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Association of Platelet Serotonin Levels in Alzheimer's Disease with Clinical and Cerebrospinal Fluid Markers

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Abstract.
Introduction: Serotonin (5-HT) is involved in the pathology of Alzheimer's disease (AD).
Objective: We aimed to measure 5-HT level in platelets in AD and explore its association with cerebrospinal fluid (CSF), AD biomarkers (amyloid-β 1-42 ($A\beta_{42}$), total tau (t-tau), and phosphorylated tau (p-tau)), and clinical symptoms.
Methods: 15 patients with AD and 20 patients with subjective cognitive impairment (SCI) were included. 5-HT metabolites were measured, in a specific fraction, using high performance liquid chromatography with electrochemical detection (HPLC-ECD).
Results: Significantly lower 5-HT concentrations were observed in AD patients compared to SCI patients both after normalization against total protein ($p = 0.008$) or platelet count ($p = 0.019$). SCI patients with lower 5-HT level have higher AD CSF biomarkers, total tau ($p = 0.026$) and tau/$A\beta_{42}$ ratio ($p = 0.001$), compared to those with high 5-HT levels.
Conclusion: AD patients have reduced platelet 5-HT levels. In SCI, lower 5-HT content was associated with a higher AD-CSF biomarker burden.

Keywords: Alzheimer's disease, amyloid, cognition, platelet, serotonin

INTRODUCTION

Alzheimer's disease (AD) is the major cause of dementia in the elderly [1]. It leads to cognitive decline, decrease in occupational and social functionality, and is a massive burden on both the family and healthcare system. Neuronal loss, extracellular

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deposition of the amyloid-β (Aβ) peptides and intracellular neurofibrillary tangles are involved in the disease neuropathology [2]. Of note, these pathological changes are reflected in the cerebrospinal fluid (CSF) concentrations of total tau, $A\beta_{42,}$ and phosphorylated tau (p-tau) proteins, respectively [3]. AD is also characterized by multiple neurotransmitter dysfunctions; for instance cholinergic, glutamatergic and serotonergic disturbances in the neocortex and hippocampus lead to a state of synaptic failure [4]. Neurochemical studies demonstrated that serotonin (5-hydroxytryptamine, 5-HT) and its breakdown metabolite 5-hydroxyindoleacetic acid (5-HIAA) are decreased in brain and CSF in AD [5, 6]. We recently showed similar results in neuronal cell models; 5-HT and 5-HIAA showed a pattern of increased turnover in cells carrying a familial mutation of AD [7]. The clinical consequences of serotonergic changes in AD are not well established; however, they likely include cognitive decline, depression, anxiety, and other behavioral changes [8, 9].

Although platelets are derived from myelocytic line, there are relevant similarities to the neuronal serotonergic system in terms of functional and morphological features such as granule secretion, signal transduction, and 5-HT transport [10–12]. Platelet activation is associated with 5-HT transporter density, which is a key regulator of synaptic 5-HT levels [13]. Accordingly, platelet 5-HT measurement was shown to be an accessible peripheral model of serotonergic activity in the brain that can add to the understanding of the underlying mechanisms of neuropsychiatric disorders [14]. Changes in the processing of the amyloid-β protein precursor (AβPP) in platelets from AD patients are similar to those typically found in the brain, thus supporting the validity of platelets as a peripheral model of AD [15]. The few available studies have been based on small samples and reported inconsistent results, with some reporting reduced levels of 5-HT in platelets of AD patients [16, 17] and other increased levels [18, 19]. Of note, 5-HT levels differ between platelet sub-populations and platelet heterogeneity in density influences 5-HT concentration [19]. In addition, reduced *in vivo* platelet activity was observed in the low-density populations of platelets derived from sporadic AD [20]. Thus, when studying 5-HT in platelets one needs to account for their density [19] and removal of plasma as a source of 5-HT and 5-HIAA. This study therefore focuses on a specific platelet density fraction to standardize the platelet population. To further control sample variation we analyzed P-selectin to control for platelets populations heterogeneity in granules content and thus 5-HT content. P-selectin is stored in the platelet's alpha granules and transported to the surface when platelets are activated [21]. Of note, P-selectin expression on the platelet surface is associated with 5-HT transporter density (SERT), which is a key regulator of synaptic 5-HT levels [13].

The aim of this study is to investigate the intracellular 5-HT and 5-HIAA platelet concentrations in AD in a specific density fraction. In addition, we wanted to explore whether 5-HT and 5-HIAA in platelets are associated with the AD specific CSF biomarkers and clinical symptoms such as cognitive impairment and depressive symptoms.

MATERIALS AND METHODS

Subjects and clinical assessments

The study cohort includes consecutive referrals to a university-based memory clinic. Two groups of patients were included: An AD group fulfilling the ICD 10 criteria for AD [22] ($n = 15$), and a comparison group with normal performance on cognitive tests and normal biomarkers but with memory complaints, i.e., subjective cognitive impairment (SCI) ($n = 20$). A structured and comprehensive clinical, biochemical and imaging workup for the patients was done as previously described [22]. This included cognitive screening using the Mini-Mental State Examination (MMSE) and neuropsychological assessment [23]. Depressive symptoms were assessed with the Cornell Scale for Depression in Dementia (CSDD), using the recommended cut-off of 8/9 [24, 25], and/or a modified version of the Geriatric Depression Scale (GDS) with 20 items, cut-off 5/6 [26]. In addition, patients were interviewed according to previous history of depression or use of anti-depressants. Depression was defined as having a depression score above the cut-off, a history of depression, or use of antidepressants. In case of discrepancy between the scales, CSDD was given priority. The clinical evaluation was performed blind to the platelet measurements by a clinician with many years of experience in clinical dementia research (VJ). Patients with a history of physical diseases, which according to the clinician influenced the cognitive functioning, were excluded. Patients signed an informed consent. This study was approved by the regional ethical committee.

Blood samples preparations and platelets fractionations

Fractionation of whole blood according to cell density was performed immediately after the venipuncture, as previously described [19] in order to obtain plasma free platelet subpopulations. A total of (7.5 mL) of venous blood was anticoagulated with 2.5 mL 0.129 M disodium citrate. A linear Percoll™ (GE Healthcare Bio-Sciences AB, Sweden) gradient was used to separate platelets according to density [27–29].

