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Homocysteine – A predictor for five year-mortality in patients with subjective cognitive decline, mild cognitive impairment and Alzheimer's dementia

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ABSTRACT

Background: Subjective Cognitive Decline (SCD) and Mild Cognitive Impairment (MCI) are preclinical stages of Alzheimer's Disease (AD), which is the most common entity of dementia. Homocysteine is an amino acid in the methionine cycle, and many studies revealed a significant association between elevated homocysteine serum levels and the progression of dementia. The primary objective of this retrospective study was to investigate whether elevated homocysteine serum levels could be associated with mortality and neuropsychological test results in individuals suffering from SCD, MCI or AD.

Methods: This study is a single-center explorative retrospective data analysis with 976 data protocols from the Memory Outpatient's Clinic of the Medical University of Vienna included. All patients underwent a neurological examination, a laboratory blood test, and neuropsychological testing to establish a diagnosis of either SCD, MCI, or AD. Data was evaluated by Kaplan-Meier functions, factor analysis, and binary logistic regression models.

Results: Patients with AD showed significantly higher mean homocysteine levels (SCD 12.15 ± 4.71, MCI 12.80 ± 4.81, AD 15.0 ± 6.44 μmol/L) compared to those with SCD and MCI ($p \leq .001$). The mean age of patients with AD (75.2 ± 7.8) was significantly older at the time of testing than of patients with MCI (69.1 ± 9.6) or SCD (66.8 ± 9.3). Since homocysteine levels increase with age, this could be a possible explanation for the higher levels of AD patients. The age at death did not differ significantly between all diagnostic subgroups, resulting in the shortest survival times for AD patients. Homocysteine levels were negatively associated with in Mini-Mental State Examination (MMSE) and Neuropsyhcological Test Battery Vienna (NTBV) factors F1-F4 (F1 = attention, F2 = memory, F3 = executive functions, F4 = naming/verbal comprehension). Moreover, higher homocysteine levels significantly predicted shorter five-year survival in the logistic regression models, even after adjusting for age, diagnostic subgroups, sex, years of education and results of neuropsychological testing.

Conclusion: The results of this study suggest that homocysteine levels are independently associated with impaired cognitive function and increased five-year mortality.

1. Introduction

Subjective Cognitive Decline (SCD) and Mild Cognitive Impairment (MCI) are preclinical stages of Alzheimer's Disease (AD), which is the most common entity of dementia (Selhub, 1999).

SCD represents the very first stage of cognitive impairment, in which individuals already subjectively notice the decline of cognitive abilities. At this point, the subjectively perceived symptoms are often not yet objectifiable by cognitive testing (Pietrzik and Brönstrup, 1998). Mild Cognitive Impairment describes an intermediate cognition stage, but in contrast to AD, MCI patients still maintain more independence in functional abilities (Sanford, 2017; Petersen et al., 2014). In addition to the individual burden, Alzheimer's disease also poses a significant challenge to society (Soria Lopez et al., 2019). According to the Austrian Alzheimer Society, about 100.000 people in Austria currently suffer from dementia. By 2050, the number is expected to rise to about 230.000. At present, about one billion Euros per year are spent on the care of dementia patients in Austria (Patscheider and Zahlen und

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Statistik, 2021). Due to demographic developments, incidence and prevalence increase, costs continue to rise, and dementia has become an important social and health policy issue (Höfler et al., 2014).

SCD and MCI, which can be seen as precursors of AD, can be detected several years before the clinical diagnosis of AD (Bäckman et al., 2003). The question of which markers or patterns of decline might predict the progress to AD are currently the subject of many studies.

Homocysteine is a non-proteinogenic amino acid, which is not a constituent of our diet, but part of the methionine cycle (Moretti and Caruso, 2019). The metabolism of homocysteine forms the interface between two different pathways, one leading back to methionine and the other to cystathionine (Finkelstein and Martin, 2000; Selhub, 1999). Apart from its important metabolic role, increased homocysteine can also be harmful. Homocysteinemia is mainly caused by a vitamin B2, B6, B12 and folate deficiency (Moretti and Caruso, 2019; Pietrzik and Brönstrup, 1998; Kim et al., 2018; Smith et al., 2010; Zhang et al., 2017). Apart from vitamin deficiencies and genetic defects, there are numerous lifestyle conditions, such as smoking, high alcohol and coffee consumption, as well as physical inactivity, that influence homocysteine levels (Bolander-Gouaille and Bottiglieri, 2007; Nygård et al., 1998; Schneede et al., 2000).

Many studies over the past 40 years have confirmed homocysteinemia as a strong risk factor for arterial and venous thromboembolism and for atherosclerotic vascular disease in the coronary, cerebral and peripheral vessels (Selhub, 1999; Nygård et al., 1999; Kalra, 2004; Wang et al., 2014; Cacciapuoti, 2011; Larsson et al., 2019; Abby et al., 1998; Marcus et al., 2007; Boers, 1997; D'Angelo et al., 1997; Maron and Loscalzo, 2006; Koller et al., 2018) and its use as a risk marker is meanwhile well established (International Conference on Homocysteine Metabolism, From Basic Science to Clinical Medicine. Ireland, July 2-6, 1995; Homocysteine Metabolism 2nd International Conference. Nijmegen, The Netherlands, 26-29 April, 1998; Proceedings of the 7th International Conference on Homocysteine Metabolism. June, 21-25, 2011; Abstracts of the 4th International Conference on Homocysteine Metabolism. Basel, Switzerland, June 29-July 3, 2003; Abstracts of the 9th International Conference On Homocysteine and One-Carbon Metabolism - HCY2013. Dublin, Ireland. September 8-12, 2013; Smith et al., 2018), especially with regard to cardiovascular mortality (Refsum et al., 1998). Moreover, homocysteine is suspected of having a carcinogenic effect (Kim et al., 2018).

