Table 1. Demographics of subjects classified into groups

PiB classification	PiB− (n = 22)	PiB+ (n = 40)			Statistics (*P*-value)
sex (M : F)	8 : 14	19 : 21			0.397
age (y)	72.1 ± 2.9	75.2 ± 4.7			0.006
APOE ε4 (%)	3/22 (13.6)	26/40 (65.0)			<0.001
Clinical classification	HC− (n = 22)	HC+ (n = 11)	MCI (n = 12)	AD (n = 17)	Statistics (*P*-value)
sex (M : F)	8 : 14	7 : 4	8 : 4	4 : 13	0.054
age (y)	72.1 ± 2.9	73.5 ± 4.7	75.7 ± 4.0	76.0 ± 5.0	0.018[a]
education (y)	11.6 ± 2.2	12.0 ± 2.8	12.2 ± 3.2	11.4 ± 2.4	0.935
MMSE	28.5 ± 1.5	28.5 ± 1.3	26.9 ± 1.4	21.6 ± 3.9	<0.001[b]
ADAS-Jcog	6.1 ± 2.4	6.0 ± 2.3	9.8 ± 3.3	15.3 ± 5.8	<0.001[c]
LM2	9.1 ± 4.4	8.7 ± 4.0	2.5 ± 3.6	0.2 ± 0.6	<0.001[d]
GDS	1.7 ± 1.5	1.5 ± 1.3	3.0 ± 1.5	3.7 ± 2.4	0.004[e]
APOE ε4 (%)	3/22 (13.6)	5/11 (45.5)	8/12 (66.7)	13/17 (76.5)	<0.001[f]

For PiB classification, the PiB− group is the same as HC− group, and the PiB+ group includes all of the HC+, MCI and AD individuals. Values are presented as mean ± SD. Statistical analyses were performed using the chi square test (sex, *APOE ε4*), Student *t*-test or one-way ANOVA (age, ADAS), and the Kruskal Wallis test (education, MMSE, LM2, GDS). Post-hoc results were as follows: [a]HC− vs AD ($P < 0.05$); [b]HC−, HC+ and MCI vs AD ($P < 0.001$), HC− vs MCI ($P < 0.05$); [c]HC− and HC+ vs AD ($P < 0.001$), MCI vs AD ($P < 0.01$), and HC− vs MCI ($P < 0.05$); [d]HC− and HC+ vs AD ($P < 0.001$), HC− and HC+ vs MCI ($P < 0.01$); [e]HC− and HC+ vs AD ($P < 0.05$); [f]HC− vs AD ($P < 0.001$) and HC− vs MCI ($P < 0.01$).
MMSE = Mini-Mental State Examination; ADAS-Jcog = Alzheimer's Disease Assessment Scale-Cognitive Component-Japanese version; LM2 = Logical Memory II from the Wechsler Memory Scale–Revised (paragraph A only); GDS = Geriatric Depression Scale; *APOE ε4* = positive for apolipoprotein E *ε4*.

applied for the post-hoc tests. Receiver operating characteristic (ROC) analyses were performed to estimate the capability of the plasma biomarkers to discriminate individuals with cortical Aβ deposition (PiB+) from those without deposition (PiB−). The area under the curve (AUC), sensitivity and specificity were calculated to assess the discriminative capability of a biomarker. The Pearson product-moment correlation coefficient analysis was conducted to evaluate the strength of the link between each biomarker and cortical Aβ deposition assessed using PiB-mcSUVR values. Multiple regression analysis and partial correlation analysis were also performed to assess the influence of possible confounders that may affect the correlation between each biomarker and PiB-mcSUVR. All the tests were two-tailed, and the significance level of difference was set at $P < 0.05$.

Results

Demographics. The demographics and clinical characteristics of the subjects are summarized in Table 1. In the PiB classification based on the visually rated PiB positivity, there were significant differences in age and allele frequency of *APOE ε4*. Therefore, we adjusted for age in the statistical analyses of the biomarkers. In the detailed classification based on the clinical category, age, the scores of MMSE, ADAS-Jcog, LM2 and GDS, and the allele frequency of *APOE ε4* were significantly different among groups. No significant differences were observed in sex ratio and educational attainment among the groups.

Measurement of Aβs and AβAPs. Plasma samples obtained from the participants were spiked with SIL-Aβ1-38 at 10 pM and subsequently subjected to IP-MS as described previously.[17] The representative mass spectra of plasma Aβs and AβAPs from PiB− (HC−) and PiB+ (AD) subjects are shown in Fig. 1. Given that the signal intensity of hydrophobic peptides such as Aβ1-42 decreases in MALDI-TOF MS, the Aβ1-42/Aβ1-40 ratio in IP-MS was smaller than that in previous studies.[13] In addition to Aβ1-40 and Aβ1-42, the peaks of various AβAPs (Aβ1-38, Aβ3-40, Aβ1-39, OxAβ1-40, and APP669-711 indicated in Fig. 2) were generally detected in all subjects. There did not appear to be a distinct difference in the relative signal intensities of most of these peptides. However, note that the ratio of the signal intensity of Aβ1-42 to that of APP669-711 in AD subjects was remarkably different from that in HC- subjects (Fig. 1, arrows).

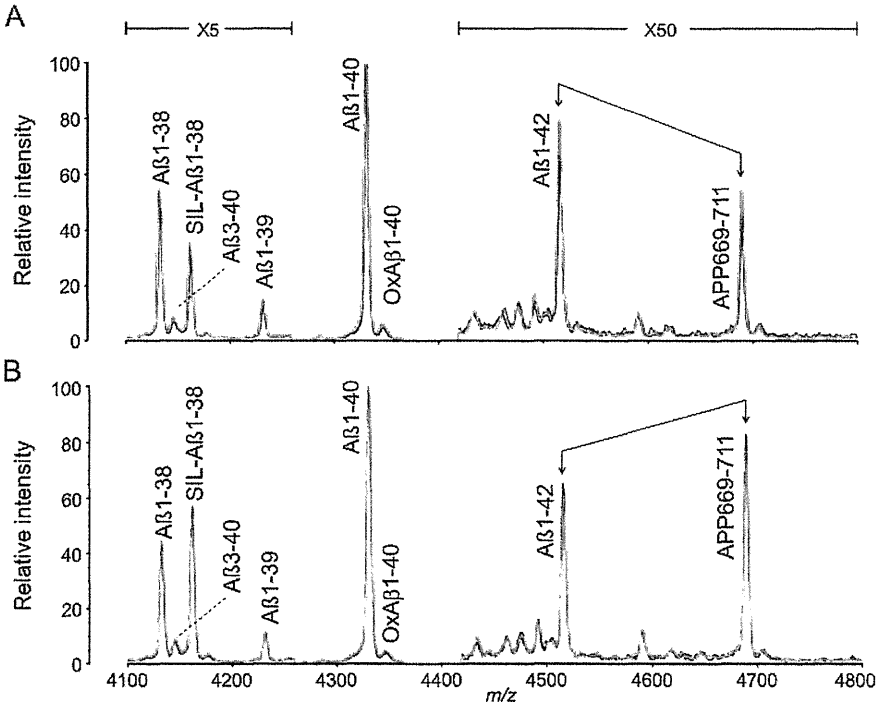

Fig. 1. MALDI-TOF mass spectra of plasma Aβs and AβAPs. Representative mass spectra obtained by IP-MS of plasma samples from the HC− (A) and AD (B) subjects are shown. In addition to Aβ1-40 and Aβ1-42, AβAPs including Aβ1-38, Aβ3-40, Aβ1-39 and APP669-711 were simultaneously measured by MALDI-TOF MS. Four mass spectra (represented in red, blue, green and orange) were obtained from one immunoprecipitation. The levels of Aβs and AβAPs were calculated by averaging the four intensity ratios of Aβs and AβAPs peak to SIL-Aβ1-38 peak. The arrows represent the difference in signal intensity between Aβ1-42 and APP669-711.

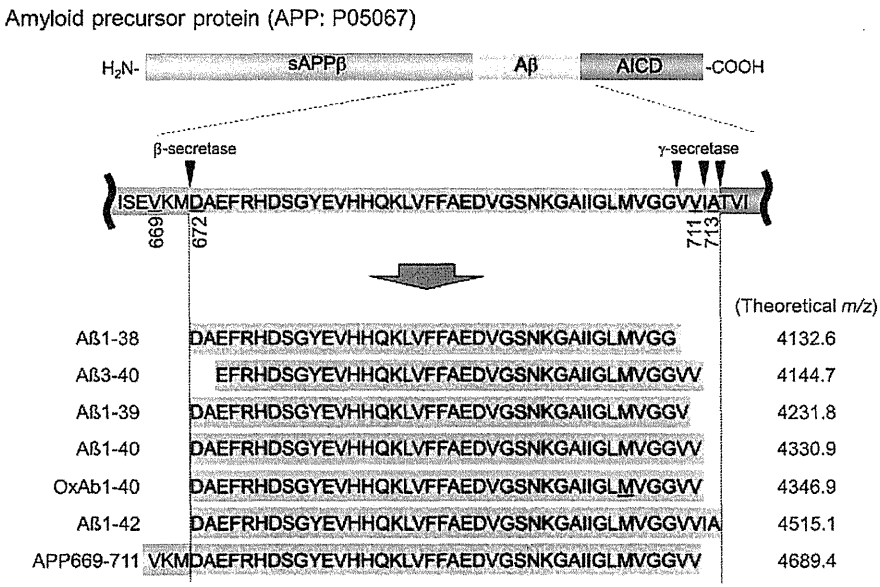

Fig. 2. Overview of Aβs and AβAPs detected by IP-MS. Seven Aβs and AβAPs were detected in plasma samples. The arrows above the sequence indicate the proteolytic processing sites of β- and γ-secretases. OxAβ1-40 represents Aβ1-40 with the oxidized methionine. sAPPβ = soluble APP; AICD = APP intracellular domain.