To standardize platelet specimen and to obtain a homogenous platelet population, we isolated platelets having a density of 1,064 kg/L, i.e., the human density fraction normally having the highest platelet counts. The separation was carried out as previously described. In brief, linear Percoll™ gradients covering the density span 1.040 to 1.090 kg/L were employed containing a blocking solution to avoid *in vitro* platelet activation and subsequent granule release. Platelet counts were determined electronically. Subsequently, platelets were lysed using a detergent (Triton X-100 final concentration 0.1%) (Sigma-Aldrich, Missouri, U.S.). Cell debris was removed by a short centrifugation and samples were stored in –70°C until further processing. To ensure platelet homogeneity determination of intracellular P-selectin as a measure reflecting platelet α-granule content, a commercial immunoassay (R&D Systems, UK) was used for soluble P-selectin [28].

Plasma–free platelets having a specific density of 1.064 kg/L are used from all cases.

Platelets 5-HT and 5-HIAA measurements

The concentrations of both 5-HT and 5-HIAA in platelet lysate were determined using high performance liquid chromatography with electrochemical detection (HPLC-ECD) platelets having a density of 1.064 kg/L as described previously [30–32]. Briefly, the HPLC system consisted of a HTEC500 (Eicom, Kyoto, Japan) and a CMA/200 Refrigerated Microsampler (CMA Microdialysis, Stockholm, Sweden) equipped with 20 μl loop and operating at +4°C. The potential of the glassy carbon working electrode was +450 mV versus the Ag/AgCl reference electrode. The separation was achieved on a 200 × 2.0 mm Eicompak CAX column (Eicom, Kyoto, Japan). The mobile phase was a mixture of methanol and 0.1 M phosphate buffer (pH 6.0) (30:70, v/v) containing 40 mM potassium chloride and 0.13 mM EDTA-2Na. Concentrations of 5-HIAA were determined by a separate HPLC system with electrochemical detection (HTEC500). The potential of the glassy carbon working electrode was +75 0 mV versus the Ag/AgCl reference electrode. The separation was achieved on a 150 × 3.0 mm Eicompak SC-5ODS column (Eicom, Kyoto, Japan). The mobile phase was a mixture of methanol and 0.1 M citrate – 0.1M sodium acetate buffer solution (pH 3.5) (16:84, v/v) containing 210 mg/L Octanesulfonic acid sodium salt and 5 mg/L EDTA-2Na. The chromatograms were recorded and integrated by use of the computerized data acquisition system Clarity (DataApex, The Czech Republic).

The limit of detection (defined as signal-to-noise ratio >3) was 10 pM (0.1 fmol/10 μl injected) for 5-HT and 20 pM (0.2 fmol/10 μl injected) for 5-HIAA. The calibration curves were linear within the range of 50 pM to 1 μM ($R = 0.999$) for 5-HT and 0.1 nM–1 μM ($R = 0.999$) for 5-HIAA.

The range of detected 5-HT was within 0.42 nM (lowest) and 432 nM (highest) and for 5-HIAA the corresponding range was 0.41–2.72 nM. Thus, the concentrations of 5-HT and 5-HIAA detected in the samples are well within the linearity range of the respective calibration curves.

CSF measurements for $A\beta_{42}$, t-tau, and p-tau

In all patients the CSF was obtained in the morning while the patient was sitting in an upright position, by lumbar puncture between the L3/L4 or L4/L5 intervertebral space, using a 25-gauge needle, and collected in 12-ml polypropylene tubes. Within 2 h, CSF samples were centrifuged at 1,000 rpm for 10 min. A small amount of CSF was used for routine analysis, including total cells (leukocytes and erythrocytes), total protein, and glucose. CSF was aliquoted in polypropylene tubes of 1 ml, and stored at –70°C until further analysis. T-tau, p-tau, and $A\beta_{42}$ concentrations were measured with xMAP technology and the INNO-BIA AkzBio3 kit (Innogenetics), as previously described in detail [33, 34]. The cutoff values for the laboratory were used to classify the result into normal or abnormal values: $A\beta_{42}$ >550 ng/l, p-tau<80 ng/l, t-tau<400 ng/l (normal values). We used the t-tau/$A\beta_{42}$ ratio as a measure of AD pathology [35].

Statistical analysis

Statistical analysis was performed using SPSS software, version 22 (Armonk, NY: IBM Corp.).

Serum concentration of 5-HT and 5-HIAA were corrected by the fraction protein concentration and fraction platelet count. For description of normality of distribution or skewness of the data, mean and standard deviation (SD) or median and interquartile range (IQR) were used, respectively. Shapiro-Walk test was applied to assess the normality of distribution for numeric variables. For unadjusted univariate comparisons between SCI and AD groups, independent samples *T*-test, Mann-Whitney U-test, Pearson Chi square and Fisher's exact tests were used for numeric, ordinal, and nominal variables wherever appropriate. As the main analysis, we applied multivariate ordinal logistic regression to adjust differences in tertiles of serum serotonin indicators between SCI and AD groups for participants' age, depression status (yes/no), and P-selectin level. Adjusted odds ratio (OR) and their 95% confidence interval (CI) were reported for each regression model. Correlations were performed using Spearman test, and adjusted for age using partial correlation. Based on inspection of the data, the SCI group was divided into two subgroups based on the median value of the protein-corrected 5-HT level. Mann-Whitney U test and linear regression model were used to compare the CSF biomarkers between the SCI with high 5-HT and low 5-HT. A p-value of <0.05 was considered statistically significant.

