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Improved Cerebrospinal Fluid-Based Discrimination between Alzheimer's Disease Patients and Controls after Correction for Ventricular Volumes

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Abstract. Cerebrospinal fluid (CSF) biomarkers may support the diagnosis of Alzheimer's disease (AD). We studied if the diagnostic power of AD CSF biomarker concentrations, i.e., $A\beta_{42}$, total tau (t-tau), and phosphorylated tau (p-tau), is affected by differences in lateral ventricular volume (VV), using CSF biomarker data and magnetic resonance imaging (MRI) scans of 730 subjects, from 13 European Memory Clinics. We developed a Matlab-algorithm for standardized automated segmentation analysis of T1 weighted MRI scans in SPM8 for determining VV, and computed its ratio with total intracranial volume (TIV) as proxy for total CSF volume. The diagnostic power of CSF biomarkers (and their combination), either corrected for VV/TIV ratio or not, was determined by ROC analysis. CSF $A\beta_{42}$ levels inversely correlated to VV/TIV in the whole study population ($A\beta_{42}$: $r = -0.28$; $p < 0.0001$). For CSF t-tau and p-tau, this association only reached statistical significance in the combined MCI and AD group (t-tau: $r = -0.15$; p-tau: $r = -0.13$; both $p < 0.01$). Correction for differences in VV/TIV improved the differentiation of AD versus controls based on CSF $A\beta_{42}$ alone (AUC: 0.75 versus 0.81) or in combination with t-tau (AUC: 0.81 versus 0.91). In conclusion, differences in VV may be an important confounder in interpreting CSF $A\beta_{42}$ levels.

Keywords: Alzheimer's disease, amyloid biomarkers, cerebrospinal fluid, lateral ventricles, tau protein

INTRODUCTION

Alzheimer's disease (AD) is a progressive and irreversible neurodegenerative disease. The neuropathological features of AD comprise the extracellular accumulation of amyloid-β (Aβ) in plaques and in the cerebrovasculature and intracellular neurofibrillary tangles containing hyperphosphorylated tau protein [1].

The diagnosis of AD is based on clinical criteria, comprising medical history, physical and neurological exams, and neuropsychological testing. However, the diagnostic accuracy of these criteria is relatively low (sensitivity: 71–88%; specificity: 44–71%) [2]. Additional tools that may help to support or refute the diagnosis of AD include amyloid positron emission tomography (amyloid PET), fluorodeoxyglucose PET (FDG PET), structural brain magnetic resonance imaging (MRI), and cerebrospinal fluid (CSF) protein analysis [3–5]. Amyloid PET imaging techniques can trace *in vivo* fibrillar Aβ accumulation in the brain by abnormal tracer retention [6]. With FDG PET, information will be obtained on the degree of neuronal degeneration or synaptic injury by visualizing reduction of glucose metabolism in cortical neurons and glial cells in AD patients [7, 8]. Structural MRI allows accurate measurement of the three-dimensional volume of brain structures. More specifically, structural MRI has revealed a specific pattern of atrophy in AD in the medial, basal, and lateral temporal lobe, and medial and lateral parietal cortices [9, 10]. Regarding CSF protein analysis, the combination of decreased concentrations of $A\beta_{42}$ and increased concentrations of both total and hyperphosphorylated tau proteins in the CSF is compatible with AD pathology [11] and may predict the progression to AD dementia in patients with mild cognitive impairment (MCI) [12, 13]. Moreover, recent studies have shown that abnormal levels of $A\beta_{42}$ can be detected already in cognitively normal individuals, 10 to 20 years before clinical symptoms occur [14].

CSF biomarkers have recently been included in diagnostic criteria for AD and may improve the accuracy of AD diagnosis to >85% [4, 15]. However, it is also well known that CSF biomarkers are influenced by several confounding factors. These confounders may include pre-analytical handling of the CSF and laboratory-specific procedures for the CSF analysis and may cause inter-laboratory variation in results and interpretation [16, 17]. Furthermore, an unstudied potential confounder in the interpretation of CSF results is the CSF volume. In aging and in AD, CSF production is known to be impaired, probably affecting the clearance of Aβ and tau and may also impact the total ventricular volume (VV) [18]. This might potentially result in an altered concentration of these proteins in the CSF. On the one hand, at a given production rate of Aβ or tau proteins, an increase in the CSF volume may lead to decreased CSF concentrations of these biomarkers. On the other hand, an increased VV is also related to increased tissue atrophy in AD. Even though changes in CSF dynamics across the AD continuum are still not completely understood, the interplay among brain atrophy, CSF production rates, and CSF protein concentrations, all known to be altered in AD [18], may contribute to dynamic changes in core AD CSF biomarker concentrations. The VV can be quantified on MRI, and used as a proxy for the total CSF volume in the brain [19, 20], which allows to study the effects of CSF volume on biomarker concentrations.

In this observational study, we investigated (1) whether there is an association between core AD CSF biomarkers and CSF volume and (2) whether correcting for this association might impact the diagnostic capacity of the CSF biomarkers. Our hypothesis is that correcting for the effect of VV may optimize clinical application of CSF biomarkers. We studied if the diagnostic power of AD CSF biomarker concentrations, i.e., $A\beta_{42}$, t-tau, and p-tau, can be improved by correction for VV as a proxy for total CSF volume, in a multi-center setting.