More than 20 years ago, the first studies were conducted to confirm the connection between elevated homocysteine levels and AD (McCaddon et al., 1998; Clarke et al., 1998). In 2018, Smith et al. concluded in an International Consensus Statement that there is clearly a causality between homocysteine levels and dementia and drawed attention to the subsequent significant public health implications. Smith et al. listed seven meta-analyses which summarized the outcomes of many prospective observational studies since 2009 that all revealed a significant association with dementia (Smith et al., 2018). In 2019, the University of Trieste reported that homocysteinemia was a marker of transition from MCI to dementia, correlating with atrophy progression (Moretti and Caruso, 2019). Hsu et al. could demonstrate a correlation between homocysteinemia and a significant decline in neuropsychological test scores in participants who had normal concentrations of vitamin B12 and folate (Hsu et al., 2016). This suggests that homocysteine itself has a neurotoxic effect and is not just a marker for vitamin deficiency and poor lifestyle. Homocystein might be neurotoxic via different mechanisms, for example agonistic modulation of the NMDA receptor, direct or indirect increase of amyloid-β (Aβ) and tau protein (P-tau), oxidative stress, or increased expression of C-reactive protein (CRP), which subsequently can lead to brain atrophy (Smith et al., 2018; Clarke et al., 1998; Hasegawa et al., 2005; Kruman et al., 2002; Selkoe, 2001; Scarpa et al., 2003; Obeid and Herrmann, 2006; Hodgson et al., 2013; Škovierová et al., 2016; Moreira et al., 2005; Pang et al., 2014; Nelson et al., 2016; Douaud et al., 2013).

The existing literature is lacking evidence concerning the connection between homocysteinemia and mortality or cognitive impairment. In the light of the potential neurotoxic effect of homocysteine, this study aims to elucidate whether homocysteine levels are associated with neuropsychological test results in patients with SCD, MCI or AD and whether it could be exploited as a predictive marker for mortality in these patient groups.

2. Methods

2.1. Subjects and procedure

This study was designed as a retrospective, single-center, cross sectional study. Data of 976 consecutive patients was collected at the Memory Outpatients Clinic of the Department of Neurology, Medical University of Vienna between 2004 and 2019. Mortality data was gathered from the RDA (Research, Documentation and Analysis) system of the Medical University of Vienna and Statistics Austria. Survival time is defined as the individual survival time of every subject after the date of neuropsychological testing until the end of the study, death or loss of follow up.

2.2. Participants

All the patients were over 50 years old and lived in the area of Vienna. They attended the Memory Outpatient's Clinic of the Medical University of Vienna due to self-reported memory problems or referrals from physicians of the Department of Neurology.

Every patient received a comprehensive clinical examination, which included a complete neurological examination, a standard laboratory blood test, and neuropsychological testing. The neuropsychological testing involved at least the Mini-Mental State Examination (MMSE), the Geriatric Depression Scale, and the Neuropsychological Test Battery Vienna (NTBV) short. The NTBV was developed by Lehrner et al. (Lehrner et al., 2007) to provide a standardized test for the early detection of dementia. Due to ethical reasons, participants with cognitive impairment were only subjected to the NTBV short, which included 15 instead of 30 tests. The NTBV can be obtained from www.psimistri.com. On the basis of the clinical examination, their neuropsychological test scores, and on the basis of current classification guidelines, all subjects assigned to either of three stadiums of cognitive impairment: SCD, MCI and AD (Jessen et al., 2010; Jessen et al., 2014; Morris et al., 2001). Patients with previous stroke or severe head injury, current psychiatric diagnosis (except mild depressive disorder), or severe physical diseases that result in cognitive impairment, or with other forms of dementia than AD, were excluded.

Homocysteine levels were quantified from blood collected within ± six months from the neuropsychological testing at the Departmentof Laboratory Medicine, Medical University of Vienna. Reference ranges were <12 μmol/L for those under 65 years of age and <16 μmol/L for those over 65 years of age. These age-dependent benchmarks divide patients into those with elevated homocysteine levels and those without.

2.3. Statistical analyses

Data were evaluated using IBM SPSS® 26.0 (Statistical Package for Social Sciences) both for descriptive and inferential statistics. An alpha-level of 5 % was set so that results $p \leq .05$ (two-tailed) would be considered statistically significant for all hypothesis testing. In the case of multiple testing, if several independent statistical analyses were conducted, Bonferroni correction was applied.

The homocysteine parameter was used as a metric variable and, if necessary, data were transformed into logarithmised (lg10) values to achieve a normal distribution.

The Kruskal-Wallis test was used to test the difference in at least ordinally scaled variables between more than two groups. Likewise, the non-parametric Mann-Whitney *U* test was used for the comparison of

two independent groups (Field, 2009). For further survival analysis, Kaplan-Meier (KM) plots were drawn and survival curves were compared by log-rank tests.

Further, a multivariate, binary logistic regression model with the homocysteine value as a predictor variable for the prognosis of five-year survival was performed. In the context of model testing, several influencing variables (quantitative as well as qualitative) can be taken into account. In the present study, the backward-stepwise-method was used, which sequentially removes predictors from the model until only predictors with explanatory value remain (Field, 2009; Bühl, 2014). In order to achieve a dimensional reduction and overview of the NTBV subscales, a principal component factor analysis (PCA) was performed (Hatzinger and Nagel, 2009; Moosbrugger and Kelaba, 2012; Kubinger and Kelaba, n.d.).

3. Results

The 976 participants were distributed among the diagnostic groups as follows: SCD (n = 90), MCI (n = 496), AD (n = 390). Sex was not differently distributed in all subgroups. Comparing age differences at time of testing, AD patients have shown to be significantly ($p < .001$) older than patients with SCD and MCI.

All diagnostic subgroups showed comparable ages at death (p = .954) for those already deceased at the end of the study. Patients with AD presented with significantly less years of education than the other subgroups (p's < .001). Table 1 displays demographic variables.

The mean homocysteine levels in each subgroup were as follows, 12.15 ± 4.71 μmol/L (SCD), 12.80 ± 4.81 μmol/L (MCI) and 15.0 ± 6.44 μmol/L (AD) and were significantly higher in the AD group compared to SCD (p = .001) and MCI ($p < .001$). When considering the clinical thresholds and thus dividing all participants into those with elevated and non-elevated homocysteine levels, it was found that 23 % of the SCD group suffered from homocysteinemia, 27 % in MCI and 39 % in AD. The ANOVA for the interaction between homocysteine levels and each diagnostic subgroup did demonstrate a significant difference with a large effect for elevated homocysteine levels with F(1, 970) = 497.110, $p < .001$ ($\eta 2 = 0.34$).

