CSF β-amyloid and white matter damage: a new perspective on Alzheimer's disease

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ABSTRACT

Objective: To assess the connection between amyloid pathology and white matter (WM) macro- and micro-structural damage in demented patients compared with controls.

Methods: Eighty-five participants were recruited: 65 with newly diagnosed Alzheimer's disease (AD), non-AD dementia or mild cognitive impairment (MCI), and 20 age- and sex-matched heatlhy controls. β-amyloid$_{1-42}$ (Aβ) levels were determined in cerebrospinal fluid (CSF) samples from all patients and 5 controls. Among patients, 42 had pathological CSF Aβ levels (Aβ+), while 23 had normal CSF Aβ levels (Aβ-). All participants underwent neurological examination, neuropsychological testing and brain magnetic resonance imaging (MRI). We used T2-weighted scans to quantify white matter (WM) lesion loads (LL), and diffusion weighted images (DWI) to assess their microstructural substrate. Non-parametric statistical tests were used for between-group comparisons and multiple regression analyses.

Results: We found an increased WM-LL in Aβ(+) compared to both, healthy controls ($p=0.003$) and Aβ(-) patients ($p=0.02$). Interestingly, CSF Aβ concentration was the best predictor patients' WM-LL ($r=-0.30$, $p<0.05$) when using age as a covariate. Lesion apparent diffusion coefficient (ADC) value was higher in all patients than in controls ($p=0.0001$), and correlated with WM-LL ($r=0.41$, $p=0.001$). In Aβ(+), WM-LL correlated with WM microstructural damage in the left peritrigonal WM ($p<0.0001$).

Conclusions: WM damage is crucial in Alzheimer's disease (AD) pathogenesis. The correlation between CSF Aβ levels and WM-LL suggests a direct link between amyloid pathology and WM macro- and microstructural damage.

INTRODUCTION

In patients with Alzheimer's disease (AD), magnetic resonance imaging (MRI) often shows focal hyperintensities in the deep and subcortical white matter (WM).[1-5] Their nature remains unclear: the main hypothesis considers them as chronic ischemic lesions caused by cerebral microangiopathy,[6,7] while neuropathological studies show evidence of demyelination and axonal loss.[5,8] Thus, other mechanisms could be implicated, including blood-brain barrier leakage, inflammation, neurodegeneration, and amyloid angiopathy.[5] A direct link between WM hyperintensities (WMHs) and the severity of cognitive decline has already been demonstrated in literature.[9,10] The incidence of WMHs is higher in patients with AD,[11-13] vascular dementia (VaD),[14] dementia with Lewy body (DLB),[14] and frontotemporal dementia (FTD)[15] (including some inherited forms of FTD,[16-18]). Moreover, the presence of WMHs seems to increase the risk for conversion from mild cognitive impairment (MCI) to AD, and to predict the progression of cognitive symptoms.[10,11,19,20] Diffusion weighted imaging (DWI) studies have demonstrated the presence of WM microstructural changes in AD brains at preclinical stages.[3] In our study, we chose to use apparent diffusion coefficient (ADC) maps, obtained from DWI scans, as metrics to state the integrity of WM at microscopic level. The Dominantly Inherited Alzheimer Network analyzed the severity and distribution of WMHs in pre-symptomatic presenilin 1, presenilin 2, and amyloid precursor protein mutation carriers, investigating the extent to which WMHs manifest genetically predisposed individuals.[21] This study found that WMHs are elevated well before symptom onset, suggesting that WMHs are a core feature of AD pathogenesis.[21]

Against this background, the contribution of WMHs to AD pathogenesis is still debated, and WMHs are mostly considered as a comorbidity rather than part of AD pathophysiology.[4,10,21]

To the best of our knowledge, only few data are available in literature on the relationship between measures of macro- and micro-structural WM damage and cerebrospinal fluid (CSF) biomarkers of neurodegeneration. Kalheim and colleagues reported a remarkable extent of WM microstructrual damage in patients with MCI who showed pathological CSF levels of β-amyloid$_{1-42}$ (Aβ).[22] Addictionally, an elegant paper by Dean III et al. has contributed in clarifying the relationship between amyloid pathology and myelin alteration in preclinical AD.[23] Measuring whole-brain longitudinal and transverse relaxation times and the myelin water fraction (MWF), a significantly negative relationship between MWF and CSF Aβ levels was observed. Concerning inherited forms of AD, Lee and colleagues reported a correlation between WMHs and CSF Aβ levels.[21] Finally, Noh and colleagues, using 11C-Pittsburgh compound B (PiB) PET imaging, demonstrated an association between WMH extension and amyloid burden.[24]

To better understand the relationship between WMHs and amyloid pathology, we aimed here at investigating how CSF Aβ and tau levels interact with measures of macro- and micro-structural WM damage and grey matter (GM) atrophy in patients with AD.

