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# Dual Phase Amyloid PET in Newly Diagnosed Multiple Sclerosis: Exploring Its Potential as a Biomarker of Myelination and Neurodegeneration

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Article

# Dual Phase Amyloid PET in Newly Diagnosed Multiple Sclerosis: Exploring Its Potential as a Biomarker of Myelination and Neurodegeneration

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## Abstract

**Background:** Amyloid positron emission tomography (PET) has been proposed as a tool to monitor myelination in multiple sclerosis (MS). We present the first study to include both early and standard phases of amyloid PET in patients with newly diagnosed MS. **Methods:** Prospective study including patients with newly diagnosed MS (Jan 2023–Feb 2024). Clinical evaluation includes neurological, disability (EDSS) and neuropsychological assessment. Brain MRI, early <sup>18</sup>F-florbetaben (FBB) PET (eFBB; 0–5', 0–10' p.i.), and standard FBB PET (sFBB; 90' p.i.) were acquired. Normal-appearing white matter (NAWM) and damaged (DWM) in MRI were segmented and co-registered with PET images. Results are presented as standardized uptake values (SUV), ratio using cerebellum as the reference region (SUVR) and percentage of change between DWM and NAWM. **Results:** Twenty patients were included (35.05±10.72 years; 75% women). We found, both “eFBB and sFBB acquisitions” a lower mean SUVR<sub>max</sub> and SUVR<sub>mean</sub> and higher mean SUVR<sub>min</sub> in DWM compared to NAWM (p<0.001) in all patients. SUV parameters in both DWM and NAWM from eFBB and sFBB PET correlated with the number of relapses and EDSS (r = -0.454 and r = -0.446, respectively; p < 0.05). Additionally, SUVR values in DWM during eFBB correlated with cognitive impairment (SDMT; r = -0.516, p < 0.01), fatigue (MFIS-5; r = -0.450, p < 0.05), and quality of life (EQ-5D; r = -0.490, p < 0.05). **Conclusion:** Quantitative analysis of dual phase FBB PET demonstrates differential uptake between DWM and NAWM, probably associated with demyelination and neurodegeneration. These preliminary findings suggest that amyloid PET may have predictive value for disease activity and progression, supporting its potential as a biomarker in MS.

**Keywords:** amyloid PET; multiple sclerosis; biomarker; myelination; neurodegeneration

## 1. Introduction

Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system characterized by inflammatory demyelinating and axonal injury, leading to secondary neurodegeneration [1]. Its estimated prevalence ranges from 5 to 300 cases per 100,000 individuals,

affecting approximately 2.8 million people globally. MS typically begins between 20 and 40 years of age, is more frequent in women (female-to-male ratio 3:1), and remains the leading cause of non-traumatic neurological disability in young adults in Europe and the United States [2]. The pathological hallmark includes focal white matter (WM) lesions with inflammation and demyelination. These lesions evolve into variable chronic states, including remyelination, unresolved inflammation, or persistent demyelination with axonal loss [1].

Relapsing–remitting MS (RRMS) is the most frequent initial phenotype (approximately 85%), characterized by clinical relapses with variable recovery, interspersed with periods of remission and disability progression independent of relapses [3]. Around 20% of RRMS patients later develop secondary progression, and approximately 15% present with progressive disease from onset. Progressive forms are associated with worse outcomes, greater disability, and fewer therapeutic options [1,3].

Disability accumulation in MS is primarily driven by inflammatory activity and neurodegenerative progression [4]. Consequently, several clinical, radiological, and biochemical tools have been developed for disease monitoring [5]. Clinically, the Expanded Disability Status Scale (EDSS) remains widely used. Brain magnetic resonance imaging (MRI) enables detection of demyelinating lesions and is essential for diagnosis and follow-up [1,5]. In recent years, neurofilament light chain (NfL) has emerged as a promising fluid biomarker associated with inflammatory activity and progression risk [6]. However, both MRI-based advanced imaging markers and NfL present limitations, such as complex post-processing images requirements or biological variability influenced by comorbidities and individual factors [6–8]. Therefore, complementary biomarkers are needed to enhance the accuracy of risk stratification and personalized treatment decisions.

