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Association between Low Vitamin D Status, Serotonin, and Clinico-Biobehavioral Parameters in Alzheimer's Disease

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Keywords

25-hydroxyvitamin D3 · Alzheimer's disease · Amyloid-beta peptide of 42 amino acids · Behavioral and psychological signs and symptoms of dementia · Serotonin

Abstract

Introduction: Studies suggest a role of vitamin D in the progression and symptomatology of Alzheimer's disease (AD), with few in vitro studies pointing to effects on serotonergic and amyloidogenic turnover. However, limited data exist in AD patients on the potential association with cognition and behavioral and psychological signs and symptoms of dementia (BPSD). In this retrospective cross-sectional study, we, therefore, explored potential correlations of serum 25-hydroxyvitamin D3 (25(OH)D3) concentrations, indicative of vitamin D status, with serum serotonin (5-hydroxytryptamine, 5-HT) levels, cognitive/BPSD scorings, and cerebrospinal fluid (CSF) biomarker levels. ***Methods:*** Frozen serum samples of 25 well-characterized AD subjects as part of a previous BPSD cohort were analyzed, of which 15 had a neuropathologically confirmed diagnosis. Serum 25(OH)D3 levels were analyzed by means of LC-MS/MS, whereas 5-HT concentrations were quantified by competitive ELISA. ***Results:*** Among AD patients, vitamin D deficiency was highly prevalent, defined as levels below 50 nmol/L. Regression analyses, adjusted for age, gender, and psychotropic medications, revealed that serum 25(OH)D3 and 5-HT levels were positively associated ($p = 0.012$). Furthermore, serum 25(OH)D3 concentrations correlated inversely with CSF amyloid-beta ($A\beta_{1\text{-}42}$) levels ($p = 0.006$), and serum 5-HT levels correlated positively with aggressiveness ($p = 0.001$), frontal behavior ($p = 0.001$), depression ($p = 0.004$), and partly with cognitive performance ($p < 0.005$). Lastly, AD patients on cholinesterase inhibitors had higher serum 25(OH)D3 ($p = 0.030$) and lower serum 5-HT ($p = 0.012$) levels. ***Conclusions:*** The molecular associations between low vitamin D status, serum 5-HT, and CSF $A\beta_{1\text{-}42}$ levels are highly remarkable, warranting further mechanistic and intervention studies to disclose potential involvement in the clinico-biobehavioral pathophysiology of AD.

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Introduction

Alzheimer´s disease (AD) is a devastating neurodegenerative disorder characterized by (non)cognitive dysfunctions, including behavioral and psychological signs and symptoms of dementia (BPSD). The proteinopathy-induced remodeling of central neurotransmitter systems from the earliest stages onwards is suggested to be a major determinant of its core symptomatology. Previous studies confirmed that reduced concentrations of both brain and cerebrospinal fluid (CSF) serotonin (5-hydroxytryptamine; 5-HT) were associated with cognitive decline [1] and some BPSD, including anxieties/phobias, depression, overactivity, and psychosis [2].

Apart from vast monoaminergic alterations, micronutrient deficiencies are frequently co-observed in AD patients. Vitamin D deficiency, usually defined as serum 25-hydroxyvitamin D3 (25(OH)D3) concentrations below 50 nmol/L, is regarded as a global burden on the general population with an estimated yearly prevalence rate of 40% in Europe [3]. Although reduced exposure to sunlight and changing dietary habits of AD patients are often pointed to as obvious causes of vitamin D deficiency, there is also increasing evidence, including from a mendelian randomization study [4], pointing to a possible role of vitamin D deficiency in the etiology of AD [5]. The potential contribution of vitamin D to alleviate cognitive deficits is underlined once more by one recent randomized controlled trial in AD patients finding improved cognitive performance after 25(OH)D3 supplementation [6]. As for BPSD, low serum 25(OH)D3 levels are correlated with depressive [7] and psychotic [8] symptoms in AD patients, whereas supplementation seems to ameliorate depressive symptoms in non-dementia patients [9].