Table 2. Summary of statistical results for biomarkers

	Aβ1-42[a]	Aβ1-42/Aβ1-40[b]	APP669-711/Aβ1-42	PiB-mcSUVR
ANCOVA group comparisons				
PiB− (n = 22) mean (95% CI)	0.21 (0.18–0.24)	0.011 (0.009–0.012)	0.72 (0.63–0.82)	1.10 (0.98–1.21)
PiB+ (n = 40) mean (95% CI)	0.14 (0.12–0.15)	0.007 (0.006–0.008)	1.13 (1.07–1.19)	1.65 (1.57–1.72)
F-value	8.19	7.89	24.98	31.62
P-value	<0.001	0.001	<0.001	<0.001
coefficient of determination (η^2)	0.298	0.290	0.564	0.621
ROC analysis (PiB− vs PiB+, n = 62)				
area under the curve (AUC)	0.808	0.798	0.969	0.975
sensitivity	0.825	0.750	0.925	0.925
specificity	0.773	0.773	0.955	1.000
Multiple regression analysis (n = 62)				
model *R*	0.424	0.317	0.687	
F-value	6.48	3.30	26.44	
P-value	0.003	0.044	<0.001	
significance (*P*-value), mcSUVR/age	<0.001/0.094	0.016/0.841	<0.001/0.826	
single correlation (*r*), mcSUVR/age	−0.374/−0.060	−0.316/−0.085	0.687/0.217	
partial correlation (*r*), mcSUVR/age	−0.421/0.216	−0.307/0.026	0.668/−0.029	

Upper part: Results of analysis of covariance (ANCOVAs) for comparison between PiB− and PiB+ groups in biomarkers and PiB-mcSUVR. Values in parentheses represent 95% confidence interval (CI). All results are adjusted for age.
Middle part: Results of the receiver operating characteristic (ROC) analysis of biomarkers and PiB-mcSUVR to discriminate visually classified PiB− and PiB+ individuals (Fig. 3). The sensitivity indicates the true positive rate as calculated by (true positive)/((true positive) + (false negative)), and the specificity indicates the true negative rate as calculated by (true negative)/((false positive) + (true negative)). The AUC (the area under the ROC curve) express a kind of overall diagnostic accuracy. The cutoff values for Aβ1-42, Aβ1-42/Aβ1-40, APP669-711/Aβ1-42, and PiB-mcSUVR are 0.183, 0.009, 0.914, and 1.271, respectively.
Lower part: Results of the multiple regression analysis of biomarkers using PiB-mcSUVR and age as predictors. Also, results of the simple analysis of correlation (single correlation) to both PiB-mcSUVR and age, and results of partial correlation analysis adjusted for age or PiB-mcSUVR are shown.
[a]The values represent by the intensity of Aβ1-42 peak relative to that of SIL-Aβ1-38 peak as an internal standard.
[b]Note that the Aβ1-42/Aβ1-40 are markedly different from the reported values in other studies, because of methodological differences (see Results, Measurements of Aβs and AβAPs).

Performances of the plasma biomarkers. Since not Aβ1-42/APP669-711 but APP669-711/Aβ1-42 was normally distributed, the latter values were used for the statistical analyses. Therefore performances of the APP669-711/Aβ1-42 were compared with those of Aβ1-42 level and Aβ1-42/Aβ1-40. PiB-mcSUVR values were also analyzed as the ideal reference. All of the statistical results are summarized in Table 2.

Group comparisons between PiB− and PiB+ individuals. The upper part of Table 2 demonstrates the results of ANCOVA adjusted for age. Using our newly established method, the Aβ1-42 level showed a highly significant difference between the groups. Aβ1-42/Aβ1-40 did not improve the significant level; however, APP669-711/Aβ1-42 markedly enhanced the group-separation capability. The *F*-value and the effect size (η^2) of APP669-711/Aβ1-42 were comparable to those of PiB-mcSUVR.

Capability to discriminate PiB+ individuals from PiB− individuals. To evaluate the capability of APP669-711/Aβ1-42 to discriminate PiB+ individuals from PiB− ones, ROC analysis was performed. The results are shown in the middle part of Table 2 and Fig. 3. APP669-711/Aβ1-42 demonstrated an extremely high AUC (0.969). The sensitivity and specificity for the discriminative capability of APP669-711/Aβ1-42 were 0.925 (3 out of 40 were false negative) and 0.955 (1 out of 22 was false positive), respectively, with a cutoff value of 0.914. The performance indices of APP669-711/Aβ1-42 were also comparable to those of PiB-mcSUVR.

Correlation between our plasma biomarkers and PiB-mcSUVR. To evaluate the strength of the link between our plasma biomarkers and PiB-mcSUVR, we performed correlation and regression analyses. The results are shown in the lower part of Table 2 and Figs. 4A and 4B. All of the plasma biomarkers

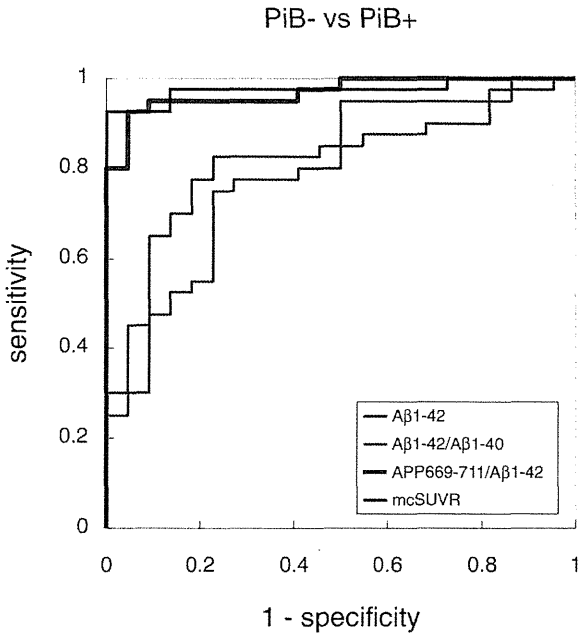

Fig. 3. ROC analysis of the biomarkers as discriminators of PiB+ and PiB− individuals. ROC curves of plasma biomarkers and PiB-mcSUVR are shown in discriminating PiB+ individuals from PiB− individuals. The associated statistical values are displayed in the middle part of Table 2.

showed statistically significant correlation with PiB-mcSUVR. In particular, APP669-711/Aβ1-42 demonstrated high correlation coefficients both in the simple correlation (single $r = 0.687$, $P < 0.001$) and the correlation adjusted for age (partial $r = 0.668$, $P < 0.001$) (Fig. 4A). Multiple regression analysis showed there were no significant ageing effects. Moreover, the regression analysis of PiB-SUVR images using biomarker levels as covariate vectors demonstrated that plasma APP669-711/Aβ1-42 significantly correlated with regional PiB retention (FWE corrected $P < 0.05$, $T = 5.18$, extent threshold $k = 200$ voxels). The visualized areas, robustly involving the frontal, precuneus, and posterior cingulate, and the parietotemporal cortices, appeared to correspond to the typical pattern of Aβ accumulation in AD (Fig. 4B, right).[29),30)] Although no significant clusters were found with the same threshold as the APP669-711/Aβ1-42 threshold, the results with a lowered threshold (uncorrected $P < 0.0001$, $T = 3.97$) suggest that the plasma Aβ1-42 level and Aβ1-42/Aβ1-40 also reflect the cortical Aβ accumulation (Fig. 4B, left and middle).

Performances across clinical categories. Additionally, the performances of the biomarkers were tested across the clinical categories. The results of ANCOVA (adjusted for age) demonstrate that APP669-711/Aβ1-42 showed highly significant group differences with a large effect size (Table 3, upper part), which were almost comparable to those of PiB-mcSUVR. The post-hoc group comparisons demonstrated that APP669-711/Aβ1-42 is sensitive in distinguishing between HC− and any other PiB+ groups (HC+, MCI and AD) (Fig. 5). On the other hand, APP669-711/Aβ1-42 appeared not so sensitive in distinguishing groups classified according to the clinical severity of AD. Within the PiB+ group, only a comparison between HC+ and AD showed a significant difference. We also conducted ROC analysis to evaluate the discriminative capability of the plasma biomarkers across the clinical categories (Table 3, lower part, and Fig. 6). APP669-711/Aβ1-42 showed very high sensitivity and specificity in discriminating HC-individuals from AD and MCI individuals. Importantly, the results demonstrated that APP669-711/Aβ1-42 could identify PiB+ individuals within a cognitively healthy group (HC− vs HC+) with 90.9% sensitivity and 90.9% specificity with a cut off value of 0.863.

