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Amyloid PET as a marker of Normal-Appearing White Matter early damage in Multiple Sclerosis: correlation with CSF β-Amyloid levels and brain volumes

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ABSTRACT

Purpose: The disease course of multiple sclerosis (MS) is unpredictable and reliable prognostic biomarkers are needed. Positron emission tomography (PET) with β-amyloid tracers is a promising tool to evaluate white matter (WM) damage and repair. Our aims were to investigate amyloid uptake in damaged (DWM) and normal-appearing WM (NAWM) of MS patients, and to evaluate possible correlations between cerebrospinal fluid (CSF) β-Amyloid$_{1-42}$ (Aβ) levels, amyloid tracer uptake and brain volumes.

Methods: Twelve MS patients were recruited and divided according to their disease activity into active and non-active groups. All participants underwent neurological examination, neuropsychological testing, lumbar puncture, brain magnetic resonance (MRI) imaging, and ^{18}F-florbetapir PET. Aβ levels were determined in CSF samples from all patients. MRI and PET images were co-registered and mean standardized uptake values (SUV) were calculated for each patient in the NAWM and in the DWM. To calculate brain volumes, brain segmentation with statistical parametric mapping software was performed. Non-parametric statistical analyses for between-group comparisons and regression analyses were conducted.

Results: We found a lower SUV in DWM compared to NAWM ($p<0.001$) in all patients. A decreased NAWM-SUV in active compared to non-active group was observed ($p<0.05$). Considering only active patients, NAWM volume correlated with NAWM-SUV ($p=0.01$). Interestingly, CSF Aβ concentration was a predictor of both NAWM-SUV ($r=0.79$; $p=0.01$) and NAWM volume ($r=0.81$, $p=0.01$).

Conclusions: The correlation between CSF Aβ levels and NAWM-SUV suggests that the predictive role of β-amyloid may be linked to myelin early damage and may reflect the disease activity and the clinical progression.

Key Words: PET, amyloid tracer, florbetapir, multiple sclerosis, amyloid, white matter

INTRODUCTION

Multiple sclerosis (MS) is the most common chronic inflammatory disease of the central nervous system (CNS) [1], whose demyelination is the pathological hallmark. MS is characterized by inflammation, axonal damage, and neurodegeneration [2]. The factors that promote spontaneous remyelination or determine axonal and neuronal loss remain poorly understood [3], and currently there are no reliable prognostic biomarkers of disease progression.

Magnetic resonance imaging (MRI) is the most widely used technique for identifying the demyelinating lesions, especially in the white matter (WM). However, no strong correlation exists between conventional MRI measures, such as T2- and T1-lesion loads, and risk of disease progression, and no MRI technique is currently specific enough to assess myelin damage and repair in vivo [4–6].

Positron emission tomography (PET) with amyloid tracers (e.g. Pittsburg Compund-B, florbetapir, florbetaben, flutemetamol) was originally developed to image amyloid deposition in neurodegenerative disorders and dementia [7], but it has been recently repurposed as an imaging marker for quantification of myelin loss and repair in MS [5,8–10]. Amyloid tracers bind extensively to WM and its uptake decreases with demyelination [8]. The usefulness of amyloid tracers in MS is traditionally considered secondary to their non-specific binding to WM, possibly being trapped into β-sheet structures of myelin proteins or being highly soluble in the myelin associated lipid bilayer [10,11]. Nevertheless, emerging evidence supports a connection between amyloid and myelin pathology. Acute WM lesions show a reduced amyloid tracer uptake compared with normal appearing WM (NAWM), reflecting a more extensive myelin loss in the lesions than in the NAWM [9,10,12,13]. Additionally, in patients with relapsing-remitting (RR) MS, an increased uptake within the lesions was found to be associated with a more benign clinical evolution [9]. In light of these data, amyloid PET tracers may

