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The factors associated with mild cognitive impairment in outpatient practice

Faktori povezani sa blagim kognitivnim oštećenjem kod ambulantno lečenih bolesnika

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Abstract

Background/Aim. Previous studies showed that mild cognitive impairment (MCI) was more common in patients with comorbidities and those using medications that disrupt the homeostasis of vitamin B12. The aim of our study was to determine which of these factors are significantly associated with MCI, as well as which are the most significant risk factors for predicting its occurrence. **Methods.** The data have been prospectively collected for 200 adults (35–65 years old) in primary care settings enrolled in the clinical study with the case-control approach. **Results.** By applying the χ^2 test for independence, we have determined that the MCIs and the use of proton-pump inhibitors (PPIs) ($p < 0.0005$), as well as metformin ($p < 0.0005$), are independent factors. In addition, a significantly higher percentage of subjects who had MCI also had a peptic ulcer and diabetes mellitus type 2 (T2DM). Direct logistic regression has been implemented in order to estimate the influence of many probability factors on whether the study patients would have the MCI. Two variables made statistically significant contributions to the model, and these are the serum concentrations of vitamin B12 [odds ratio (OR) = 0.953; 95% confidence interval (CI) 0.936–0.971; $p < 0.001$] and T2DM (OR = 6.681; 95% CI 1.305–34.198; $p = 0.023$). **Conclusion.** The absolute and relative risk associations of exposure to medicines and MCI is lower than those of comorbidities and MCI. Serum concentrations of vitamin B12, as well as the presence of T2DM, have the greatest statistically significant influence on predicting MCI.

Key words:
cognitive dysfunction; comorbidity; diabetes mellitus, type 2; risk assessment; risk factors; vitamin b 12.

Apstrakt

Uvod/Cilj. Prethodne studije pokazale su da je blago kognitivno oštećenje (BKO) češće kod bolesnika sa komorbiditetima, kao i kod bolesnika koji koriste lekove koji remete homeostazu vitamina B12. Cilj rada bio je da se utvrdi koji od tih faktora su značajno povezani sa BKO i koji su najznačajniji za predviđanje njegovog nastanka. **Metode.** Podaci o 200 odraslih osoba (starosti 35–65 godina) uključenih u kliničku studiju po tipu anamnestičke studije u ustanovi primarne zdravstvene zaštite su prikupljeni prospektivno. **Rezultati.** Primenom χ^2 testa nezavisnosti, utvrđeno je da da su BKO i upotreba inhibitora protonske pumpe (IPP) ($p < 0{,}0005$), kao i metformina ($p < 0{,}0005$), nezavisni faktori. Takođe, značajno viši procenat ispitanika koji su imali BKO su imali peptički ulkus i tip 2 dijabetes melitus (T2DM). Da bi se procenio uticaj mnogih faktora verovatnoće na to da li će ispitani bolesnici imati BKO primenjena je direktna logistička regresija. Dve varijable dale su statistički značajan doprinos modelu, a to su koncentracije vitamina B12 u serumu [*odds ratio* (OR) = 0,953; 95% *confidence interval* (CI) 0,936–0,971; $p < 0{,}001$] i T2DM (OR = 6,681; 95% CI 1,305–34,198; $p = 0{,}023$). **Zaključak.** Apsolutna i relativna povezanost rizika izloženosti lekovima sa BKO niža je od povezanosti komorbiditeta sa BKO. Koncentracije vitamina B12 u serumu i prisustvo T2DM imaju najveći statistički značajan uticaj na predviđanje BKO.

Ključne reči:
saznanje, disfunkcija; komorbiditet; dijabetes mellitus, insulin nezavisni; rizik, procena; faktori rizika; vitamin b12.

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Introduction

Mild cognitive impairment (MCI) is a clinical-cognitive syndrome that includes concern regarding a change in cognition, impairment in one or more cognitive domains, and preservation of independence in functional abilities without dementia [1]. MCI does not always precede dementia; it can revert to normal cognition or remain stable. It is characterized by cognitive dysfunction in different domains including the memory domain. It does not disable everyday independent functioning, but the deteriorations are inappropriate for a patient's age and education [2].

Some of the clinical syndromes associated with MCI can be cured, but, on the other hand, drugs used for treating these conditions can also have adverse effects opposing the improvement of the existing features of MCI [3, 4].

One of the possible causes of the appearance of MCI can be the deficiency of vitamin B12, as its insufficient body levels can adversely affect brain functions. If there is a deficiency in an organism of the nutrient, it is necessary to compensate for it by intaking appropriate dietary supplements. The causes of vitamin B12 deficiency are in a wide spectrum of pathological conditions such as reduced consumption because of stomach pathology, which includes pernicious anemia, atrophic gastritis caused by chronic inflammation of *Helicobacter (H.) pillory* infection, gastrectomy, Zollinger-Ellison syndrome, bowel diseases, such as Crohn's disease, celiac disease, tropical sprue, bowel resections, congenital selective malabsorption of vitamin B12 with proteinuria, or Imerslund-Grasbeck syndrome.