Regarding the baseline performance in neuropsychological test results, as expected, MMSE performances display significant ($p < .001$) differences between the diagnostic subgroups (SCD > MCI > AD). Likewise, significant differences in the test results of the individual subtests of the NTBV short were observed when comparing the diagnostic groups (SCD > MCI > AD). In contrast, the groups did not differ significantly (p = .114) in the assessment of depression with GDS (see Table 2).

In order to quantify neuropsychological performance, a dimensional reduction of the NTBV short subscales was performed. A conducted PCA, using the orthogonal rotation method (Varimax-Kaiser Normalisation, KMO = 0.903) revealed that NTBV subtest performance can be

Table 1
Characteristic values (M ± SD, Md, IQR) of demographic variables regarding diagnostic subgroups.

	Diagnostic subgroup			Total (N = 976)
	SCD (n = 90)	MCI (n = 496)	AD (n = 390)	
Age at testing	66.8 ± 9.3 65.4 (60.7; 73.5)	69.1 ± 9.6 70.2 (61.9; 76.4)	75.2 ± 7.8 76.7 (70.2; 80.9)	71.3 ± 9.5 72.4 (64.6; 78.2)
Years of education	12.3 ± 3.7 12.0 (8.8; 15.0)	12.4 ± 4.1 12.0 (8.0; 16.0)	10.6 ± 3.4 9.0 (8.0; 12.0)	11.7 ± 3.9 11.0 (8.0; 14.0)
Age at death	82.6 ± 9.8 82.2 (75.2; 89.3)	81.7 ± 8.2 83.2 (75.7; 88.0)	82.2 ± 7.5 82.8 (77.5; 88.1)	82.1 ± 7.8 82.9 (77.2; 88.1)
Estimated survival time	13.8 ± 0.4 –	12.2 ± 0.2 –	8.2 ± 0.3 7.4 (6.6; 8.2)	11.0 ± 0.2 13.4 (11.7; 15.1)
Estimated five-years survival rate	97.7 %	90.0 %	72.5 %	83.72 %
Homocysteine [μmol/L]	12.2 ± 4.7 11.4 (9.2; 13.8)	12.8 ± 4.8 11.9 (9.7; 14.7)	15.0 ± 6.4 14.2 (11.1; 17.5)	13.6 ± 5.6 12.6 (10.1; 16.1)
Patients with homocysteinemia	21 (23.3 %)	134 (27.0 %)	152 (39.0 %)	307 (31.5 %)
MMSE (0–30)	28.7 ± 1.1 (90) 29 (28; 29)	27.7 ± 1.8 (496) 28 (27; 29)	20.4 ± 4.7 (390) 21 (2; 24)	24.9 ± 4.9 (976) 26 (2; 30)
GDS (0–15)	3.2 ± 2.8 (88) 3.0 (1.0; 4.8)	4.1 ± 3.5 (480) 3.0 (1.0; 6.0)	3.72 ± 3.1 (288) 3.0 (1.0; 5.0)	3.89 ± 3.3 (856) 3.0 (1.0; 5.3)

Table 2
Characteristics and key values [M ± SD (n); Md, IQR] of MMSE, GDS and NTBV short subtests with respect to diagnostic subgroups (n = cases with valid protocols).

	Diagnostic subgroup			p-Value
	SCD (n = *90*)	MCI (n = *496*)	AD (n = *390*)	
NTBV-subtest				
AKT (time)	28.9 ± 7.4 *(90)* 28 (24; 34)	38.7 ± 15.6 *(495)* 35 (28; 45)	65.4 ± 28.2 *(328)* 59 (43; 82)	<.001**
AKT (total score)	1.99 ± 0.54 *(90)* 1.96 (1.62; 2.29)	1.57 ± 0.52 *(425)* 1.50 (1.18; 1.93)	0.95 ± 0.42 *(324)* 0.89 (0.63; 1.22)	<.001**
Symbols counting (C.I.)	18.0 ± 4.4 *(90)* 18 (15; 21)	22.5 ± 8.0 *(495)* 21 (17; 26)	33.7 ± 12.4 *(316)* 30 (25; 40)	<.001**
Psychomotor proc. speed (TMT A)	34.3 ± 10.1 *(90)* 34 (28; 40)	47.5 ± 20.4 *(495)* 44 (34; 58)	90.9 ± 42.3 *(327)* 80 (57; 118)	<.001**
Animals	25.1 ± 5.2 *(90)* 24 (22; 28)	20.0 ± 6.0 *(494)* 20 (15; 24)	11.6 ± 4.5 *(341)* 12 (9; 14)	<.001**
PWT-Letter f	12.5 ± 3.8 *(90)* 12 (9; 15)	9.3 ± 4.1 *(495)* 9 (6; 12)	5.7 ± 3.2 *(299)* 6 (3; 8)	<.001**
Boston naming test (BNT)	14.5 ± 0.7 *(90)* 15 (14; 15)	13.8 ± 1.5 *(493)* 14 (13; 15)	11.8 ± 2.4 *(336)* 12 (10; 14)	<.001**
Verbal memory immediate recall	8.3 ± 1.9 *(90)* 8 (7; 10)	7.1 ± 2.1 *(494)* 7 (5; 9)	4.0 ± 1.9 *(327)* 4 (3; 5)	<.001**
Verbal memory total recall	52.9 ± 8.5 *(90)* 53 (47; 60)	43.8 ± 11.4 *(494)* 44 (36; 51)	25.3 ± 7.9 *(322)* 25 (20; 30)	<.001**
Verbal memory delayed recall	11.4 ± 2.6 *(90)* 12 (10; 13)	8.4 ± 3.5 *(494)* 9 (6; 11)	2.9 ± 2.4 *(321)* 3 (1; 4)	<.001**
Verbal memory recognition	14.7 ± 0.6 *(90)* 15 (14.5; 15)	13.4 ± 2.4 *(493)* 14 (13; 15)	9.3 ± 4.0 *(318)* 10 (6.5; 12.5)	<.001**
Labyrinth (time)	30.9 ± 9.7 *(90)* 30 (23.2; 37)	47.3 ± 24.9 *(494)* 41 (30; 56)	78.5 ± 33.2 *(314)* 75 (50; 120)	<.001**
Labyrinth total/time	0.54 ± 0.19 *(90)* 0.50 (0.41; 0.65)	0.39 ± 0.20 *(493)* 0.36 (0.26; 0.50)	0.23 ± 0.16 *(308)* 0.18 (0.13; 0.29)	<.001**
Interference test (C.I.)	20.2 ± 4.1 *(90)* 19 (17; 23)	25.7 ± 8.1 *(494)* 24 (20; 29)	40.0 ± 13.6 *(319)* 37 (28; 53.5)	<.001**
Interference total/time (C.I.)	1.75 ± 0.38 *(90)* 1.78 (1.48; 2.00)	1.43 ± 0.42 *(490)* 1.42 (1.13; 1.70)	0.90 ± 0.38 *(301)* 0.84 (0.57; 1.15)	<.001**