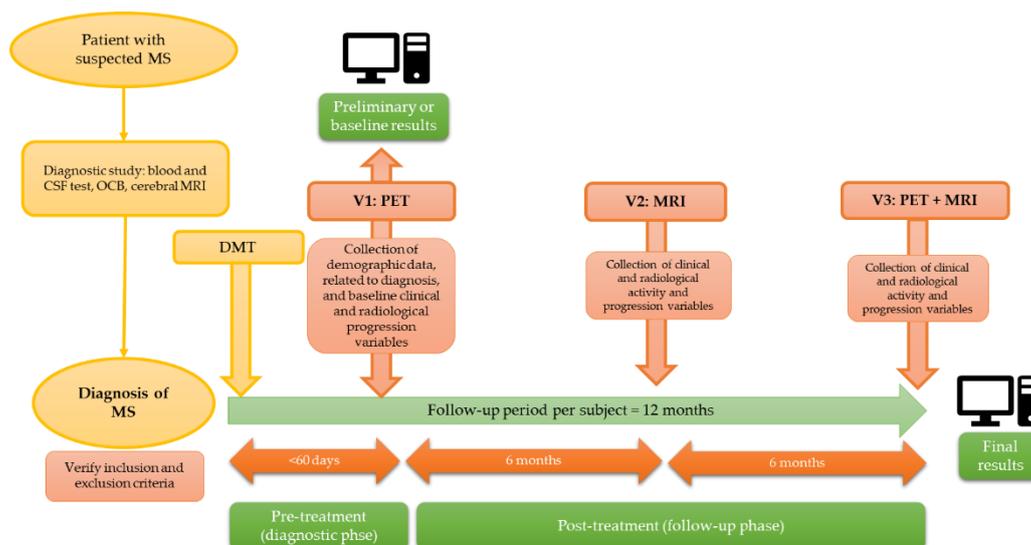
Amyloid positron emission tomography (PET) imaging has been proposed as a noninvasive technique for assessing myelin integrity in MS patients [9]. Amyloid tracers display selective affinity for aggregated  $\beta$ -sheet structures, such as the myelin basic protein, which is abundant in WM. When myelin is damaged, the loss of  $\beta$ -sheet conformation leads to reduced tracer binding [10]. Initial studies using amyloid PET in MS demonstrated lower uptake in damaged white matter (DWM) compared to normal-appearing white matter (NAWM) in both MS patients and healthy controls [11]. Subsequent investigations also detected reduced tracer uptake in MRI-defined NAWM, particularly in patients with active RRMS and progressive phenotypes, when compared to stable RRMS cases [9,12,13]. Moreover, lower baseline amyloid uptake in NAWM has been associated with a higher risk of cognitive decline and greater disability burden [14,15].

However, most existing studies are limited by cross-sectional designs, heterogeneous patient cohorts, not including newly diagnoses patients, and not analysing the early PET phase [9,12–14,16]. Here, we present the first study to include both early and standard phases of amyloid PET in patients with newly diagnosed MS. The primary objective is to evaluate its potential role as a biomarker of disease activity and progression risk.

## 2. Materials and Methods

### *Study Design and Population*

We conducted a multicenter and prospective study in subjects with a recent diagnosis of RRMS, divided into two phases: a pre-treatment or diagnostic phase, in which the correlation between PET findings and baseline variables was analyzed; and a post-treatment or follow-up phase, involving a new follow-up PET scan at 12 months, in which the correlation between baseline and follow-up PET findings and clinical/radiological markers of disease activity and progression was assessed. This article presents preliminary findings from the pre-treatment phase (Figure 1).



**Figure 1.** Baseline and follow-up period for the included participants. OCB (oligoclonal bands); MS (Multiple sclerosis); CSF (cerebrospinal fluid); PET (positron emission tomography); MRI (magnetic resonance imaging); DMT (disease-modifying treatment); V1-3 (sequential visits: V1: baseline; V2: 6-month; V3: 12-month visit).