MATERIALS AND METHODS

Subjects

Sixty-five patients with cognitive deficits were consecutively recruited at the Alzheimer Center of the University of Milan, Policlinico Hospital (Milan, Italy). All patients underwent a clinical interview, neurological and neuropsychological examination, routine blood tests, brain MRI, and lumbar puncture (LP) for quantification of the CSF biomarkers Aβ, total tau (tau), and tau phosphorylated at position 181 (Ptau). Cut-off thresholds of normality were: Aβ ≥600 pg/ml; tau ≤500 pg/ml for

individuals older than 70 and ≤450 pg/ml for individuals aged between 50 and 70 years; Ptau ≤61 pg/ml.[25] For the purpose of this study, patients with CSF Aβ levels <600 pg/ml were classified as Aβ(+), while patients with CSF Aβ levels within the normal range were classified as Aβ(-). Similar classification was applied to tau and Ptau CSF levels.

Fourty-two patients were diagnosed with AD, as confirmed by their pathological CSF Aβ levels, according to the criteria of the International Working Group guidelines.[26] Twenty-three patients (all with normal CSF Aβ levels) were diagnosed with a non-AD form of neurodegenerative dementia.

To minimize the risk of confounding variables associated with vascular comorbidities (i.e. subcortical vascular dementia), we selected only patients with a Hachinski Ischaemic score (HIS) <3[27], a periventricular and deep white matter Fazekas score ≤2[1], without any relevant history or risk factor for cardiovascular disease. In particular, patients suffering from diabetes, atrial fibrillation, arterial hypertension or with a history of stroke or myocardial infarction were excluded from the study.

Twenty age- and sex-matched controls who volunteered to undergo neuropsychological assessment and brain MRI were also enrolled. Among them, 5 individuals agreed to undergo LP. All these subjects were clinically followed-up for almost three years and none of them developed any symptom or sign suggestive for cognitive decline.

The main demographic and clinical characteristics of all recruited subjects are summarized in Table 1.

The current study was approved by the Institutional Review Board of the Fondazione Cà Granda, IRCCS Ospedale Maggiore Policlinico (Milan, Italy). All patients (or their legal guardians) and controls gave their written informed consent for this research before entering the study.

CSF collection and Aβ and tau determination

CSF samples were collected by LP in the L3/L4 or L4/L5 interspace. The LP was done between 8 and 10 a.m. after one-night fasting. Then, CSF samples were centrifuged in 8000 rpm for 10 minutes. The supernatants were aliquoted in polypropylene tubes and stored at – 80 °C until use. CSF cell counts, glucose, and proteins were determined. CSF Aβ, tau and Ptau were measured using, respectively, three commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kits (Fujirebio, Ghent, Belgium).

MRI acquisition

All patients underwent a MRI examination on Achieva 3T scanner (Philips, The Netherlands). The acquisition protocol included: 1) a 3D T1-weighted scan (TR 9.90 ms; TE 4.61 ms; Flip angle 8°; slices thickness 1 mm; gap 0) 2) a T2-weighted scan (TR 2492 ms; TE 78 ms; Flip angle 90°; slices thickness 4 mm; gap 0); 3) a Fluid attenuated inversion recovery (FLAIR) scan (TR 11000 ms; TE 125 ms; Flip angle 90°; slices thickness 2 mm; gap 0); 4) a Diffusion weighted imaging (DWI) scan (b-value 1000 s^2/mm ; TR 2733 ms; TE 53 ms; Flip angle 90°; slices thickness 4 mm; gap 0).

WM macrostructual damage

To quantify the macroscopic load, WMHs were first identified on FLAIR scans by consensus of three independent observers (MS; PB; TC). WMHs were then outlined using a semi-automated local threshold contouring technique (Jim 7.0, Xinapse System, Leicester, UK, http://www.xinapse.com/). For each dataset, the WM lesion load (WM-LL) was calculated and used for correlation analyses. Additionally, as explained below, WM lesion masks were overlapped to diffusion imaging data to obtain a measure of microscopic tissue damage.