According to in vitro and animal studies, vitamin D might control serotonergic synthesis and metabolism in the brain by interfering with 5-HT-related gene expression. For example, vitamin D's active metabolite, i.e., 1,25-dihydroxyvitamin D3 ($1,25(OH)_2D3$), might activate the transcription of tryptophan hydroxylase 2, the rate-limiting enzyme of neuronal 5-HT synthesis. In addition, suppression of the 5-HT transporter and the degradative monoamine-oxidase A (MAO-A) enzyme was observed [10]. These effects may result in higher brain 5-HT levels. So far, only one human intervention study has been performed, in which vitamin D´s role on the 5-HT neurotransmitter system and accompanied depression severity were assessed. Apparently, in individuals supplemented with vitamin D3 for 3 months, serum 5-HT levels increased, next to a decrease in depression scores [9]. More surprisingly, there is a lack of human studies investigating this proposed relationship, specifically in AD patients.

Moreover, a shift in cerebral Aβ homeostasis towards increased production and reduced degradation of the insoluble Aβ peptide of 42 amino acids ($A\beta_{1-42}$) was observed during vitamin D-deficient conditions, following one in vitro study [11]. Possibly, this may result in increased brain $A\beta_{1-42}$ levels, which are more prone to oligomerization and plaque formation. In line with this assumption, a recent study revealed that worse cognitive functioning in AD patients was probably mediated by a vitamin D deficiency, having significant impact on CSF $A\beta_{1-42}$ concentrations [12].

Despite the potential relevance of vitamin D status to both 5-HT and $A\beta_{1-42}$ levels in key events related to cognitive maintenance and BPSD evolution in AD, there are hardly any biochemical data available from patients with a clear pre- and/or post-mortem diagnosis. Therefore, the primary aim of this study was to investigate the association between serum 25(OH)D3 and 5-HT levels in well-characterized AD patients with particular interest for (individual) correlations with cognition and BPSD, such as aggressiveness and depression. Secondary, this study aimed to unveil a possible monotonic association between serum 25(OH)D3 and CSF $A\beta_{1-42}$ levels.

Materials and Methods

Study Population

Serum samples along with demographic, clinical, neurocognitive, BPSD, and CSF AD biomarker data of 25 well-characterized probable AD patients were retrospectively acquired from the Neurobiobank of the Institute Born-Bunge (Antwerp, Belgium; FAGG registration ID 190113). Patient samples were part of a BPSD cohort (1996–2015) [2]. In total, 15 out of 25 patients had additional neuropathological confirmation of their clinical diagnosis following standardized post-mortem immunohistochemical analysis, as published previously [13]. Sample selection took into account (i) equal gender distribution; (ii) age at onset (>65 years); (iii) similar disease stages (score of 5–6 on the Global Deterioration Scale); (iv) availability of neurocognitive ratings (Mini-Mental State Examination, Boston Naming Test (BNT), and Verbal Fluency Test (VFT)); (v) BPSD assessment ratings, with predefined cut-offs for aggressiveness and depression; (vi) time interval between serum sampling and BPSD rating of less than 14 days; (vii) similar total storage times; and (viii) minimal number of freeze-thaw cycles.

Behavioral Assessment

BPSD assessment was performed by means of a battery of behavioral assessment scales, including the Middelheim Frontality Score (MFS), Behavioral Pathology in Alzheimer´s Disease Rating

Scale, Cohen-Mansfield Agitation Inventory (CMAI), and Cornell Scale for Depression in Dementia (CSDD), according to protocol. Patients with at least one out of ten individual CMAI cluster 1 items scoring ≥3 (indicating *´at least once or twice a week´*) and additionally having at least a minimum CMAI cluster 1 subscore of ≥12 were defined as aggressive. Similarly, CSDD scores of ≥8 indicated significant depression [13].

(Neuro)chemical Analyses

Serum and CSF sampling were executed on the same day during baseline visits. CSF AD biomarker concentrations of $A\beta_{1\text{-}42}$, total tau (T-tau), and tau phosphorylated at threonine ($P\text{-}tau_{181P}$) were analyzed by means of ELISA, as reported previously (data already available) [14]. In-house validated cut-offs of the CSF biomarker values from the UAntwerp BIODEM Lab have been used [15].

Blood was originally collected from included AD subjects, sampled into two serum gel tubes coated with clotting activator (S-Monovette 7.5 mL Z-gel), and centrifuged at 3,000 rpm for 10 min at room temperature. Serum aliquots were subsequently distributed to marked polypropylene vials, immediately snap frozen in liquid nitrogen, and stored at −80°C until neurochemical analyses.