** $p \leq .01$.

attributed to four underlying dimensions, which could be named *attention* (F1), *memory* (F2), *executive function* (F3) and *naming/verbal comprehension* (F4). All four dimensions together cover more than three quarters (77 %) of the total variability of NTBV subtests scoring. All factor loadings and communalities of the NTBV items in a four-structured solution can be seen in Table 3. The resulting loadings of the 15 NTBV short items are given in a four-factorial solution. The items are arranged hierarchically descending according to their loading per factor. The loading corresponds to Pearson's product-moment correlation r of the respective item in the factor, ranging between −1 and + 1. Additionally, the communality hi2, the sum of the squared loadings per item in the extracted factors, is given. The communality reaches values ≤1, corresponding up to 100 %. The column square sum per component (factor), the so-called eigenvalue Lambda (λ), is given. This should be >1, since a factor should explain more variance than individual items are able to. The principal component analysis (PCA), using to orthogonal rotation method (Varimax-Kaiser Normalisation), converged in 10 iterations; KMO = 0.903 (Bartlett's Test of Sphericity, χ^2 (105) = 10,602.7, $p < .001$) displays a sufficient information content of the underlying correlation matrix. It should also be noted that the factor analysis could only be performed with complete data protocols (n = 826).

The four extracted factors are able to cover 77.0 % of the total item variability. The following keywords for the four components, as latent constructs, can be suggested on the basis of high loading items (*marker variables*).

1) Vigilance/concentration/perceptual speed
2) Memory
3) Executive functions
4) Verbal comprehension

The weighted factor scores were calculated in order to be able to use four categorized neuropsychological performances as predictor variables for the subsequent model tests taking mortality into account. Accordingly, cognitive performance in the four NTBV factor scores was compared with respect to the three diagnostic groups, as shown in Table 4 below.

Using Kruskal-Wallis' procedure all three diagnostic subgroups were compared, and each result showed a significant difference in performance on NTBV factor scores, p's < .001. The performance level of the groups can be categorized as SCD > MCI > AD. Pairwise post-hoc comparisons by means of U-testing, each carried out between the subgroups taking into account the Bonferroni correction (α* = 0.0167), also revealed significant differences in performance between subgroups, except factor score F3 executive functions revealed a non-significant result comparing SCD vs. MCI, $p = .037$.

As already mentioned, while the age at death in the three groups at the time of death is comparable, the age at the time of testing is significantly different, implying that a shorter survival time can be assumed for AD patients (see Fig. 1). Hence, the estimated mean survival time for SCD patients is 13.8 years, for MCI 12.2 years and for AD patients 8.2 years. Accordingly, the estimated survival rates for the five-year survival are as follows: SCD (97.7 %), MCI (90.0 %), AD (72.5 %) (p ≤ .010).

To evaluate the association between mortality and elevated homocysteine levels, a multivariate binary logistic regression model for the criterion five-years mortality was conducted. Nagelkerke's R^2 = 29.1 % and Hosmer-Lemeshow test $p = .598$ indicated a suitable model fit. Considering only those cases that were neuropsychologically tested at least five years before the end of the study, data from 624 patients were used. The study-relevant parameters, diagnostic subgroups, homocysteine (metric or categorized), sex, age at testing, years of education, MMSE score, GDS score, and the 4 NTBV short factors were used as predictors for five- year survival and treated with the backward stepwise method. Furthermore, it is important to mention that the diagnostic subgroup AD (first comparison with SCD, second comparison with MCI) was used as the reference group.

In the adjusted model, homocysteine values maintained their predictive value for the five-year survival. Of the assessed predictors, homocysteine levels, whether calculated metrically logarithmized or categorized, presented as most relevant predictor of mortality within five years after diagnosis. More specifically, homocysteine levels were exponentially associated with mortaly risk, increasing by 11 % with each logarithmic step (OR 11.20, 95 %-CI [2.03; 61.86]) (see Table 4a).

In a second model, which homocysteine levels dichotomized at the clinical threshold for homocysteinemia, those with homocysteinemia had an OR 1.84, 95 % CI [1.08; 3.11] compared to those within the normal range ($p = .024$) (see Table 4 b for details).

For both models, the confounders showed similar explanatory values

Table 3
Factor loadings and communalities of the NTBV items in a four-structured solution corresponding a varimax rotated component matrix (n = 826).

NTBV subtests	Component				Communality
	1	2	3	4	h_i^2
AKT (time)	−0.802	−0.236	−0.256	−0.051	0.766
Interference time (C. I.)	−0.782	−0.225	−0.160	−0.329	0.797
Symbols counting (C. I.)	−0.757	−0.177	−0.207	−0.162	0.674
Interference total/ time (C.I.)	0.747	0.265	0.138	0.325	0.753
AKT (total score)	0.745	0.309	0.301	0.122	0.756
Psychomotor processing speed (TMT A)	−0.674	−0.258	−0.385	−0.182	0.702
Verbal memory delayed recall (VSRT)	0.234	0.866	0.156	0.170	0.857
Verbal memory total recall (VSRT)	0.340	0.854	0.148	0.209	0.910
Verbal memory immediate recall (VSRT)	0.301	0.799	0.107	0.171	0.770
Verbal memory recognition (VSRT)	0.118	0.779	0.166	0.103	0.659
Labyrinth total/time	0.396	0.214	0.776	0.125	0.820
Labyrinth (time)	−0.491	−0.193	−0.767	−0.128	0.883
PWT - Letter f	0.414	0.165	−0.014	0.737	0.742
Boston naming test (BNT)	0.044	0.277	0.474	0.659	0.738
Animals	0.449	0.451	0.171	0.538	0.723
Eigenvalue (λ)	4.44	3.48	1.92	1.70	–
Explained proportion of variance	29.6 %	23.2 %	12.8 %	11.4 %	77.0 %

Table 4
Key values of NTBV z-factor scores according to diagnostic subgroups (N = 607 valid cases).