Deficiency of vitamin B12, besides hematological and gastrointestinal disorders, can lead to neuropsychiatric disorders and symptoms such as neuropathy, cerebellar ataxia, dementia, and disorders of mood occurring as a result of insufficient intake, inadequate absorption, and reduced function. Cyanocobalamin participates in the metabolism of amino acids, myelin regeneration, and growth and differentiation of bone marrow cells. Due to the deficiency of vitamin B12, degeneration of lateral and dorsal columns of the spinal cord with consequent neuropathy occurs. Cognitive dysfunction of these patients is often followed by irritability, depression, and in some cases, psychotic behavior [5].

Among the causes of vitamin B12 deficiency are some medicaments such as biguanides (metformin) and proton-pump inhibitors (PPI), which significantly disturb its metabolism. That can have secondary consequences to hematopoiesis, gastrointestinal system and nervous system, including cognitive consequences, which it has on healthy patients [6, 7]. The aim of our research was to investigate the factors which are associated with MCI in patients within primary healthcare settings, particularly including comorbidities and drugs. We proposed that insufficient vitamin B12 body levels play a prominent role in predicting MCI existence because of the influence of many factors commonly encountered in ambulatory patients which disturb the homeostasis of that essential nutrient.

Methods

In this clinical research, we implemented the case-control approach on the level of primary health care. We conducted the research at the Health Center "Dr. Milenko Marin" in Loznica, Serbia, with the approval of the Ethics Committee, number 1117/1 / V-1, issued on 8 July 2021. In the case group, there were patients with MCI (MCI+), as confirmed using appropriate screening tests, while in the control group, there were patients without MCI (MCI-).

The subjects were selected from the population of adult patients of the Health Center Loznica who fulfilled the inclusion criteria and did not have exclusion criteria. The inclusion criteria were the following: male or female gender, 35–65 years of age, and those who gave consent for participating in the study and were previously fully informed.

The exclusion criteria were the following: patients younger than 35 or older than 65 years of age (since physiological aging is associated with the weakening of cognitive functions, we did not include people over 65 years of age in our research), patients with a confirmed diagnosis of MCI before this study, presence of other neuropsychiatric illness for which MCI was diagnosed, the respondents who did not give consent for participation or who already participate in another study, and the presence of any illness or condition which disrupts the participation in the study.

All the respondents who satisfy the criteria for participation in the study were given to fill in a screening test by educated doctors in order to reveal the presence of cognitive deficiency, and the scores were measured by Mini-Mental State Examination (MMSE), Montreal Cognitive Assessment (MoCA), and Lawton Instrumental Activities of Daily Living (IADL) scale. The participants who had values lower than 26 points for MoCA, 20 to 24 points for MMSE, which indicates MCI, and values higher than 8 points for women and 5 points for men for Lawton IADL scale, which marks that the patients were functional in doing everyday activities, but with a present cognitive impairment, were referred to a neurologist for the confirmation of the diagnosis of MCI.

MoCA is a test for the fast assessment of cognition. The test includes the domain of concentration and attention, executive functions, language, memory visuoconstructive skills, conceptualization, and calculation of orientation. In this work, the Serbian version of the test was used with a structure and description, scheme, and instructions for the application and scoring of the test. The Serbian version of MMSE consists of questions in which the following cognitive functions are examined: memory, attention and calculation, orientation in space and time, naming, recalling, and complex actions. It represents the most frequently and widely used screening test, which correlates well with other neuropsychological tests. Testing time is less than 10 min for a trained clinician (4–21 min). Lawton IADL is an appropriate instrument to assess independent living skills [8–10]. These skills are considered more complex than the basic activities of daily living as measured by the Katz Index of activities of

daily living. The instrument is most useful for identifying how a person is functioning at present and for identifying improvement or deterioration over time. There are eight domains of function measured with the Lawton IADL scale. The Lawton IADL is an easy-to-administer assessment instrument that provides self-reported information [8–10].

The study subjects, for which the diagnosis of MCI was confirmed by the consulted neurologist, were allocated to the case group while the others, without the presence of MCI, were allocated to the control group in the proportion 1 : 3. While allocating to the study groups, the participants were matched according to their gender and age group.

The primary dependent variable (outcome) was the presence of MCI, which was demonstrated as a binary variable (present/absent). The primary independent variable was the presence of deficiency of vitamin B12 expressed as its concentration in the serum of an examinee under the given limit value. In serum samples of study subjects, the concentration of vitamin B12 was measured using a routine method of clinical biochemistry at the authorized laboratory of the Health Center. The limit values and the concentration units of vitamin B12 were determined according to the local laboratory standards. They were determined by taking blood samples and analyzing the serum with chemiluminescent microparticle immunoassay (CMIA) using the analyzer Abbott Alinity; its reference values were from 138.00 to 652.00 pmol/L.