represent a new tool to monitor MS progression and to provide outcome measures [8]. Amyloid PET tracer WM uptake in MS has raised questions about its utility as a biomarker of demyelination. Moreover, amyloid-precursor protein (APP) accumulates in damaged axons in MS [14,15], suggesting that it may constitute a reliable marker of axon demyelination. High APP immunoreactivity has been found in actively demyelinating MS lesions but not in chronic ones, indicating a peculiar modification of APP metabolism across disease stages [16]. Moreover, β-amyloid (Aβ) might be involved in remyelination: the β-site APP-cleaving enzyme 1 (BACE1), the enzyme that process APP to generate Aβ, is also involved in the cleavage of neuregulin 1, a protein that plays a crucial role in oligodendrocytes differentiation and remyelination. Indeed, the genetic deletion of BACE1 during development leads to hypomyelination [17]. Several studies have aimed to determine how remyelination and MS are affected by APP and the proteins expressed via APP proteolytic processing, and whether amyloid PET can provide an in vivo molecular diagnosis of this process, but the question is still open.

Reduced cerebrospinal fluid (CSF) Aβ levels have been reported in MS patients [18–22] and have recently been suggested as prognostic biomarker [22,23].

Given these premises, aims of the current study are: 1) to assess amyloid tracer uptake in damaged WM (DWM) and NAWM of MS patients divided according to their disease activity; 2) to investigate possible correlations between amyloid tracer uptake and CSF Aβ levels, WM brain volumes, and clinical markers of disease progression.

MATERIALS AND METHODS

Subjects

Twelve patients with a diagnosis of MS according to the 2010 revised McDonald criteria were recruited [24]. Seven patients had RRMS, 3 patients had secondary

progressive (SP) subtype, and 2 patients had primary progressive (PP) MS. Then, patients were divided according to their disease activity in the last year before the recruitment [25]: 8 patients were considered active (active MS) based on clinical relapses and/or MRI findings (contrast-enhancing lesions; new or unequivocally enlarging T2 lesions), whereas 4 patients were judged stable, with no evidence of acute inflammation (non-active MS). The main demographic and clinical characteristics of all recruited subjects are summarized in **Table 1**.

All patients underwent clinical assessment, neuropsychological evaluation, brain MRI, ^{18}F-florbetapir PET, and lumbar puncture (LP). LP was always performed before starting any treatment (i.e. corticosteroids). All patients underwent brain MRI for diagnostic purposes and ^{18}F-florbetapir PET within a maximum of four weeks after brain MRI. For each recruited patient, we assessed the Expanded Disability Status Scale (EDSS) score [26] and we calculated the Bayesian Risk Estimate for MS at Onset (BREMSO). The BREMSO score has been created in order to assess an individual risk score calculated from demographic and clinical variables collected at disease onset. The higher the BREMSO score, the higher the risk of future disability [27].

The neuropsychological evaluation was assessed on the same day of the ^{18}F-florbetapir PET using the Italian translation of the Rao Brief Repeatable Battery (BRB-N) of Neuropsychological Tests in Multiple Sclerosis [28]. Tests were administered by a trained neuropsychologist in a standardized manner, during daytime, in a quiet room, and in a fixed order. The administration of the whole battery took about 30 minutes. The neuropsychological battery included: selective reminding test (SRT), consistent long term retrieval (SRT-CLTR), spatial recall test, oral symbol digit modalities test (SDMT), paced auditory serial addition test at 3 (PASAT3) and 2 seconds (PASAT2), selective reminding test–delayed recall (SRT-D), and spatial recall test-delayed. Neuropsychological tests included in the analyses were adjusted for age, gender,

and/or education, according to the Italian validation study [28].

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

CSF collection and Aβ determination

CSF samples were collected by LP in the L3/L4 or L4/L5 interspace. Following LP, 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. Albumin was measured by rate nephelometry. Oligoclonal bands (OCB) were evaluated by isoelectrofocusing. CSF Aβ1-42 was measured by using a commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kit (Fujirebio, Ghent, Belgium).

MRI acquisition

All patients underwent a MRI examination on Achieva 3T scanner (Philips, The Netherlands). The protocol included: 1) a T1-weighted scan (TR 9.90 ms; TE 4.61 ms; Flip angle 8°; slices thickness 1 mm; gap 0); 2) Fluid attenuated inversion recovery (FLAIR) images (TR 11000 ms; TE 125 ms; Flip angle 90°; slices thickness 1 mm; gap 0); 3) a T2-weighted scan (TR 2492 ms; TE 78 ms; Flip angle 90°; slices thickness 4 mm; gap 0).