NTBV-subtest	SCD (n = *90*)	MCI (*n* = *485*)	AD (*n* = *251*)	*p*-value
F1 Vigilance	0.588 ± 0.523	0.215 ± 0.750	−0.627 ± 1.227	
Md (IQR)	0.549 (0.150; 0.889)	0.148 (−0.370; 0.662)	−1.173 (−2.118; 82)	<.001**
F2 Memory	0.707 ± 0.586	0.276 ± 0.858	−0.787 ± 0.895	
Md (IQR)	0.491 (−0.139; 1.006)	−0.077 (−0.793; 0.665)	−0.324 (−1.013; 0.250)	<.001**
F3 Executive funct.	0.351 ± 0.657	0.081 ± 0.980	−0.282 ± 1.072	
Md (IQR)	0.578 (0.011; 0.959)	0.194 (−0.527; 0.693)	−0.959 (−1.730; −0.248)	<.001**
F4 Verbal compr.	0.561 ± 0.724	0.092 ± 0.930	−0.378 ± 1.079	
Md (IQR)	0.386 (−0.300; 0.990)	−0.154 (−0.674; 0.594)	−0.353 (−1.069; 0.197)	<.001**

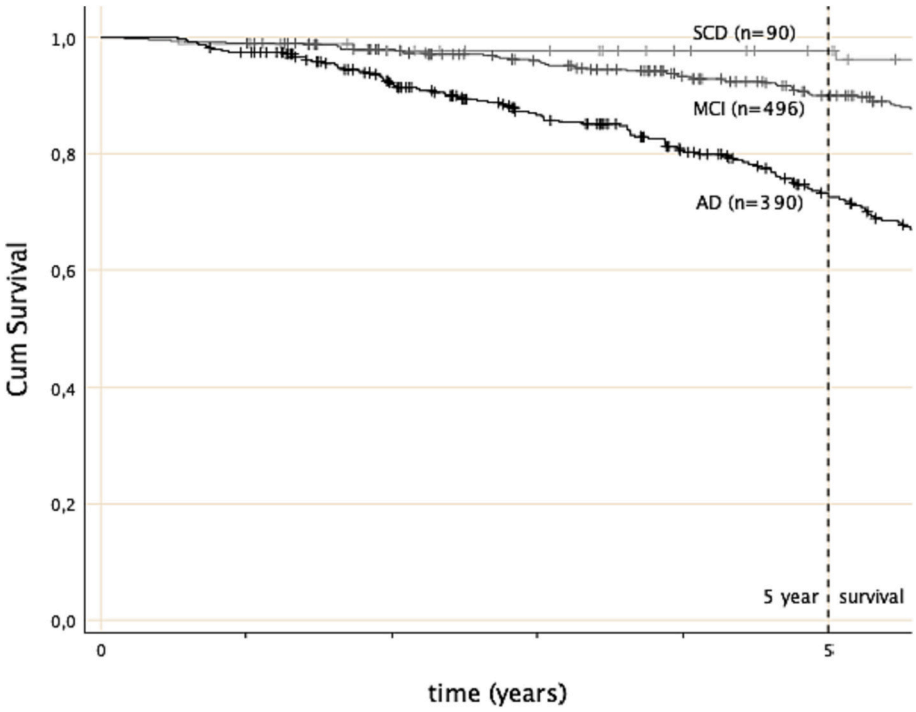

Fig. 1. Kaplan-Meier Survival Functions for diagnostic subgroups.

for five years survival. Increasing age at time of testing showed an OR of 1.09 per year, 95 % CI [1.047; 1.124] and a female sex showed an OR of 0.51, 95 %-CI [0.30; 0.85] in the first model. In contrast, belonging to a diagnostic subgroup or a worse cognitive performance in the Mini-Mental State Examination did not represent a risk factor for earlier death.

Depression (GDS) can be considered a weak but significant predictor of death, with an OR of 1.16, 95 %-CI [1.07; 1.25]. Normal attention with OR 0.66, 95 %-CI [0.52; 0.85] and normal memory with OR 0.58, 95 %-CI [0.43; 0.78] were associated with a better five-year survival. However, no significant predictive value was found for the other two NTBV short factors, executive functions and verbal comprehension.

Finally, with a uni-directional Spearman rank correlation analyse revealed that a higher homocysteine level was related to poorer neuropsychological test results. As presented in Tables 5a and 5b, cognitive performance (MMSE, NTBV short factors attention (F1), memory (F2), executive functions (F3) and naming/verbal comprehension (F4)), but not depression were negatively associated with increased homocysteine levels (See Table 6 for details).

4. Discussion

The present study aimed to investigate the effect of elevated homocysteine levels on mortality and cognitive performance of cognitively impaired participants. Using a logistic regression model, we were able to show that an increased homocysteine level was independently associated with 5-year survival.

There may be several reasons for this observation. Firstly, numerous studies and International Consensus Statements from the past 40 years have confirmed that homocysteinemia is a risk factor for cardiovascular events and diseases (Selhub, 1999; Nygård et al., 1999; Kalra, 2004; Wang et al., 2014; Cacciapuoti, 2011; Larsson et al., 2019; Abby et al., 1998; Marcus et al., 2007; Boers, 1997; D'Angelo et al., 1997; Maron and Loscalzo, 2006). Already in 1998, Refsum et al. showed that elevated homocysteine levels are a strong predicting factor for cardiovascular mortality (Refsum et al., 1998) and Koller et al. implicated in 2018 that homocysteine itself causes endothelial damage (Koller et al., 2018). Secondly, according to Kim et al., homocysteinemia is also associated with many other diseases, e.g. malignant diseases (Kim et al., 2018). Thirdly, homocysteine levels have been shown to be increased in individuals consuming alcohol or tobacco, keeping a poor diet, and lacking exercise. These unhealthy lifestyle habits might contribute to the

Table 5a
Variables in the Equation (step 8), criterion 5 years survival (0 = survived, 1 = deceased; $n = 624$; metric homocysteine value).