Discussion

This study shows that APP669-711/Aβ1-42 in plasma has a big potential as a biomarker precisely surrogating cerebral amyloid deposition. Our biomarker clearly discriminated between PiB− and PiB+ groups with a large effect size ($\eta^2 = 0.56$), and the sensitivity and specificity in discriminating PiB+ individuals from PiB− individuals were very high (0.925 and 0.955, respectively). Furthermore, APP669-711/Aβ1-42 significantly correlated with cortical PiB retention with a high correlation coefficient (age-adjusted partial $r = 0.668$). As a surrogate marker for cerebral amyloid deposition, the performances of APP669-711/Aβ1-42 were far beyond those of reported plasma biomarkers[13),31),32)] and were comparable to those of CSF biomarkers.[27),28),33)] Considering invasiveness and cost, the clinical, as well as social, impact of our novel plasma biomarker would be very significant.

A great deal of effort has been made to determine whether plasma Aβs can be diagnostic and/or predictive biomarkers for AD; however, so far the results were contradictory and, in most studies, there was a broad overlap in the levels of plasma Aβs between controls and patients. This may be due to the difficulties in Aβ measurement in plasma, which are likely caused by various factors,[14)] including low

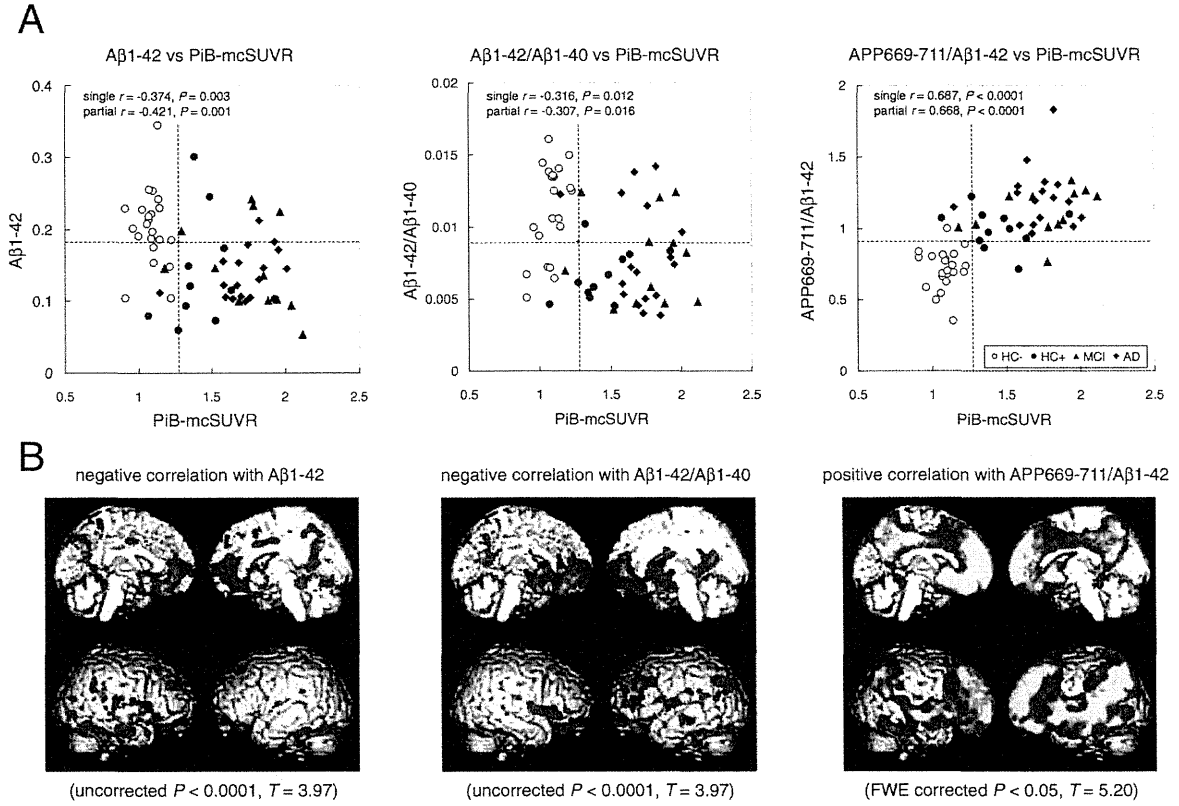

Fig. 4. Correlations between plasma biomarkers and PiB-mcSUVR. A) Scatter plots for biomarkers and PiB-mcSUVR. The open and closed symbols in the scatter plots indicate PiB− and PiB+ groups, respectively. The dashed lines represent cut-off values estimated by the ROC analyses as shown in Fig. 3 and Table 2. B) Regression analysis of PiB-SUVR images for each biomarker adjusted for age. Brain areas that showed statistically significant correlation between regional PiB retention and each biomarker are visualized. Please note that the height threshold of APP669-711/Aβ1-42 is different from the others. The extent thresholds of all are the same ($k = 200$ voxels).

Aβ concentrations and unavoidable Aβ binding to other proteins, which may mask the antibody epitope of Aβ.[34] The utility of the plasma Aβs as AD biomarkers may also be complicated by the fact that plasma Aβs can originate from peripheral organs.[2),35] In addition to these technical and biological aspects of plasma Aβs, the development of biomarkers for AD is generally hampered by a difficulty in correctly recruiting "control subjects" because a large proportion of cognitively normal aged individuals exhibit AD pathologic features, including cerebral amyloid deposition,[19)–21] which has recently been corroborated by amyloid PET studies.[36),37]

To overcome these obstacles, we designed this study as follows. First, to increase detection sensitivity and specificity, we employed our novel IP-MS system.[16] In this system, we used hetero-F(ab′) fragments of two monoclonal antibodies (6E10 and 4G8), which are specific to different Aβ epitopes, that were coupled to PEG on magnetic beads [hetero-F(ab′)-$(PEG)_{24}$ beads],[16] and then, the molecular species of the captured Aβs and AβAPs were precisely and simultaneously determined by MALDI-TOF MS. Compared with the performance of conventional sandwich ELISA measurements, the efficiency and accuracy of Aβ detection in our IP-MS system were markedly high.[17] Indeed, the performances of Aβ1-42 in the present study (Table 2, Figs. 3 and 4) were much higher than those in previous studies.[13),31),32] Second, to decipher the pathological significance of unpredictable changes of Aβ1-42 in plasma, we used APP669-711 as a reference against Aβ1-42. Using the APP669-711 to Aβ1-42 ratio, the performances were extremely high compared with those obtained from Aβ1-42 and Aβ1-40 (Table 2, Figs. 3 and 4). Third, to correctly classify participants in terms of cerebral amyloid deposition, we carried out PiB amyloid imaging by PET of all the subjects. By objective

Table 3. Summary of statistical results for biomarkers for discriminating across clinical categories

	Aβ1-42	Aβ1-42/Aβ1-40	APP669-711/Aβ1-42	PiB-mcSUVR
ANCOVA group comparisons				
HC− (n = 22) mean (95% CI)	0.21 (0.18–0.24)	0.011 (0.009–0.012)	0.72 (0.64–0.81)	1.10 (0.99–1.20)
HC+ (n = 11) mean (95% CI)	0.14 (0.11–0.17)	0.007 (0.005–0.009)	0.99 (0.88–1.09)	1.45 (1.33–1.57)
MCI (n = 12) mean (95% CI)	0.14 (0.10–0.17)	0.008 (0.006–0.010)	1.11 (1.01–1.21)	1.74 (1.61–1.86)
AD (n = 17) mean (95% CI)	0.14 (0.11–0.17)	0.008 (0.006–0.009)	1.24 (1.15–1.33)	1.74 (1.63–1.84)
F-value	5.21	3.52	15.22	19.23
P-value	<0.001	0.003	<0.001	<0.001
coefficient of determination (η^2)	0.403	0.313	0.664	0.714
ROC analysis				
HC− vs HC+ (AUC/sensitivity/specificity)	0.789/0.818/0.818	0.876/0.909/0.773	0.930/0.909/0.909	0.934/0.909/1.000
HC− vs MCI	0.746/0.667/0.909	0.803/0.750/0.773	0.966/0.917/1.000	0.989/0.917/1.000
HC− vs AD	0.864/0.941/0.773	0.743/0.706/0.727	0.997/1.000/0.955	0.992/0.941/1.000

Upper part: Results of the ANCOVA for comparisons among classified groups for biomarkers and PiB-mcSUVR. Values in the parentheses represent 95% confidence interval (CI). All results are adjusted for age. The post-hoc results are displayed in Fig. 5.
Lower part: Results of the receiver operating characteristics (ROC) analyses for each biomarker and PiB-mcSUVR to discriminate across the clinical categories; HC− vs. HC+, MCI, and AD. The cutoff value for each analysis was determined by the nearest point in the curve from the left upper corner. ROC curves are shown in Fig. 6.