RESULTS

5-HT and 5-HIAA in AD and SCI

Demographic and clinical characteristics of the cohort are shown in Table 1. There were no significant differences between SCI and AD groups for gender, medication, or APOEε4 genotype. As expected, the AD group had a lower MMSE score ($p < 0.001$), but was also significantly older compared to the SCI group ($p = 0.006$), and depression was more common ($p = 0.037$) in the SCI group. Age was inversely correlated with the platelet 5-HT content corrected for protein concentration in the entire study population ($r = –0.36$, $p = 0.034$), and accordingly, age adjusted multivariate analyses were performed. As expected AD patients had higher t-tau, p-tau, and t-tau/Aβ_{42} values ($p = 0.001$, 0.009 and <0.001, respectively) and lower Aβ_{42} levels ($p < 0.001$), compared to the SCI group. The unadjusted analyses showed that the AD group had significantly lower levels of 5-HT compared to SCI, both for crude and corrected values (Fig. 1 and Table 2). In addition, significantly higher crude concentrations of 5-HIAA and higher 5-HIAA per platelet counts compared to SCI were observed (Fig. 1D, F and Table 2) but not following correction for protein concentration (Fig. 1E). Our analyses show no correlation between 5-HT and 5-HIAA levels (data not shown). In addition, lower levels of P-selectin were observed in the AD group after adjustment for age, sex, and depression scales (Supplementary Material).

In the multivariate regression analyses, AD patients had significantly lower 5-HT tertiles after adjustment for age, P-selectin, and presence of depression for both corrected levels by protein concentration [OR = 0.11 (95% CI: 0.01–0.82), $p = 0.032$] and numerically lower when corrected to the platelet counts [OR = 0.18 (95% CI: 0.03–1.21), $p = 0.077$]. In addition, tertiles of the platelet count and protein-corrected 5-HIAA levels were higher in AD group compared to the SCI after adjustment for age, P-selectin, and depression [OR = 24.64 (95% CI: 2.55–238.48), $p = 0.006$] and

Table 1
Baseline, laboratory, clinical, and medication characteristics of the two study groups

Characteristic	SCI ($n = 20$)	AD ($n = 15$)	p-value
Gender-n (%)			
Female	11 (55.0%)	7 (46.7%)	0.625[#]
Age (y)-median (IQR)	61 (14)	74 (17)	**0.006**[¶]
MMSE score-median (IQR)	29 (2)	24 (9)	**<0.001**[¶]
Medications-n (%)			
SSRIs	3 (15.0%)	4 (26.7%)	0.430[§]
ASA	3 (15.0%)	2 (13.3%)	1[§]
Anticoagulants	2 (10.0%)	0	0.496[§]
Statins	5 (25.0%)	2 (13.3%)	0.672[§]
Antihypertensive	5 (25.0%)	6 (40.0%)	0.344[#]
Anti-diabetes	0	1 (6.7%)	0.429[§]
Neuroleptics	0	0	–
APOE ε4-n (%)			
One allele	5 (29.4%)	7 (50.0%)	0.242[#]
Depression			
Frequency-n (%)	12 (60.0%)	3 (20.0%)	**0.037**[§]
CSF Biomarkers-mean (SD)			
Aβ_{42}	1274.0 (280.2)	548.5 (171.0)	**<0.001**[*]
Total tau (T-tau)	250.0 (110.2)	600.1 (309.4)	**0.001**[*]
Phosphorylated tau (P-tau)	47.2 (16.7)	79.2 (36.0)	**0.009**[*]
T-tau/Aβ_{42} ratio	0.19 (0.07)	1.24 (0.80)	**<0.001**[*]

SCI, subjective cognitive impairment; AD, Alzheimer's disease; IQR, interquartile range; SD, standard deviation; SSRIs, selective serotonin reuptake inhibitors; ASA, acetylsalicylic acid; CSF, cerebrospinal fluid. Shapiro-Wilk test was used to check for the normality of the distribution of numeric variables in each study group. Statistical significant differences (two-tailed p-value<0.05) are bolded. [#]Pearson Chi Square statistics; [¶]Mann-Whitney *U*-test; [§]Fisher's Exact test; [*]Independent Samples *T*-test.