Predictor	B	SE	Wald χ^2 (*df*)	*p*-Value	OR	95 % CI OR	
						LL	UL
Homocysteine [lg10]	2.416	0.872	7.674 (1)	.006**	11.198	2.027	61.862
gender (female/male)	−0.683	0.268	6.489 (1)	.011*	0.505	0.299	0.854
age at testing (years)	0.081	0.018	20.198 (1)	<.001**	1.085	1.047	1.124
GDS	0.146	0.039	14.152 (1)	<.001**	1.157	1.072	1.248
NTBV F1 attention	−0.415	0.126	10.917 (1)	.001**	0.660	0.516	0.845
NTBV F2 memory	−0.549	0.152	13.090 (1)	<.001**	0.578	0.429	0.778
Constant	−10.974	1.566	49.139 (1)	<.001**	0.000		

Note: Hosmer-Lemeshow Test $p = .598$, Nagelkerke $R^2 = 29.1$ %.
** $p \leq .01$.
* $p \leq .05$

Table 5b
Variables in the Equation (step 8), criterion 5 years survival (0 = survived, 1 = deceased; n = 624; categorized homocysteine value).

Predictor	B	SE	Wald χ^2 (*df*)	*p*-Value	OR	95 % CI OR	
						LL	UL
Homocysteine [cat]	0.607	0.269	5.105 (1)	.024**	1.836	1.084	3.109
gender (female/male)	−0.667	0.268	6.184 (1)	.013*	0.513	0.304	0.868
age at testing (years)	0.088	0.018	25.381 (1)	<.001**	1.093	1.056	1.131
GDS	0.143	0.039	13.721 (1)	<.001**	1.154	1.070	1.245
NTBV F1 attention	−0.426	0.125	11.665 (1)	<.001**	0.653	0.511	0.834
NTBV F2 memory	−0.551	0.150	13.455(1)	<.001**	0.576	0.429	0.774
Constant	−9.005	1.344	44.899 (1)	<.001**	0.000		

Note: Hosmer-Lemeshow Test $p = .167$, Nagelkerke $R^2 = 28.4$ %.
** $p \leq .01$.
* $p \leq .05$.

Table 6
Spearman's rank correlation for the correlation between test performance and homocysteine level.

	Neuropsych. test	Depression	NTBV short factor score			
	MMSE	GDS	F1	F2	F3	F4
Correlation coefficient r_s	−0.23**	−0.03	−0.20**	−0.22*	−0.06*	−0.06*
n (valid data protocols)	976	856	826	826	826	826

Note:
** $p \leq .01$.
* $p \leq .05$.

increased mortality (Bolander-Gouaille and Bottiglieri, 2007). Fourthly, it must also be mentioned that homocysteine levels increase with age, as is also shown by the age-dependent reference values. This fact is also evident from the present data, because the significant older group of AD patients showed significantly higher values compared to patients with SCD and MCI.

Concerning five-year survival, a significant explanatory value for mortality was found for lower attention (F1) and decreased memory (F2). This raises the question of whether increased homocysteine levels are also associated with severe cognitive impairment among the participants.

Smith et al. established a causal link between homocysteine levels and dementia in the International Consensus Statement from 2018 (Smith et al., 2018). That cognitive performance is influenced by higher homocysteine levels, has already been shown by the University of Trieste in 2019, which postulated homocysteinemia as a marker of transition from MCI to dementia (Moretti and Caruso, 2019).

Likewise, the results of correlation analyses of the present study also confirm a negative, weak association between lower cognitive performances in MMSE or NTBV short factors F1-F4 and increased homocysteine levels. However, this negative correlation of poorer test performance with higher homocysteine values was much more evident in the attention (F1) and memory (F2) factors than for the executive functions (F3) and the naming/verbal comprehension (F4).

In contrast, we observed no correlations between elevated homocysteine levels and depressive symptoms, as assessed by the GDS.

Additionally, a recent study with comparable patient data, conducted by Đapić et al. (Đapić et al., 2022), analysed the effects of thyroid hormones on 5-year mortality. Similarly to our study, they demonstrated comparable effects on mortality in dependence of neuropsychological testing. However, thyroid hormones did not have any explanatory effect on the 5-year survival. These findings highlight the potential role of homocysteine as a life-shortening predictor in cognitive impaired patients (Đapić et al., 2022).

As already mentioned, the homocysteine levels are primarily determined by dietary intakes of vitamin B12, folate, and methionine (Moretti and Caruso, 2019; Pietrzik and Brönstrup, 1998). Furthermore, in the International Consensus Statement, Smith et al. also listed a number of studies implicating that vitamin B12 substitution lowers homocysteine levels and would, therefore, improve the test results (Smith et al., 2010; Zhang et al., 2017; Smith et al., 2018; Douaud et al., 2013). In the future, vitamin B12 and folic acid substitution could have beneficial effects on patients with a risk of cognitive decline and possibly prolong their survival.

However, the extent to which elevated homocysteine levels are causally inducing faster progression and even earlier mortality in Alzheimer's disease remains a question for future studies.

The results of this study may serve as a basis for further hypothesis generation for subsequent studies. In particular, it should be investigated whether homocysteine itself actually has a neurotoxic effect, as hypothesized in numerous articles (Smith et al., 2018; Clarke et al., 1998; Hasegawa et al., 2005; Kruman et al., 2002; Selkoe, 2001; Scarpa et al., 2003; Obeid and Herrmann, 2006; Hodgson et al., 2013; Škovierová et al., 2016; Moreira et al., 2005; Pang et al., 2014; Nelson et al., 2016; Douaud et al., 2013), and thus causes rapid progression of dementia. This should be followed up, and larger, prospective studies should be conducted. One envisaged future perspective could contain standardized screening programmes for early detection of cognitive decline and for homocysteine levels. Consequently, simple vitamin supplementation could be a potential way to slow cognitive decline.