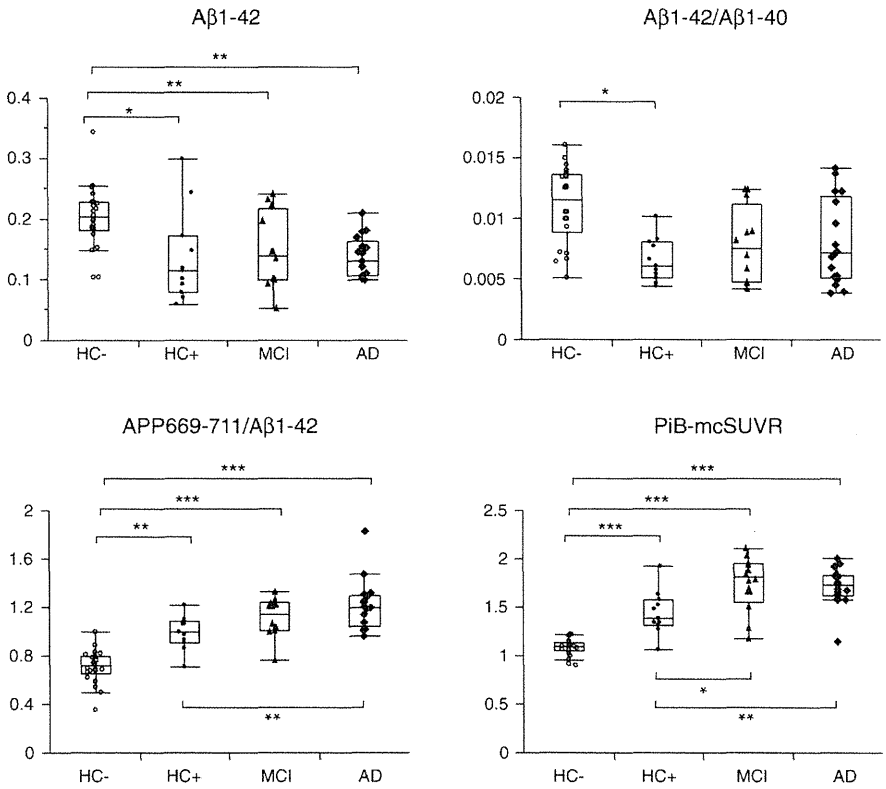

Fig. 5. Group comparisons of the plasma biomarkers and PiB-mcSUVR across clinical categories. Distribution of each value is shown by a box-whisker plot. The boxes represent the 25th, 50th (median) and 75th percentiles of the data. The ends of whiskers represent the lowest (or highest) datum within 1.5-times interquartile range from the 25th (or 75th) percentile. The plotted values were original, but the results of the multiple comparisons were adjusted for age. All p-values were Bonferroni corrected, and the significance levels are represented by the number of asterisks: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

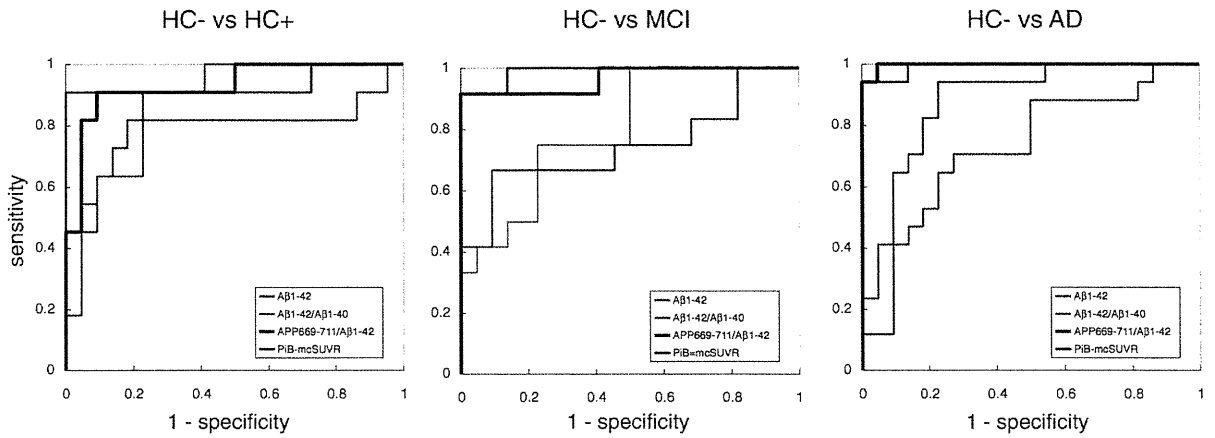

Fig. 6. ROC curves to discriminate across the clinical categories. ROC curves of the plasma biomarkers and PiB-mcSUVR to discriminate between HC− vs. HC+ (left), HC− vs. MCI (middle), and HC− vs. AD (right). The associated statistical values are displayed in the lower part of Table 3.

determination of the state of cerebral amyloid deposition in each participant, we were able to confirm the usefulness of our biomarkers.

It remains to be elucidated why the APP669-711/Aβ1-42 ratio in the plasma showed the highest significance in correlation with the presence of cerebral amyloid deposition. One possible explanation is as follows. APP669-711 has almost the same size and amino acid sequence as Aβ1-42; thus, these two peptides may show the same metabolism rate, binding tendency to other molecules in the brain and plasma, and also penetration capability through the blood-brain barrier. In contrast, the aggregation tendency of APP669-711 may be extremely lower than that of Aβ1-42 because of the difference in both amino- and carboxyl-termini. Collectively, the coexistence of similar and dissimilar characteristics of Aβ1-42 likely allows APP699-711 in plasma to function as a good reference against Aβ1-42.

In conclusion, we found that APP669-711/Aβ1-42 is a highly sensitive plasma biomarker that precisely surrogates cerebral amyloid deposition. This simple and minimally invasive biomarker should be beneficial in the clinical diagnoses of AD, possibly substituting the CSF examination. In addition, our biomarker can be a very powerful screening tool to identify people at risk of AD development from a community, and thereby likely facilitates development of disease-modifying clinical trials for AD.

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LOWER VITAMIN D IS ASSOCIATED WITH WHITE MATTER HYPERINTENSITY IN ELDERLY WOMEN WITH ALZHEIMER'S DISEASE AND AMNESTIC MILD COGNITIVE IMPAIRMENT

To the Editor: White matter hyperintensity (WMH) on brain magnetic resonance imaging (MRI) is prevalent in the aging brain and is associated with cognitive decline and mobility impairment.[1,2] It is a clinical challenge to establish a prevention strategy for WMH. Although believed to be of vascular origin, the exact etiology of WMH remains unclear. Age and hypertension are consistent risk factors for WMH. High homocysteine levels, diabetes mellitus, hyperlipidemia, smoking, obesity, low vitamin B12 levels, and alcohol consumption are likely to increase WMH.[3]

There is growing evidence of potential roles of vitamin D in sustaining healthy brain function.[4] Low serum vitamin D has been reported in Alzheimer's disease (AD), which is often associated with WMH,[5] but little is known about the link between vitamin D and WMH in elderly adults with AD. This study aimed to clarify the interaction between vitamin D, WMH, brain atrophy in elderly adults with AD and amnestic mild cognitive impairment (aMCI).

Two hundred fifty-three women aged 65 and older diagnosed with aMCI (n = 39) or AD (n = 214) were recruited. AD was diagnosed as possible or probable AD according the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association, and aMCI was diagnosed based on previously defined criteria.[2,6] Individuals with severe cardiac failure, renal disorder, liver dysfunction, or musculoskeletal disease or with cortical lesions on MRI were excluded.

Cognitive function was evaluated using the Mini-Mental State Examination (MMSE). Hypertension, diabetes mellitus, lipid abnormalities, and chronic kidney disease were defined as having a history of these diseases or use of medication to treat them. Information on current smoking and drinking habits was obtained from clinical charts. Vitamin D insufficiency was assessed according to serum concentration of 25-hydroxyvitamin D (25(OH)D).

A standard series of axial T2-weighted (repetition time (TR), 3,800 ms; echo time (TE), 93 ms) and fluid-attenuated inversion recovery (TR, 8,000 ms; TE, 101 ms; inversion time, 2,500 ms; a 256 × 256 matrix) MR sequences were obtained using a 1.5-T MR scanner (Siemens Avanto, Munich, Germany). Scans in parallel with the anterior commissure–posterior commissure line were performed with 6-mm-thick slices and an interslice gap of 1.2 mm.[2] MRI data were processed to measure the total volume of the intracranial space (IC), parenchyma, ventricles, and WMH using a fully automatic segmentation program (Software for Neuro-Image Processing in Experimental Research).[7]

Statistical analysis was performed using SPSS 19.0 for Windows (SPSS, Inc., Chicago, IL). Because WMH were not normally distributed, their values were converted to rank variables. The association between WMH and clinical variables was analyzed using partial Spearman rank order correlation analysis and multivariate regression (stepwise). Differences were considered significant at $P < .05$.

Table 1. Clinical Profile and Magnetic Resonance Imaging Results of Study Participants (N = 253)

Characteristic	Value
Demographic characteristics and biochemical measurements	
Age, mean ± SD	77.5 ± 5.0
Education, years, mean ± SD	10.0 ± 2.1
Mini-Mental State Examination score, mean ± SD	19.9 ± 5.0
Hypertension, %	56.9
Diabetes mellitus, %	21.6
Lipid abnormality, %	41.7
Chronic kidney disease, %	32.3
Current smoking, %	2.0
Drinking habit, %	7.7
Systolic blood pressure, mmHg, mean ± SD	150.1 ± 21.2
Diastolic blood pressure, mmHg, mean ± SD	80.9 ± 11.9
Body mass index, kg/m^2, mean ± SD	21.8 ± 3.6
Glycosylated hemoglobin, %, mean ± SD	6.1 ± 0.8
Total cholesterol, mg/dL, mean ± SD	221.5 ± 37.5
High-density lipoprotein cholesterol, mg/dL, mean ± SD	63.5 ± 15.7
Triglyceride, mg/dL, mean ± SD	126.5 ± 71.9
Estimated glomerular filtration rate, mL/min per 1.73 m^2, mean ± SD	68.5 ± 18.4
Cystatin C, mg/L, mean ± SD	1.0 ± 0.4
25-hydroxyvitamin D, ng/mL, mean ± SD	23.6 ± 7.2
Homocysteine, nmol/mL, mean ± SD	11.1 ± 5.6
Vitamin B12, pg/mL, mean ± SD	674.2 ± 476.3
Cranial magnetic resonance imaging, mean ± SD	
IC, mL	1,324.2 ± 96.4
WMH, mL	17.2 ± 18.1
WMH/IC, %	1.3 ± 1.4
Parenchyma, mL	991.1 ± 78.3
Parenchyma/IC, %	74.9 ± 3.0

SD = standard deviation; IC = intracranial space; WMH = white matter hyperintensity.