PET acquisition

PET scans were obtained with a Biograph Truepoint 64 PET/CT scanner (Siemens, Erlangen, Germany). All patients underwent ^{18}F-florbetapir PET scanning at

rest after intravenous injection of 370 MBq. Patients were positioned comfortably in a quiet room for at least 50 min. Each acquisition included a CT transmission scan of the head (55 mAs lasting 10 s) followed by a 20-minute PET list-mode acquisition. PET sections were reconstructed with 4 frames of 5 minutes to verify the absence of movement of the patients during the acquisition and then with one frame of all 20 minutes in the form of transaxial images of 168 × 168 pixels of 2 mm, using iterative 3D TrueX algorithm with 8 iterations and 14 subsets, with a Gaussian filter with full width at half maximum of 4 mm, and corrected for scatter and for attenuation using density coefficients derived from the low-dose CT scan of the head obtained with the same scanner, with the proprietary software.

Neuroimaging Data Analysis

The statistical analyses were performed using Statistical Parametric Mapping (SPM12, Wellcome Department of Cognitive Neurology, London, UK). Using ImcCalc of SPM, Standardized uptake value (SUV) PET maps were derived as SUV= AC/(radiotracer dose/BW), where AC represents activity concentration in a given voxel [kBq/ml], radiotracer dose is the injected florbetapir dose corrected for residual activity in the syringe [MBq], and BW is the body weight [kg].

FLAIR- weighted images and SUV-PET images were coregistered to individual volumetric T1-weighted images.

To quantify the macroscopic WM lesion load, lesions were segmented by the lesion growth algorithm [29] as implemented in the Lesion Segmentation Tool (LST) toolbox version 2.0.15 (www.statistical-modelling.de/lst.html) for SPM. The algorithm first segments the T1 images into the main tissue classes. This information is then combined with the coregistered FLAIR intensities in order to calculate lesion belief maps. By thresholding these maps with a threshold K value of 0.2 (determined by visual

inspection of the results of the patients) an initial binary lesion map is obtained which is subsequently grown along voxels that appear hyperintense in the FLAIR image and the region of interest (ROI) for the DWM was created. For each dataset, the WM lesion load was calculated, visually inspected to exclude the presence of macroscopic artifacts and used for correlation analyses.

Lesions in T1-weighted images were previously filled using the lesion filling tool provided by LST toolbox. The lesion-filled T1-series images were segmented according to GM, WM and CSF tissue probability maps to generate the normalization deformation field into the Montreal Neurological Institute (MNI) space to be applied to the coregistered SUV-PET scan.

Using the WM and GM probability map of segmentation of the individual lesion-filled T1-images, we extracted the mean SUV of each patient's total WM by the coregistered SUV-PET images. To calculate the mean SUV of NAWM, the volume of interest (VOI) representing NAWM was calculated by subtracting the DWM VOI from the total WM VOI (**Fig. 1**).

GM was evaluated also using the Siemens PET Amyloid Plaque Quantification software of the package Molecular Imaging Neurology with syngo.via server, using the cortical ROI of anterior cingulated gyrus, orbital part of frontal lobe, superior parietal lobule, posterior cingulated gyrus, precuneus, temporal lobe, and of the average of these six regions.

The whole cerebellum was used as the reference region for the standardized uptake values relative ratios (SUVR).

Finally, to obtain brain volumetrics, brain segmentation using SPM12 was performed. For each scan, we derived the GM fraction, the total WM fraction, the NAWM fraction and the DWM fraction, calculated as the ratio of GM, total WM, NAWM and DWM volumes to total intracranial volume (TIV), respectively. Data were

subsequently converted to percentages.

Statistical analysis

All statistical analyses were performed using SPSS 21.0 for Windows (SPSS Inc., Chicago, IL, USA), Graph Pad PRISM 6.0 and SPM12.

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 (Mann-Whitney U test and Wilcoxon test for paired T-test). We used a paired t-test for the comparison of the amyloid tracer mean SUV between DWM and NAWM.