4.1. Limitations

The present study had several limitations. First, the univariate analyses were performed using the available cases with documented values, while listwise case exclusion was applied in the model tests. According to this, sample size variations were noted for some neuropsychological test results. Second, it should be noted that the data underwent heterogeneous technical adjustments and availabilities, which were necessary due to the long period of data collection of 15 years. Third, an important limitation is that the retrospective study does not contain a control group. Nevertheless, the comparatively large number of cases, despite clinical limitations, and the inclusion of a profound neuropsychological examination of all patients represent absolute strengths of the study.

5. Conclusion

In this retrospective, monocentric study, we show that, despite taking into account all confounders, such as age at testing, higher homocysteine levels are associated with earlier death and poorer test results. Regarding five-years mortality, an OR of 11.20 could be recorded for elevated homocysteine levels. In addition, an inverse correlation between cognitive performances in MMSE or NTBV short factors attention (F1), memory (F2), executive functions (F3) and the naming/verbal comprehension (F4) and increased homocysteine levels was observed.

References

Abby, S.L., Harris, I.M., Harris, K.M., 1998. Homocysteine and cardiovascular disease. J. Am. Board Fam. Pract. 11, 391–398.

Abstracts of the 4th International Conference on Homocysteine Metabolism. Basel, Switzerland, June 29-July 3, 2003. J. Inherit. Metab. Dis. 26 (Suppl. 1), 2003, 1–136.

Abstracts of the 9th International Conference On Homocysteine and One-Carbon Metabolism - HCY2013. Dublin, Ireland. September 8-12, 2013. J. Inherit. Metab. Dis. 36 (Suppl. 1), 2013, S1–S54.

Bäckman, L., Jones, S., Small, B.J., Agüero-Torres, H., Fratiglioni, L., 2003. Rate of cognitive decline in preclinical Alzheimer's disease: the role of comorbidity. J. Gerontol. B. Psychol. Sci. Soc. Sci. 58, P228–P236.

Boers, G.H., 1997. Hyperhomocysteinemia as a risk factor for arterial and venous disease. A review of evidence and relevance. Thromb. Haemost. 78, 520–522.

Bolander-Gouaille, C., Bottiglieri, T., 2007. Related Vitamins And Neuropsychiatric Disorders. Springer-Verlag, France.

Bühl, A., 2014. SPSS 22, Einführung in die moderne Datenanalyse. Pearson.

Cacciapuoti, F., 2011. Hyper-homocysteinemia: a novel risk factor or a powerful marker for cardiovascular diseases? Pathogenetic and therapeutical uncertainties. J. Thromb. Thrombolysis 32, 82–88.

Clarke, R., et al., 1998. Folate, vitamin B12, and serum total homocysteine levels in confirmed Alzheimer disease. Arch. Neurol. 55, 1449–1455.

D'Angelo, A., Mazzola, G., Crippa, L., Fermo, I., Viganò D'Angelo, S., 1997. Hyperhomocysteinemia and venous thromboembolic disease. Haematologica 82, 211–219.

Đapić, B., Schernhammer, E., Haslacher, H., Stögmann, E., Lehrner, J., 2022 Apr. No effect of thyroid hormones on 5-year mortality in patients with subjective cognitive decline, mild cognitive disorder, and Alzheimer's disease. J. Neuroendocrinol. 34 (4), e13107. https://doi.org/10.1111/jne.13107. Epub 2022 Feb 25.

Douaud, G., et al., 2013. Preventing Alzheimer's disease-related gray matter atrophy by B-vitamin treatment. Proc. Natl. Acad. Sci. U. S. A. 110, 9523–9528.

Field, A., 2009. Discovering Statistics Using SPSS. Sage Publications, UK.

Finkelstein, J.D., Martin, J.J., 2000. Homocysteine. Int. J. Biochem. Cell Biol. 32, 385–389.

Hasegawa, T., et al., 2005. Homocysteic acid induces intraneuronal accumulation of neurotoxic Abeta42: implications for the pathogenesis of Alzheimer's disease. J. Neurosci. Res. 80, 869–876.

Hatzinger, R., Nagel, H., 2009. SPSS Statistics Statistische Methoden und Fallbeispiele. Pearson Education Deutschland GmbH.

Hodgson, N., Trivedi, M., Muratore, C., Li, S., Deth, R., 2013. Soluble oligomers of amyloid-β cause changes in redox state, DNA methylation, and gene transcription by inhibiting EAAT3 mediated cysteine uptake. J. Alzheimers Dis. 36, 197–209.

Höfler, S., Bengough, T., Winkler, P., Giebler, R., Österreichischer Demenzbericht, 2014. Bundesministerium für Gesundheit und Sozialministeri- um (2015).

Homocysteine Metabolism 2nd International Conference. Nijmegen, The Netherlands, 26-29 April 1998. Abstracts. Neth. J. Med. (52 Suppl), 1998, S1–S63.

Hsu, W.-C., et al., 2016. Digit symbol substitution test score and hyperhomocysteinemia in older adults. Medicine (Baltimore) 95, e4535.

International Conference on Homocysteine Metabolism, From Basic Science to Clinical Medicine. Ireland, July 2-6, 1995. Abstracts. Ir. J. Med. Sci. 164 (Suppl. 15), 1995, 2–36.

Jessen, F., et al., 2010. Prediction of dementia by subjective memory impairment: effects of severity and temporal association with cognitive impairment. Arch. Gen. Psychiatry 67, 414–422.

Jessen, F., et al., 2014. A conceptual framework for research on subjective cognitive decline in preclinical Alzheimer's disease. Alzheimers Dement. J. 10, 844–852. Alzheimers Assoc.

Kalra, D.K., 2004. Homocysteine and cardiovascular disease. Curr. Atheroscler. Rep. 6, 101–106.

Kim, J., Kim, H., Roh, H., Kwon, Y., 2018. Causes of hyperhomocysteinemia and its pathological significance. Arch. Pharm. Res. 41, 372–383.

Koller, A., et al., 2018. Coronary microvascular and cardiac dysfunction due to homocysteine pathometabolism; a complex therapeutic design. Curr. Pharm. Des. 24, 2911–2920.