Table 1 shows the demographic characteristics, biochemical measurements, and results of MRI analyses of the participants. Serum 25(OH)D levels in individuals receiving vitamin D supplementation (n = 20) were significantly lower than in those who were not ($P = .03$).

Partial Spearman rank order correlation revealed a possible correlation between WMH/IC and age ($r_s = 0.252$, $P < .001$), hypertension ($r_s = 0.179$, $P = .001$), MMSE score ($r_s = -0.157$, $P < .001$), cystatin-C ($r_s = 0.166$, $P = .004$), 25(OH)D ($r_s = -0.171$, $P = .006$), and homocysteine ($r_s = 0.146$, $P = .01$), whereas the other clinical indices were not correlated (data not shown). Multivariate regression analysis using these possible factors, together with education, as variables revealed that older age ($\beta = 0.208$, $P = .001$), hypertension ($\beta = 0.162$, $P = .009$), and lower 25(OH)D ($\beta = -0.163$, $P = .008$) were independently associated with WMH/IC. In contrast, 25(OH)D was not related to parenchyma/IC as an index of global brain atrophy (data not shown).

The present study clearly demonstrates that 25(OH)D is negatively correlated with WMH volume in elderly women with aMCI or AD, even after adjusting for classic risk factors for WMH, whereas 25(OH)D was not

associated with brain atrophy. A previous study suggested that individuals with 25(OH)D deficiency had greater WMH volume.[8] A correlation between vitamin D and WMH in multiple sclerosis has been demonstrated.[9] Another study recently reported an association between WMH and low 25(OH)D in independent outpatients.[10] The current study indicates that hypovitaminosis D is a predictor of WMH in individuals with AD or aMCI.

WMH has been found to have clinical relevance in cognitive decline and several geriatric syndrome conditions,[1,2] and vitamin D plays central roles in muscle weakness and physical frailty in elderly individuals. Taken together, the results of the current study suggest that vitamin D controls two pathways toward cognitive decline and frailty by means of white matter burden and sarcopenia in elderly adults with dementia. Prospective studies are needed to clarify the effects of vitamin D supplementation in prevention of WMH in individuals with AD.

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IMPROVING CARE TRANSITIONS IN INDIVIDUALS FREQUENTLY ADMITTED TO THE HOSPITAL

To the Editor: Individuals who are frequently admitted to the hospital have high levels of multimorbidity, polypharmacy, and functional impairment,[1] and the sudden reduction in intensity of care in the posthospital period may contribute to high rates of hospital readmission.[2–5] A multidisciplinary follow-up clinic for frequently admitted patients was piloted to address demonstrated gaps in transitional care.[6] This report describes the patient perspective of care in this model.

METHODS

Participants were recruited from general medical wards of a metropolitan hospital in Brisbane, Australia, from June to December 2012. Consecutive inpatients aged 60 and older with at least one unplanned hospitalization in the previous 6 months were identified through the admissions database. Individuals were ineligible if they lived outside Brisbane, were discharged to residential care, were receiving palliative care, could not consent, or were receiving care from the heart failure service. Eligible individuals were invited to participate if their treating team agreed. The study received ethical approval.

Participants were scheduled for clinic review within 2 weeks of discharge. The first visit included comprehensive assessment of social and medical services, symptoms, care goals, activities of daily living, malnutrition, and depression by a registered nurse. A clinical pharmacist undertook review of medication use after discharge, current medication understanding, and adherence. The physician then reviewed medical conditions and treatments and recommended a treatment plan, including referrals as needed. A physiotherapist or exercise physiologist assessed each participant and prescribed an individualized exercise program in a supervised weekly group exercise class or at home. A comprehensive summary with recommendations was sent to the general practitioner within 5 days, and at least two further reviews were scheduled over 12 weeks to assess progress and adjust management, with telephone support as required.

Participant perceptions were measured using the 15-item Care Transitions Measure (CTM-15), a reliable measure of quality of transitional care.[7,8] The CTM-15 was administered at the initial visit (reflecting hospital discharge) and at 12 weeks (reflecting clinic experience). Items were scored from 1 (strongly disagree) to 4 (strongly agree), and the CTM-15 score was calculated by averaging and linear transformation as originally described.[8] Baseline and 12-week CTM-15 scores were compared using paired *t*-tests; mean nontransformed scores for each item were compared using paired *t*-tests.

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ORIGINAL ARTICLE

Left ventricular diastolic dysfunction is associated with cerebral white matter lesions (leukoaraiosis) in elderly patients without ischemic heart disease and stroke

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Aim: Cerebral white matter lesions (WML) are known to increase with age, as is left ventricular (LV) diastolic dysfunction with normal contraction. Although aging is a common risk factor, the link between these diseases is not fully understood. The aim was to clarify this relationship, using the ratio between early diastolic mitral inflow and early diastolic mitral annular tissue velocity (E/E'). E/E' measured by tissue Doppler echocardiography offers an indicator of the severity of LV diastolic dysfunction, reflecting both diastolic LV stiffness and diastolic LV filling pressure.

Methods: Participants comprised 75 patients aged between 65 and 75 years with normal LV contraction and no signs or history of symptomatic heart failure, ischemic heart diseases, atrial fibrillation, stroke, or cognitive dysfunction. The volume of WML was quantified on brain magnetic resonance imaging.

Results: The participants were classified into three groups: Low E/E', E/E' ≤ 8; Middle E/E', 8 < E/E' < 15; and High E/E', E/E' ≥ 15. WML volume was 3.6 ± 3.0 mL in Low E/E', 5.4 ± 6.5 mL in Middle E/E' and 12.0 ± 11.0 mL in High E/E', increasing significantly with increased diastolic LV stiffness (Low *vs* High, $P = 0.034$; Middle *vs* High, $P = 0.016$). Linear regression analysis showed the positive association between the volume of WML and E/E' ratio ($r = 0.377$, $P = 0.0009$).

Conclusions: This investigation identified an association between LV diastolic dysfunction and WML. Further investigations are required to clarify whether there is a direct association between the two diseases. **Geriatr Gerontol Int 2014; 14 (Suppl. 2): 71–76.**

Keywords: cerebral white matter lesions, left ventricular diastolic dysfunction, ratio of early diastolic mitral inflow to early diastolic mitral annular tissue velocity.

Introduction

Cerebral white matter lesions (WML) on magnetic resonance imaging (MRI) consisting of nerve axons or glia have been shown to exist in older adults. Cerebral WML have been established to increase with age, and are associated with heightened risks of stroke,[1,2] cognitive decline[3,4] and depressive disorder.[5]

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Left ventricular (LV) diastolic dysfunction with normal contraction also increases with age.[6,7] This dysfunction is characterized by increased stiffness of the left ventricle caused by proliferation of the extracellular matrix and progression of myocardial fibrosis, and by decreased elasticity of cardiomyocytes as a result of abnormalities in calcium dynamics, energy metabolism or the cytoskeleton of cardiomyocytes.[8]

Although studies have not reached consistent conclusions, some studies have suggested an association between LV systolic dysfunction and brain abnormalities, such as WML[9] and cognitive decline.[10] At present, cerebral hypoperfusion resulting from LV systolic dysfunction is speculated to contribute to the brain abnormalities.[11] In contrast, no reports have described the relationship between LV diastolic dysfunction with

normal contraction and brain abnormalities. The purpose of the present study was thus to clarify this relationship by focusing on WML and brain function.

We applied the ratio between early diastolic mitral inflow and early diastolic mitral annular tissue velocity (E/E') as an indicator of the severity of LV diastolic dysfunction, and evaluated the relationship between E/E' ratio and cerebral WML volume among elderly patients with normal LV contraction and without symptomatic heart failure.

Methods

Participants

Outpatients aged 65–75 years (mean age 69.3 ± 3.4 years) treated by the Department of Cardiology at the National Center for Geriatrics and Gerontology were enrolled. Among these, patients with symptomatic heart failure, ischemic heart disease, valvular heart disease, atrial fibrillation, stroke, neurodegenerative disorder or clinically diagnosed dementia were excluded. To exclude patients with prior myocardial infarction, angina or stroke, prespecified criteria were used to define those diseases using a combination of self-report of a doctor diagnosis, World Health Organization chest pain questionnaire and 12-lead exercise electrocardiography.[12] We also excluded those patients with ejection fraction (EF) < 50% or LV end-diastolic volume index (LVEDVI) ≥ 97 mL/m^2 on echocardiography, major brain infarction resulting from major cerebral artery lesions detected on brain MRI, ≥50% stenosis in the carotid arteries on ultrasonography with 2-D and Doppler analysis, or cognitive dysfunction (Mini-Mental State Examination score [MMSE] <24). The study protocol was approved by the ethics/conflict of interest committee at the National Center for Geriatrics and Gerontology. Written informed consent was obtained from all participants before participation.