Serum 25(OH)D3 levels, the accepted biomarker of vitamin D status, were analyzed using LC-MS/MS at the Clinical Chemistry and Haematology Laboratory of Gelderse Vallei Hospital (Ede, the Netherlands). Hereby, levels of 25(OH)D3 below 25 nmol/L were defined as severely deficient, between 25 and 50 nmol/l as moderately deficient, between 50 and 75 nmol/l as insufficient, between 75 and 100 nmol/l as optimal, and above 250 nmol/l as toxic [3]. This specific subdivision and the 50 nmol/L cut-off from which to define deficiency agree with the investigated relationship between vitamin D deficiency and Alzheimer's disease risk assessment [4, 16]. To quantify serum 5-HT concentrations, competitive ELISA analyses were conducted in duplicate, according to the manufacturer's protocol (Enzo Life Sciences, ADI-900-175).

Statistical Analysis

SPSS statistical software (v.28) was used, and in all cases, a p value <0.05 was considered significant, apart from the correlation analyses that necessitated total Bonferroni correction for multiple comparisons. Adjusted p values are: (i) BPSD ratings (0.05/17 repeated tests = 0.0029), (ii) neurocognitive scorings (0.05/4 repeated tests = 0.0125), and (iii) CSF AD biomarkers (0.05/4 repeated tests = 0.0125). Spearman rank-order correlation tests were executed to examine potential correlations of serum 25(OH)D3 with serum 5-HT or CSF AD biomarker levels, next to individual correlations with neurocognitive and behavioral scorings. Furthermore, multiple linear regression analyses were applied to unveil whether serum 25(OH)D3 levels might be a possible predictor of serum 5-HT levels, and vice versa. All regression analyses were adjusted for age and gender (model 1), then additionally for psychotropic medications including the intake of anti-Alzheimer medications, antidepressants, and antipsychotics (model 2), and finally for seasonal effects and total storage time (model 3). Results were indicated as standardized beta coefficients (β) along with their standard errors (SE). Additionally, Mann-Whitney U tests were performed to reveal probable effects of psychotropic medications on serum 25(OH)D3 or 5-HT levels.

Results

Baseline demographic, clinical, behavioral, neurocognitive, and (neuro)chemical data of AD patients are summarized in Table 1, whereas significant correlations of serum 5-HT and serum 25(OH)D3 levels with the aforementioned clinico-biobehavioral variables are shown in Table 2. Vitamin D deficiency was highly prevalent in our study population, with 8 out of 25 patients labeled as severely deficient (<25 nmol/L), 10 as moderately deficient (25–50 nmol/L), four as insufficient (50–75 nmol/L), and only three having an optimal status (75–100 nmol/L). As expected, serum 25(OH)D3 levels correlated inversely with age at baseline (p = 0.027). Moreover, an inverse correlation between serum 25(OH)D3 and CSF $A\beta_{1\text{-}42}$ was found (p = 0.006; shown in Fig. 1). Correlations with CSF T-tau or $P\text{-}tau_{181P}$ remained insignificant (p = 0.498 and p = 0.418, respectively).

In contrast to the positive correlation of cognitive performance with serum 5-HT (p = 0.002 and p = 0.003 for BNT and VFT scorings, respectively), similar correlations with serum 25(OH)D3 were not observed. As for BPSD ratings, correlations with serum 25(OH)D3 rendered insignificant after Bonferroni corrections, and only strong positive correlations between serum 5-HT and MFS total, CMAI cluster 2, cluster 3, and total scores (p = 0.001, p = 0.002, p < 0.001, p = 0.001, respectively) were found. Furthermore, a positive correlation of serum 5-HT levels with CSDD scorings was noticed (p = 0.004), albeit insignificant following Bonferroni correction.

According to Kruskal-Wallis tests, neither serum 25(OH)D3 (p = 0.307) nor serum 5-HT (p = 0.623) nor BPSD ratings (range; p = 0.264–0.990) depended on the season of sampling or scoring. Moreover, correlations between sample storage time and serum 5-HT (p = 0.333) or 25(OH)D3 concentrations (p = 0.718) remained insignificant.