A comprehensive and consecutive recruitment of patients was carried out after being diagnosed with RRMS (according to the 2017 revised McDonalds criteria), from January 2023 to March 2024, in two public hospitals in southern Spain (Hospital Universitario Clínico San Cecilio and Hospital Universitario Virgen de las Nieves in Granada) [17]. The inclusion and exclusion criteria for MS patients were listed in Supplementary table S1.

#### *Clinical and Cognitive Assessments*

Demographic, clinical, radiological, and neuropsychological variables were collected from each subject during the pre-treatment (diagnostic) phase and subsequently during the post-treatment phase. The study variables are summarized in Supplementary table S2.

Each subject underwent a clinical examination, including the assessment of physical disability through the Expanded Disability Status Scale (EDSS), the Timed 25-Foot Walk (T25FW) and the Nine-Hole Peg Test (9HPT) to assess walking speed and upper extremity dexterity, respectively [5,18]. Cognitive and neurobehavioral evaluation was assessed including evaluation of information processing speed, verbal memory and learning and visual memory through the Symbol Digit Modalities Test (SDMT), California verbal learning test (CVLT) and the brief visuospatial memory test (BVMT), respectively [19]. Additional tests included in the neuropsychological battery are summarized in Supplementary table S2. Each patient also completed Modified Fatigue Impact Scale (MFIS-5), the Beck Depression Inventory (BDI-II) to assess depressive symptoms and EuroQoL (EQ-5D) for the health-related quality of life [20].

Clinical assessments and scale administration were conducted by neurologists from the Demyelinating Diseases Units of the participating hospitals, who were blinded to data collection and analysis. Neuropsychological evaluation and the BICAMS battery were administered by a neuropsychologist.

#### *Brain Magnetic Resonance Imaging*

All patients underwent two MRI examinations: one at baseline and one during follow-up. Both diagnostic and follow-up MRI studies were performed on a Philips Ingenia CX 3T system, with a total acquisition time of approximately 20 minutes. Sequence-specific MRI parameters are detailed in Supplementary table S3. All the three-dimensional sequences were acquired with an isotropic voxel size. The images obtained were evaluated by a neuroradiologist with 3 years of experience,

considering target lesions those with enhancement or with high signal in T2/FLAIR and a diameter above 3 mm, with a maximum of 5 lesions of each type per patient.

#### *Positron Emission Tomography with $^{18}\text{F}$ -florbetaben*

Amyloid PET images were acquired in a Siemens Biograph Vision 600 Positron Emission Tomography/ Computed Tomography (PET/CT) digital scanner. The tracer used,  $^{18}\text{F}$ -florbetaben (FBB) (Neuraceq; Piramal Pharma), was administered intravenously at a mean dose of 300 MBq. Amyloid PET was acquired with a dual-point protocol including two acquisitions: early FBB PET (eFBB; 0–5', 0–10' p.i.), and standard acquisition FBB PET (sFBB; 90' p.i.). Specific details about the imaging protocol are given in Supplementary table S4.

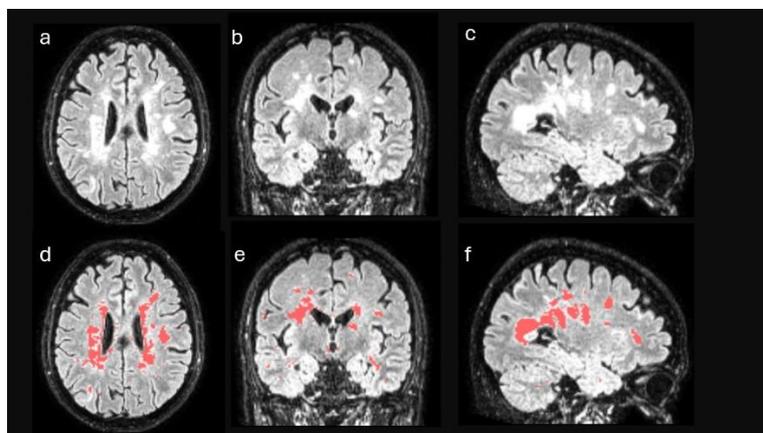
#### *Neuroimaging Preprocessing and Analysis*

Image processing was performed using a structured workflow, which included lesion segmentation, spatial registration of the data and calculation of Standardized Uptake Value (SUV) values in different brain regions.