Study design

The registration period was from April 2010 to August 2012. In the present study, WML volume, E/E', left ventricular EF (LVEF) and cognitive functions (MMSE, Logical Memory 1 and 2 of the revised Wechsler Memory Scale test, Trail-Making Test [TMT] [A] and [B], Raven's Colored Progressive Matrices [RCPM], and Geriatric Depression Scale [GDS]) were evaluated within 1 month of enrolment. In addition, blood pressure, body mass index (BMI), pulse wave velocity (PWV), carotid intimal media thickness (IMT), and levels of plasma B-type natriuretic peptide (BNP) and hemoglobin (Hb) A1c were also determined. Tests of cognitive functions were carried out by two clinical psychologists. A total of 82 patients were initially registered, but seven patients were excluded because of atrial fibrillation in three patients, cerebral infarction resulting from a major cerebral artery lesion in one patient, valvular disease (mitral valve stenosis) in one patient and systolic impairment shown by EF < 50% on echocardiography in two patients. The number of participants in the final analysis was therefore 75.

Neuroimaging studies

Brain MRI was used for the quantification of WML volume. A standard series of axial T1-weighted (repetition time [TR], 485 ms; echo time [TE], 11 ms), T2-weighted (TR, 3800 ms; TE, 93 ms) and fluid-attenuated inversion recovery (TR, 8000 ms; TE, 101 ms; inversion time, 2500 ms; matrix, 256 × 256) MRI sequences were carried out using a 1.5-T MR system (Siemens Avanto, Muenchen, Germany). Scans were carried out parallel with the anterior commissure-posterior commissure line, with 6-mm thick slices and an interslice gap of 1.2 mm. MRI data were processed to measure total volumes of the intracranial space, parenchyma, ventricles and WML using a fully automatic segmentation program (Software for Neuro-Image Processing in Experimental Research: SNIPER) developed in the Department of Radiology at Leiden University Medical Center (Leiden, the Netherlands). Detailed procedures for MRI post-processing using SNIPER have been described elsewhere.[13]

Echocardiographic examination

Echocardiography was carried out using an ACUSON SC2000 volume imaging ultrasound system (Siemens Medical Solutions, Tokyo, Japan). LVEF was estimated using Teichholz's method. To determine E/E', pulse wave tissue Doppler echocardiography was applied to the apical four-chamber view at both septal and lateral mitral annuli by two experienced cardiosonographers.[14]

Criteria for analysis

Hypertensive patients were defined as patients already undergoing regular antihypertensive treatment, patients with blood pressure in the examination room exceeding 140/90 mmHg on two separate occasions during outpatient visits or patients with mean blood pressure exceeding 135/85 mmHg on 24-h ambulatory blood pressure monitoring. Diabetic patients were defined as patients who were already undergoing regular treatment for diabetes or with HbA1c ≥ 6.5%.

Statistical analysis

Values are shown as mean ± standard deviation unless otherwise stated. When patients were stratified

according to E/E', differences among the three groups were analyzed using analysis of variance, followed by the Tukey–Kramer multiple comparison test or Kruskal–Wallis multiple comparison test, and differences among the two groups were analyzed using χ^2-square test or Fisher's exact test, Student's *t*-test and Welch's test. Values of $P < 0.05$ were considered significant. Data were analyzed using SPSS version 17.0 software (SPSS, Chicago, IL, USA).

Results

Three-group comparison of age, sex, hypertension or diabetes based on E/E' ratio

The ratio between early diastolic mitral inflow (E) and early diastolic mitral annular tissue velocity (E') as estimated by echocardiography has recently been identified as a useful indicator of the severity of LV diastolic dysfunction. E/E' has been shown to reflect both diastolic LV stiffness and diastolic LV filling pressure. More precisely, E/E' > 8 has been identified as a marker of elevated diastolic LV stiffness,[15] whereas E/E' ≥ 15 also shows a diastolic LV filling pressure is definitely elevated.[16] For classification based on the severity of LV diastolic dysfunction, we used E/E' from echocardiography and classified participants into three groups: E/E' ≤ 8 (Low E/E'); 8 < E/E' < 15 (Middle E/E'); and E/E' ≥ 15 (High E/E'). This classification was established based on the European Heart Journal Guideline 2007.[17] Differences in background factors between the three groups are presented in Table 1. Importantly, no significant differences were detected between groups in terms of age, sex, hypertension or diabetes.

Cerebral WML volume increases with severity of LV diastolic dysfunction

In the present study, WML were classified as periventricular WML (PVL) and deep WML (DWML). Table 2 summarizes cerebral WML volume, PVL volume and DWML volume. All cerebral WML were found to increase in volume as E/E' increased. Although no significant differences were seen between Low E/E' and Middle E/E', significant differences were found in comparisons between each of these groups and High E/E'. Then, we carried out linear regression analysis to clarify the relationship between WML volume and E/E' ratio. As a result, we confirmed a positive association between WML volume and E/E' ratio ($r = 0.377$, $P = 0.0009$) (Fig. 1).

Table 1 Patients' characteristics

	Total	E/E' ≤ 8	8 < E/E' < 15	15 ≤ E/E'
n	75	10	51	14
Males	36	8	22	6
Age (years)	69.3 ± 3.4	69.1 ± 2.1	69.2 ± 3.6	69.6 ± 3.3
Hypertension (*n*)	55	6	37	12
Diabetes mellitus (*n*)	10	0	6	4
IMT (mm)	0.67 ± 0.11	0.65 ± 0.11	0.66 ± 0.10	0.73 ± 0.15
PWV (cm/s)	1816 ± 307	1665 ± 267	1835 ± 308	1853 ± 319
BMI (kg/m^2)	23.4 ± 3.9	22.3 ± 2.3	23.2 ± 3.4	24.6 ± 3.9
Echocardiographic data				
EF (%)	65.7 ± 4.3	64.2 ± 1.6	65.6 ± 4.8	67.1 ± 3.4
E/E'	11.8 ± 3.5	7.1 ± 0.4*	11.1 ± 1.9*	17.4 ± 2.2*
BNP (pg/mL)	29.9 ± 36.0	23.1 ± 20.2	26.2 ± 29.7	47.7 ± 57.1

*$P < 0.01$. BMI, body mass index; BNP, plasma B-type natriuretic peptide; E/E', ratio of early diastolic mitral inflow (E) to early diastolic mitral annular tissue velocity (E'); EF, ejection fraction; IMT, carotid intimal media thickness; PWV, pulse wave velocity.

Table 2 The relationship between LV diastolic dysfunction and lesion volume

	Total	E/E' ≤ 8	8 < E/E' < 15	15 ≤ E/E'
WML (mL)	6.4 ± 7.6	3.6 ± 3.0*	5.4 ± 6.5*	12.1 ± 11.0*
PVL (mL)	5.6 ± 6.8	3.4 ± 2.7*	4.8 ± 5.8*	10.2 ± 10.1*
DWML (mL)	0.8 ± 1.4	0.2 ± 0.3*	0.6 ± 1.0*	1.9 ± 2.2*

*$P < 0.05$. DWML, deep subcortical white matter lesions; PVL, periventricular white matter lesions; WML, white matter lesions.

Relationship between LV diastolic dysfunction and brain function

As shown in Table 3, although performance of brain functional tests worsened with the severity of LV diastolic dysfunction, no significant differences were seen among the three groups except for GDS. We then re-divided the studied population into two groups based on the presence of LV diastolic dysfunction (E/E' ≤ 8 and 8 < E/E') and re-analyzed. As results, significant differences were identified between the severity of LV diastolic dysfunction and MMSE (29.0 ± 0.9, 28.2 ± 2.0, respectively; $P = 0.040$) or GDS (1.2 ± 0.9, 3.6 ± 2.6, respectively; $P < 0.001$), and associated tendencies were also found between severity of LV diastolic dysfunction and TMT (B) data (96.5 ± 27.8, 119.6 ± 48.9, respectively; $P = 0.151$), TMT (B–A) data (51.9 ± 26.7, 71.6 ± 41.5, respectively; $P = 0.151$) and RCPM (29.7 ± 2.4, 28.0 ± 4.6, respectively; $P = 0.091$). No significant differences were detected between the two groups in terms of age, sex, hypertension or diabetes. In addition, we carried out regression analysis between WML volume and scores from brain function tests, but no significant relationships were observed (data not shown).

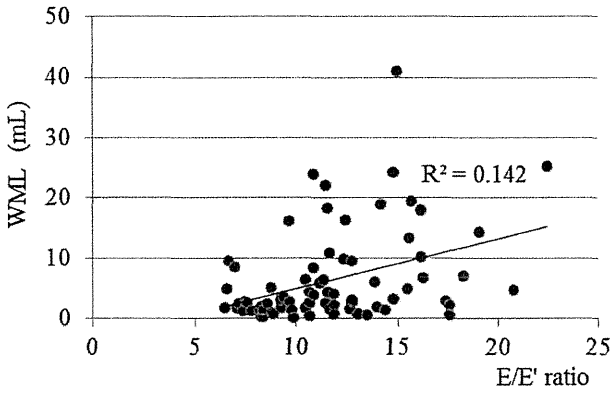

Figure 1 Linear regression analysis of cerebral white matter lesions (WML volume and the ratio between early diastolic mitral inflow and early diastolic mitral annular tissue velocity (E/E'). A positive correlation is apparent between WML volume and E/E' ratio ($r = 0.377$, $P = 0.0009$).