Although we could not observe a direct monotonic correlation between serum 5-HT and 25(OH)D3 levels (p = 0.088), regression analyses confirmed a significant prediction of serum 5-HT by 25(OH)D3, and vice versa. Based on p/R^2-values, model 2, including adjustments for age, gender, and psychotropic medications, seemed to fit the best with our data (p = 0.007, R^2 = 0.729, and p = 0.006, R^2 = 0.733 for the predictions of 25(OH)D3 and 5-HT, respectively). Both age (β = −0.515, SE = 0.260, p = 0.006) and serum 5-HT levels (β = 0.656, SE = 0.026, p = 0.012) were significant determinants of serum 25(OH)D3 concentrations. An increase of 5-HT by 1 ng/mL increased 25(OH)D3 levels by 0.076 nmol/L, and, additionally, there was a decrease of 25(OH)D3 levels by

Table 1. Baseline characteristics of Alzheimer's disease patients (n = 25)

Demographical data	Gender, male/female, *n*	13/12
	Age at baseline, years	81.2±8.0 (65–92)
Clinical data	Definite/*probable* AD, *n*	15/10
	GDS score (/7)	5.6±0.5 (5–6)
	Taking/not taking antidepressants, *n*	12/13
	Taking/not taking antipsychotics, *n*	12/13
	Taking/not taking anti-Alzheimer drugs, *n*	12/13
Behavioral data	MFS total score (/10)	4.6±1.8 (1–8)
	Behave-AD total score (/75)	12.6±9.1 (3–40)
	CMAI cluster 1 score (/70)	15.4±9.1 (10–45)
	CMAI cluster 2 score (/77)	21.7±8.6 (11–48)
	CMAI cluster 3 score (/56)	18.3±8.1 (8–38)
	CMAI total score (/203)	55.4±18.6 (31–101)
	CSDD total score (/38)	8.1±3.7 (1–14)
Neurocognitive data	MMSE score (/30)	14.0±4.8 (7–23), *n* = 24
	VFT score	16.4±9.7 (0–35), *n* = 17
	BNT score (/60)	21.3±11.6 (4–47), *n* = 19
(Neuro)chemical data	Serum 25(OH)D3, nmol/L	37.5±23.5 (12–99)
	Serum 5-HT, ng/mL	195.3±111.3 (47–380), *n* = 19
	CSF $A\beta_{1\text{-}42}$, pg/mL	389.5±157.1 (114–710), *n* = 20
	CSF T-tau, pg/mL	515.6±206.2 (151–908), *n* = 19
	CSF P-tau_{181P}, pg/mL	74.6±42.2 (23–215), *n* = 21
	CSF $A\beta_{1\text{-}42}$/P-tau_{181P} ratio	6.9±4.6 (1.4–20.7), *n* = 21
Additional information	Interval serum sampling and behavioral scoring, days	1.0±2.8 (0–11)
	Total serum storage time at −80°C, years	18.0±2.6 (12–21)
	1/no freeze-thaw-cycles of serum samples, *n*	2/23

Baseline data are presented as mean ± standard deviation with minimum-maximum ranges between parentheses or as frequency of occurrence (*n*) for all nominal variables. One outlier each for CSF $A\beta_{1\text{-}42}$ and CSF T-tau and six outliers for serum 5-HT were excluded from statistical analysis. 25(OH)D3, 25-hydroxyvitamin D3; 5-HT, 5-hydroxytryptamine/serotonin; $A\beta_{1\text{-}42}$, amyloid-beta peptide of 42 amino acids; Behave-AD, Behavioral Pathology in Alzheimer's Disease Rating Scale; BNT, Boston Naming Test; CMAI, Cohen-Mansfield Agitation Inventory; CSDD, Cornell Scale for Depression in Dementia; CSF, cerebrospinal fluid; GDS, Global Deterioration Scale; MFS, Middelheim Frontality Score; MMSE, Mini-Mental State Examination; P-tau_{181P}, tau protein phosphorylated at threonine 181; T-tau, total tau protein; VFT, Verbal Fluency Test.

0.859 nmol/L for each 1 year increase in age. Moreover, serum 25(OH)D3 concentrations added significantly to the prediction of serum 5-HT levels (β = 0.646, SE = 1.867, p = 0.012). Thereby, an increase of 25(OH)D3 levels by 1 nmol/L increased 5-HT levels by 5.547 ng/mL. Lastly, we noticed that patients on anti-Alzheimer medications (mainly cholinesterase inhibitors) had higher mean serum 25(OH)D3 levels (p = 0.030) in addition to lower mean serum 5-HT levels (p = 0.012).

Discussion

The high prevalence of vitamin D deficiency within our study population is in line with several observational studies and a recent mendelian randomization study showing an association between vitamin D status and the risk of AD or dementia in general, also after adjustment for sunlight exposure, genetic factors (APOE4 gene), and diet [4]. At the same time, other investigators warn for caution because of risk for residual cofounders and the ambiguity of outcomes from intervention studies [17].