First, structural MRI FLAIR images of each patient were processed in order to identify disease-specific lesions. These images were registered to the standard Montreal Neurological Institute (MNI) space and segmented using the unified segmentation algorithm implemented in Statistical Parametric Mapping (SPM), version 12 [21]. After the process, the gray matter and white matter of each patient were selected in MNI space, discarding the remaining tissues. From these data and using a semi-supervised thresholding procedure, a mask with the lesions of each patient was generated (Figure 2). Using these masks, the lesion volume of each patient was obtained, both globally in the whole brain and in isolation in the WM. Likewise, the masks were used to estimate the volume of the non-damaged white matter of each patient, that is, the white matter excluding the areas affected by the disease.

On the other hand, Amyloid PET images were spatially registered to MNI space to ensure anatomical correspondence with the processed MRI images. The registration was performed using rigid alignment transformations and nonlinear alignment transformations, optimized to preserve the anatomical relationship between modalities [22].

Once the spatial registration was completed, we proceeded to the quantitative analysis of the PET images by calculating SUV in different regions of interest (ROIs). ROIs were defined based on the masks obtained in the previous steps and comprised the following: DWM, NAWM, non-damaged gray matter (GM), total GM, and cerebellum. The gray matter and WM masks were derived from two complementary sources: the segmentation of FLAIR MRI images and the segmentation of structural T1 MRI images from the same patients, allowing to compare the robustness of both approaches.



**Figure 2.** DWM segmentation of FLAIR MRI images. Axial (a), coronal (b), and sagittal (c) slices of the FLAIR MRI sequence. Following a semi-supervised thresholding procedure, a lesion mask was generated for each patient. Images (d), (e), and (f) show the segmentation (in red) of the damage white matter.

The following SUVs were calculated for both the DWM and the NAWM:

- SUVmax (Maximum Standardized Uptake Value): The highest SUV value within a defined region of interest (ROI). It reflects the point of greatest radiotracer uptake and is commonly used as an indicator of peak metabolic activity.
- SUVmean (Mean Standardized Uptake Value): The mean SUV within the ROI, representing the average value of all voxel SUV measurements. It provides a robust measure that is less influenced by outliers compared to SUVmax.
- SUVmin (Minimum Standardized Uptake Value): The lowest SUV value within the ROI. While less frequently used on its own, it can be helpful in assessing areas of low tracer uptake or evaluating lesion heterogeneity.
- SUVR (SUV relative to the cerebellum): ratio using cerebellum as the reference region.
- % of change SUV: Percentage of change between DWM and NAWM calculated, according to previous studies, as follows: DWM uptake minus NAWM uptake, divided by NAWM uptake and multiplied by 100 [9].

The delineation of the cerebellum was performed from the Automated Anatomical Labeling (AAL) anatomical atlas, registered to the same MNI space [23]. All masks were adjusted to the resolution and dimensions of the PET images before the calculation of SUVs, to avoid interpolation errors or incorrect overlapping between modalities.

#### *Standard Protocol Approvals, Registrations, and Patient Consents*

This study was performed in accordance with Good Clinical Practice and the Helsinki Declaration and was approved by the Biomedical Research Ethics Committee of the Province of Granada, under the code AMPET-MS22 (107-N-22). Informed consent was obtained from all study participants after reviewing the inclusion and exclusion criteria.