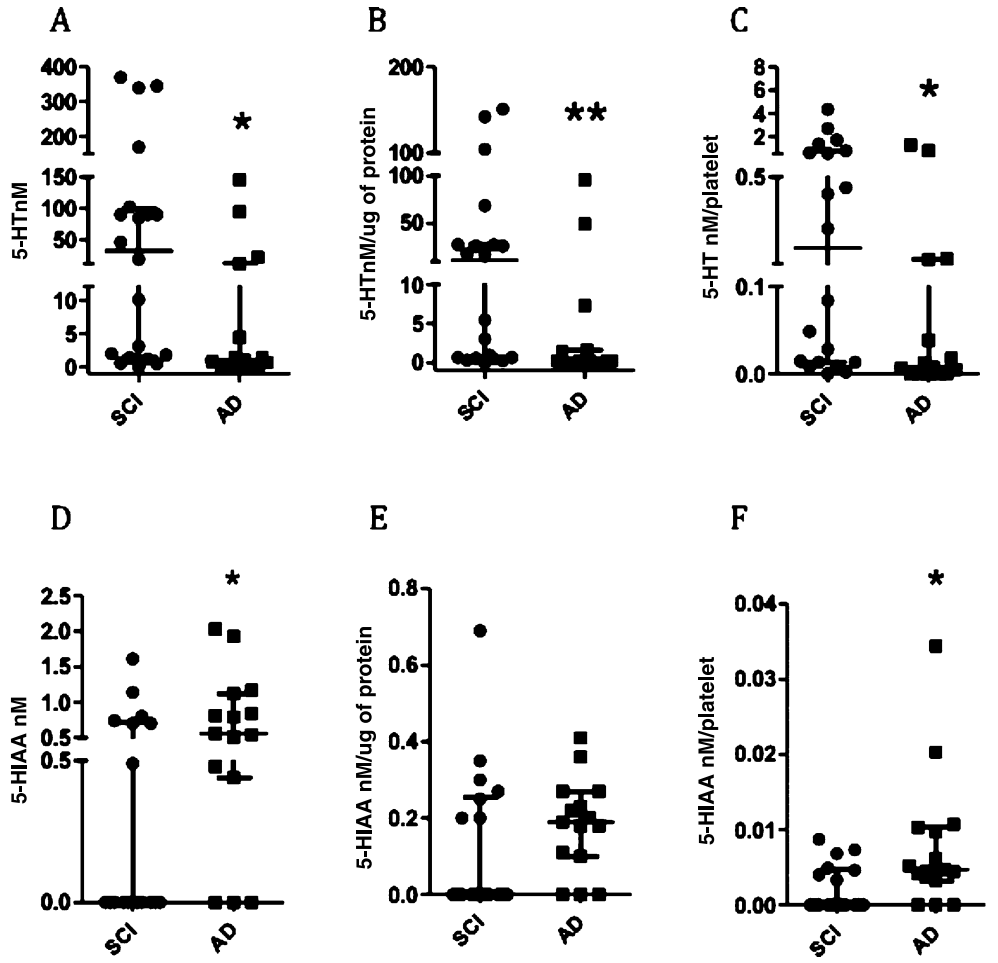

Fig. 1. Serum serotonin metabolites levels in the two study groups. Comparison between 5-HT and 5-HIAA levels in AD and SCI groups are shown. 5-HT crude levels (A) adjusted to protein concentration (B) and to platelets count (C) and 5-HIAA crude levels (D), adjusted to protein concentration (E) and to platelets count (F) are shown. Data is represented as median and interquartile range (IQR). The statistical significance grades are represented by asterisk $^{*}p < 0.05$ and $^{**}p < 0.001$. SCI, subjective cognitive impairment; AD, Alzheimer's disease; IQR, interquartile range; 5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid.

[OR = 10.55(95% CI: 1.23–90.63, $p = 0.032$], respectively (Table 2).

Associations between 5-HT and CSF and clinical markers

Bivariate unadjusted and adjusted correlations between AD-CSF biomarkers and 5-HT and 5-HIAA levels in each group are summarized in Table 3. In unadjusted correlations, the SCI group showed significant inverse correlations between the t-tau/Aβ_{42} ratio and both protein concentration- and platelet count-corrected 5-HT levels [$r = -0.61$ ($p = 0.006$) and $r = -0.51$ ($p = 0.024$), respectively] (Fig. 2A), i.e., reduced 5-HT levels were associated with more AD-like CSF values. However, these correlations became statistically non-significant after adjustment for age (Table 3).

Since the distribution of 5-HT in SCI showed one group with high and one with low values (See Fig. 1 with distribution), and people with SCI have a higher risk of progressing to AD than people without SCI [36], we hypothesized that SCI patients with low 5-HT may have prodromal AD which will be reflected in abnormal CSF values. As shown in Fig. 3, SCI subjects with a low 5-HT level had a significantly higher CSF level of t-tau (Fig. 3A) and higher t-tau/Aβ_{42} ratio (Fig. 3D) compared to those with high 5-HT. These differences remained statistically significant even after adjustment for age and depression

Table 2
Univariate and age-adjusted comparisons of serum serotonin level between the two study groups

Variables	SCI ($n = 20$)	AD ($n = 15$)	Univariate p-value[#]	Adjusted Comparison
Platelet Serotonin (5-HT)				B = –9.4[§]
Crude level (nM/mL)-median (IQR)	65.600 (117.575)	1.000 (12.200)	**0.016**	(95% CI: –126.2–107.3) (p-value = 0.867)
5-HT nM/ug of protein-median (IQR)	17.615 (37.165)	0.238 (1.630)	**0.007**	
Tertiles Category-n (%)				
1st tertile	2 (10.0%)	10 (66.7%)		OR = 0.11*
2nd tertile	8 (40.0%)	3 (20.0%)	**0.002**	(95% CI: 0.01–0.82)
3rd tertile	10 (50.0%)	2 (13.3%)		**(p-value = 0.032)**
5-HT nM/platelet count-median (IQR)	0.342 (0.908)	0.008 (0.121)	**0.017**	
Tertiles Category-n (%)				
1st tertile	3 (15.0%)	8 (53.3%)		OR = 0.18*
2nd tertile	6 (30.0%)	5 (33.3%)	**0.008**	(95% CI: 0.03–1.21)
3rd tertile	11 (55.0%)	2 (13.3%)		(p-value = 0.077)
Platelet 5-HIAA				B = 0.44[§]
Crude level (nM/mL)-median (IQR)	0.000 (0.710)	0.560 (0.680)	**0.044**	(95% CI: –0.25–1.13) (p-value = 0.195)
5-HIAA nM/ug of protein-median (IQR)	0.000 (0.255)	0.193 (0.169)	0.178	
Tertiles Category-n (%)				
1st tertile	11 (61.1%)	3 (20.0%)		OR = 10.55*
2nd tertile	2 (11.1%)	6 (40.0%)	0.093	(95% CI: 1.23–90.63)
3rd tertile	5 (27.8%)	6 (40.0%)		**(p-value = 0.032)**
5-HIAA nM/platelet count-median (IQR)	0.000 (0.005)	0.005 (0.007)	**0.016**	
Tertiles Category-n (%)				
1st tertile	11 (61.1%)	3 (20.0%)		OR = 24.64*
2nd tertile	3 (16.7%)	5 (33.3%)	**0.044**	(95% CI: 2.55–238.48)
3rd tertile	4 (22.2%)	7 (46.7%)		**(p-value = 0.006)**

SCI, subjective cognitive impairment; AD, Alzheimer's disease; IQR, interquartile range; OR, odds' ratio; CI, confidence interval. *Shapiro-Wilk* test was used to check for the normality of the distribution of numeric variables in each study group. Multivariate linear and ordinal regression models were applied to adjust between-group comparisons for participants' age, depression, and p-selectin level. Statistical significant differences (two-tailed p-value <0.05) are bolded. [#]Mann-Whitney U-test; [§]Multivariate linear regression; *Multivariate ordinal logistic regression.