MATERIALS AND METHODS

Subjects

T1 weighted MRI scans and AD CSF biomarker data of 800 subjects were acquired from 13 research centers within the Biomarkers for Alzheimer's and Parkinson's Disease (BIOMARKAPD) project, a consortium of the European initiative Joint Program for Neurodegenerative Diseases (JPND). The study was approved by the local ethics committee or the institutional review board of each center. The participants or their legal representatives gave written informed consent.

All subjects underwent clinical and neurological assessment, lumbar puncture, MRI scanning, and CSF analysis at their local laboratory. The diagnostic criteria used in the different groups are presented in Supplementary Table 1; the locally applied cut-off

values for the use of the CSF biomarkers are shown in Supplementary Table 2. The patient groups comprised: 180 neurological controls, including healthy controls and subjective memory complainers, 336 MCI patients, 185 AD patients, 61 frontotemporal dementia (FTD) patients, and 38 patients with other dementias (e.g., vascular dementia, dementia with Lewy bodies, dementia not otherwise specified, corticobasal ganglionic degeneration, progressive supranuclear palsy).

The data of the subjects fulfilled the following requirements: the time between the lumbar puncture and the T1 weighted MRI scan was less than 6 months, and the T1 weighted MRI scans had a maximum voxel size of 2 mm × 2 mm × 2 mm. Additionally, information about the height, age, gender, diagnosis according to internationally accepted criteria (AD: [4, 21–24], MCI: [5, 25, 26], FTD: [27, 28]), scanner type, and magnetic field strength, were recorded and analyzed as covariates. In Supplementary Tables 3 and 4, information on the MRI scanner type, acquisition parameters, and whether a center used a specific protocol or not, can be found.

Segmentation algorithm for ventricular volume

Existing atlas-based algorithms were tried to segment the CSF volume in the brain, but did not provide robust segmentations in extremely enlarged ventricles [29]. To overcome this limitation, we developed a ventricle segmentation algorithm to be applied on a CSF segmentation of the MRI scans. The target ventricular region of interest (ROI) consisted of the lateral and third ventricles. The algorithm is based on a mixed region growing and atlas based approach and implemented in MATLAB using the tissue segmentation tool in the VBM8 toolbox of SPM8 (http://dbm.neuro.uni-jena.de/vbm8/). Briefly, the MRI scans were spatially normalized to the Montreal Neurological Institute (MNI) atlas using the DARTEL algorithm implemented in SPM8. In the normalized CSF *a priori* image, 'seed points' were placed in the lateral and third ventricles and the main anatomical boundaries of the ventricles were manually delineated. Then, the seed points and the boundaries in the normalized space were brought to the individual space by applying the inverse spatial normalization field. In the CSF segmented image, the ventricular ROI, was created by adding CSF-classified voxels adjacent to the seed points and this process was iterated by adding more CSF contiguous voxels to the ventricular ROI. This iterative process stops when no more voxels can be added to the ROI, either because contiguous voxels are not classified as CSF or because they were beyond the specified morphological boundaries. All the ventricular masks underwent visual quality control by an experienced reader. The ventricular segmentation algorithm is available at https://github.com/jdgispert/Ventricular-segmention under a GNU license.

The algorithm was validated using publicly available datasets (https://sites.google.com/site/mrilateralventricle/) and following the methodology described in [29]. Accuracy of the lateral ventricle segmentation was determined for healthy young adults and AD patients. Test-retest reproducibility was estimated with repeated acquisitions in the same scanner, as well as for different scanners and pulse sequences. Accuracy and reproducibility were quantified using the intraclass correlation coefficient (ICC) measure (single measure, 2-way mixed model, consistency). For accuracy results, the ICC quantifies how well the automated segmentations agree with respect to the gold standard measures (average of two manual segmentations by the same rater). For reproducibility, the ICC value quantifies the consistency of the segmentations. For further details on the validation methodology and characteristics of the test datasets, please see [29].

Total intracranial volume

The total intracranial volume (TIV) was determined by tissue segmentation, to correct the ventricular volume measurements. Voxels in MRI images were assigned to white matter, grey matter, or CSF in the brain using the algorithm included in the SPM8 suite. In the spatial normalization processes, the individual image was deformed to a template, in this case the MNI atlas. The template resembles a normal image with information about whether a voxel is more likely to represent a particular tissue depending on its position in the brain. The information of tissue types acquired is then applied to subject's space by quantifying the probabilities based on location in the subject's image rather than the template and thus the deformation is undone. The segmentation combines the latter information as well as the voxel intensity one in a Bayesian framework to define the tissue type. This process is repeated for all images and collected for each subject in our study population.

AD CSF biomarkers

The ELISA's from Fujirebio (Gent, Belgium) were used according to the manufacturers' protocol for the determination of $A\beta_{42}$ (INNOTEST® β-AMYLOID (1–42)), t-tau (INNOTEST® hTAU Ag), p-tau (INNOTEST® PHOSPHO-TAU (181P)) at each site separately. In all participating laboratories, the CSF samples were collected in polypropylene tubes. The CSF samples were directly transported to the laboratory, centrifuged, and measured or stored at –80°C until use.