#### *Statistical Analysis*

Descriptive data are shown as means  $\pm$  standard deviations or as frequencies and percentages. To compare the SUV parameters in different regions within the same patient, a paired Student's T-test was used after confirming normal distribution with the Kolmogorov-Smirnov test; for non-normally distributed variables, the Wilcoxon signed-rank test was applied. A repeated measures ANOVA was performed to evaluate the evolution of SUV parameters from the early phases (5 and 10 minutes) to the standard phase. The assumption of sphericity was tested using Mauchly's test and found to be violated; therefore, the Greenhouse-Geisser correction was applied. Correlations between neuroimaging measures and clinical and neuropsychological tests were calculated using Spearman's coefficient. SPSS 29.0 (IBM SPSS, Armonk, NY, USA) and R version 4.2.0 (R Foundation for Statistical Computing, Vienna, Austria) were used for data analyses. Statistical significance was set at  $p < 0.05$ .

### 3. Results

#### *3.1. Baseline Clinical and Neuroimaging Characteristics*

Between January 2023 and February 2024, 20 patients were included (75% female), with a mean age at diagnosis of  $35.05 \pm 10.72$  years. The mean number of relapses and EDSS score at diagnosis were  $1.95 \pm 1.15$  and  $1.90 \pm 1.09$  (median 2), respectively (Table 1). Additional baseline demographic, clinical and neuropsychological variables are summarized in Supplementary table S5.

**Table 1.** Clinical-demographic characteristics and neuropsychological testing of the study cohort.

Characteristic (n= 20)	Mean (SD) or n (%)
MS onset age (y)	35.05 (10.72)
Sex	
Male	5 (25)

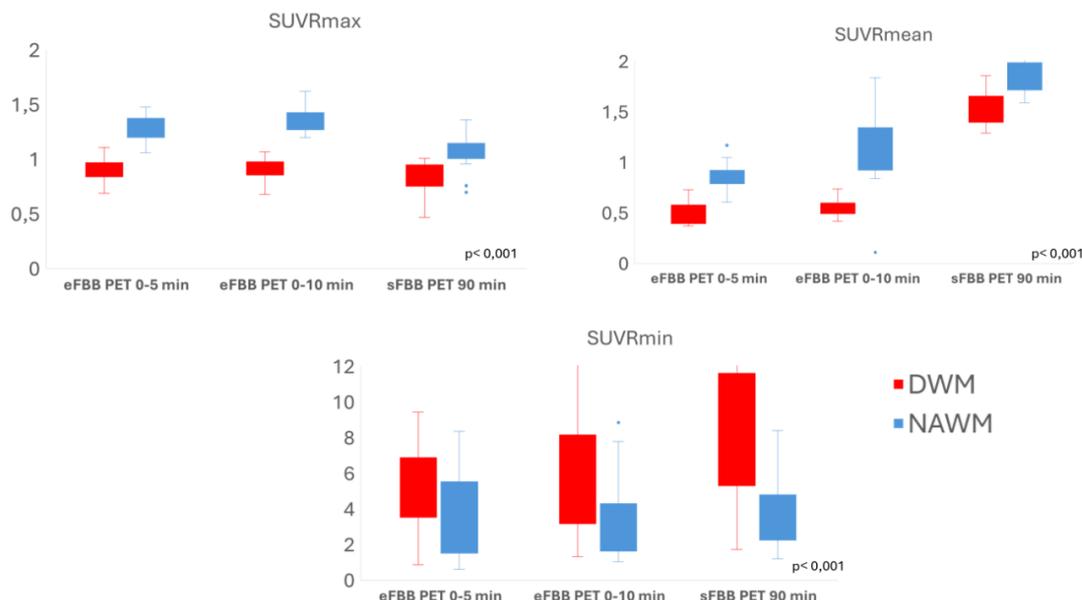
Female	15 (75)
Initial clinical presentation	
Optic neuritis	6 (30)
Myelitis	7 (35)
Hemispheric syndrome	2 (10)
Brainstem syndrome	5 (25)
N° of relapses	1.95 (1.15)
EDSS score	1.90 (1.09)
Other progression disease scales	
T25FW (seconds)	5.62 (1.19)
9HPT-D (seconds)	23.66 (5.06)
9HPT-ND (seconds)	24.07 (3.49)
Fatigue: MFIS-5 score	8.95 (6.37)
Cognitive functions	
SDMT (z-score)	-1.13 (0.96)
CVLT-II (z-score)	-1.35 (1.18)
BVMT (z-score)	-0.68 (1.51)
Beck Depression Inventory-II (points)	16.55 (12.97)
Quality of life: EQ-5D (points)	68.75 (22.35)