The prospectively collected data for the study subjects were analyzed by the methods of descriptive statistics, hypothesis testing, and logistic regression. The descriptive statistics include determinations of central tendency measures (e.g., the mean, median) and variability values [e.g., standard deviation (SD), confidence limits]. Continuous variables, such as age and vitamin B12 value, were presented using minimum and maximum values, mean, and SD. Categorical variables, such as MCI, the use of certain medications, and the presence of certain comorbidities, were presented using absolute and relative frequencies.

We analyzed the data in relation to whether the patients have MCI in the following way: when analyzing continuous variables, we used the *t*-test for independent samples, and when analyzing categorical variables, we used the χ^2 test. We analyzed the data in relation to vitamin B12 values in patients in the following way: we used linear correlation and regression methods for analyzing continuous variables, and we used the *t*-test for independent samples when analyzing categorical variables.

Standard multiple regression was used to assess the ability to predict the value of vitamin B12 in patients. In order to evaluate the influence of several factors on the probability that the respondents will have MCI, a direct logistic regression was conducted. We used the receiver operating curve (ROC) to determine the size of the influence of the administered drugs and associated diseases on the occurrence of MCI. The results were considered statistically significant if the *p*-value was less than or equal to 0.05.

Results

The study population consisted of 200 adult persons. The main characteristics of the patients are presented in Table 1.

The values of the level of vitamin B12 varied from 73.8–1,476.0 pmol/L for the total number of respondents, 173.8–326.6 pmol/L for the case group of the study subjects, and 198.0–1,476.0 pmol/L for the control group of the patients. The average value and SD of vitamins for all the respondents, for the patients with the diagnosed MCI, and patients with preserved cognition are shown in Table 1. Charslon's comorbidity index (CCI) gives us two results, a total score and an estimate of the probability that a person will live for the next ten years according to the groups presented in Table 1.

The sociodemographic characteristics of the study population and the data of the χ^2 test for the descriptive characteristics of the population were presented in Table 2, where the value of $p > 0.05$ for the entire category was variable, so these variables did not prove to be significant for that research.

Table 3 shows the numerical and percentage frequencies of the subjects' associated diseases and the results of the χ^2 test for comorbidities for MCI. The results were not statistically significant ($p > 0.005$) for sideropenic anemia and other types of anemia, hypertension, angina pectoris, acute myocardial infarction, heart failure, vascular diseases, diseases of the thyroid gland, diseases of cerebral blood vessels, asthma, and other chronic obstructive lung diseases, liver diseases, diseases of the musculoskeletal system and joint tissue, chronic kidney diseases, and benign prostatic hyperplasia, while for the peptic ulcer ($\chi^2 = 24.000$, df = 1, $p < 0.001$) and diabetes mellitus (DM) ($\chi^2 = 21.258$, df = 1, $p < 0.001$), χ^2 test results were found to be significant.

Figures 1 and 2 present the distribution of disease frequency of peptic ulcer and DM, respectively, with the appearance of MCI. By applying the χ^2 test, we have determined that a significantly higher percentage of respondents with MCI also had peptic ulcer and DM.

Table 1

Basic characteristics of study subjects

Variable	All subjects	Case group	Control group
Age (years)	54.18 ± 5.90 (35–65)	55.34 ± 3.85 (45–60)	53.79 ± 6.41 (35–65)
Vitamin B12 (pmol/L)	372.2 ± 153.1 (73.8–1476.0)	229.9 ± 55.5 (73.8–326.6)	419.6 ± 145.8 (198.0–1476.0)
CCI-score	2.03 ± 1.26 (0–5)	2.66 ± 1.27 (0–5)	1.82 ± 1.18 (0–5)
CCI-10-year survival rate	83.95 ± 17.19 (21–98)	75.24 ± 23.65 (21–98)	86.85 ± 13.30 (21–98)

CCI – Charslon Comorbidity Index. All values are expressed as mean ± standard deviation (minimum-maximum).