One major finding of our study was the positive association between serum 25(OH)D3 and 5-HT concentrations following regression analyses, which could be related to vitamin D's capability to regulate the expression of genes involved in 5-HT synthesis, transport, and catabolism. According to a study by Sabir et al. [10], adequate 25(OH)D3 levels might reduce 5-HT degradation by repressing the expression of MAO-A localized in neuronal and peripheral tissues. To our knowledge, the present study is the first confirming this association in

Table 2. Significant correlations of serum 25-hydroxyvitamin D3 and serotonin with demographical, behavioral, neurocognitive and/or (neuro)chemical parameters in Alzheimer's disease patients

Serum 25(OH)D3, nmol/L	
Demographical data	
Age at baseline	$n = 25$, $r = -0.388$, $\mathbf{p = 0.027}$
Behavioral data	
MFS total score	$n = 25$, $r = +0.342$, $p = 0.047$
Behave-AD cluster G score	$n = 25$, $r = -0.355$, $p = 0.041$
Neurochemical data	
CSF $A\beta_{1\text{-}42}$ (pg/mL)	$n = 20$, $r = -0.552$, $\mathbf{p = 0.006}$
Serum 5-HT, ng/mL	
Behavioral data	
MFS total score	$n = 19$, $r = +0.664$, $\mathbf{p = 0.001}$
Behave-AD cluster AB score	$n = 19$, $r = +0.442$, $p = 0.029$
Behave-AD cluster D score	$n = 19$, $r = +0.573$, $p = 0.005$
Behave-AD cluster F score	$n = 19$, $r = -0.401$, $p = 0.044$
Behave-AD total score	$n = 19$, $r = +0.510$, $p = 0.013$
Behave-AD global score	$n = 19$, $r = +0.504$, $p = 0.014$
CMAI cluster 1 score	$n = 19$, $r = +0.539$, $p = 0.009$
CMAI cluster 2 score	$n = 19$, $r = +0.638$, $\mathbf{p = 0.002}$
CMAI cluster 3 score	$n = 19$, $r = +0.745$, $\mathbf{p < 0.001}$
CMAI total score	$n = 19$, $r = +0.684$, $\mathbf{p = 0.001}$
CSDD total score	$n = 19$, $r = +0.596$, $p = 0.004$
Neurocognitive data	
BNT score	$n = 16$, $r = +0.670$, $\mathbf{p = 0.002}$
VFT score	$n = 14$, $r = +0.686$, $\mathbf{p = 0.003}$

Only statistically significant ($p < 0.05$) Spearman rank-order correlations (sample size (n), correlation coefficient (r), p value) are included in the table, and those remaining significant following Bonferroni correction for (i) behavioral ratings (0.05/17 repeated test = 0.0029), (ii) neurocognitive scorings (0.05/4 repeated test = 0.0125), and (iii) CSF AD biomarkers (0.05/4 repeated tests = 0.0125) are displayed in bold. 25(OH)D3, 25-hydroxyvitamin D3; 5-HT, 5-hydroxytryptamine/serotonin; $A\beta_{1\text{-}42}$, amyloid-beta peptide of 42 amino acids; Behave-AD, Behavioral Pathology in Alzheimer's Disease Rating Scale; BNT, Boston Naming Test; CMAI, Cohen-Mansfield Agitation Inventory; CSDD, Cornell Scale for Depression in Dementia; CSF, cerebrospinal fluid; GDS, Global Deterioration Scale; MFS, Middelheim Frontality Score; VFT, Verbal Fluency Test. Behave-AD cluster: AB (psychosis)/D (aggressiveness)/F (affective disturbances)/G (anxieties/phobias); CMAI cluster 1 (aggressive behavior)/cluster 2 (physically nonaggressive behavior)/cluster 3 (verbally agitated behavior); CSDD score for depression.