SD: standard deviation; EDSS: Expanded Disability Status Scale; T25FW: Timed 25-foot Walk, 9HPT-D: 9-hole Peg dominant side, 9HPT-ND: 9-hole Peg non-dominant side Test, MFIS: Modified Fatigue Impact Scale; SDMT: Symbol Digit Modalities Test; CVLT-II: California Learning Verbal Test – Second Edition; BVMT-R: Brief Visuospatial Memory Test-Revised; PVF: phonemic verbal fluency; SVF: semantic verbal fluency; EQ-5D: index and visual analogy scale.

In the neuroimaging analysis, patients showed a mean ( $\pm$  standard deviation) MRI volume ( $\text{cm}^3$ ) of  $13.94 \pm 14.98$  in DWM,  $540.98 \pm 38.59$  in NAWM, and  $968.63 (\pm 72.70)$  in GM. Regarding DWM lesion load, 2 patients had a low lesion burden ( $\leq 9$  lesions on T2/FLAIR), 8 had a high non-confluent lesion burden ( $>9$  lesions on T2/FLAIR), and 10 presented with a high confluent lesion burden ( $>9$  lesions on T2/FLAIR with extensive, confluent and/or uncountable lesions). Coregistration of MRI and FBB PET yielded a total of 54 variables derived from the analysis of SUV parameters in DWM and NAWM across both phases of the FBB PET. These are summarized in Supplementary Table S6.

### 3.2. Comparison of Tracer Uptake Intensity in DWM Versus NAWM

The mean SUV<sub>max</sub> and SUV<sub>mean</sub> values in DWM were significantly lower than those in NAWM in both eFBB and sFBB of FBB PET acquisition. However, the mean SUV<sub>min</sub> values in DWM were significantly higher than those in NAWM. Similarly, DWM showed significantly lower SUVR<sub>max</sub> and SUVR<sub>mean</sub> (cerebellum-referenced SUV<sub>max</sub> and SUV<sub>mean</sub>, respectively) compared to NAWM, while SUVR<sub>min</sub> values were higher in DWM than in NAWM (Figure 3; Supplementary table S7).



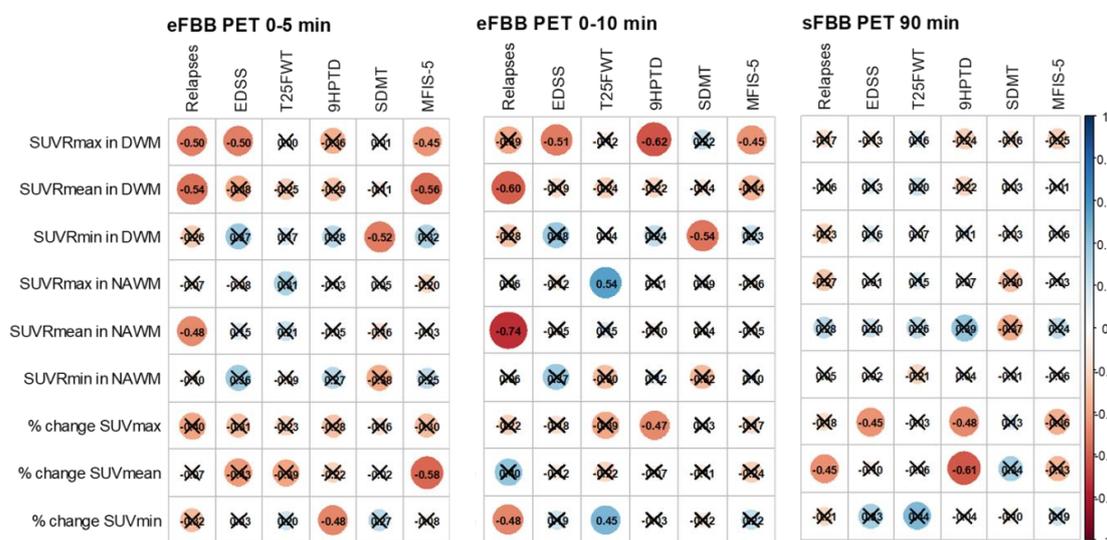
**Figure 3.** Box plot showing the comparative analysis of the Standardized Uptake Value ratios (SUVR) between damage white matter (DWM) and normally-appearing white matter (NAWM) in the early (eFBB) y standard (sFBB) phases of <sup>18</sup>F-florbetaben (FBB) PET.