Discussion

The present study investigated the relationship between severity of LV diastolic dysfunction and the volume of WML in elderly patients, showing a clear association between the two. Importantly, the present results were obtained from analyses of elderly patients with neither ischemic heart disease nor symptomatic stroke. In addition, the present results were obtained from analyses of groups showing no significant differences statistically in age, hypertension or diabetes mellitus, all of which represent common risk factors for the development and progression of both WML[18] and LV diastolic dysfunction. Considering this background, there might be a mechanism that directly connects WML and LV diastolic dysfunction. Further prospective investigations are required to clarify it.

Theoretically, LV diastolic dysfunction causes systemic hypoperfusion as a result of the reduction of LV stroke volume, which is caused by decreased LV end-diastolic volume and decreased LV inflow from the left atrium as a result of the increased LV stiffness. LV diastolic dysfunction with normal contraction thus also causes systemic hypoperfusion the same as LV systolic dysfunction. Considering the common effect to systemic perfusion of LV systolic and diastolic dysfunction, the relationships between brain abnormalities and LV systolic or diastolic dysfunction might be therefore expected to be the same.

The relationship between LV systolic dysfunction and brain abnormalities has been discussed, but remains unclear. The present findings suggest that the severity of LV diastolic dysfunction is associated with WML development and progression. Such results are consistent with previous studies that investigated

Table 3 The relationship between LV diastolic dysfunction and brain function

	Total	E/E' ≤ 8	8 < E/E' < 15	15 ≤ E/E'
MMSE	28.3 ± 1.9	29.0 ± 0.9	28.2 ± 1.8	27.9 ± 2.7
Logical memory (1)	21.4 ± 7.0	22.0 ± 5.2	21.8 ± 6.8	19.0 ± 8.6
Logical memory (2)	20.0 ± 7.6	20.8 ± 6.5	20.6 ± 7.4	17.1 ± 9.1
RCPM	28.3 ± 4.4	29.7 ± 2.4	28.4 ± 4.5	26.5 ± 4.7
TMT-A (s)	47.4 ± 14.4	44.6 ± 10.8	47.1 ± 14.8	51.3 ± 15.7
TMT-B (s)	116.3 ± 47.0	96.5 ± 27.8	117.7 ± 48.9	126.6 ± 50.6
TMT(B-A)(s)	69.0 ± 40.2	51.9 ± 26.7	70.6 ± 42.4	75.3 ± 39.0
GDS	3.2 ± 2.6	1.2 ± 0.9*	3.4 ± 2.5*	4.2 ± 3.0*

*$P < 0.01$. GDS, Geriatric Depression Scale; Logical Memory, logical memory in the revised Wechsler Memory Scale test; MMSE, Mini-Mental State Examination; RCPM, Raven's Colored Progressive Matrices; TMT, Trail-Making Test.

associations between LV systolic dysfunction and WML volume,[9,19] but are inconsistent with the others.[20,21] One of the reasons why the results of previous studies have been controversial could be the study populations investigated. Participants in previous studies have comprised patients with LV systolic dysfunction, and, as a result, a large number of ischemic heart disease patients were included as study participants. Needless to say, ischemic heart disease is known to be associated with a high risk of cerebrovascular complications. Therefore, these patients might have impacted the results of previous studies and prevented consistent results. The present study excluded patients with ischemic heart disease from participating in the study as much as possible, which might have facilitated the identification of a clear association between LV diastolic dysfunction and WML.

The most important, but difficult question is how LV diastolic dysfunction is associated with WML development and progression. We cannot answer this question based only on the results of the present cross-sectional study. However, considering that LV diastolic dysfunction itself reduces systemic perfusion in the same manner as LV systolic dysfunction, systemic hypoperfusion as a result of the progression of LV diastolic dysfunction might impair the system for the autoregulation of cerebral blood flow, disrupt cerebral perfusion, and cause WML development and progression, as previously speculated.[22] Further studies are required to clarify these issues.

It has been postulated that WML are associated with cognitive dysfunction and depressive mood.[23,24] In the present study, we could not show a significant association between severity of LV diastolic dysfunction and cognitive decline in three-group comparisons, whereas a clear association was shown for GDS. When we carried out two-group comparison analysis between $E/E' \leq 8$ and $8 < E/E'$ to test the association of LV diastolic capacity with cognition, a significant difference in global brain function (MMSE) was identified.

In this connection, most previous studies that detected clear associations between WML and cognitive decline comprised a study population of at least 100 participants, including patients with cognitive decline.[25,26] The present sample was thus thought to be relatively small to detect the association between LV diastolic function and cognitive function, even if an association was actually present. Regarding the effect of WML on physical functions or cognition, a "threshold effect" has been suggested.[27–29] Recent studies show that regional distribution of WML, rather than total volume of WML, seems more important to manifest functional implication of WML.[24,30] In addition, WML are known to be one of the risk factors for cognitive impairment, but other factors, such as years of education, medication and smoking status, are also known to affect cognitive function. Other factors might to thus obscure the association between the two diseases in the present study.

We therefore believe that the present results do not preclude an association between the diseases, but rather fail to confirm one. Further detailed and prospective analysis is required to settle this matter.

Previous studies have already shown that increases in WML volume are associated with the development of dementia and stroke, and with increased mortality. At present, no effective treatments for WML have been established. Suppression of the development and progression of WML is therefore crucial. From this perspective, the finding that the progress of LV diastolic dysfunction is associated with the development and progression of WML is important. Further investigations are required to clarify whether there is a direct association between LV diastolic dysfunction and WML.

The number of participants in the present study was small, at just 75. Therefore, even though an association between WML volume and severity of LV diastolic dysfunction was able to be confirmed, we could not identify an association between cognitive function and LV diastolic function or WML volume. In addition, because the present study represented a cross-sectional analysis, the mechanism by which WML develop in patients with LV diastolic dysfunction could not be clarified. Further investigation with a larger number of patients is therefore warranted.

The present results suggest the possibility that LV diastolic dysfunction is associated with the development and progression of WML. Future studies will need to investigate in a greater number of patients whether LV diastolic dysfunction directly associates with the progression of WML.

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Disclosure statement

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

Differential subtypes of diabetic older adults diagnosed with Alzheimer's disease

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Aim: The clinical management of diabetic elderly patients with Alzheimer's disease (AD) is hindered by several difficulties. The present study aimed to clarify the clinical characteristics and pathophysiological properties of AD in diabetic older adults.

Methods: A total of 91 patients with type 2 diabetes mellitus and 161 non-diabetic individuals who were diagnosed with AD were recruited. Diabetic patients were classified into two groups with glycated hemoglobin (HbA1c) <7.0% or ≥7.0%. The demographics, cognition, daily-life function, metabolic changes, treatment, and behavioral and psychological symptoms of dementia (BPSD), as well as brain pathophysiology, were compared among the three groups.

Results: Patients with higher HbA1c had increased diabetic vascular complications and impaired activities of daily living with decreased levels of serum high-molecular-weight adiponectin and 25-hydroxyvitamin D. Although cognitive status was similar among the three groups, BPSD, including apathy, overeating and excessive daytime sleeping appeared to be increased in the patients with HbA1c ≥7.0%. The frequency of apolipoprotein E4 carriers and of posterior cerebral hypoperfusion (AD-pattern) on single-photon emission computed tomography in poorly controlled diabetic subjects was similar to that in non-diabetic AD patients, whereas diabetic patients with HbA1c <7.0% included fewer apolipoprotein E4 carriers and fewer patients with an AD pattern on single-photon emission computed tomography.

Conclusion: Subtypes of older diabetic patients with AD were identified based on clinical features and brain pathophysiology. Physical and psychological complications of dementia are prevalent in patients with higher HbA1c. It seems likely that difficulties in the management of diabetes with AD are due not only to non-adherence to diabetes treatment, but also several symptoms and pathophysiological characteristics of dementia. **Geriatr Gerontol Int 2014; 14 (Suppl. 2): 62–70.**

Keywords: Alzheimer's disease, behavioral and psychological symptoms of dementia, diabetes, glycemic control, pathophysiology.

Introduction

Diabetes increases the risk of dementia, including Alzheimer's disease (AD) and vascular dementia.[1] Once an older diabetic patient begins to experience cognitive decline, treatment of diabetes becomes difficult despite intensive care and education. Physical exercise and dietary changes are feasible treatment options, but adherence to diabetic medicine is usually impaired, even in the early course of AD. When serious hyperglycemia continues, the more powerful antidiabetic medicines are prescribed, which in turn increases the risk of hypoglycemia. Hyperglycemia and hypoglycemia, as well as acute fluctuation of glucose, further worsen cognitive impairment.[2–4] Behavioral and psychological symptoms of dementia (BPSD) also cause difficulties in the management of diabetes. To overcome these problems, a coordinated treatment plan that addresses both the AD and diabetes is required.