AD-CSF-indices [30, 31] were calculated applying the following formulas:

$$AD-CSF-Index\,(t-tau) = \frac{A\beta_{max}-A\beta_{42}}{A\beta_{max}-A\beta_{min}} + \frac{ttau-ttau_{min}}{ttau_{max}-ttau_{min}} \quad (1)$$

$$AD-CSF-Index\,(p-tau) = \frac{A\beta_{max}-A\beta_{42}}{A\beta_{max}-A\beta_{min}} + \frac{ptau-ptau_{min}}{ptau_{max}-ptau_{min}} \quad (2)$$

where $A\beta_{max}$, $ttau_{max}$, and $ptau_{max}$ represent the 95th percentile of the respective values; $A\beta_{min}$, $ttau_{min}$, and $ptau_{min}$ represent the 5th percentile of the distribution values; and $A\beta_{42}$, ttau, and ptau represent the biomarker values for every individual. Derivation of 'minimum' and 'maximum' values of the biomarkers was based on the 5th and 95th percentiles of their respective distributions after pooling the different sample data (inter-site).

MTA scoring

Medial temporal lobe atrophy (MTA) scoring was used as measure for hippocampal atrophy and was used in a subset of patients to study the correlation between hippocampal atrophy (as a proxy for disease progression) and VV/TIV. In 34 AD patients and 13 controls from the Radboud University Medical Center the MTA was scored visually on the coronal T1 weighted images throughout the hippocampus at the level of the anterior pons. This score ranged from 0 (no atrophy) to 4 (severe atrophy) and assessed the width of the choroid fissure, width of the temporal horn of the lateral ventricle and the height of the hippocampus [32].

Data and statistical analysis

Statistical analyses were performed using IBM SPSS Statistics 22 (Armonk, NY, USA) and Graphpad Prism 5.03 (La Jolla, CA, USA). Significant differences between AD patients and control subjects were tested using a non-parametric Mann-Whitney test, because the data was not normally distributed according to the D'Agostino and Pearson omnibus normality test. The Chi-square test was used to check gender differences between the diagnostic groups. Significant differences between the CSF biomarkers in the diagnostic groups were estimated by non-parametric Kruskal-Wallis test. Bivariate correlations were determined using Spearman correlation coefficient. Models were constructed using forward (conditional) logistic regression analysis.

The diagnostic power of CSF biomarkers (or combinations) was determined by receiver operating characteristic (ROC) analyses. The area under the curve (AUC) in the ROC analyses was determined and the sensitivity at 85% specificity along with the positive likelihood ratio (LR+ = sensitivity/(1-specificity)) were compared between different CSF biomarkers and combinations in models. MedCalc version 16.2.1 (Mariakerke, Belgium) was used to check whether the ROC curves were significantly different.

RESULTS

Validation of ventricular volume segmentation algorithm

The ventricular segmentation algorithm successfully segmented all images in the accuracy and reproducibility datasets. Regarding accuracy, the algorithm achieved an ICC of 0.9895 for healthy young adults and 0.9893 for AD patients. The ICC for the test-retest reproducibility was of 0.9995 for the between-scanner and 0.9990 for the pulse sequence reproducibility. Average time to process each scan was 10 min approximately in a standard laptop (CPU 3.0 GHz 64-bit). Individual ventricular masks and volumetric results of the validation process can be found in (https://github.com/jdgispert/Ventricular-segmention).

All ventricular masks obtained with the segmentation algorithm were visually checked for each patient and revealed correct segmentation for the small and large ventricular volume in most cases (Fig. 1). Unfortunately, 70 subjects had to be excluded from the study group (Supplementary Table 5), since in these subjects the visual inspection of the segmentation showed an underestimation or overestimation