All correlation analyses were performed assessing the Pearson correlation coefficient. Linear regressions analyses between WM-SUV and NAWM volume as dependent variables and CSF Aβ levels as explanatory variable were performed. Each regression model was adjusted in order to control for the potential effect of age and gender.

For all analyses the statistical threshold was set to $p < 0.05$, except for correlation between NAWM-SUV and clinical assessment, which was set to $p < 0.025$ after Bonferroni correction for multiple comparisons ($\alpha = 0.05/2 = 0.025$), and for correlation between CSF Aβ levels and clinical assessment, which was set to $p < 0.016$ after Bonferroni correction for multiple comparisons ($\alpha = 0.05/3 = 0.016$).

RESULTS

The main CSF, PET and MRI findings are summarized in **Table 2** and in **Additional Table**.

Concerning the GM tracer uptake, all patients were categorized as amyloid negative by quantitative cut-off because their GM-SUVR average was less than 1.10

[30]. No correlation between CSF Aβ levels and GM-SUVR average was found (for all SUVR see **Additional Table**).

Concerning the WM tracer uptake, DWM-SUV was lower compared to NAWM-SUV (1.032±0.112 vs. 1.128±0.122; p=0.0005; **Fig. 2a**) in each subject. Comparing patients according to their clinical course (RR vs. SP/PP), no differences in NAWM-SUV and DWM-SUV were observed. Conversely, when dividing patients according to their disease activity (active group vs. non-active group), we found a reduced NAWM-SUV in active group compared to non-active group (1.077±0.119 vs. 1.231±0.023; p=0.028; **Fig. 2b**), whereas no significant differences in DWM-SUV were observed.

NAWM-SUV correlated with both total WM volume (r=0.73; p=0.006) and NAWM volume (r=0.82; p=0.01; **Fig. 3**), but not with DWM volume (r=-0.45; p>0.05) in all MS patients.

Focusing on the active group, CSF Aβ levels were lower in patients with WM-SUV less than 1.05 compared to those with WM-SUV greater than or equal to 1.05 (698.8±32.57 vs. 1033±226.4; p=0.029; **Fig. 4a**). Neither DMW-SUV nor NAWM-SUV correlated with patients' age. CSF Aβ levels correlated with NAWM-SUV (r =0.79; p=0.017; **Fig. 4b**). Particularly, the lower the CSF Aβ concentration, the lower the tracer uptake in the NAWM. The linear regression analysis showed CSF Aβ levels as a predictor of NAWM-SUV (r =0.79; p=0.01). NAWM-SUV correlated with NAWM volume (r =0.82; p =0.01; **Fig. 4c**), but not with DWM volume (r =-0.64; p>0.05). The multiple regression analysis showed NAWM volume as the best predictor of NAWM-SUV (r =0.87; p=0.007). CSF Aβ levels correlated with NAWM volume (r=0.81; p=0.01; **Fig. 4d**), but not with DWM volume (r =-0.36; p>0.05). The multiple regression analysis showed CSF Aβ levels as the best predictor of NAWM volume (r=0.81; p=0.007).

As regards clinical data, NAWM-SUV showed a trend towards significance in active patients as regards the correlation with BREMSO score (r =-0.75; p=0.03),

although data did not maintain the statistical significance after Bonferroni correction.

CSF Aβ levels correlated with PASAT2 test (r =0.85; p=0.007), whereas the correlations with BREMSO score (r=-0.73; p=0.04), EDSS score (r=-0.72; p=0.04) and PASAT3 test (r=0.71; p=0.04) lost their significance after Bonferroni correction.

DISCUSSION

In this study, we report WM-SUV data of amyloid PET in active and non-active MS patients with the aim of investigating possible differences between the two groups. Furthermore, to the best of our knowledge this is the first attempt to correlate CSF Aβ levels and amyloid PET in MS reported so far.

In line with previous studies [9,10], we find that the amyloid tracer uptake in the largest lesion of each patient is reduced compared to their NAWM, confirming the role of amyloid PET as a biomarker of myelin loss.