There is currently no consensus as to the pathophysiology of AD in diabetes; studies have alternatively reported that AD-associated pathology is increased,

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unchanged or even decreased in diabetic older adults.[5–8] Other studies have found that cerebral vascular disease (CVD) is more likely to be involved in diabetes.[9,10] Metabolic factors of diabetes might have a profound impact on the clinical course of AD, resulting in a variety of clinical pictures.[11–14] Because of the complex nature of AD, the true reasons for the difficulties in managing diabetic elderly patients with AD have remained unclear.

The purpose of the present study was to clarify the clinical characteristics of diabetic older adults with AD from the standpoint of demographics, cognition, activities of daily living (ADL), complications of dementia, metabolic changes, treatment and pathophysiology of the brain. We hypothesized that clinical symptoms related to AD would depend largely on glycemic control and brain pathophysiology. We therefore compared these variables among three patient groups: diabetic patients with AD and good glucose control, diabetic patients with AD and poor glucose control, and non-diabetic patients with AD. The present study was designed to identify subtypes of dementia with differential clinical properties and pathophysiology in diabetic patients with AD.

Methods

Participants

The study protocol was approved by the institutional review board of the National Center for Geriatrics and Gerontology (NCGG), Japan. Candidate patients and their caregivers submitted informed consent before participation in the study.

A total of 252 elderly patients (age 65–85 years) who had been diagnosed with AD and treated in the NCGG were enrolled consecutively. The total Barthel Index score for each of the 252 patients was 80 or over.[15] Patients with severe cardiac failure, renal disorder, liver dysfunction or other neurological and psychiatric disorders, such as depression or alcohol abuse, and patients with symptomatic cerebral infarction or cortical lesions on brain magnetic resonance imaging (MRI) were excluded from the present study.

The final participant groups thus consisted of 91 patients with type 2 diabetes and 161 non-diabetic individuals. Diabetic patients were classified into two groups based on whether their glycated hemoglobin (HbA1c) was <7.0% or ≥7.0%. All diabetic participants had a history of diabetes, and were receiving pharmacological treatment for diabetes that included oral antihyperglycemic agents and/or insulin.

All participants underwent the standardized and reliable diagnostic procedures for dementia disorders.[16] AD was diagnosed as probable AD or possible AD according to the criteria from the National Institute of Neurological and Communicative Disorders and Stroke, and the Alzheimer's Disease and Related Disorders Association.[17]

Comprehensive assessment

Information about previous diseases and medication was obtained from the clinical charts. Polypharmacy was defined as taking five or more types of oral medicine.[18] The Barthel Index and the Lawton Index were used to evaluate basic and instrumental ADL, respectively.[15,19] Cognitive status was measured by using a psychiatric assessment battery that included the Mini-Mental State Examination (MMSE), Alzheimer's Disease Assessment Scale (ADAS), Digit Span Forward and Backward trials, Frontal Assessment of the Brain (FAB), Raven's Colored Progressive Matrices (RCPM), and Logical Memory I and II subtests from the Wachster Memory Scale-Revised.[20–24] Depressive mood and BPSD were estimated by the Geriatric Depression Scale-15 (GDS) and Dementia Behavior Disturbance Scale (DBD), respectively.[25,26] The Zarit burden interview (ZBI) was used for measurement of the caregivers' burden.[27] Risks for falls were evaluated by the Fall Risk Index (FRI).[28] Geriatric syndrome was assessed by administering a questionnaire to patients and their caregivers; the questionnaire included questions on dyspnea, cough, chest oppression, edema, fatigue, nausea, abdominal pain, diarrhea, constipation, dysphasia, numbness, tremor, syncope, dizzying, chewing troubles, polyuria, incontinence of urine, decubitus ulcer, itching, lumbago, back pain, lower limb pain, upper limb pain and sleeping problems.

Laboratory measurements

Apolipoprotein (Apo) E phenotypes were determined in plasma specimens by using isoelectric electrophoresis and immunoblotting methods.[29] Vitamin D insufficiency was assessed by the serum concentration of 25-hydroxyvitamin D, as currently recommended.[30] Levels of high-molecular-weight adiponectin were analyzed by enzyme immunoassay as described elsewhere.[31]

Neuroimaging studies

Brain MRI and single-photon emission computed tomography (SPECT) were used to elucidate the pathophysiology of dementia. A standard series of axial T1-weighted (repetition time [TR], 485 ms; echo time [TE], 11 ms), T2-weighted (TR, 3800 ms; TE, 93 ms) and fluid-attenuated inversion recovery (TR, 8000 ms; TE 101 ms; inversion time, 2500 ms; a 256×256 matrix). MR sequences were carried out in a 1.5T MR system (Siemens Avanto, Munich, Germany). Scans were in parallel with the anterior commissura–posterior commissura line, with 6-mm thick slices and an interslice gap of 1.2 mm.

MRI data were processed to measure the total volumes of the intracranial space (IC), parenchyma, ventricles and white matter regions (WML) by a fully automatic segmentation program (Software for Neuro-Image Processing in Experimental Research: SNIPER), which was developed at the Department of Radiology, Leiden University Medical Center, Leiden, the Netherlands. Detailed procedures of the MRI post-processing by SNIPER have been described elsewhere.[32]

SPECT scanning was carried out by using a two-head rotating GCA 7200DI gamma-camera (Toshiba, Otabara, Japan). Imaging was started 15–45 min after injection of 222 MBq (6 mCi) of N-isopropyl-p-[^{123}I] iodoamphetamine (Nihon Mediphysics, Tokyo, Japan), while the participants rested in a supine position with their eyes closed. The data were acquired in 128 × 128 matrices through an 18° rotation at an angle interval of 4°. The projection data were prefiltered and reconstructed, and Chang's attenuation and scattering corrections were applied.[33]

SPECT data were processed using the three-dimensional stereotactic surface projection (3D-SSP) method (Neurostat Software Library; Department of Internal Medicine, University of Michigan, Ann Arbor, MI, USA).[34] To assess perfusion deficits, the normalized brain activity of each patient was compared with that of 18 normal participants by using a pixel-by-pixel z-score analysis.[33] Qualitative z-score image analysis was carried out by two specialists without any knowledge of the clinical data. An image was defined as showing an AD pattern if the perfusion was decreased in the bilateral parietal association areas and posterior cingulate cortices, with relative sparing of the sensorimotor cortex, occipital cortex and cerebellum. The 3D-SSP technique, together with SPECT and positron emission tomography, provide a high diagnostic accuracy for AD.[34]

Statistical analysis

Statistical analysis was carried out using SPSS 19.0 for Windows (SPSS, Chicago, IL, USA). Comparisons of variables among the three patients groups were carried out by χ^2-test and analysis of covariance (ANCOVA), followed by post-hoc analysis (Bonferroni) to detect statistically significant differences. The association between BPSD and HbA1c was analyzed by Spearman's correlation analysis. Independent risks for BPSD were analyzed by multivariate logistic regression. Differences were considered significant at $P < 0.05$.

Results

Clinical profiles of the study participants

Age and education level were similar among the three groups of patients, whereas male sex was more prevalent in diabetic patients with HbA1c <7% (Table 1). The Barthel Index was lower in diabetic patients with HbA1c ≥7.0% than those with HbA1c <7.0%. Impaired dressing ability and urinary incontinence were apparent in diabetic patients with higher HbA1c (data not shown). Depressive mood and vitality, as well as caregivers' burden were not different among the three patient subgroups. The FRI was significantly increased in diabetic individuals with HbA1c ≥7.0%, although the incidence of falls in the previous year was unchanged. Polypharmacy was prevalent in diabetic participants. Use of sulfonylurea and insulin was increased in patients with HbA1c ≥7.0%, whereas biguanide was used more frequently in patients with HbA1c <7.0% (Table 1).

Apo E4 carriage, a genetic risk for AD, was seen in 52.5% ($n = 122$) of non-diabetic AD patients, which was compatible with the previous reports. However, among diabetic patients, the frequency of Apo E4 carriage was 39.4% and 47.7% in those with HbA1c <7% ($n = 33$) and ≥7.0% ($n = 44$).

As for physical complications, numbness was significantly increased in patients with HbA1c ≥7.0%, compared with non-diabetic individuals (25.0% and 11.8%, respectively). Dysphagia and diarrhea/constipation were also increased in poorly controlled diabetic participants (data not shown).

Biochemical properties

Blood glucose and HbA1c were significantly elevated in diabetic individuals (Table 2). Serum alkaliphosphatase tended to be increased in diabetic patients, and a significant increase was seen in diabetic patients with HbA1c ≥7.0%. Serum creatinine and estimated glomerular filtration rate were not changed among the three subgroups, whereas persistent proteinuria (>1 g protein/gCr) tended to be prevalent in patients with higher HbA1c ($P = 0.056$). The serum level of adiponectin was significantly reduced in patients with HbA1c ≥7.0%. 25-Hydroxyvitamin D, which reflects the activity of vitamin D, was significantly decreased in poorly controlled diabetic participants.

Cognitive impairment

Global brain function as measured by MMSE, ADAS and RCPM was substantially impaired in all three groups, but the degree of impairment was not significantly different among them (Table 3). Verbal fluency was significantly impaired in diabetic patients with HbA1c ≥7.0%. Performance on the recent memory and digit span tests, the latter of which is used as a measure of attention, was not different among the groups.

BPSD

The total score of DBD was significantly elevated in patients with HbA1c ≥7.0% (Table 4). DBD is a