AD, while a recent intervention study had a similar outcome in nondemented participants [9]. However, a limitation of our finding is that the monotonic relationship was not significant using a Spearman rank-order correlation test. Possibly, (i) the intake of anti-Alzheimer medication might account for this discrepancy, since patients using such medications had significantly higher 25(OH)D3 opposed to lower 5-HT concentrations. There might be additional interfering factors, e.g., (ii) differences in renal function affecting biotransformation of 25(OH)D3 into $1,25(OH)_2D3$, despite sufficient 25(OH)D3 concentrations; or (iii) genetically determined differences in the vitamin D receptor-mediated regulation of target genes, including those involved in 5-HT synthesis and metabolism. A more general explanation is that (iv) the inverted U-shaped concentration-effect relationship, typical for micronutrients like vitamin D, will result in effect sizes which are dependent on the existing status, i.e., when vitamin D is in the optimal range, 5-HT levels are regulated effectively. Additionally, these factors (i–iv) might have masked potential correlations between vitamin D and BPSD in our study population. Nevertheless, we noticed a trend towards an inverse correlation of serum 25(OH)D3 and the severity of anxieties/phobias, which is in line with dietary studies in mice [18].

The positive correlations of serum 5-HT levels with BNT/VFT might reflect the importance of 5-HT for cognitive functioning. Of note, 5-HT itself is most likely not able to cross the blood-brain barrier, at least not in excessive amounts [19], so a direct quantitative

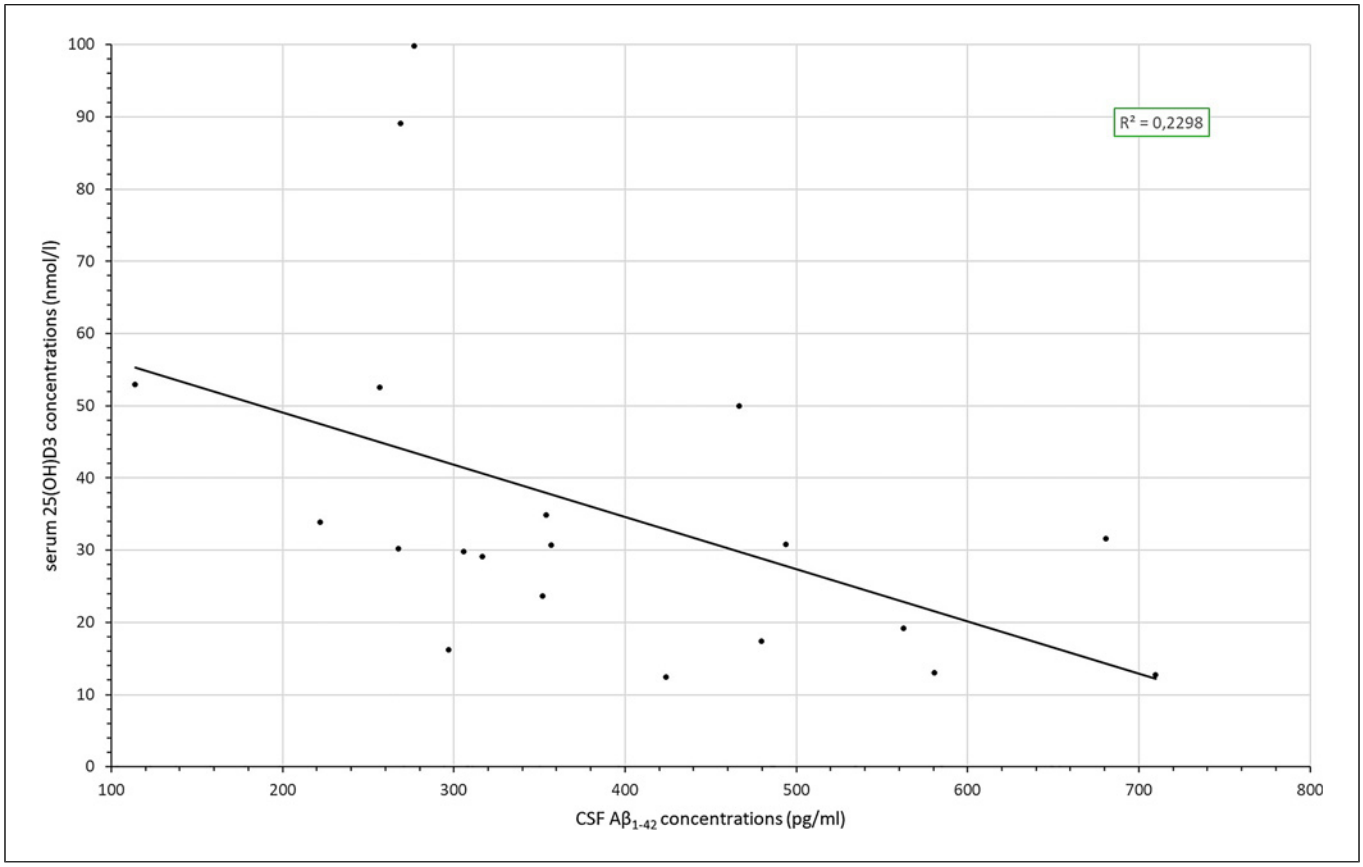

Fig. 1. Significant inverse correlation between serum 25(OH)D3 and CSF $A\beta_{1\text{-}42}$ concentrations in 20 Alzheimer's disease patients (Spearman rank-order statistics: $p = 0.006$).