Table 3
Spearman unadjusted (first row) and partial age-adjusted (second row) correlations between serum serotonin level and CSF biomarkers and cognitive status in each of the two study groups [data is presented as correlation coefficient (p-value)]

Variables	$A\beta_{42}$	Total tau (T-tau)	Phosphorylated tau (P-tau)	T-tau/$A\beta_{42}$ ratio	MMSE score
Subjective Cognitive Impairment (SCI)					
5-HT nM/μg of protein	–0.12 (0.613)	–0.44 (0.062)	–0.35 (0.139)	**–0.61 (0.006)**	–0.35 (0.127)
	–0.14 (0.616)	–0.17 (0.541)	–0.15 (0.575)	–0.14 (0.601)	–0.28 (0.273)
5-HT nM/platelet count	–0.16 (0.513)	–0.38 (0.109)	–0.31 (0.191)	**–0.51 (0.024)**	–0.27 (0.255)
	–0.23 (0.382)	–0.16 (0.554)	–0.17 (0.530)	–0.08 (0.769)	–0.20 (0.434)
5-HIAA nM/μg of protein	0.28 (0.281)	–0.02 (0.948)	0.08 (0.763)	–0.06 (0.824)	0.23 (0.362)
	0.31 (0.239)	0.19 (0.472)	0.21 (0.443)	–0.14 (0.601)	0.24 (0.353)
5-HIAA nM/platelet count	0.23 (0.380)	–0.02 (0.926)	0.07 (0.801)	–0.10 (0.714)	0.25 (0.308)
	0.15 (0.570)	0.06 (0.819)	0.07 (0.797)	–0.11 (0.690)	0.27 (0.296)
Alzheimer's Disease (AD)					
5-HT nM/μg of protein	–0.18 (0.530)	0.52 (0.058)	0.51 (0.077)	0.47 (0.091)	–0.01 (0.974)
	–0.02 (0.950)	0.16 (0.606)	0.19 (0.560)	0.06 (0.847)	0.25 (0.391)
5-HT nM/platelet count	–0.15 (0.619)	0.48 (0.084)	0.50 (0.081)	0.44 (0.117)	–0.02 (0.948)
	–0.03 (0.912)	0.21 (0.483)	0.34 (0.282)	0.10 (0.749)	0.26 (0.363)
5-HIAA nM/μg of protein	0.00 (0.994)	0.14 (0.635)	0.44 (0.136)	0.17 (0.551)	0.15 (0.588)
	0.24 (0.427)	0.22 (0.464)	0.48 (0.111)	0.04 (0.907)	0.02 (0.943)
5-HIAA nM/platelet count	0.00 (1)	0.27 (0.348)	0.38 (0.206)	0.34 (0.238)	–0.37 (0.180)
	0.15 (0.618)	0.53 (0.061)	0.55 (0.064)	0.26 (0.389)	–0.38 (0.175)

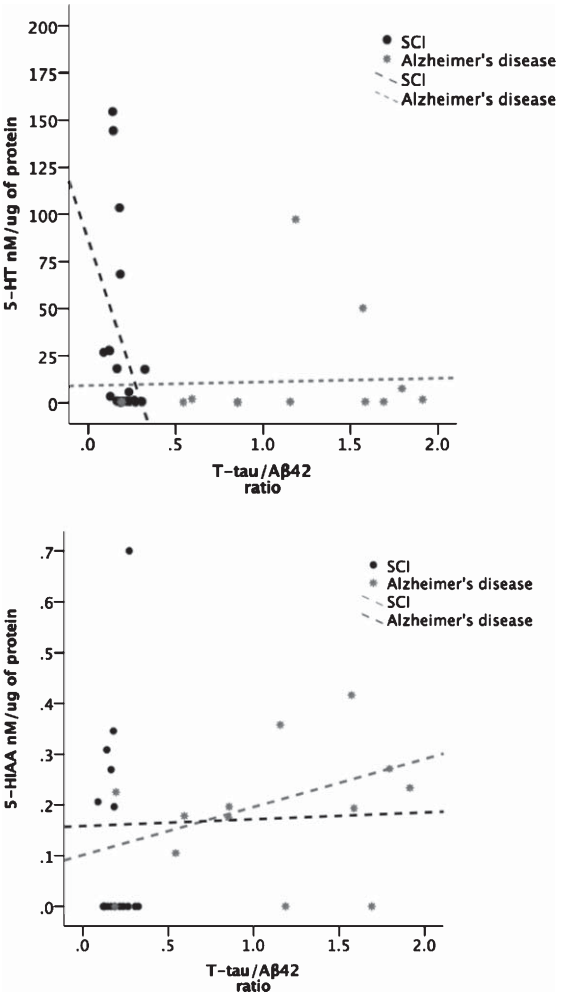

Fig. 2. Correlation between 5-HT, 5-HIAA, and CSF biomarkers. Scatter plot of the association between serum serotonin and CSF biomarker levels in each of the two study groups. The T-tau/Aβ ratio is plotted against (A) 5-HT and (B) 5-HIAA corrected to the fraction's protein concentration. Multivariate analysis model is adjusted to age and presence of depression.

[adjusted mean difference for CSF total tau = 102.7 pg/mL (95% CI: 8.2–197.2), $p = 0.035$; adjusted mean difference for CSF tau/Aβ_{42} ratio = 0.08 (95% CI: 0.03–0.13), $p = 0.005$].

In the AD group, neither the unadjusted nor age-adjusted analyses showed a significant correlation between platelet 5-HT concentrations and CSF t-tau/Aβ42 ratio (Fig. 2A, Table 3). Corrected concentrations of 5-HIAA had no significant correlation with t-tau/Aβ_{42} neither in the AD nor SCI groups (Fig. 2B). Neither MMSE scores nor depression were significantly associated with platelet serotonin indicators in SCI or AD groups (results not shown).

DISCUSSION

The main finding in this study was that platelets of a medium-density (1.064 kg/L) subtype had lower levels of 5-HT together with higher 5-HIAA in AD compared to individuals with SCI (Fig. 1, Table 2). These lower 5-HT levels were independent of age or presence of depression. In addition, in the SCI group those with low platelet 5-HT values had higher t-tau and t-tau/Aβ_{42} ratio, raising the possibility that these patients may have prodromal AD and that this can be detected by measuring platelet 5-HT (Fig. 2).

Previous reports have shown different results of peripheral platelet serotonin levels in AD. Most of the published data support the reduction of 5-HT in AD platelets [16, 17, 37, 38]. In contrast, other studies reported higher 5-HT platelet content in AD [18, 19]. The previous studies have however had methodological limitations. For example, very few patients were male, thus there is a risk for gender bias. In addition, in all previous studies the diagnosis was purely clinical without biomarker information, thus there is a risk of misdiagnosis, and in one study no specific dementia diagnosis was made [18]. In another study, there was no information regarding disease duration or severity [16]. In one study, significant reduced platelet 5-HT was only found in the group with severe disease, with an extremely low mean MMSE of 2.2, and these patients were also much older (mean age 82.0) than the control subjects (74.7) [16]. Our results, based on patients with relatively mild dementia (median MMSE score 24), well characterized clinical and biomarker diagnosis, and mild dementia severity, support the conclusion that platelet 5HT is reduced in AD. However, more studies are needed to clarify the association between platelet 5-HT in AD, and, in particular, its relationship to clinical and biological markers.