Kruman, I.I., et al., 2002. Folic acid deficiency and homocysteine impair DNA repair in hippocampal neurons and sensitize them to amyloid toxicity in experimental models of Alzheimer's disease. J. Neurosci. 22, 1752–1762.

K. D. Kubinger Psychologische Diagnostik: Theorie und Praxis psychologischen Diagnostizierens. (Hogrefe Verlag). n.d.

Larsson, S.C., Traylor, M., Markus, H.S., 2019. Homocysteine and small vessel stroke: a mendelian randomization analysis. Ann. Neurol. 85, 495–501.

Lehrner, J., Gleiß, A., Auff, E., Dal-Bianco, P., 2007. Demenzdiagnostik mit Hilfe der Vienna Neuropsychologischen Testbatterie (VNTB): Standardisierung, Normierung und Validierung. Psychol. Österreich 4, 5.

Marcus, J., Sarnak, M.J., Menon, V., 2007. Homocysteine lowering and cardiovascular disease risk: lost in translation. Can. J. Cardiol. 23, 707–710.

Maron, B.A., Loscalzo, J., 2006. Homocysteine. Clin. Lab. Med. 26 (591–609), vi.

McCaddon, A., Davies, G., Hudson, P., Tandy, S., Cattell, H., 1998. Total serum homocysteine in senile dementia of Alzheimer type. Int. J. Geriatr. Psychiatry 13, 235–239.

Moosbrugger, H., Kelaba, A., 2012. Testtheorie und Fragebogenkonstruktion. Springer-Lehrbuch.

Moreira, P.I., et al., 2005. Oxidative stress and neurodegeneration. Ann. N. Y. Acad. Sci. 1043, 545–552.

Moretti, R., Caruso, P., 2019. The controversial role of homocysteine in neurology: from labs to clinical practice. Int. J. Mol. Sci. 20, E231.

Morris, J.C., et al., 2001. Mild cognitive impairment represents early-stage Alzheimer disease. Arch. Neurol. 58, 397–405.

Nelson, A.R., Sweeney, M.D., Sagare, A.P., Zlokovic, B.V., 2016. Neurovascular dysfunction and neurodegeneration in dementia and Alzheimer's disease. Biochim. Biophys. Acta 1862, 887–900.

Nygård, O., Refsum, H., Ueland, P.M., Vollset, S.E., 1998. Major lifestyle determinants of plasma total homocysteine distribution: the Hordaland Homocysteine Study. Am. J. Clin. Nutr. 67, 263–270.

Nygård, O., Vollset, S.E., Refsum, H., Brattström, L., Ueland, P.M., 1999. Total homocysteine and cardiovascular disease. J. Intern. Med. 246, 425–454.

Obeid, R., Herrmann, W., 2006. Mechanisms of homocysteine neurotoxicity in neurodegenerative diseases with special reference to dementia. FEBS Lett. 580, 2994–3005.

Pang, X., et al., 2014. Homocysteine induces the expression of C-reactive protein via NMDAr-ROS-MAPK-NF-κB signal pathway in rat vascular smooth muscle cells. Atherosclerosis 236, 73–81.

Patscheider, C.C., Zahlen und Statistik, 2021. Österreichische Alzheimer Gesellschaft Vereinigung zur Erforschung der Alzheimer-Krankheit und verwandter Demenzformen. http://www.alzheimer-gesellschaft.at/informationen/zahlen-statistik/.

Petersen, R.C., et al., 2014. Mild cognitive impairment: a concept in evolution. J. Intern. Med. 275, 214–228.

Pietrzik, K., Brönstrup, A., 1998. Vitamins B12, B6 and folate as determinants of homocysteine concentration in the healthy population. Eur. J. Pediatr. 157 (Suppl. 2), S135–S138.

Proceedings of the 7th International Conference on Homocysteine Metabolism. June, 21-25, 2009. Prague, Czech Republic; and the Annual International Meeting on Inborn Errors of Metabolism. Fulda, Germany. J. Inherit. Metab. Dis. 34, 2011, 1–157.

Refsum, H., Ueland, P.M., Nygård, O., Vollset, S.E., 1998. Homocysteine and cardiovascular disease. Annu. Rev. Med. 49, 31–62.

Sanford, A.M., 2017. Mild cognitive impairment. Clin. Geriatr. Med. 33, 325–337.

Scarpa, S., Fuso, A., D'Anselmi, F., Cavallaro, R.A., 2003. Presenilin 1 gene silencing by S-adenosylmethionine: a treatment for Alzheimer disease? FEBS Lett. 541, 145–148.

Schneede, J., Refsum, H., Ueland, P.M., 2000. Biological and environmental determinants of plasma homocysteine. Semin. Thromb. Hemost. 26, 263–279.

Selhub, J., 1999. Homocysteine metabolism. Annu. Rev. Nutr. 19, 217–246.

Selkoe, D.J., 2001. Presenilin, notch, and the genesis and treatment of Alzheimer's disease. Proc. Natl. Acad. Sci. U. S. A. 98, 11039–11041.

Škovierová, H., et al., 2016. The molecular and cellular effect of homocysteine metabolism imbalance on human health. Int. J. Mol. Sci. 17, E1733.

Smith, A.D., et al., 2010. Homocysteine-lowering by B vitamins slows the rate of accelerated brain atrophy in mild cognitive impairment: a randomized controlled trial. PloS One 5, e12244.

Smith, A.D., et al., 2018. Homocysteine and dementia: an international consensus statement. J. Alzheimers Dis. 62, 561–570.

Soria Lopez, J.A., González, H.M., Léger, G., 2019. C. Alzheimer's disease. Handb. Clin. Neurol. 167, 231–255.

Wang, C., et al., 2014. Elevated plasma homocysteine level is associated with ischemic stroke in Chinese hypertensive patients. Eur. J. Intern. Med. 25, 538–544.

Zhang, D.-M., Ye, J.-X., Mu, J.-S., Cui, X.-P., 2017. Efficacy of vitamin B supplementation on cognition in elderly patients with cognitive-related diseases. J. Geriatr. Psychiatry Neurol. 30, 50–59.