Moreover, we show that ^{18}F-florbetapir uptake in active patients is lower than in non-active patients, suggesting an interesting link between early WM damage and disease activity. A previous study described a more marked reduction in the amyloid tracer uptake in both DWM and NAWM in patients with progressive MS compared to RR MS patients [10]. Authors speculated that such findings may be associated with the reduced remyelination present in the progressive forms of the disease [10,31].

We find that active patients have a lower amyloid tracer uptake compared to non-active patients, regardless of their disease form, and that the lower uptake is not only in the DWM, but also in the NAWM.

The most interesting finding of our study is that the tracer uptake in the NAWM correlates with CSF Aβ levels: the lower the uptake, the lower the CSF Aβ concentration. We hypothesize that active patients, i.e. those with lower uptake in both DWM and NAWM and with lower CSF Aβ levels, may have a reduced capacity of

remyelination and consequently a higher risk of disease progression. To strengthen this hypothesis, we also find a lower uptake in the NAWM and lower CSF Aβ levels in those patients with a smaller NAWM volume.

We recently described a relationship between low CSF Aβ levels and worse prognosis in MS [22,23]. In line with these findings and hypothesis [22], the correlation we describe between amyloid tracer uptake and CSF Aβ concentration suggests that amyloid plays a role in the progression of WM damage in MS. Thus, ^{18}F-florbetapir PET may represent a marker of early WM damage and a useful prognostic tool. As part of this speculation, we had already hypothesized that lower CSF Aβ levels could be associated with a decreased ability to remyelinate CNS axons, with an early WM and GM damage, resulting in a higher probability of disease progression [22]. Nevertheless, the exact role played by Aβ remains to be determined. Further studies are necessary to draw a definitive picture.

There are some limitations when considering our study. First, we acknowledge that this represents an exploratory study and that a larger cohort of patients will be needed to confirm our findings. The number of patients included was unfortunately limited due to the removal of trade of the radiopharmaceutical 18F-florbetapir in Europe in January 2018. Second, all patients underwent 18F-florbetapir PET scanning following an acquisition protocol validated for AD patients to assure the absence of movements during the acquisition. All patients underwent 18F-florbetapir PET scanning following an acquisition protocol validated for AD patients to assure the absence of movements during the acquisition. Our study was not aimed to optimise the protocol for MS patients, but rather to apply the best protocol used to 18F-florbetapir PET acquisition. Because all patients, including those with active disease, were very cooperating and none of them moved their head during the entire acquisition protocol, static images using for measurements were always the 20-minute ones.

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In conclusion, this study provides evidence on the role of amyloid PET in the assessment of MS, particularly in relation to disease activity and early prognosis. Moreover, these findings suggest a predictive role of CSF Aβ levels in MS. A replication in a larger cohort of patients is required to confirm these preliminary data.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest: the authors declare that they have no conflict of interest.

Statement of human rights: all procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards

Statement of the welfare of animals: This article does not contain any studies with animals performed by any of the authors.

Informed consent: informed consent was obtained from all individual participants included in the study.

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FIGURE LEGENDS

Fig. 1: **a)** T1-weighted MRI; **b)** coregistrated ^{18}F-florbetapir PET and **c)** T1-weighted MRI/PET fusion images (using the hot iron map); **d)** T2-weighted FLAIR MRI; **e)** lesion elaboration from Lesion Segmentation Tool (yellow); **f)** normal appearing white matter (blue) and damaged white matter (red) segmentation on T2-weighted FLAIR MRI.

