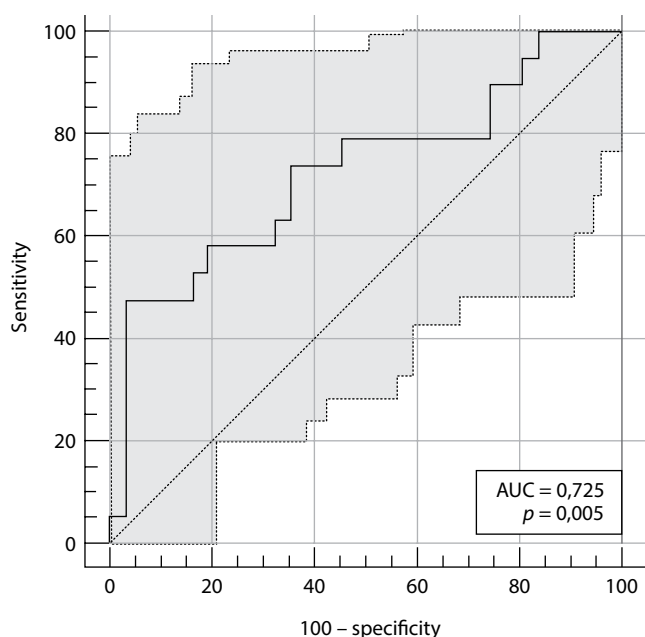


Fig. 3. ROC curve assessing the sensitivity and specificity of serum pChE level for the diagnosis of MCI.

Therefore, an inverse correlation was identified between MoCA scores and pChE levels. Higher enzyme levels were found to be associated with a lower score on this scale: OR = -1.0005; 95% CI 1.0001–1.009;  $p = 0.01$ , confirming the role of cholinergic deficiency in developing the cognitive impairment.

Although there is a correlation between pChE level and MoCA score, no significant association was found between changes in pChE level and MMSE score or FAB. In clinical practice, the MoCA scale is more sensitive than MMSE in diagnosing different variants of MCI in patients over 60 years of age [18]. We can therefore conclude an association of pChE level and MCI. A ROC analysis was performed for a more detailed evaluation (Figure 3).

According to the ROC-analysis, the sensitivity of the ChE threshold level of 9978 U/L (according to the Youden index) for detecting MCI was 47% (95% CI 24.4–71.1); specificity 97% (95% CI 83.3–99.9); AUC = 0.725;  $p = 0.005$ . The positive predictive value of the test was 55.6%; negative predictive value of the test -83%; diagnostic accuracy - 78%; likelihood ratio for the positive test - 14.68; for the negative test - 0.54; Cohen's kappa - 48.6%. The low sensitivity of pChE level does not allow the use of this enzyme level as a laboratory screening for MCI, but the high specificity allowed to suspect MCI in patients with pChE level > 9978 U/L due to a very low risk of false-positive results.

Despite the correlation between pChE levels, MoCA scores, and Age Is Not a Hindrance questionnaire scores, no correlation was found between the MoCA scores and the Age Is Not a Hindrance questionnaire scores.

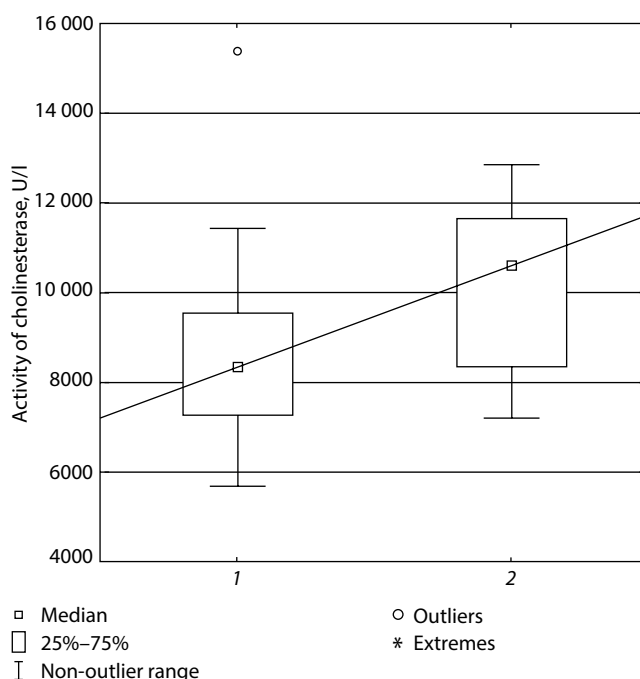


Fig. 4. Cholinesterase level in group of patients without T2DM (1) and with T2DM (2).