Table 2

Sociodemographic characteristics of the subjects

Variable	All subjects	Case group	Control group	χ^2 test	*p*-values
Gender					
male	98 (49.0)	24 (48.0)	74 (49.3)	0.027	0.870
female	102 (51.0)	26 (52.0)	76 (50.7)		
Marital status					
single	19 (9.5)	3 (6)	16 (10.7)		
married	147 (73.5)	39 (78)	108 (72)	1.083	0.582
widower	34 (17)	8 (16)	26 (17.3)		
Education					
elementary school	20 (10)	4 (8)	16 (10.7)		
high school	129 (64.5)	35 (70)	94 (62.7)	0.921	0.820
college	38 (19)	8 (16)	30 (20)		
master's degree	13 (6.5)	3 (6)	10 (6.7)		
Monthly income					
minimal	31 (15.5)	5 (10)	26 (17.3)		
middle	96 (48)	31 (62)	65 (43.3)	5.343	0.069
high	73 (36.5)	14 (28)	59 (39.3)		
Settlement					
city	96 (48)	21 (42)	75 (50)	0.962	0.327
countryside	104 (52)	29 (58)	75 (50)		
Occupation					
employed	140 (70)	33 (66)	107 (71.3)		
unemployed	35 (17.5)	10 (20)	25 (16.7)	0.510	0.775
retired	25 (12.5)	7 (14)	18 (12)		
Tobacco	101 (50.5)	26 (52)	75 (50)	0.060	0.806
Alcohol	81 (40.5)	21 (42)	60 (40)	0.062	0.803
Immunized	132 (66)	33 (66)	99 (66)	< 0.001	1.000
COVID-19 positive	60 (30)	15 (30)	45 (30)	< 0.001	1.000

***p* – probability. All values are expressed as numbers (percentages).**

Table 3

Associated diseases of the subjects

Variable		All subjects	Case group	Control group	χ^2 test	*p*-values
D50	yes	12 (6)	3 (6)	9 (6)	0.000	1.000
D51-64	yes	3 (1.5)	2 (4)	1 (0.7)	2.820	0.093
I10	yes	128 (64)	34 (68)	94 (62.7)	0.463	0.496
N18	yes	5 (2.5)	3 (6)	2 (1.3)	3.350	0.067
I20	yes	30 (15)	7 (14)	23 (15.3)	0.052	0.819
J45	yes	4 (2)	1 (2)	3 (2)	0.000	1.000
E00-07	yes	9 (4.5)	4 (8)	5 (3.3)	1.900	0.168
N40	yes	22 (11)	6 (12)	16 (10.7)	0.068	0.794
I21	yes	11 (5.5)	2 (4)	9 (6)	0.289	0.591
I50	yes	8 (4)	3 (6)	5 (1.3)	0.694	0.405
I70-89	yes	86 (43)	25 (50)	61 (40.7)	1.333	0.248
I60-69	yes	1 (0.5)	1 (2)	0 (0)	3.015	0.082
J44	yes	4 (2)	1 (2)	3 (2)	0.000	1.000
M00-99	yes	3 (1.5)	1 (2)	2 (1.3)	0.113	0.737
K27	yes	40 (20)	22 (44)	18 (12)	24.000	< 0.001
K70-77	yes	1 (0.5)	1 (2)	0 (0)	3.015	0.082
E10-14	yes	42 (21)	22 (44)	20 (13.3)	21.258	< 0.001

D50 – Iron deficiency anemia; D51-64 – Non-Iron deficiency anemia; I10 – Essential (primary) arterial hypertension; N18 – Chronic kidney disease; I20 – Angina pectoris. J45 – Asthma; E00-07 – Diseases of the thyroid gland; N40 – Prostate enlargement; I21 – Acute heart attack; I50 – Heart insufficiency; I70-89 – Diseases of arteries, veins, small blood vessels, lymphatic vessels, and lymph nodes; I60-69 – Diseases of blood vessels of the brain; J44 – Chronic obstructive pulmonary disease; M00-99 – Diseases of the musculoskeletal system and connective tissue; K27 – Peptic ulcer; K70-77 – Liver disease; E10-14 – Diabetes mellitus. *p* – probability. All values are expressed as numbers (percentages).

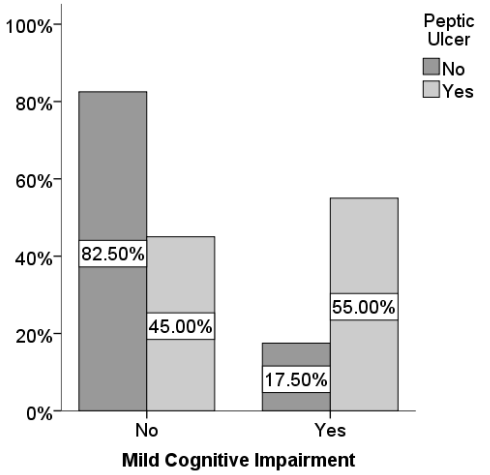

Fig. 1 – Influence of peptic ulcer on the occurrence of mild cognitive impairment.