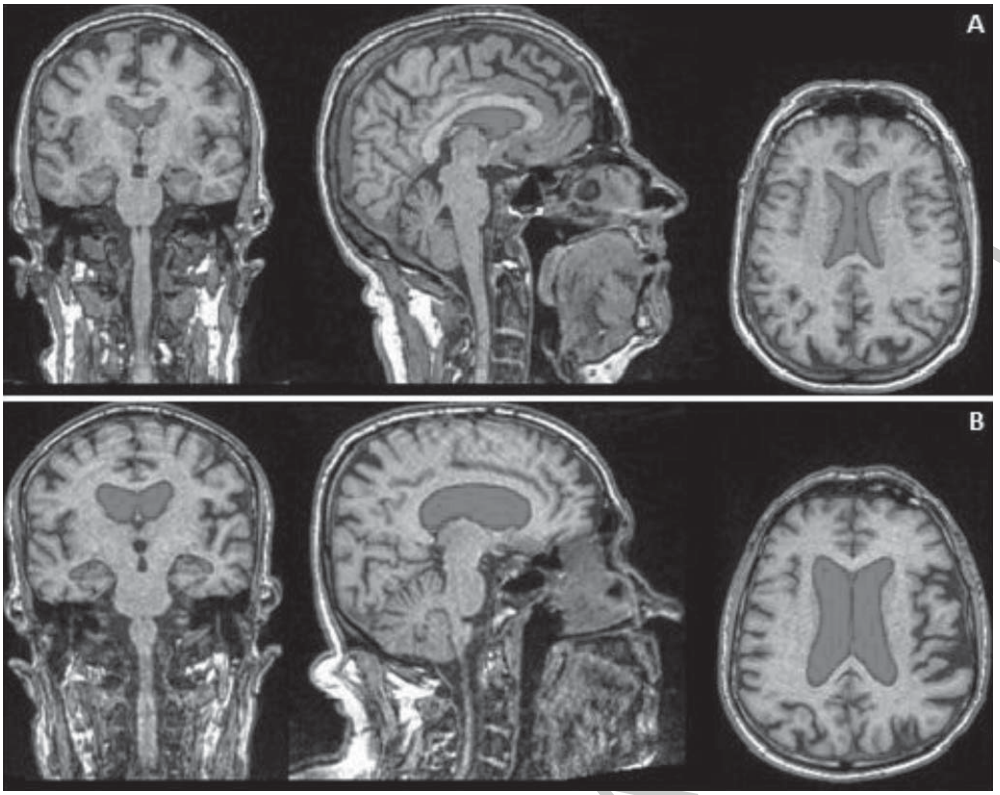

Fig. 1. Segmentation of ventricular volume (VV). (A) and (B) From left to right: coronal, sagittal, and transverse plane. The segmentation of the lateral and third ventricles were included in the region of interest (ROI) by the ventricle segmentation algorithm. Red indicates the segmentation mask on a T1 weighted MRI scan of cognitive healthy person (A) and Alzheimer's disease patient (B).

of the ventricle size. The excluded subjects were measured on different scanners: 7 subjects were measured on 1.0 T MRI scanners, 32 subjects on 1.5 T scanners, and 31 subjects on 3.0 T scanners. Thus 730 subjects remained in the study and their demographics are shown in Table 1 and the number of included and excluded patients per center and per disease group can be found in Supplementary Table 5. Control subjects had significantly ($p < 0.0001$) lower VV/TIV ratios compared to AD patients (Table 1).

Clinical validation of AD CSF biomarkers

The $A\beta_{42}$ concentrations were significantly decreased ($p < 0.0001$) in AD patients (mean: 495 pg/mL) compared to control subjects (mean: 703 pg/mL) (Fig. 2A). The mean t-tau (Fig. 2B) and p-tau (Fig. 2C) concentrations were significantly increased ($p < 0.0001$) in AD patients (t-tau: 720 pg/mL, p-tau: 98 pg/mL) compared to control subjects (t-tau: 296 pg/mL, p-tau: 57 pg/mL). Table 1 provides an overview of the CSF analysis data of all patient groups.

Correlations between CSF biomarkers and VV

Bivariate correlations between the CSF biomarkers and VV, corrected for the size of the head (TIV) as VV/TIV ratio, are displayed in Fig. 3A, B, C for all patients. $A\beta_{42}$ negatively, albeit weakly, correlated with the VV/TIV ratio ($r = -0.28$; $p < 0.0001$), but both t-tau and p-tau did not correlate with the VV/TIV ratio (t-tau: $r = 0.04$; p-tau: $r = -0.03$; both $p > 0.34$). Similar results were found for correlations of VV/TIV with $A\beta_{42}$ ($r = -0.35$; $p < 0.0001$), t-tau ($r = 0.11$; $p > 0.17$), and p-tau ($r = -0.11$; $p > 0.17$) for the group of controls only (Fig. 3D-F). When considering only AD and MCI patients, we found significant correlations of VV/TIV with both $A\beta_{42}$ ($r = -0.23$; $p < 0.0001$), t-tau ($r = -0.15$; $p < 0.01$), and p-tau ($r = -0.13$; $p < 0.01$) (Fig. 3G-I), although it should be noted that r values of these latter correlations are low.

Table 1
Demographic data and CSF biomarker concentrations across the diagnostic groups

	Control	AD	MCI	FTD	Other	*p-value*
Sample size: *n*	157	175	308	57	33	
Gender: female *n* (%)	98 (62)	107 (61)	151 (49)	24 (42)	15 (45)	<0.01[a]
Age: years mean (SD)	63.6 (8.6)	67.4 (8.7)	69.8 (7.4)	65.1 (10.5)	68.5 (10.1)	<0.0001[b]
$A\beta_{42}$: mean in pg/mL (SD)	703 (250)	495 (238)	630 (305)	774 (256)	643 (243)	<0.0001[b]
t-tau: mean in pg/mL (SD)	296 (223)	723 (454)	519 (328)	342 (228)	371 (307)	<0.0001[b]
p-tau: mean in pg/mL (SD)	57 (39)	98 (64)	78 (48)	43 (24)	61 (34)	<0.0001[b]
VV: mean in cm^3 (SD)	26 (17)	40 (19)	40 (22)	52 (30)	67 (50)	<0.0001[b]
VV/TIV: mean (SD)	0.019 (0.011)	0.030 (0.013)	0.029 (0.015)	0.039 (0.021)	0.047 (0.033)	<0.0001[b]

CSF, cerebrospinal fluid; AD, Alzheimer's disease; MCI, mild cognitive impairment; FTD, frontotemporal dementia; Other, other dementias; $A\beta_{42}$, amyloid-β; t-tau, total tau; p-tau, phosphorylated tau; SD, standard deviation.[a]Chi-square test.[b]Kruskal-Wallis test.