Brain volumetrics

All 3D T1-weighted scans were first visually inspected to exclude the presence of macroscopic artefacts. Data were processed using an optimized voxel-based morphometry (VBM) protocol in Statistical Parametric Mapping 8 (SPM8; Wellcome Department of Imaging Neuroscience; www.fil.ion.ucl.ac.uk/spm/). Segmentation and normalization produced a GM probability map[28] in Montreal Neurological Institute coordinates. To compensate for compression or expansion during warping of images to match the template, GM maps were modulated by multiplying the intensity of each voxel by the local value derived from the deformation field.[29] All data were then smoothed using a 8-mm full width half maximum (FWHM) Gaussian kernel. Then, GM maps were analysed in SPM8, using a sample t-test for the comparison between groups and a regression model to assess possible associations between patients' regional GM volumes and other variables of interest. We derived for each scan the GM fraction, calculated as the ratio of total GM volume to total intracranial volume (TIV). Age, gender, disease duration and Mini Mental State Examination (MMSE) scores were always entered as covariates of no interest. For every T-contrast, we applied the family wise error (FWE) correction for multiple comparisons, and we accepted as significant p values < 0.05 (corrected at cluster level).

WM microstructural damage

FLAIR images were coregistered to ADC maps using ANTs[30] after having skullstripped both images. The same transformation was applied to the lesion mask in order to project them into the ADC space. Finally, for each subject, we derived the mean value of ADC inside the lesions. This analysis aimed at investigating whether specific neurobiological substrates existed across groups, and whether there was any

association between them and the CSF biomarkers. To this purpose, ADC maps were analysed in SPM8, using a regression model to assess possible associations between WM microstructural damage and other variables of interest (WM-LL, GM fraction and CSF biomarkers). For every T-contrast, we applied the family wise error (FWE) correction for multiple comparisons, and we accepted as significant p values < 0.05 (corrected at cluster level).

Statistical analyses

Statistical analyses were performed using SPSS 20.0 for Windows (SPSS Inc., Chicago, IL, USA) and SPM8. Due to the non-normal distribution of data (as preliminary assessed by Shapiro-Wilk test), all between-group comparisons were tested by non-parametric inferential statistical analyses (Kruskal-Wallis test and Mann-Whitney U test). For all analyses, the statistical threshold was set to $p < 0.017$ after Bonferroni correction for multiple comparisons ($\alpha = 0.05/3 = 0.017$).

Spearman correlation coefficient between WM-LL and GM fraction was assessed in all patients.

Hierarchical multiple regression analyses between WM-LL as dependent variable and CSF Aβ, tau and Ptau levels as explanatory variables were conducted in the patient group. Each regression model was adjusted in order to control for the potential effect of age, gender, MMSE score, disease duration and GM fraction. Hierarchical multiple regression analyses were also investigated in the patient group by entering the GM fraction as dependent variable and CSF Aβ, tau and Ptau levels as explanatory variables. Again, each regression model was adjusted to control the potential effect of age, gender, MMSE and disease duration. CSF data from controls were not used for statistical analyses due to the smal sample size small (n=5).

RESULTS

WM macrostructural damage

Taken altogether, patients showed higher WM-LL than controls (U=16,000 p< 0.001). In none of the groups, WM-LL correlated with the correspondent total GM fraction (r=-0.15, p=0.33). Using the Kruskal-Wallis test, we compared WM-LL across all groups, obtaining the following statistical values: 1) controls (mean rank 23.95); Aβ(+) patients (mean rank 54.25), Aβ(-) patients (mean rank 40.24) (χ^2 20.54, df = 2, p < 0.001). The pairwise comparisons showed higher WM-LL in Aβ(+) patients as compared to controls (p=0.003), and in Aβ(+)as compared to Aβ(-) patients (p=0.020). No significant differences were found between Aβ(-) patients and controls *(*p=0.078*;* Figure 1). Regarding WM-LL, no significant differences were observed either in tau(+) compared with tau(-) patients (p=0.43), or in Ptau(+) compared with Ptau(-) patients (p=0.06).

Multiple regression analysis showed CSF Aβ concentration to be a predictor of patients' WM-LL (r=-0.30, p < 0.05, Figure 2). The percentage of variability of the regression model explained by CSF Aβ levels was 41% (p < 0.05). Patient's age was a significant predictor (r=0.32, p < 0.05) of WM-LL, while, interestingly, disease duration and the level of global cognition (assessed by the MMSE score) were not (p > 0.05).

Brain volumetrics

As expected, total GM fraction was significantly lower in all patients compared to controls (34.35%±3.36% vs 41.40%±5.3%, p < 0.001). No significant differences in total GM fraction were observed between patients' groups (34.76%±3.95% vs

34.03%±2.84%, p=0.398). When comparing patients against controls in a voxel-wise fashion to assess the regional GM atrophy, the two groups showed two distinct patterns. In Aβ(+) patients, GM atrophy involved, mainly, the medial temporal lobes. Conversely, in Aβ(-) patients, GM loss was prominent in the orbitofrontal cortices. Finally, when stratifying the Aβ(+) group for the severity of cognitive impairment (MMSE cut-off score=24), most impaired patients showed a trend towards significance of higher atrophy in their hippocampal and parahippocampal regions ($p < 0.01$ unc.; $p < 0.05$ unc., respectively).