relationship between serum and brain 5-HT levels seems rather unlikely. Nevertheless, this finding adds to earlier findings indicating decreased serotonergic neurotransmission as a putative marker of accelerated cognitive decline in AD [1].

Our results also showed that serum 5-HT concentrations correlated positively with aggressiveness and frontal behavior, next to a trend for depression. In line with our finding, one study in nondemented participants revealed a similar positive correlation of serum 5-HT with aggressiveness [20]. Of note, the majority of previous studies in AD patients examined cerebral or CSF 5-HT concentrations, such as in our previous observation, where cerebral 5-HT levels correlated inversely with BPSD severity [13]. As far as we know, our current study is the first testing in serum. Hypothetically, an elevated inflammatory state in AD patients might stimulate the secretion of 5-HT from platelets into serum. Furthermore, it seems plausible that this state of low-grade inflammation also enhances the breakdown of tryptophan – the precursor of both 5-HT and kynurenine – via the neuroinflammatory kynurenine pathway. This might reduce tryptophan's availability for cerebral 5-HT synthesis, crucial for controlling behavior. Although our study did not collect data on serum tryptophan, kynurenine, or cerebral 5-HT concentrations, Comai et al. [20] confirmed that aggressive nondemented participants had increased serum 5-HT but lower serum tryptophan levels. In contrast to our results, a human intervention study identified improved depression scores with increasing serum 5-HT levels [9]. However, those patients were not diagnosed with AD.

The inverse correlation between serum 25(OH)D3 and CSF $A\beta_{1\text{-}42}$ levels is another important finding of the present study. It could be hypothesized that hypovitaminosis D might disrupt the homeostasis of cerebral Aβ synthesis and degradation, mediated by stimulation and preference of the amyloidogenic pathway through increased activity of β-/y-secretase next to a reduced expression of the $A\beta_{1\text{-}42}$ degradative

enzyme, i.e., neprilysin [11]. Hence, cerebral $A\beta_{1-42}$ levels might be elevated, thus making them prone to form plaques. Regarding the advanced disease stage of our study population, we assume that due to a conceivable saturation effect of $A\beta_{1-42}$ plaque formation in the brain, an efflux of excess cerebral $A\beta_{1-42}$ levels into the CSF might be possible, explaining the inverse correlation. Of note, such a rise is still relative because CSF $A\beta_{1-42}$ levels in our AD population were almost all well below the in-house validated cut-off of 638.50 pg/mL [15]. This inverse correlation is in contrast with the only other existing study in AD patients investigating the association of CSF $A\beta_{1-42}$ with 25(OH)D3, measured in plasma [12]. This could be due to different sample matrices for the 25(OH)D3 and $A\beta_{1-42}$ quantifications, or the more severe disease stages and even worse vitamin D status in our study population. For instance, Soares et al. [21] found that patients with positive AD core biomarkers had low CSF 25(OH)D3 levels, despite sufficient serum levels. Our results thus would be strengthened by future studies, including also CSF 25(OH)D3 analyses and CSF $A\beta_{1-40}$ measures to investigate the CSF $A\beta_{1-42}/A\beta_{1-40}$ ratio or analyses of shorter, more truncated Aβ variants, confirming that the non-amyloidogenic pathway becomes less activated in vitamin D-deficient conditions. At this point, it must also be mentioned that Lai et al. [22] identified that the usual genomic vitamin D receptor (VDR)-retinoid X receptor (RXR) pathway is compromised in a dose-dependent manner by $A\beta_{1-42}$, and instead the formation of a VDR/p53 complex is stimulated, potentially activating autophagic apoptosis in AD. Future investigations should focus more intensively on the relationship between 25(OH)D3 and $A\beta_{1-42}$ concentrations in terms of their competition for VDR, since vitamin D's attributed benefits on AD development and symptomatology depend on the well-functioning of the VDR-RXR signaling pathway.