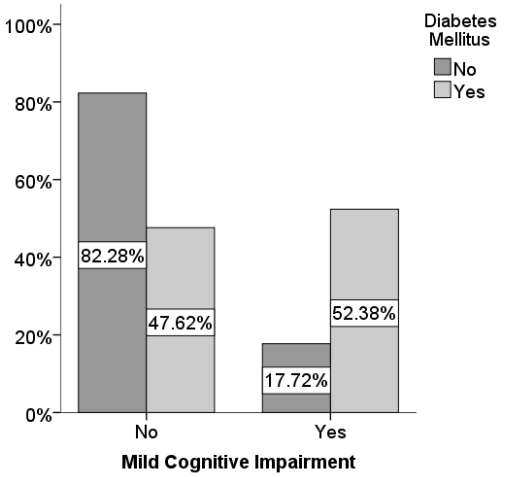

Fig. 2 – Influence of diabetes mellitus on the occurrence of mild cognitive impairment.

In Table 4, we have presented absolute values and percentage frequencies of drug use among the study subjects, and by using the χ^2 test, the distribution between drug use and the occurrence of MCI was examined. For mesalazine, the combination of progestin and estrogen, estrogen, tetracycline, penicillin, erythromycin, phenobarbital, methotrexate, diuretics, iron, vitamin B1, vitamin B12, folic acid, levothyroxine sodium, bronchodilators, angiotensin-converting enzyme (ACE) inhibitors, antiarrhythmic agents, beta-adrenergic receptor blockers, trimetazidine, calcium channel blockers, isosorbide mononitrate, allopurinol, acetylsalicylic acid, clopidogrel, hydroxymethylglutaryl-CoA (HMG-CoA) reductase inhibitors (statins), glyceryl trinitrate, the results were not statistically significant ($p > 0.005$), while for PPIs ($\chi^2 = 22.256$, df = 1, $p < 0.001$) and metformin ($\chi^2 = 21.258$, df = 1, $p < 0.001$), high statistical significance was found.

Table 4

Drugs used by subjects

Drug		All subjects	Case group	Control group	χ^2 test	*p*-values
Proton pump inhibitors	yes	53 (26.5)	26 (52)	27 (18)	22.256	< 0.001
Mesalazine	yes	3 (1.5)	0 (0)	3 (2)	1.015	0.314
Metformin	yes	42 (21)	22 (44)	20 (13.3)	21.258	< 0.001
Progestogens and estrogens	yes	6 (3)	2 (4)	4 (2.7)	0.229	0.632
Estrogens	yes	14 (7)	4 (8)	10 (6.7)	0.102	0.749
Tetracycline	yes	13 (6.5)	3 (6)	10 (6.7)	0.027	0.868
Penicillins	yes	40 (20)	11 (22)	29 (19.3)	0.167	0.683
Erythromycin	yes	6 (3)	2 (4)	4 (2.7)	0.229	0.632
Phenobarbital	yes	1 (0.5)	1 (2)	0 (0)	3.015	0.082
Methotrexate	yes	4 (2)	1 (2)	3 (2)	<0.001	1.000
Diuretics	yes	79 (39.5)	20 (40)	59 (39.3)	0.007	0.933
Iron	yes	13 (6.5)	4 (8)	9 (6)	0.247	0.619
Vitamin B1	yes	15 (7.5)	4 (8)	11 (7.3)	0.024	0.877
Vitamin B12	yes	15 (7.5)	4 (8)	11 (7.3)	0.024	0.877
Folic acid	yes	8 (4)	2 (4)	6 (4)	<0,001	1.000
Levothyroxine sodium	yes	8 (4)	4 (8)	4 (2.7)	2.778	0.096
Bronchodilators	yes	4 (2)	1 (2)	3 (2)	<0.001	1.000
ACE inhibitors	yes	95 (47.5)	25 (50)	70 (46.7)	0.167	0.683
Antiarrhythmics	yes	15 (7.5)	4 (8)	11 (7.3)	0.024	0.877
Beta blockers	yes	73 (36.5)	19 (38)	54 (36)	0.065	0.799
Trimetazidine	yes	14 (7)	2 (4)	12 (8)	0.922	0.337
Anti-cholinesterase	yes	1 (0.5)	0 (0)	1 (0.7)	0.335	0.563
Tamsulosin	yes	31 (15.5)	8 (16)	23 (15.3)	0.013	0.910
Calcium channel blockers	yes	64 (32)	16 (32)	48 (32)	<0,001	1.000
Isosorbide mononitrate	yes	19 (9.5)	5 (10)	14 (9.3)	0.019	0.889
Allopurinol	yes	13 (6.5)	3 (6)	10 (6.7)	0.027	0.868
Acetasalicylic acid	yes	86 (43)	21 (42)	65 (43.3)	0.027	0.869
Clopidogrel	yes	9 (4.5)	2 (4)	7 (4.7)	0.039	0.844
HGM inhibitors	yes	89 (44.5)	23 (46)	66 (44)	0.061	0.805
Glyceryl trinitrate	yes	7 (3.5)	2 (4)	5 (3.3)	0.049	0.824

ACE – angiotensin-converting enzyme; HGM – hydroxymethylglutaryl.