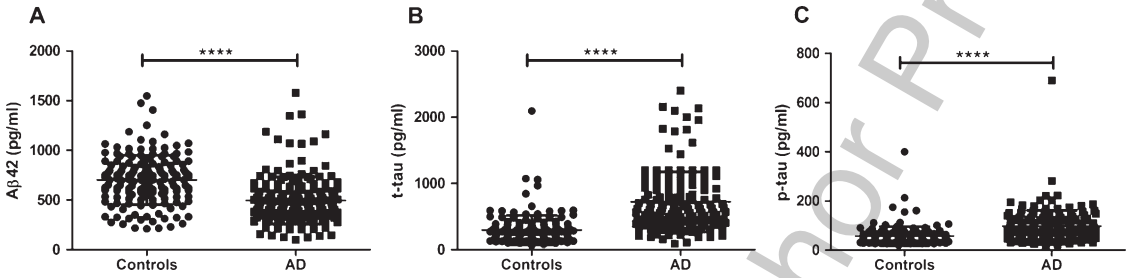

Fig. 2. Clinical validation of AD CSF biomarkers: $A\beta_{42}$ ($n = 332$), t-tau ($n = 306$), and p-tau ($n = 331$). Clinical validation of (A) $A\beta_{42}$ with Controls ($n = 157$), and Alzheimer's disease (AD) patients ($n = 175$), (B) t-tau with Controls ($n = 156$) and AD patients ($n = 150$). and (C) p-tau with Controls ($n = 156$) and AD patients ($n = 175$). $^{****}p < 0.0001$.

Comparison of diagnostic power

Next, we compared whether correction for VV/TIV improved the biomarker performance of $A\beta_{42}$, t-tau, and p-tau in differentiating AD from controls (Table 2, index 1–6). The diagnostic power increased when correcting $A\beta_{42}$ for the VV/TIV, but an opposite result was observed for both t-tau and p-tau. For differentiation of AD and controls, the AUC for $A\beta_{42}$ (0.75, 95% confidence interval (CI): 0.70–0.81), but not for t-tau and p-tau, increased significantly ($p < 0.01$) after correction for VV/TIV (AUC: 0.81, 95% CI: 0.77–0.86). The full list of AUCs and 95% CIs can be found in Table 2. Additionally, the sensitivity for $A\beta_{42}$ improved from 49% to 57% at a fixed specificity of 85% and the positive likelihood ratio increased (3.7 versus 3.2) after correction for VV/TIV.

We also compared whether correction for VV/TIV improved the biomarker performance of $A\beta_{42}$, t-tau, and p-tau in differentiating AD from FTD and other dementias. No improvement in differentiation could be found in correcting either of the three AD CSF biomarkers for VV/TIV (data not shown).

Next, we determined the diagnostic power of combined AD CSF biomarker concentrations in differentiating AD from controls (Table 2, index 7–10). The AD-CSF-Index t-tau (AUC: 0.86) showed no significantly different ($p = 0.97$) result compared to t-tau (AUC: 0.87). The AD-CSF-Index p-tau (AUC: 0.83) showed no significantly different ($p = 0.09$) result compared to p-tau (AUC: 0.79). The sensitivities, at a fixed specificity of 85%, and positive likelihood ratios were equal for the AD-CSF-Index of t-tau or p-tau compared to t-tau or p-tau alone, respectively. Logistic regression modeling using the three AD CSF biomarkers resulted in model 1, in which p-tau was not included. The combination of $A\beta_{42}$ and t-tau (Model 1) improved the diagnostic value (AUC: 0.88, 95% CI: 0.84–0.92) to discriminate AD from controls, compared to $A\beta_{42}$ alone. This diagnostic power (AUC: 0.91, 95% CI: 0.88–0.94) was even higher when CSF $A\beta_{42}$ was normalized to the VV/TIV ratio in combination with the CSF t-tau analysis (Model 2). The AUC of Model 2 was significantly ($p < 0.01$) higher than that of Model 1. Additionally, the sensitivity of Model 2 (83%) was higher than that of model 1 (71%) at a fixed specificity of 85%. The positive likelihood ratio was also higher in Model 2 than Model 1 (5.7 versus 4.8) (see Table 2). Height, age, gender, scanner type, and magnetic field strength were analyzed as covariates, but did not affect the above-mentioned results.