3.3. Comparison of SUVR Values in DWM and NAWM Between Early and Late FBB PET Phases

Repeated-measures analysis revealed that mean SUVR values in both DWM and NAWM, as well as the % of change between DWM and NAWM (% of change SUV), changed significantly from eFBB to sFBB acquisition. The SUVRmax values increased during eFBB and then decreased in the sFBB, while SUVRmean and SUVRmin showed a continuous upward trend from eFBB to sFBB, predominantly in DAWM (Table S8; Figure S1, Supplementary Material).

3.4. Correlations Between FBB PET Quantitative Parameters and Clinical/Neuropsychological Scales

Correlations with clinical variables related to disease activity, progression, and neuropsychological function are summarized in Figure 4 and Supplementary table S9. The number of relapses within one year showed a significant negative correlation with SUVRmean values in both DWM and NAWM in the eFBB ( $r = -0.541$  and  $r = -0.596$ , respectively;  $p < 0.05$ ), and with the % of change SUVmean in the sFBB phase ( $r = -0.454$ ,  $p < 0.05$ ).



**Figure 4.** Correlogram visualizing correlation between SUV parameters calculated in eFBB and standard sFBB phases of FBB PET and clinical variables related to disease activity and progression. Correlation with  $p$ -value  $> 0.05$  are considered insignificant and are indicated with a cross (X) over the corresponding circle. Color and size of the circles are proportional to the correlation coefficients. Positive correlations are shown in blue (different shades; dark blue with the strongest correlation) whereas negative correlations in red (ranging from light red to red; dark red with the weakest correlation).

EDSS scores were significantly and negatively correlated with SUVRmax in DWM in the eFBB phases ( $r = -0.501$  and  $r = -0.513$ ,  $p < 0.05$ ) and with the % of change SUVRmax in DWM in the sFBB phase ( $r = -0.446$ ,  $p = 0.049$ ) (Supplementary Figure S2). Manual dexterity, as measured by the 9HPTD, was significantly and inversely correlated with the % of change SUVRmax in both eFBB and sFBB ( $r = -0.472$  and  $r = -0.477$ ,  $p < 0.05$ , respectively). Gait speed, assessed using the T25FW, was positively correlated with the % of change SUVRmin in both eFBB and sFBB ( $r = 0.453$  and  $r = 0.440$ ,  $p < 0.05$ , respectively).

Regarding neuropsychological evaluation, a significant negative correlation was found between information processing speed (SDMT) and SUVRmin in DWM in eFBB phases ( $r = -0.516$  and  $r = -0.539$ ,  $p < 0.05$ , respectively). Verbal memory and learning, measured by the CVLT, also showed significant negative correlations with SUVRmin in both DWM and NAWM in eFBB ( $r = -0.564$  and  $r = -0.649$ , respectively;  $p < 0.05$ ). Symptoms of depression (BDI-II), were significantly and positively correlated with SUVRmin in DWM in eFBB ( $r = 0.570$  and  $r = 0.444$ ,  $p < 0.05$ , respectively).