All values are expressed as numbers (percentages).

Applying the χ^2 test of independence, we determined that the values of MCI and the use of PPI ($p < 0.0005$) were dependent variables. An increase in the use of PPIs was associated with an increase in the incidence of MCI (Figure 3). We also found that MCI and the use of metformin ($p < 0.0005$) were related, too. The increase in the use of metformin was associated with an increase in the incidence of MCI (Figure 4).

By analyzing the comorbidities and use of medications of the respondents, we have determined that the MCI was influenced by two comorbidities and the application of two medications. We shall consider which of these four factors had a greater influence on the presence of the MCI (Table 5).

Using the ROC curve, it has been determined which risk factors (DM, peptic ulcer, and use of the medicaments of metformin and the PPI) had the greatest influence on the MCI, as shown in Figure 5.

Since the test area under the curve was occupied by the PPI, we concluded that the PPI had the greatest influence on the presence of the MCI; however, it should be noticed that all the areas had approximately similar values.

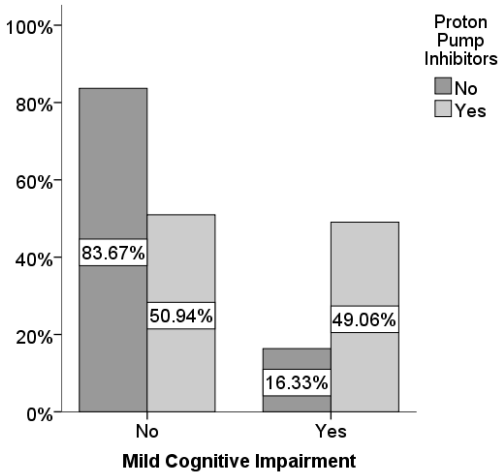

Fig. 3 – Influence of proton pump inhibitors on the occurrence of mild cognitive impairment.

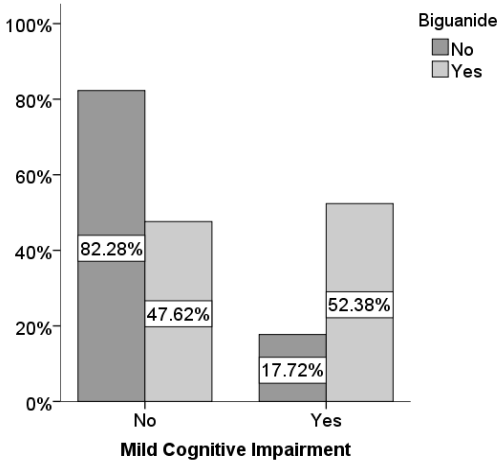

Fig. 4 – Influence of biguanide (metformin) on the occurrence of mild cognitive impairment.

Table 5

Influence of drugs and diseases on the occurrence of mild cognitive impairment

Variable	AUC	SE	*p*	95% confidence interval	
				lower bound	upper bound
Proton pump inhibitors	0.670	0.047	< 0.001	0.578	0.762
Metformin	0.653	0.048	0.001	0.559	0.748
Peptic ulcer	0.660	0.048	0.001	0.5660	0.754
Diabetes mellitus	0.653	0.048	0.001	0.559	0.748

AUC – area under the curve; SE – standard error; *p* – probability.

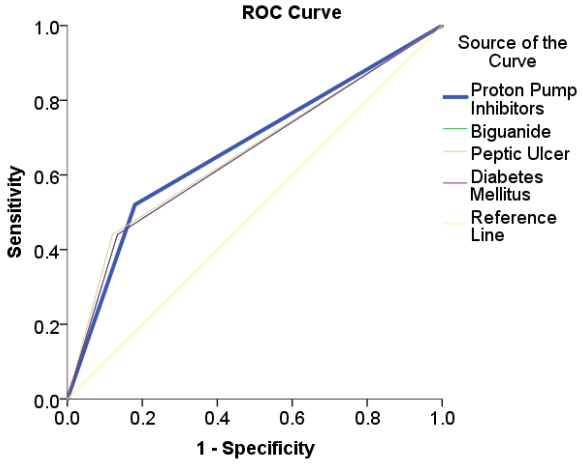

Fig. 5 – Influence of drugs and diseases on the occurrence of mild cognitive impairment. ROC – receiver operating characteristic curve.