Multiple regression analysis showed CSF tau levels to be a predictor of patients' GM atrophy ($r=-0.27$, $p < 0.05$). The percentage of variability of the regression model explained by CSF tau levels was 36% ($p < 0.05$). No other significant predictors were found.

WM microstructural damage

DWI analysis showed a significant increase of ADC values obtained by averaging all WM lesions in the brain, in patients versus controls ($p < 0.0001$, Figure 3a). Moreover, lesion ADC values in patients were significantly correlated with the correspondent WM-LL ($r=0.41$; $p=0.001$, Figure 3b). Conversely, no significant correlation was found between patients' lesion ADC values and CSF Aβ and tau levels ($p > 0.01$). Concerning Aβ(+) and Aβ(-) patients, no significant difference about ADC values was found between-group. When considering Aβ(+) patients in isolation, their WM-LL was significantly correlated with the ADC values in the left peritrigonal area ($p < 0.0001$).

DISCUSSION

In this study, we recruited a group of patients with cognitive decline, classifying them as Aβ(+) and Aβ(-). Both groups were globally more atrophic than controls, but with a different pattern of regional GM atrophy. Consistent with previous findings[31], AD patients showed a prominent atrophy in the medial temporal lobes, while non-AD patients presented with a more pronounced pattern of orbitofrontal GM atrophy.

With respect to macroscopic WM involvement, we first confirmed previous findings[32] demonstrating that total WM-LL is significantly higher in patients with dementia than in healthy elderly individuals. Furthermore, we demonstrated that, even accounting for the aging effect, CSF Aβ levels are the best predictor for the accumulation of WMHs: the lower the CSF Aβ levels, the higher the total WMHs. Consistently, Aβ(+) patients showed significantly higher WM-LL when compared to either group, controls or Aβ(-) patients. These data support the hypothesis that the macroscopic WM damage is likely to reflect a pathogenic mechanism which is part of AD pathophysiology rather than expression of concomitant comorbidities. Whether this WM damage is strictly related to GM degeneration or is an independent process is still a matter of debate. According to our data, we suggest that WMHs are not necessarily associated to GM degeneration. We did not observe any significant difference in WM-LL when stratifying AD patients for disease duration and global cognition; so, we might argue that WMHs may occur at an early pathophysiological stage of AD. Notably, patients with Fazekas ≥2 were excluded in order to likely exclude any bias due to the presence of cerebrovascular disease. Moreover, patients included had a HIS <3. In any case, our findings confirm, along with previously published data,[9,11,12,19,20] that WMHs should be regarded as a crucial feature of AD.

Concerning the evidence here reported that CSF Aβ levels are the best predictor for WM-LL, age excluded, our results are in accordance with Lee et al., who found an association between the increase of WM-LL and the reduction of CSF Aβ levels in

autosomal dominant genetic forms of AD.[21] This evidence suggests that WMHs and Aβ pathology may share some degree of correlation and, probably, some pathophysiological mechanism.

In this framework, our findings suggest that CSF Aβ reduction might be associated with the occurrence of WM metabolic damage due to Aβ deposition, possibly caused by impairment of pathways implicated in myelination and myelin repairing processes.[33,34]

As argued by Prins and Scheltens, WMHs may represent only the extreme end of a continuous spectrum of WM injury, creating a need for imaging approaches able to detect subtler changes.[8] Diffusion imaging is one of the most suitable techniques to assess WM integrity in vivo. The LADIS study showed that ADC values within the normal-appearing white matter (NAWM) of patients with cognitive impairment are associated to WM-LL.[35] More recently, ADC changes in the NAWM were also demonstrated to precede the development of WMHs.[36] In the current study, we used ADC maps to characterize the microstructural substrate of WM lesions in a Aβ(+) and Aβ(-) patients as compared to healthy elderly individuals. We found that the average ADC value across all WMHs was higher in both groups of patients compared to controls, thus indicating a different pathogenesis for lesions occurring in the brain of patients suffering from neurodegenerative dementia. Moreover, we found out that lesion ADC values in patients' brains correlate with WM-LL. This result may not be surprising, but corroborates the speculation that there is a connection between the type and severity of WM microstructural damage and the macroscopic WM lesions themselves. Interestingly, our data revealed that WM-LL correlates with WM microstructural damage in the left peritrigonal WM in the Aβ(+) group compared to controls. These findings are consistent with the hypothesis that one of the early features of AD is WM microstructural damage, particularly in the left peritrigonal WM, a crucial area for AD pathology due to

its strong connections with the precuneus. As known, parietal areas represent the main node of integration between structural and functional brain networks.[37] The precuneus is a notable area, not only due to its location deep in the postero-medial cortex of the parietal lobe, but also because of its possible role in fundamental cognitive functioning.[38] Interestingly, it also shows exceptionally high levels of energy requirement.[38]