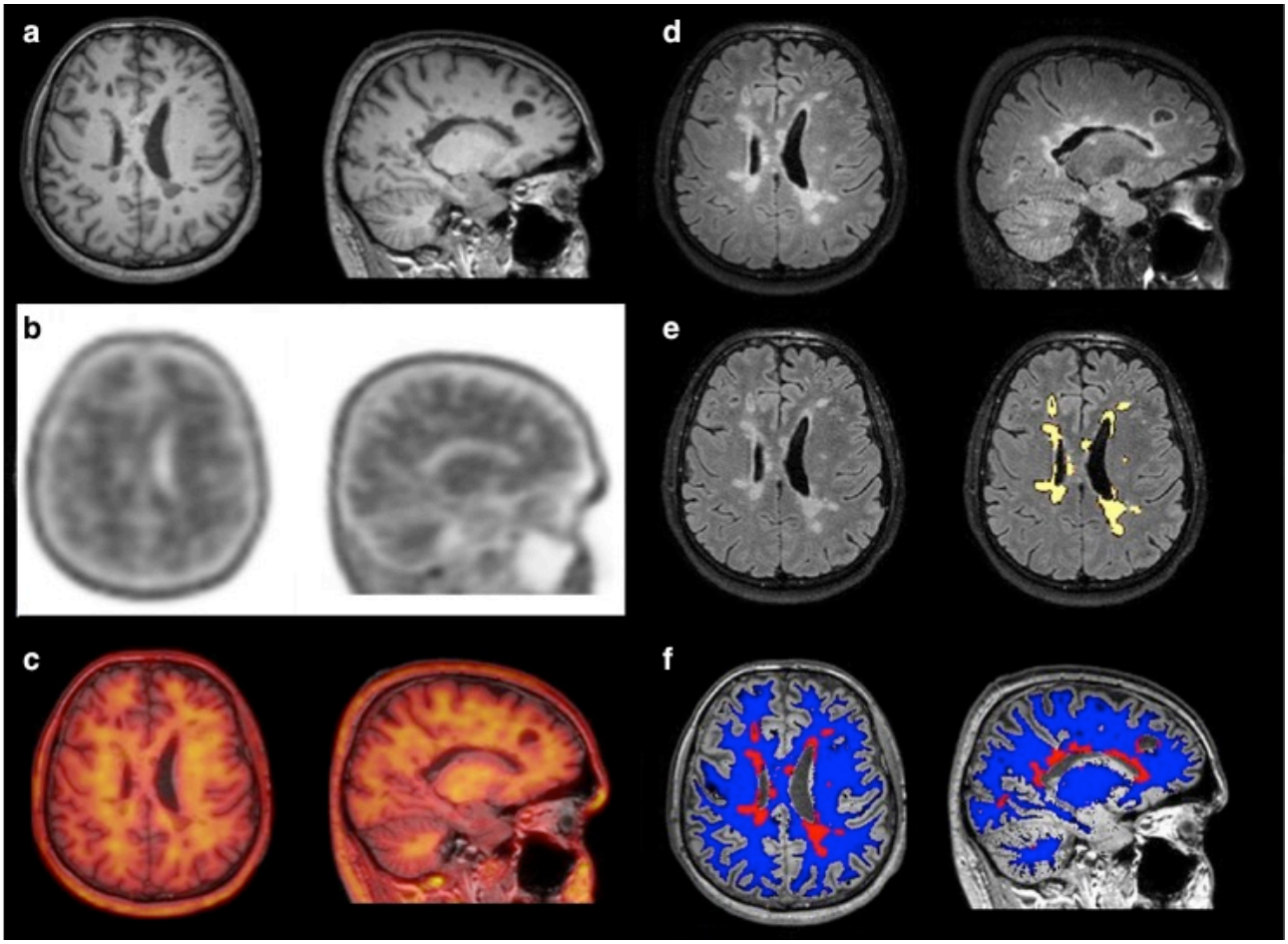

Fig. 2: **a)** comparison of the amyloid tracer mean standard uptake value (SUV) in damaged white matter (DWM) and normal appearing white matter (NAWM) of all patients (p=0.0005). **b)** comparison of the amyloid tracer mean SUV in the NAWM of active and non-active patients (p=0.028).