This study is novel in the sense that it examines the association between the platelet 5-HT and AD-CSF biomarker. In addition to securing the diagnosis, these CSF markers allow us to explore the association between 5-HT and key pathological features of AD. Although there was no significant association between the markers and 5HT levels in patients with established AD, in the SCI group, reduction of 5-HT metabolite was associated with increased t-tau/Aβ_{42} ratio. Some of these SCI patients likely have early preclinical AD [39, 40], and thus this observation suggests that the very early pathological changes are associated with reduced platelet 5-HT levels. This observation suggests that 5-HT measure-

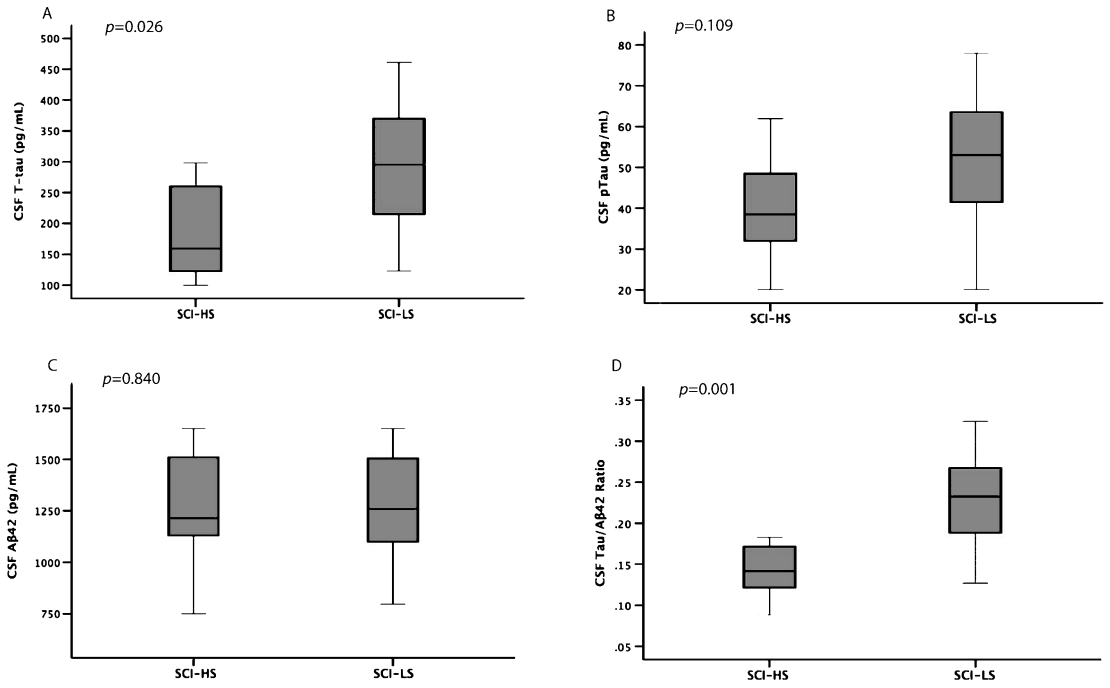

Fig. 3. CSF biomarkers and SCI group divided according to platelet serotonin levels. Comparison of CSF biomarkers between SCI subjects with high (SCI-HS) $N=9$ and low protein-corrected 5HT level (SCI-LS) $N=11$ (p-values are from univariate Mann-Whitney u test.). Statistical significance level is $p<0.05$.

ment in platelets could serve as an early biomarker for AD even before the MCI stage. Prospective studies are needed to test this hypothesis.

Platelet 5-HT has been shown to be associated with behavioral changes and mood symptoms during the disease course. For example, patients with involuntary emotional expression disorder (IEED) showed lower platelet 5-HT content compared to AD with other behavioral disturbances like aggression disorder [38]. Another report linked the change of platelet 5-HT to psychotic symptoms in AD. They showed that AD with psychotic symptoms have lower platelet 5-HT compared to those without psychotic symptoms [37].

Together, these studies indicate that drugs with serotonergic activity may positively influence psychiatric and possibly cognitive symptoms in AD and related dementias, consistent with recent evidence in Parkinson's disease [41].

5-HT is stored in the dense granules in the platelets [42]. Although platelets lack the MAO-A, MAO-B could be the key player in degrading 5-HT into 5-HIAA. Higher 5-HIAA levels in this study could be due to the increased activity of the MAO-B [43]. In addition, the fact that there is no correlation between 5-HT and 5-HIAA in this study indicates that 5-HIAA is affected by the 5-HT transmission inside platelets and released from them. The intracellular availability of stored or free serotonin metabolites depends on the trans-membrane transport from and into plasma. This transport is affected in AD. Studies investigating the serotonin uptake sites (Vmax) in AD platelets, showed increased uptake in mild and moderate AD, but a trend toward reduction in severe AD [44]. Other studies showed higher Vmax and affinity (Km) in platelets of female AD patients [45].

The low sample size and absence of patients with more severe cognitive impairment are some of the limitations of this study, and thus not all multivariate adjusted analyses had enough statistical power to show the between-groups and between-subgroups differences. There were also substantial variations in the measurements, with several AD patients having very low 5HT levels, whereas a few had levels in the normal range. This could be due to biological heterogeneity, to assay issues, or both. A number of statistical analyses were made without adjusting for the risk of false positives. Ideally a longitudinal approach with follow-up sampling of SCI patients should be used to confirm our findings. Thus our

findings should be viewed cautiously and need confirmation in independent studies with larger sample sizes. The early stage of mild cognitive impairment (MCI) is a stage of cognitive decline intermediate between SCI and dementia [46], which represent a more accurate prodromal phase of AD, and thus future studies of platelet 5-HT as a marker of prodromal AD should include patients with MCI. Another methodological limitation is the inclusion of a single platelets sub-fraction, based of the report showing the differential comparison between fractions in AD and controls [19]. This strategy lacks the advantages of demonstrating the 5-HT in all different fractions and relate 5-HT to platelet density in AD and SCI. On the other hand, controlling for platelets density is a desirable method when measuring 5-HT, since different platelets densities exhibit different 5-HT concentrations and activity [19, 20].