#### Relationship between cholinesterase level and metabolic parameters

Data on the effect of a number of metabolic factors on serum pChE level have recently been published and should be considered when assessing the relationship between this enzyme level and indicators of cognitive function. The main source of pChE is the liver, and evaluation of the effect of key metabolic parameters (including liver disease) on this enzyme level plays an important role in the development of a diagnostic model. Specifically, patients with T2DM had significantly higher pChE level compared to patients without T2DM: 10,614 (8337–11,646) and 8346 (7279–9535) U/L, respectively;  $p_{MW} < 0.05$  (Fig. 4). No correlation was found between pChE level and Charlson Comorbidity Index scores ( $r_s = 0.21$ ;  $p > 0.05$ ). Also, the median pChE level was not significantly different between the group of patients with postmenopausal osteoporosis (8465 U/L) and the group without osteoporosis (8741 U/L),  $p_{MW} > 0.05$ .

pChE activity correlated with alanine aminotransferase (ALT:  $r_s = 0.43$ ;  $p < 0.05$ ) but not aspartate aminotransferase (AST) levels, confirming the relationship between liver functional status and pChE levels. Despite this association, pChE level in patients with nonalcoholic fatty liver disease (NAFLD), 9110 U/L (8173–9880), was not significantly different from that in patients without NAFLD, 8465 U/L (7242–9733);  $p_{MW} = 0.43$ . However, it should be noted that a small number of patients with confirmed NAFLD ( $n = 5$ ) could not reliably exclude a false-negative result.

pChE activity did not correlate with total cholesterol ( $r_s = -0.21$ ;  $p > 0.05$ ), alkaline phosphatase ( $r_s = 0.11$ ;  $p > 0.05$ ), triglyceride

**Table 3. Multiple linear regression analysis of socio-demographic and clinical factors, laboratory parameters, and peripheral cholinesterase level**

Parameter	$\beta$ -Coefficient	<i>p</i>
Age	0.56	0.764
Higher education	0.183	0.659
Body mass index	0.272	0.621
Hypertension	-0.213	0.702
Type 2 diabetes mellitus	0.095	0.897
Non-alcoholic fatty liver disease	0.489	0.589
Postmenopausal osteoporosis	-0.336	0.548
Charlson Comorbidity Index	0.333	0.485
Statins	0.547	0.593
Alanine aminotransferase	-0.651	0.411
Aspartate aminotransferase	0.673	0.395
Total cholesterol	-0.142	0.874
Triglycerides	0.073	0.865
Total protein	0.251	0.754

**Table 4. Multiple logistic regression analysis to examine the correlation between social and clinical factors, laboratory parameters, and mild cognitive impairment**

Parameter	Odds ratio	95% CI
Age	0.941	0.736–1.203
Higher education	0.674	0.099–4.559
Body mass index	0.987	0.741–1.312
Hypertension	13.038	0.968–187.399
Type 2 diabetes mellitus	3.102	0.097–99.57
Non-alcoholic fatty liver disease	2.006	0.073–55.388
Postmenopausal osteoporosis	0.423	0.06–2.981
Charlson Comorbidity Index	2.263	0.961–5.33
Statins	0.89	0.109–7.25
Alanine aminotransferase	0.827	0.613–1.115
Aspartate aminotransferase	1.091	0.922–1.291
Total cholesterol	1.136	0.618–2.089
Triglycerides	0.996	0.159–6.253
Total protein	0.929	0.718–1.204
Peripheral cholinesterase	1.0008	1.0001–1.0015

( $r_s = -0.03$ ;  $p > 0.05$ ), or total protein ( $r_s = -0.11$ ;  $p > 0.05$ ) levels. pChE level did not significantly depend on the intake of lipid-lowering agents of the statin group either ( $p_{MW} = 0.66$ ). A multiple linear regression model was constructed to assess the multicollinearity of the factors under study and pChE (Table 3). Under the influence of various parameters, the correlation between pChE and ALT or T2DM became statistically insignificant.