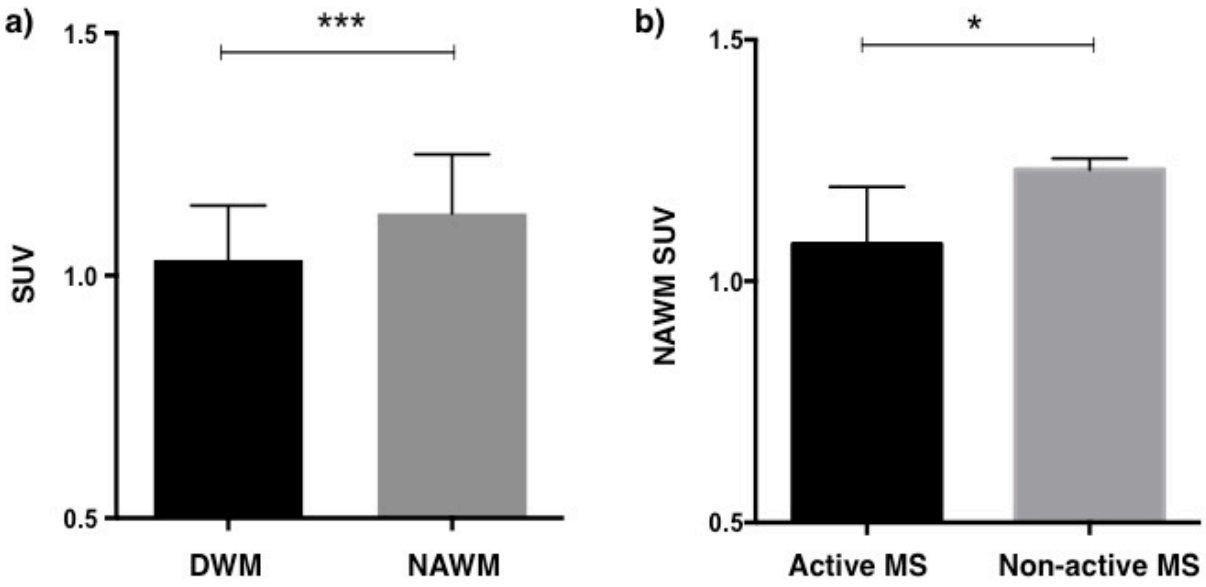

Fig. 3: correlation between the normal appearing white matter (NAWM) amyloid tracer mean standard uptake value (SUV) and NAWM volume in all patients. NAWM volume is expressed as percentage, calculated as the ratio of NAWM volume to total intracranial volume, per cent (r=0.82; p=0.01).

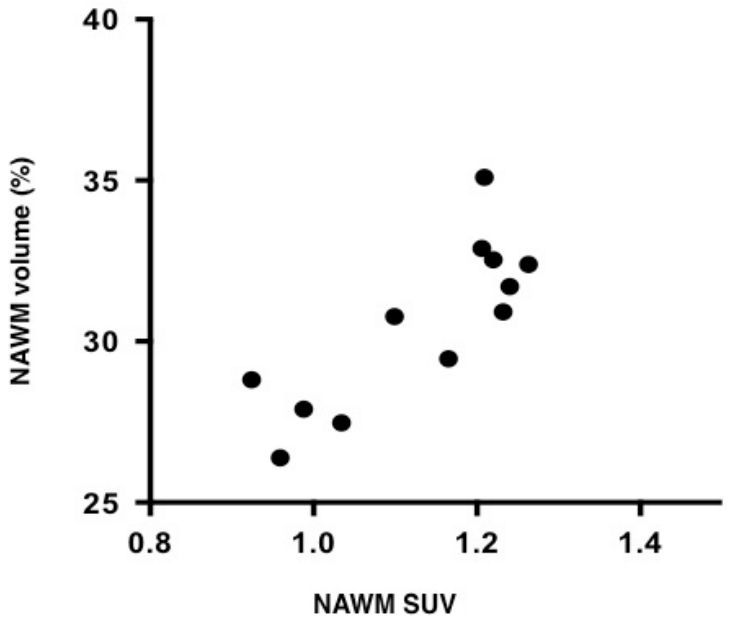

Fig. 4: **a)** comparison of CSF levels of β-amyloid in active patients according to their normal appearing white matter (NAWM) amyloid tracer mean standard uptake value (SUV) (p=0.029). **b)** correlation between the NAWM amyloid tracer mean SUV and the CSF levels of β-amyloid in active patients (r =0.79; p=0.017). **c)** correlation between the NAWM amyloid tracer mean SUV and the NAWM volume in active patients (r =0.82; p =0.01). **d)** correlation between CSF levels of β-amyloid and the NAWM volume in active patients (r=0.81; p=0.01). NAWM volume is expressed as percentage, calculated as the ratio of NAWM volume to total intracranial volume, per cent.