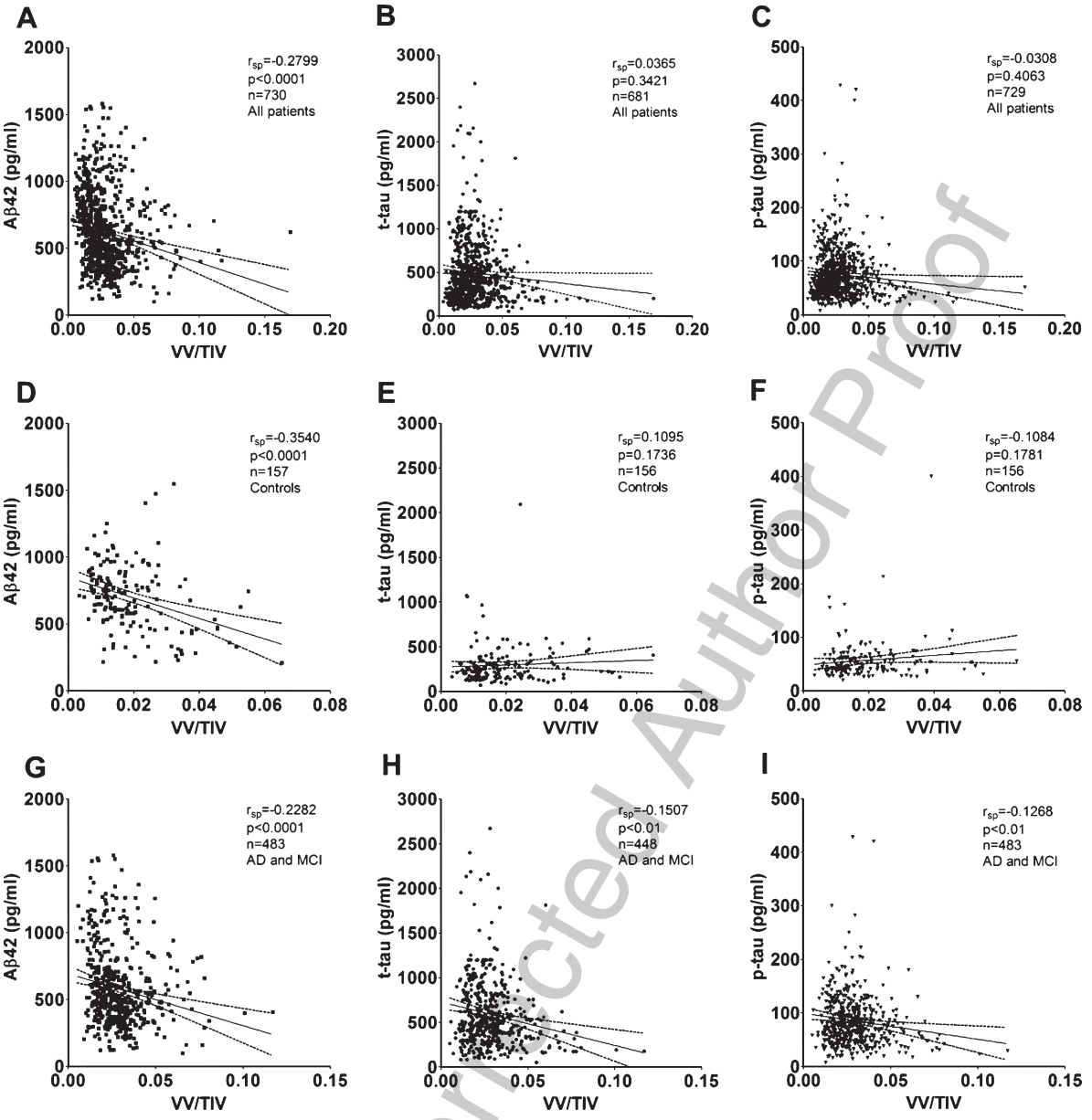

Fig. 3. Correlation of ventricular volume with CSF $A\beta^{42}$, t-tau, and p-tau in all patients. Ventricular volume corrected for total intracranial volume (VV/TIV) versus CSF $A\beta_{42}$ (A, D, G), t-tau (B, E, H), and p-tau (C, F, I) concentrations of all patients combined (A-C; i.e., controls, Alzheimer's disease (AD), mild cognitive impairment (MCI), frontotemporal dementia (FTD), and other dementias ($A\beta_{42}$: $n = 730$; t-tau: $n = 681$; p-tau: $n = 729$)), of controls only (D-F; $A\beta_{42}$: $n = 157$; t-tau: $n = 156$; p-tau: $n = 156$) and AD plus MCI patients only (G-I; $A\beta_{42}$: $n = 483$; t-tau: $n = 448$; p-tau: $n = 483$). A significant ($p < 0.0001$) correlation was found between VV/TIV and CSF $A\beta_{42}$ concentrations (A, D), but not t-tau (B, E) or p-tau (C, F) for all patients combined and the control group only. For AD plus MCI patients, a significant correlation was found between VV/TIV and both CSF $A\beta_{42}$ concentrations ($p < 0.0001$) (G), t-tau ($p < 0.01$) (H), and p-tau ($p < 0.01$) (I). For p-tau in panel C and I, one data point (690 pg/mL) is outside the axes limit. r_{sp} = spearman r.