To test the hypothesis regarding the relevance of serum pChE level as a potential biomarker of cognitive impairment, a multiple logistic regression model was constructed taking into account the main metabolic parameters: T2DM, hypertension, NAFLD, BMI, ALT, AST, triglycerides, total cholesterol, total protein levels, and intake of statins. Even in the presence of a direct relationship with ALT (the primary liver function test), pChE level was significantly associated with MCI:



**Table 5. Multiple linear regression analysis to examine the correlation between social and clinical factors, laboratory parameters, and MoCA scores**

Parameter	$\beta$ -Coefficient	<i>p</i>
Age	-0.082	0.871
Higher education	0.004	0.311
Body mass index	-0.389	0.676
Arterial hypertension	0.205	0.394
Type 2 diabetes mellitus	-0.038	0.458
Non-alcoholic fatty liver disease	-0.138	0.148
Postmenopausal osteoporosis	0.042	0.13
Charlson Comorbidity Index	-0.07	0.454
Statins	-0.06	0.175
Alanine aminotransferase	0.306	0.825
Aspartate aminotransferase	-0.263	0.724
Total cholesterol	0.031	0.753
Triglycerides	-0.175	0.718
Peripheral cholinesterase	-1.15	0.01

OR = -1.0008; 95% CI 1.0001–1.0015 (Table 4). Alkaline phosphatase level was not included in the model due to insufficient data. Furthermore, when a multiple linear regression model was constructed to assess the effect of socio-demographic and metabolic factors on the MoCA score, only pChE level was significantly associated with this cognitive assessment score (Table 5). The coefficient of determination ( $R^2$ ) is 0.42, indicating a moderate degree of effect of the characteristic. The normal distribution of residuals (Shapiro–Wilk test > 0.05) and the acceptable model quality according to analysis of variance ( $F = 2.69$ ;  $p = 0.34$ ) confirm the effect of pChE level on MoCA scores.

Therefore, even when metabolic factors are considered, pChE level may serve as a potential laboratory marker of cognitive impairment, as evidenced by the regression analysis.

## Discussion

There is currently an ongoing search for cost-effective and available biomarkers for laboratory diagnosis of frailty and cognitive impairment. Specifically, in patients with established frailty, the primary laboratory diagnostic focus is on the evaluation of hematologic (hemoglobin level) and endocrinologic (thyroid-stimulating hormone, T3, T4) parameters. The role of vitamin D and changes in the level of inflammatory markers such as C-reactive protein and interleukin-6 have also been studied [19]. A small study by R.E. Hubbard et al. performed on 30 hospitalized patients is published, indicating an inverse relationship between the severity of frailty and the level of AChE, BChE, and benzoylcholinesterase [11]. The authors mention that malnutrition in elderly age, particularly in patients with frailty, may be a potential mechanism for the decrease in esterase level. These findings are consistent with the obtained data on the inverse correlation between the Age Is

Not a Hindrance questionnaire and serum pChE activity. The higher the risk of frailty, the lower the pChE activity. Nevertheless, the potential diagnostic utility of pChE in the diagnosis of frailty remains incompletely understood.

Frailty is significantly associated with the risk of development and progression of cognitive impairment, and the study of blood pChE levels in patients with various cognitive status scores is a key area of investigation [20]. It is established that cholinergic deficiency plays a pivotal role in the progression of cognitive impairment, including in Alzheimer's disease [21]. Cholinergic system dysfunction may be associated with increased serum cholinesterase activity and central nervous system, resulting in high levels of acetylcholine catabolism and impaired cholinergic transmission. R.C. Smith et al. found a 100% increase in plasma pseudocholinesterase (BChE) level in patients with Alzheimer's disease compared to controls [22]. The paper by M. Hosoi et al. points to the potential role of pChE (mainly BChE) as a biomarker for Alzheimer's disease. Activation of neuroinflammation and hyperexpression of BChE by astrocytes and microglia are accompanied by changes in the permeability of the blood-brain barrier, suggesting a relationship between increased BChE level in the central nervous system and serum pChE level [23].

High BChE level is detected in amyloid plaques and neurofibrillary tangles. Increased accumulation of  $\beta$ -amyloid in the hippocampus, thalamus and amygdala is associated with the modulating effect of BChE [13, 24]. Therefore, BChE level plays a pivotal role in the processes of amyloidogenesis and formation of neurofibrillary tangles, which permits the consideration of this enzyme not only in the context of laboratory diagnostics, but also as a potential therapeutic target. For example, when BChE level is high, rivastigmine is the preferred antedementia agent because, unlike donepezil, it also inhibits BChE [23].