We found that in AD patients, medium density platelets have lower 5-HTcontent compared to SCI and that SCI patients with low 5-HT have an AD-like CSF biomarker profile. Further clinical studies with larger sample sizes and using different platelets population are needed to further characterize the relationship between AD pathology and platelet 5-HT levels.

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SUPPLEMENTARY MATERIAL

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REFERENCES

[1] Ballard C, Gauthier S, Corbett A, Brayne C, Aarsland D, Jones E (2011) Alzheimer's disease. *Lancet* **377**, 1019-1031.

[2] Montine TJ, Phelps CH, Beach TG, Bigio EH, Cairns NJ, Dickson DW, Duyckaerts C, Frosch MP, Masliah E, Mirra SS, Nelson PT, Schneider JA, Thal DR, Trojanowski JQ, Vinters HV, Hyman BT, National Institute on Aging, Alzheimer's Association (2012) National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease: A practical approach. *Acta Neuropathol* **123**, 1-11.

[3] Blennow K, Zetterberg H (2009) Cerebrospinal fluid biomarkers for Alzheimer's disease. *J Alzheimers Dis* **18**, 413-417.

[4] Selkoe DJ (2002) Alzheimer's disease is a synaptic failure. *Science* **298**, 789-791.

[5] Reinikainen KJ, Paljarvi L, Huuskonen M, Soininen H, Laakso M, Riekkinen PJ (1988) A post-mortem study of noradrenergic, serotonergic and GABAergic neurons in Alzheimer's disease. *J Neurol Sci* **84**, 101-116.

[6] Blennow K, Wallin A, Gottfries CG, Lekman A, Karlsson I, Skoog I, Svennerholm L (1992) Significance of decreased lumbar CSF levels of HVA and 5-HIAA in Alzheimer's disease. *Neurobiol Aging* **13**, 107-113.

[7] Tajeddinn W, Persson T, Maioli S, Calvo-Garrido J, Parrado-Fernandez C, Yoshitake T, Kehr J, Francis P, Winblad B, Hoglund K, Cedazo-Minguez A, Aarsland D (2015) 5-HT1B and other related serotonergic proteins are altered in APPswe mutation. *Neurosci Lett* **594**, 137-143.

[8] Lai MK, Tsang SW, Francis PT, Keene J, Hope T, Esiri MM, Spence I, Chen CP (2002) Postmortem serotoninergic correlates of cognitive decline in Alzheimer's disease. *Neuroreport* **13**, 1175-1178.

[9] Rodriguez JJ, Noristani HN, Verkhratsky A (2012) The serotonergic system in ageing and Alzheimer's disease. *Prog Neurobiol* **99**, 15-41.

[10] Moreno J, Campos MG, Lara C, Torner C (2005) The platelet as biological marker of the neural serotonergic function. *Salud Mental* **28**, 79-87.

[11] Pletscher A (1986) Blood platelets as neuronal models: Use and limitations. *Clin Neuropharmacol* **9**(Suppl 4), 344-346.

[12] Goubau C, Buyse GM, Van Geet C, Freson K (2014) The contribution of platelet studies to the understanding of disease mechanisms in complex and monogenetic neurological disorders. *Dev Med Child Neurol* **56**, 724-731.

[13] Frankhauser P, Baranyai R, Ahrens T, Schloss P, Deuschle M, Lederbogen F (2008) Platelet surface P-selectin expression is highly correlated with serotonin transporter density in human subjects. *Thromb Haemost* **100**, 1201-1203.

[14] Camacho A, Dimsdale JE (2000) Platelets and psychiatry: Lessons learned from old and new studies. *Psychosom Med* **62**, 326-336.

[15] Cattabeni F, Colciaghi F, Di Luca M (2004) Platelets provide human tissue to unravel pathogenic mechanisms of Alzheimer disease. *Prog Neuropsychopharmacol Biol Psychiatry* **28**, 763-770.

[16] Kumar AM, Sevush S, Kumar M, Ruiz J, Eisdorfer C (1995) Peripheral serotonin in Alzheimer's disease. *Neuropsychobiology* **32**, 9-12.

[17] Muck-Seler D, Presecki P, Mimica N, Mustapic M, Pivac N, Babic A, Nedic G, Folnegovic-Smalc V (2009) Platelet serotonin concentration and monoamine oxidase type B activity in female patients in early, middle and late phase of Alzheimer's disease. *Prog Neuropsychopharmacol Biol Psychiatry* **33**, 1226-1231.

[18] Meszaros Z, Borcsiczky D, Mate M, Tarcali J, Szombathy T, Tekes K, Magyar K (1998) Platelet MAO-B activity and serotonin content in patients with dementia: Effect of age, medication, and disease. *Neurochem Res* **23**, 863-868.

[19] Milovanovic M, Eriksson K, Winblad B, Nilsson S, Lindahl TL, Post C, Jaremo P (2014) Alzheimer and platelets: Low-density platelet populations reveal increased serotonin content in Alzheimer type dementia. *Clin Biochem* **47**, 51-53.

[20] Jaremo P, Milovanovic M, Buller C, Nilsson S, Winblad B (2012) Low-density platelet populations demonstrate low *in vivo* activity in sporadic Alzheimer disease. *Platelets* **23**, 116-120.

[21] Binder FP, Ernst B (2011) E- and P-selectin: Differences, similarities and implications for the design of P-selectin antagonists. *Chimia (Aarau)* **65**, 210-213.

[22] Kramberger MG, Jelic V, Kareholt I, Enache D, Eriksdotter Jonhagen M, Winblad B, Aarsland D (2012) Cerebrospinal fluid Alzheimer markers in depressed elderly subjects with and without Alzheimer's disease. *Dement Geriatr Cogn Dis Extra* **2**, 48-56.