Parietal dysfunction might contribute to the cognitive deficits that can be observed in the earliest stages of AD.[39] In clinical practice, medial parietal areas hypometabolism is an accurate tool to differentiate cognitively healthy elderly individuals from early AD patients.[40] Several studies in early AD patients have shown loss of WM volume or integrity, particular affecting medial parietal regions,[39] resulting in metabolic dysfunction.[39] Emerging evidence indicates that AD vulnerability is strongly associated with hyperconnectivity, augmented synaptic and metabolic activities, as well as functional plasticity. Speculations can be made about recent higher cortical functions carrying a risk for cognitive decline. We hypothesize that the development of newer cortical areas and the concomitant increase of synapse network might result in a higher structural instability, because of a major regional metabolic burden. This could result first in WM microstructural changes, and then in WM macrostructural damage. In light of this, the causal and temporal relationship between WM microstructural alterations and neurodegeneration needs to be further investigated, possibly in longitudinal studies.

With regard to tau and Ptau proteins, no significant correlations were found, but considering the borderline p value (p=0.06), further studies would be needed to confirm these data. Conversely, in our cohort of patients, CSF tau protein levels resulted as a predictor of GM volume fraction.

In conclusion, this study suggests that WM lesions and their micro-structural substrate, particularly in the left parietal areas, represent a crucial feature in AD,

independent of vascular risk factors and disease stage. Moreover, the correlation between CSF Aβ levels and total WMH volume seems to confirm a link between Aβ pathology and WM macro- and microstructural damage. Limitations of the study include the cross-sectional design, the small sample size, the absence of statistical analysis with healthy controls and the lack of more detailed neuropsychological description. Therefore, replication in a larger cohort of patients is required to confirm these data.

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Conflict of interest statement

None declared.

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LIST OF THE ACRONYMS

Aβ: β-amyloid$_{1-42}$

Aβ(+): patients with pathological CSF Aβ levels

Aβ(-): patients with normal CSF Aβ levels

AD: Alzheimer's disease

ADC: apparent diffusion coefficient

CSF: cerebrospinal fluid

DWI: diffusion weighted imaging

FTD: frontotemporal dementia

FWE: family wise error

GM: grey matter

HIS: Hachinski ischaemic score

DLB: dementia with Lewy body

LL: lesion load

LP: lumbar puncture

MCI: mild cognitive impairment

MMSE: mini mental state examination

MRI: magnetic resonance imaging

MWF: myelin water fraction

NAWM: normal appearing white matter

PiB: 11C-Pittsburgh compound B

Ptau: tau phosphorylated at position 181

Tau: total tau

TIV: total intracranial volume

VaD: vascular dementia

VBM: voxel based morphometry

WM: white matter

WMHs: white matter hyperintensities

Table 1: Clinical and demographic characteristics of healthy controls, AD patients with low CSF Aβ levels (Aβ(+)) and non-AD patients with high CSF Aβ levels (Aβ(-)).

* Data available for five out of twenty subjects. Abbreviations: MMSE = mini mental state examination; WM-LL = white matter lesion load; CSF = cerebrospinal fluid; Aβ = β-amyloid; Ptau = 181-phospho-tau.

	Healthy controls (n = 20) mean±SD	Aβ(+) – AD patients (n = 42) mean±SD	Aβ(-) – non-AD patients (n = 23) mean±SD
Age, y	72.3 ± 9.8	74.22 ± 7.5	74.9 ± 4.8
Female/male	11/9	24/18	12/11
Disease duration, m	-	47 ± 42	43 ± 23
MMSE, raw score	29.10 ± 0.79	20.36 ± 6.04	20.08 ± 5.71
WM-LL, mm^3	3302.95 ± 3964.11	8604.00 ± 6550.32	6876.86 ± 8025.32
CSF Aβ, pg/ml	1214.00 ± 186.92*	486.43 ± 111.06	843.74 ± 241.65
CSF tau, pg/ml	209.40 ± 93.73*	609.48 ± 371.12	430.48 ± 315.35
CSF Ptau, pg/ml	30.80 ± 15.48*	79.24 ± 30.79	60.43 ± 30.58