Since the liver is involved in the synthesis of pChE, esterase (particularly BChE) level serves as a dynamic indicator of liver synthetic function and lipid metabolism. Reduced pChE levels may be observed in liver pathology [25]. In this study, metabolic parameters did not significantly affect the association between increased pChE level and the prevalence of MCI. Nevertheless, in the study sample, a markedly elevated pChE level was found in patients with T2DM substantiating earlier hypotheses regarding the hyperfunction of this enzyme in experimentally-induced diabetes mellitus [26]. Common mechanisms of increased pChE level in patients with T2DM and MCI include defects in insulin signaling, mitochondrial metabolism, SIRT-PGC-1 $\alpha$  axis, Tau signaling, autonomic function, and neuroinflammatory pathways [27]. Furthermore, elevated levels of pChE are associated with the development of diabetic retinopathy [9]. Thus, high cholinesterase level (specifically BChE) may be a predictor of the development of T2DM and MCI (including of the Alzheimer type) [28]. The lack of a notable correlation between pChE, osteoporosis prevalence, and Charlson Comorbidity Index scores permits the consideration of this parameter as a specific marker of cognitive impairment. However, larger studies involving additional population groups are required.

Notwithstanding the findings of this study, R.C. Smith et al. [22], and M. Hosoi et al. [23], which indicate an increase in serum pChE level with the progression of neurodegenerative processes, there are published papers that demonstrate an inverse relationship. M.X. Dong et al. found a decrease in BChE level in patients with Parkinson's disease (PD) compared to controls [29]. The optimal cut-off point for BChE of 6864.08 U/L allows for distinguishing PD patients with a sensitivity of 61.8% and specificity of 72.1%. A reduction in BChE level to a value below 6550 U/L is significantly associated with a high probability of dementia, with a sensitivity of 70.6% and specificity of 76.3% [29]. Y.C. Chen et al. revealed a decrease in serum pChE, AChE, and BChE activities in patients with post-stroke vascular dementia [30]. Similar findings of lower plasma cholinesterase levels in patients with Alzheimer's disease and dyscirculatory encephalopathy were published by Russian authors [31, 32].

The disparate outcomes of studies examining pChE level in MCI, Alzheimer's disease, Parkinson's disease, and vascular dementia may be attributed to the heterogeneity of the samples and the varied methodological and research approaches employed. Furthermore, there is evidence of a markedly reduced BChE level in patients with dementia with Lewy bodies in comparison to patients with AD and controls. This does not preclude an association between reduced pChE level and synucleinopathies [29, 33]. A parabolic change in the pChE level cannot be excluded with a gradual level increase with the development of MCI and a subsequent level decrease as the dysfunction of the cholinergic system progresses with dementia manifestation. A potential role is played by the notable advancement of comorbid conditions, particularly

alterations in metabolic status and synthetic liver function in patients with dementia and synucleinopathies (Parkinson's disease, dementia with Lewy bodies).

Limitations and advantages of the study. The single-time cross-sectional study did not yield sufficient evidence to establish a causal relationship between the studied parameters. Furthermore, the sample was comprised of female outpatients over the age of 60, which precludes the possibility of extrapolating the results to other populations. Cognitive impairment was diagnosed based on the common validated scales without additional in-depth neuropsychological examination or additional stratification.

To determine the frailty risk, the screening Age Is Not a Hindrance questionnaire was applied without additional comprehensive geriatric examination (phenotype model, frailty index) and the use of objective methods of assessment of various geriatric domains (dynamometry, Timed Up and Go test), which does not allow to reliably exclude the subjective nature of the assessment.

Patients with dementia were not included in the study. Total serum pChE levels were measured without verification of AChE and BChE. Further evaluation of pChE levels in the context of additional comorbidities and laboratory markers (including systemic inflammatory parameters) is advisable.

However, the homogeneous nature of the sample, the sufficiently strict selection criteria, the availability of standardized assessment tools, and the consistency of the identified trends with the data of other researchers allow us to anticipate more significant results when studying a larger sample that includes male patients.

## Conclusion

A significant correlation was identified between pChE activity and changes in MoCA scores and the prevalence of MCI. The high degree of specificity of the test, coupled with the exceedingly low probability of a false-positive result, renders it feasible to suspect MCI at a pChE level of 9978 U/L or above. However, the low level of sensitivity implies a high risk of false-negative results. The data obtained from the multiple linear and logistic regression analyses corroborate the established relationship between pChE activity, MoCA scores, and MCI, even when accounting for the potential influence of metabolic parameters and comorbidities. Despite the correlation between the Age Is Not a Hindrance questionnaire scores and pChE activity, no significant differences in this enzyme levels were identified between patients at low and high risk of frailty. A study on a larger sample size is needed to reliably assess the association of pChE and frailty risk parameters and to further investigate pChE level changes in patients with cognitive impairment of different origin.

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