Hippocampal atrophy

The improvement of correcting $A\beta_{42}$ for VV/TIV could be due to a general dilutional effect, but could also be related to disease progression. We performed a pilot study to test this hypothesis. We measured hippocampal atrophy (MTA score) as a measure for disease progression in 34 AD patients and 13 controls and analyzed the correlation with VV/TIV. The VV/TIV ratio raised significantly with increasing MTA score (Fig. 4), suggesting that the improved biomarker functioning after correction for VV/TIV

Table 2
Comparison of CSF biomarker (combinations) with and without correction for VV/TIV ratio

Index		AUC (95% CI)	Sensitivity (%) at 85% specificity	LR+	Patients C + AD *(n)*
1	Aβ42	0.754 (0.701–0.808)	49.1	3.2	332
2	Aβ42/(VV/TIV)	0.813 (0.765–0.861)	57.1	3.7	332
3	t-tau	0.867 (0.826–0.908)	75.3	5.1	306
4	t-tau/(VV/TIV)	0.637 (0.574–0.699)	35.3	2.4	306
5	p-tau	0.793 (0.743–0.842)	66.9	4.4	331
6	p-tau/(VV/TIV)	0.503 (0.440–0.565)	23.4	1.5	331
7	AD-CSF-Index t-tau (pooled)	0.864 (0.821–0.907)	77.0	4.9	306
8	AD-CSF-Index p-tau (pooled)	0.829 (0.783–0.875)	66.3	4.3	331
9	Model 1 ($A\beta_{42}$ and t-tau)[a]	0.880 (0.840–0.920)	71.3	4.8	305*
10	Model 2 ($A\beta_{42}$/(VV/TIV) and t-tau)[b]	0.912 (0.880–0.944)	83.3	5.7	305*

AUC, area under the curve; CI, confidence interval; LR+, positive likelihood ratio; Aβ42, amyloid-β; t-tau, total tau; p-tau, phosphorylated tau; VV, ventricular volume; TIV, total intracranial volume; AD, Alzheimer's disease; C: controls. [a]Y = (–0.650118) + (–0.002705)*[AB42] + (0.005129)*[T-Tau] Covariates: $A\beta_{42}$, p-tau, t-tau. [b]Y = (–0.568419) + (–0.000055)*[AB42]/(VV/TIV) + (0.005053)*[T-Tau] Covariates: $A\beta_{42}$/(VV/TIV), p-tau, t-tau.*Input data was $n = 332$, but $n = 26$ are missing t-tau and $n = 1$ is missing p-tau. These $n = 27$ patients were not used in creating the model.

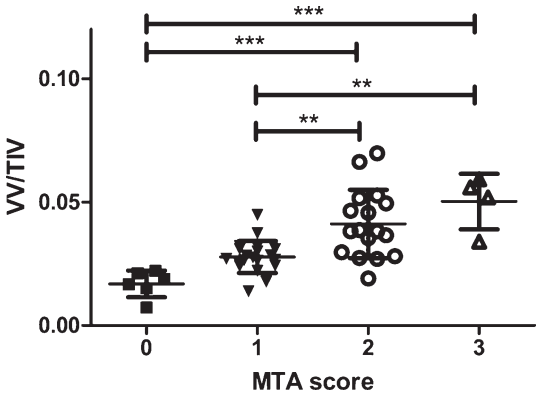

Fig. 4. Correlation of ventricular volume with hippocampal atrophy ($n = 47$). Ventricular volume corrected for total intracranial volume (VV/TIV) versus medial temporal lobe atrophy (MTA) score as measure for hippocampal atrophy in Controls and AD patients (pilot: total $n = 47$). Significant differences in VV/TIV were found between the different MTA scores ($^{**}p \leq 0.01$ and $^{***}p \leq 0.001$).

may, at least in part, be related to differences in disease progression.

DISCUSSION

Quantification of CSF biomarker concentrations may be a valuable addition for early diagnosis of AD, because these biomarkers reflect the ongoing process of amyloid deposition and neuronal changes. The added value of CSF biomarkers can be optimized if as many confounding factors as possible are excluded. One of these confounders may be variation in CSF volume, and normalizing CSF biomarker results for the VV/TIV ratio may correct for this confounding factor.

Validation of ventricular volume segmentation algorithm

In this study, we developed and validated a novel algorithm for automated measurement of VV in T1 images. The algorithm showed excellent accuracy and reproducibility and was capable of segmenting all the images in the validation dataset, thus, also showing appropriate robustness. This is advantageous compared to other existing segmentation algorithms, which did not provide robust segmentations in extremely enlarged ventricles, as are often seen in severe cases of AD [29]. However, the ventricular segmentation algorithm failed in a non-negligible percentage of the MRI scans available for this study. To this regard, it has to be noted that the images in this study come from a previously-acquired convenience sample in a multi-center setting in which no previous MRI protocol harmonization was conducted. Therefore, images were acquired in a variety of scanners with a wide range of parameters in the pulse-sequences. This resulted in significant contrast variation within the dataset, a situation that is challenging for automatic segmentation algorithms. This fact can account for the relatively high number of failures in the study dataset, even though the algorithm was able to process all the scans in the validation dataset and the resulting accuracy and test-retest variability were excellent. This behavior highlights the relevance of protocol harmonization in multicenter studies aiming at obtaining quantitative measurements using MRI.