[23] Folstein MF, Folstein SE, McHugh PR (1975) Mini-mental state. A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* **12**, 189-198.

[24] Alexopoulos GS, Abrams RC, Young RC, Shamoian CA (1988) Cornell scale for depression in dementia. *Biol Psychiatry* **23**, 271-284.

[25] Barca ML, Engedal K, Selbaek G (2010) A reliability and validity study of the cornell scale among elderly inpatients, using various clinical criteria. *Dement Geriatr Cogn Disord* **29**, 438-447.

[26] Gottfries CG (1997) Recognition and management of depression in the elderly. *Int Clin Psychopharmacol* **12**(Suppl 7), S31-S36.

[27] Jaremo P (1995) Computerised method for recording platelet density distribution. *Eur J Haematol* **54**, 304-309.

[28] Milovanovic M, Lysen J, Ramstrom S, Lindahl TL, Richter A, Jaremo P (2003) Identification of low-density platelet populations with increased reactivity and elevated alpha-granule content. *Thromb Res* **111**, 75-80.

[29] Milovanovic M, Lotfi K, Lindahl T, Hallert C, Jaremo P (2010) Platelet density distribution in essential thrombocythemia. *Pathophysiol Haemost Thromb* **37**, 35-42.

[30] Yoshitake T, Kehr J, Todoroki K, Nohta H, Yamaguchi M (2006) Derivatization chemistries for determination of serotonin, norepinephrine and dopamine in brain microdialysis samples by liquid chromatography with fluorescence detection. *Biomed Chromatogr* **20**, 267-281.

[31] Kehr J, Ichinose F, Yoshitake S, Goiny M, Sievertsson T, Nyberg F, Yoshitake T (2011) Mephedrone, compared with MDMA (ecstasy) and amphetamine, rapidly increases both dopamine and 5-HT levels in nucleus accumbens of awake rats. *Br J Pharmacol* **164**, 1949-1958.

[32] Yoshitake S, Kuteeva E, Hokfelt T, Mennicken F, Theodorsson E, Yamaguchi M, Kehr J, Yoshitake T (2014) Correlation between the effects of local and intracerebroventricular infusions of galanin on 5-HT release studied by microdialysis, and distribution of galanin and galanin receptors in prefrontal cortex, ventral hippocampus, amygdala, hypothalamus, and striatum of awake rats. *Synapse* **68**, 179-193.

[33] Vanmechelen E, Vanderstichele H, Davidsson P, Van Kerschaver E, Van Der Perre B, Sjogren M, Andreasen N, Blennow K (2000) Quantification of tau phosphorylated at threonine 181 in human cerebrospinal fluid: A sandwich ELISA with a synthetic phosphopeptide for standardization. *Neurosci Lett* **285**, 49-52.

[34] Andreasen N, Hesse C, Davidsson P, Minthon L, Wallin A, Winblad B, Vanderstichele H, Vanmechelen E, Blennow K (1999) Cerebrospinal fluid beta-amyloid(1-42) in Alzheimer disease: Differences between early- and late-onset Alzheimer disease and stability during the course of disease. *Arch Neurol* **56**, 673-680.

[35] Samgard K, Zetterberg H, Blennow K, Hansson O, Minthon L, Londos E (2010) Cerebrospinal fluid total tau as a marker of Alzheimer's disease intensity. *Int J Geriatr Psychiatry* **25**, 403-410.

[36] Lam LC, Lui VW, Tam CW, Chiu HF (2005) Subjective memory complaints in Chinese subjects with mild cognitive impairment and early Alzheimer's disease. *Int J Geriatr Psychiatry* **20**, 876-882.

[37] Mimica N, Muck-Seler D, Pivac N, Mustapic M, Dezeljin M, Stipcevic T, Presecki P, Radonic E, Folnegovic-Smalc V (2008) Platelet serotonin and monoamine oxidase in Alzheimer's disease with psychotic features. *Coll Antropol* **32**(Suppl 1), 119-122.

[38] Prokselj T, Jerin A, Muck-Seler D, Kogoj A (2014) Decreased platelet serotonin concentration in Alzheimer's disease with involuntary emotional expression disorder. *Neurosci Lett* **578**, 71-74.

[39] Linn RT, Wolf PA, Bachman DL, Knoefel JE, Cobb JL, Belanger AJ, Kaplan EF, D'Agostino RB (1995) The preclinical phase of probable Alzheimer's disease. A 13-year prospective study of the Framingham cohort. *Arch Neurol* **52**, 485-490.

[40] Small BJ, Fratiglioni L, Viitanen M, Winblad B, Backman L (2000) The course of cognitive impairment in preclinical Alzheimer disease: Three- and 6-year follow-up of a population-based sample. *Arch Neurol* **57**, 839-844.

[41] Cummings J, Isaacson S, Mills R, Williams H, Chi-Burris K, Corbett A, Dhall R, Ballard C (2014) Pimavanserin for patients with Parkinson's disease psychosis: A randomised, placebo-controlled phase 3 trial. *Lancet* **383**, 533-540.

[42] Holmsen H (1985) Platelet metabolism and activation. *Semin Hematol* **22**, 219-240.

[43] Adolfsson R, Gottfries CG, Oreland L, Wiberg A, Winblad B (1980) Increased activity of brain and platelet monoamine oxidase in dementia of Alzheimer type. *Life Sci* **27**, 1029-1034.

[44] Arora RC, Emery OB, Meltzer HY (1991) Serotonin uptake in the blood platelets of Alzheimer's disease patients. *Neurology* **41**, 1307-1309.

[45] Kumar AM, Kumar M, Sevush S, Ruiz J, Eisdorfer C (1995) Serotonin uptake and its kinetics in platelets of women with Alzheimer's disease. *Psychiatry Res* **59**, 145-150.

[46] Gauthier S, Reisberg B, Zaudig M, Petersen RC, Ritchie K, Broich K, Belleville S, Brodaty H, Bennett D, Chertkow H, Cummings JL, de Leon M, Feldman H, Ganguli M, Hampel H, Scheltens P, Tierney MC, Whitehouse P, Winblad B, International Psychogeriatric Association. Expert Conference on mild cognitive impairment (2006) Mild cognitive impairment. *Lancet* **367**, 1262-1270.