Table 6

Prediction of mild cognitive impairment

Variable	B	SE	Wald	df	*p*	OR	95% confidence interval of OR	
							lower limit	upper limit
Vitamin B12	-0.048	0.009	26.565	1	0.000	0.953	0.936	0.971
Peptic ulcer	0.950	1.096	0.751	1	0.386	2.585	0.302	22.130
Diabetes mellitus type 2	1.899	0.833	5.197	1	0.023	6.681	1.305	34.198
CCI	-0.147	0.314	0.218	1	0.640	0.863	0.466	1.599
PPI	-0.072	0.987	0.005	1	0.942	0.930	0.134	6.438
Constant	12.592	2.684	22.003	1	0.000	294,278.263		

CCI – Charlson Comorbidity Index; PPI – proton pump inhibitors; df – degree of freedom; *p* –probability; OR – odds ratio; SE – standard error; B – regression slope (unstandardised coefficient).

The absolute and relative risk of being exposed to medications was lower than the risk of comorbidities and conditions for the presence of the MCI at the same time in patients in primary health protection.

The following variables had a statistically important influence on the prediction of the MCI: the serum level of vitamin B12 ($p < 0.001$; Wald = 26.565) and type 2 DM (T2DM) ($p = 0.023$; Wald = 5.197). Considering that the highest value of the Wald coefficient had the serum level of vitamin B12, we came to the conclusion that this factor had the highest and statistically important influence on the prediction of the MCI. Besides this result, in this model, T2DM also had an important influence on the prediction of the MCI (Table 6).

The whole model (with all the predictors) was statistically important, c2 (8, H = 200) = 12.352, $p < 0.0005$, which shows that the model could help successfully in distinguishing the patients who have or do not have the MCI: the model, as a whole, explains between 52.7% (R squared Cox-Snell) and 78.1% (R squared Nagelkerke) variance of the MCI. Two variables have given statistically important contributions to the model, and these were the serum values of vitamin B12 and T2DM.

The serum value of vitamin B12 is the strongest factor in predicting the MCI, of which the quotient probability is 0.953. That means that the study patients who had a value of vitamin B12 higher by one unit had 4.7% less frequent MCI compared to those who had a value of vitamin B12 lower by one unit.

Discussion

Our study presents evidence that low serum concentrations of vitamin B12 and the presence of T2DM are associated independently with the clinical features of MCI in patients of primary healthcare settings. Our statistical models, which also included CCI, the use of PPI, and peptic ulcer disease (which did not show significant association with the MCI), were fairly reliable, explaining between 52.7% and 78.1% variability of the MCI appearances. Other researchers, by using a standard approach for estimating global cognition in examining Parkinson's disease (PD), investigated previously the relationships between cognitive status, comorbidities, metabolic variables, and lifestyle factors of 533 participants with PD from the data of the COPPADIS study. They found that the cognitive outcome was negatively connected to the levels of interleukin (IL)-2, IL-6, iron, and homocysteine ($p < 0.05$) and positively connected to the levels of vitamin B12. The results of this study are similar to our research, as they establish a connection between the increase in the presence of MCI and vitamin B12 deficiency. They also do not indicate the greater importance of the impact of iron deficiency on cognitive abilities [11].

The estimation of the potential value of some microRNAs as diagnostic biomarkers for MCI among patients with T2DM and the identification of other risk factors for MCI was the aim of the research of another study. In this trial, researchers included 163 adult persons with the disease, and they found significantly excessive expression of microR-132 among the study subjects with T2DM with MCI compared to those with normal cognition. In this research, the association between T2DM and MCI was proven, which also proved to be a significant result in our final research model [12]. Other research groups tried to estimate the associations between CCI, polypharmacy, inappropriate use of medications, and cognitive impairments of 105 patients from institutions for long-term care. An important difference was reported between genders, CCI, and cognitive impairment, while every increase of the CCI by one point added the risk of cognitive impairment 3.1 times (95% CI 1.8–5.4), hypertension increases the risk 12 times (95% CI 2.5–67.8). The study conducted by these researchers examines the impact of CCI on the occurrence of MCI, which was also part of our study, but this variable did not stand out as significant compared to the other variables we looked at in our study [13]. The methodological differences, including the sample sizes, could contribute to the differences between the results of the two studies.

The deficiency of cobalamin (vitamin B12) could be connected to the *H. pylori* infection. It had been reported that the serum levels of hemoglobin and cobalamin were significantly increased after the treatment of *H. pylori* infections, regardless of the status of its eradication. This research points to the direct and/or indirect relationships between *H. pylori* gastrointestinal infection, vitamin B12 homeostasis, and PPI use, which was also the assumption in our research that turned out to be correct [14].

It is also known that homocysteine is a risk factor for brain atrophy, cognitive impairment, and dementia and that vitamin B12 and folate are necessary for the methylation of

homocysteine. The previous study, enrolling elderly adult Chinese people, demonstrated that the low level of folates and vitamin B12 in the blood increased serum levels of homocysteine, which were, in turn, significantly related to MCI and Alzheimer's disease. This is another study that proves the connection between MCI and vitamin B12 deficiency, which was the basic hypothesis of our study [15].