Finally, AD patients on cholinesterase inhibitors had significantly higher serum 25(OH)D3 levels, which was also observed by Shah et al. [23]. Due to biotransformation of anti-Alzheimer drugs by the cytochrome P450 (CYP) 3A4 enzyme in the liver, the hepatic elimination of 25(OH)D3 by the same enzyme might become reduced. It might also be related to the possible inhibitory properties of cholinesterase inhibitors on the 25(OH)D3-converting enzyme, i.e., CYP27B1.

Our study population was rather small, and hence it should be considered explorative. At the same time, all participants were clinically well characterized with 15 out of 25 patients having a definite AD diagnosis following neuropathologic investigation and up to 21 having (additional) positive CSF AD biomarkers, corroborating the uniformity of our study population. Serum sampling and BPSD scoring were time-linked, and it is unlikely that our results were influenced by seasonal effects. Next to that, serum 5-HT and 25(OH)D3 levels remained unaffected by prolonged storage time at −80°C. Serum sample quality was further guaranteed by the absence of hemolysis, the low number of previous freeze-thaw cycles, and the low intra-assay variability of the measured 5-HT concentrations (mean = 5.1%). Except the impact of anti-Alzheimer medication on serum 5-HT/25(OH)D3 levels, pharmacological confounding by neither antidepressants nor antipsychotics was found. Nevertheless, potential biases by other psychotropic medications that have not been extensively examined in our study, supplement use, or other dietary factors might still have influenced our findings. Importantly, vitamin D status should be regarded as a marker of good health, implicating that lifestyle factors like physical activity, diet, and maintenance of a healthy body weight may contribute to the study results [24]. Further limitations might be the lacking information on educational levels, genetic alterations, co-morbidities, and renal functioning, all of which could be influencing factors of serum 5-HT/25(OH)D3 and cognitive/behavioral assessment scores. Although emerging evidence points to a potential positive correlation between central and peripheral 5-HT levels [25], hypothetical explanations linking our findings in serum to cerebral 5-HT status must be evaluated with caution. Nevertheless, by quantifying both 5-HT and 25(OH)D3 in serum, we ensured to avoid matrix biases. With regard to the multiple linear regression analyses, the additional statistical determination of the variance inflation factor for each included possible predictor is allowed to exclude multicollinearity. Lastly, by performing Bonferroni correction, type I errors were avoided but potentially might have introduced type II errors and thus false preclusion of some significant findings.

In conclusion, the findings of this research highlight that serum 25(OH)D3 is positively associated with serum 5-HT but inversely with CSF $A\beta_{1-42}$ levels in AD patients with a moderately severe disease stage. Therefore, vitamin D status might possibly affect the progression and symptomatology by altering serotonergic and amyloidogenic synthesis/metabolism. However, solely based on our data, this supposition should be interpreted cautiously. We suggest that an altered serum serotonergic neurotransmitter system might explain some BPSD, particularly aggression, frontal behavior, and depression. In future investigations, a large study in mild-moderate AD patients compared with age- and

gender-matched controls, with the inclusion of more vitamin D-nondeficient subjects and adjustments for renal impairments and genetic predispositions (e.g., mutations of the vitamin D receptor), could provide more definite evidence of whether there might be an inverted U-shaped relationship between serum 25(OH) D3 and the regulation of 5-HT levels. Moreover, metabolic quantifications of serum tryptophan, kynurenine, and cerebral 5-HT levels (e.g., PET imaging) would allow establishing a greater degree of accuracy on the complex molecular mechanisms.

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Statement of Ethics

The study was approved by the Local Ethics Committees of the University Hospital Antwerp and Middelheim General Hospital (Antwerp, Belgium; approval numbers 2805 and 2806). All procedures were performed according to the Declaration of Helsinki for medical research involving human subjects. All patients and/or their relatives gave written informed consent before enrollment.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Conceptualization: Y.V., R.F.W., and S.E.; investigation, methodology, and formal analysis: A.-L.R., M.D.-B., M.G.J.B., and Y.V.; statistical and data analyses and original manuscript drafting: A.-L.R. and Y.V.; manuscript revision, editing, and final approval: all authors; resources, funding acquisition, and provision: Y.V., R.F.W., P.P.D., and S.E.; supervision and project administration: Y.V., L.C.P.G.M.G., M.D.-B., and R.F.W.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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