Clinical validation of AD CSF biomarkers

We decided to pool the healthy control subjects and SMC in one group as neurological controls. A recent study showed that SMC are very similar to healthy controls in terms of prevalence of amyloid pathology [14]. The diagnostic value of $A\beta_{42}$ for the discrimination of AD versus these controls was slightly, but significantly improved ($p < 0.01$) by correction for VV/TIV. CSF $A\beta_{42}$ slightly decreased when VV/TIV increased, which is compatible with a dilutional effect, as described previously [19], but could also be explained by more advanced brain atrophy related to enlarged VV, which is a measure of disease progression [33, 34]. Indeed, it has been suggested that CSF $A\beta_{42}$ concentrations decrease steadily with advancing disease progression [10, 35–38]. It should be noted, however, that the variation in the biomarker concentrations between the participating groups was larger than would be expected and, possibly, could be smaller when all biomarker results were obtained from the same laboratory. It is therefore possible that the diagnostic gain is higher when the biomarker analyses are more harmonized. Furthermore, it could be argued that there is a circularity in testing the diagnostic capacity of core AD CSF biomarkers when, in some of the participating centers, these have also been used as a support for the diagnosis of AD. This would likely lead to an increased observed discriminative power of the CSF biomarkers in these selected samples. In this article, however, our main interest was to test whether correcting for VV increased their discriminative power as compared to non-corrected values, and not to estimate the absolute diagnostic capacity of the core AD biomarkers in a clinical setting. Since both approaches were compared in the same selection of samples, we do not expect our main results to be affected by any potential biases in the absolute discrimination power.

Correlations between CSF biomarkers and VV

In contrast, the CSF t-tau and p-tau concentrations did not correlate with VV or the VV/TIV ratio when analyzed for all patients together, and normalization for this ratio did not affect the diagnostic accuracy of these parameters. This lack of correlation may be explained by a combination of events with opposite effects. On one hand, t-tau and p-tau concentrations could be lower at higher VV because of dilution, but this effect may be counteracted by higher rates of tau protein release in the CSF during neurodegeneration. The latter may especially be true for p-tau, since CSF p-tau, as well as VV, are strongly associated with disease progression in MCI and AD patients [10, 38–40]. A biomarker dilution effect is supported by the significant negative correlation of t-tau and p-tau with VV/TIV in the combined group of MCI and AD patients, however, we did not observe such a correlation between t-tau or p-tau levels and VV/TIV in the control group, where neurodegeneration is not expected to occur. This argues not necessary against such a dilution effect, because both tau levels and VV displayed a reduced range of variation in the control group in comparison to that observed in the combined group of MCI and AD patients as a consequence of the neurodegenerative process in the latter group. In the combined MCI and AD groups, however, the disease progression is linked to VV.

Limitations

Although promising, the results of this study should be interpreted with caution for a number of reasons. Firstly, the measurement of CSF ventricular volume is a proxy for the total CSF volume in the brain and only globally indicates brain atrophy. Furthermore, it is important to note that CSF biomarker concentrations were determined in different laboratories and, despite that the same assays were used, variation in the pre-analytical and analytical procedures used will influence our data [31]. Efforts for more standardized methods are needed to measure CSF biomarkers, and standardized guidelines are developed using uniform reference materials within the JPND-BIOMARKAPD project (http://biomarkapd.org/). Additionally, there was neither a standardized MRI protocol used for this study nor patients were scanned using the same scanner, therefore variations in scanning protocol could have influenced our data. Furthermore, we have not evaluated the possible correlations between CSF biomarker levels (in particular CSF $A\beta_{42}$) and disease progression of AD in the entire group, but only in a small subgroup. Patients at a more advanced stage of AD may have larger ventricular volumes, and—according to our results—disease progression may explain part of the relation we found between $A\beta_{42}$ and VV/TIV ratio. In controls, both VV and t-tau showed normal population variability (i.e., not linked with disease progression). Just the opposite was found when we compared the combined MCI and AD groups, in which the disease progression is

correlated to VV. The only way to untangle variations in VV from tissue atrophy would be to have concentrations of a CSF biomarker known not to vary with AD, but unfortunately we do not have such a biomarker in this study. Also in this study, we did not have access to APOE genotyping data and therefore we could not determine the effects of APOE genotype on either CSF biomarkers or on the ventricular volume. However, despite these limitations we found that correction for differences in ventricular volume improved the differentiation of AD versus controls based on CSF $A\beta_{42}$ alone or in combination with t-tau.

CONCLUSION

In summary, we studied if the differences in CSF volume may act as a confounding factor for interpretation of CSF biomarkers. We used VV as a surrogate marker of brain atrophy. For this purpose, we developed and validated a novel algorithm for automated measurement of VV in T1 MRI images available to accurately segment normal as well as abnormally large ventricular volumes. CSF $A\beta_{42}$ concentrations decreased with increasing VV; correction for differences in this volume improved the differentiation of AD versus controls based on CSF $A\beta_{42}$ alone or in combination with t-tau. The correlation between VV and hippocampal atrophy gives an indication that the dilutional effect could be partly explained by ongoing brain atrophy in AD, as an indirect measurement of disease progression. Dilution of CSF biomarkers by increased VV may affect interpretation of the results of CSF biomarker analysis.

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SUPPLEMENTARY MATERIAL

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