In our study, besides the PPI, the use of metformin was significantly associated with the presence of MCI in the univariate analysis, but this connection disappeared when other factors had been analyzed simultaneously within the final statistical model. In the previously published observational study of 200 patients with T2DM treated with metformin, the deficiency of vitamin B12 and diabetic neuropathy were very frequent, and they were related to the increased anti-gastric parietal cell antibodies, tumor necrosis factor (TNF)-α, and dyslipidemia [16]. While using the screening for the B12 deficiency by two markers, methylmalonic acid and homocysteine, one research group noticed a prevalence of 23.3% of the deficiency of vitamin B12 within 490 hospitalized patients. In this study, it was shown that the prolonged use of metformin and PPI was significantly related to vitamin B12 deficiency [17]. The use of these drugs also has a more significant impact in our study on vitamin B12 deficiency compared to other drugs.

The importance of metabolic functions on vitamin B12 deficiency and, consequently, its association with MCI was the subject of our research. Our results are in concordance with many previously published pieces of evidence, either directly or indirectly. The highest risk of weak cognitive function was noticed in the comorbidity group (study subjects with depressive symptoms and metabolic syndrome) in our study, as well as in the previously published study [18].

T2DM was the key factor independently associated with the MCI in our study. In the meta-analysis, this disease was also identified as the key risk factor for dementia and MCI, so the growing prevalence of glycemic disorders had the potential to additionally increase the prevalence of cognitive impairment in the general population [19]. Other researchers reported similar results, and some of them pointed to the mitochondrial abnormalities in patients with DM as the pathogenic pathways for neuronal damage and subsequent cognitive impairment [20, 21]. Our research showed that vitamin B12 deficiency leads to MCI, which suggests that vitamin B12 supplementation can prevent this damage. Some scientists have already dealt with this topic, investigating the proposed neuroprotective effects of vitamin B12 for cognition. There are reports that vitamin B12 affects the function of memory, especially in patients with MCI, so they also point out that supplements of vitamin B12 can be efficient strategies for the prevention and/or treatment of Alzheimer's dementia [22].

Again, the researchers focused on the relationships between the deficiency of vitamin B12 and the use of metformin, including the long-term prescribing settings. Some of them reported that anemia was more frequent in patients using metformin but without significant effects on the status of vitamin B12. On the other hand, the prevalence of neuropathy was higher in the patients using metformin, which, at the same time, had low levels of vitamin B12. This research recommends measures to be applied when using metformin, which, in our research, presented as a significant factor in the occurrence of MCI [23].

Furthermore, the results of another study showed that the levels of vitamin B12 in the serum had a strong negative correlation with the duration of using metformin. At the same time, the status of vitamin B12 did not influence the presence and severity of polyneuropathy, which is one of the important complications of long-standing, uncontrolled DM [24].

Overall, it seems that the use of PPI and metformin, as well as peptic ulcer disease, could be indirectly associated with MCI. The presence of damaged stomach mucosa caused by *H. pylori* infection or other aggressive agents and conditions necessitates the prescription of gastroprotective medications, such as PPIs. On the other hand, gastric mucosal injury disturbs vitamin B12 homeostatic pathways, which, on its own, has a plethora of negative effects on nervous system functions, including cognitive impairment. Similar circumstances probably exist for the use of metformin which represents the first choice of the majority of patients with T2DM. In addition, there is a possibility that metformin could interfere directly with vitamin B12 intestinal absorption, although the exact mechanism is yet to be clarified [25]. Some inconsistency of results between different studies about the relative importance of various risk factors for MCI and/or vitamin B12 deficiency is probably influenced by the heterogeneity of enrolled patient populations. A wide spectrum of drug prescription patterns, disease clinical features, and healthcare settings exist, thus making direct comparisons rather difficult. Nevertheless, we consider that our study contributed significantly to the novel scientific knowledge in the field, quantifying exactly the associations with MCI for at least two factors commonly encountered in primary health care patients, serum concentration of vitamin B12 and T2DM.

Conclusion

The results of this study revealed that there is an association between the low serum level of vitamin B12 and the presence of MCIs in patients in primary health care. In addition, we have found that a significantly higher percentage of the study patients who had MCI also used PPI and metformin and had peptic ulcer disease, but that associations could have indirect pathways. On the other hand, the increasing number of patients with T2DM also manifested the symptoms of MCI. Overall, the absolute and relative risk of being exposed to the medications and having MCI was lower than the corresponding risks of comorbidities and conditions for the presence of MCI at the same time. The low levels of vitamin B12 and T2DM had the greatest, most unique, and statistically significant influence on predicting the outcome of MCI. These results could contribute to the realization of additional research and medical protective measures for preventing the occurrence of MCI.

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