Fatigue, as assessed using the MFIS-5, showed a significant negative correlation with SUVRmax in DWM in the eFBB phases ( $r = -0.450$ ,  $p < 0.05$  at both 5 and 10 minutes). Finally, quality of life (EQ-5D) was negatively correlated with SUVRmin in DWM in eFBB phases ( $r = -0.468$  and  $r = -0.490$ ,  $p < 0.05$ ).

#### 4. Discussion

Amyloid PET has been proposed to monitor myelination changes in patients with MS, showing reduced SUVR in DWM compared to NAWM on MRI in MS patients [11]. Greater NAWM demyelination has been observed in more active patients or progressive forms, and lower baseline SUVR in NAWM has been associated with increased risk of disability progression (EDSS) and cognitive impairment (SDMT) [9,12–15].

We present preliminary findings from our study, based on co-registration of FBB PET and MRI, and their association with clinical variables at diagnosis. Our work is the first to include a homogeneous cohort of newly diagnosed RRMS patients [9,12–16]. This is particularly relevant, as acute inflammatory activity is highest during the early stages of MS, allowing us to analyze baseline PET findings and identify patients at greater risk of long-term disease activity and progression [24]. Finally, this is the first work to physiologically interpret the eFBB and the SUV parameters, exploring their potential utility in detecting neurodegeneration- and inflammation-related changes [25].

##### *Myelination and Neurodegeneration*

At diagnosis, RRMS patients showed lower SUVRmax and SUVRmean values in DWM compared to NAWM. These findings can be explained by several factors: the affinity of amyloid tracers for  $\beta$ -sheet structured protein such as myelin basic protein; the greater degree of demyelination observed in DWM in pathological studies; and similar results from previous amyloid PET studies in MS, which demonstrated lower tracer uptake in DWM areas compared to NAWM in both MS patients and healthy controls [9–11,26]. Altogether, these results reinforce the potential use of FBB PET to detect dynamic in vivo changes in myelination.

On the other hand, our study found that SUVRmin was higher in DWM compared to NAWM. This finding has not been previously described, as most studies have focused on SUVRmax and the % of change SUV between DWM and NAWM, with lower values associated with greater demyelination [9,14,15]. Several studies suggest a potential role for amyloid in the pathogenesis of

MS, based on the detection of amyloid precursor protein within axons of demyelinating and remyelinating plaques, and the presence of soluble amyloid- $\beta$ 1-42 oligomers (A $\beta$ ) in the brain and cerebrospinal fluid (CSF) of MS patients [25,27]. A study including 60 MS patients with a 3–5-year follow-up found that lower CSF A $\beta$  levels were associated with a higher risk of disease progression as measured by EDSS [28]. Similarly, in a cohort of 103 RRMS patients, CSF A $\beta$  levels were associated with increased proinflammatory cytokines and decreased anti-inflammatory molecules [29]. Therefore, SUVR<sub>min</sub> may be interpreted as the basal or minimal detectable SUV value, reflecting amyloid accumulation secondary to axonal damage and/or inflammation. This hypothesis is consistent with our findings, as a greater degree of neurodegeneration would be expected in DWM compared to NAWM. Therefore, SUVR<sub>min</sub> may be valuable for identifying states of neurodegeneration, with or without concurrent inflammation.

#### *Early Phase of Amyloid PET*

This is the first study to include the eFBB in patients with MS. Based on our findings, we propose an interpretation grounded in the temporal evolution of SUVR values from the eFBB to the sFBB (Table S8; Figure S1, Supplementary Material). SUVR<sub>max</sub> tends to decrease from eFBB to sFBB, which would be the expected evolution considering the tracer's pharmacokinetics, with lower SUVR in sFBB likely reflecting greater demyelination [9,15].

In contrast, both SUVR<sub>mean</sub> and SUVR<sub>min</sub> exhibit an increasing trend from eFBB to sFBB. These findings are more challenging to interpret, as no previous studies have assessed both eFBB and sFBB of amyloid PET in MS patients. Initially our hypothesis was that eFBB uptake could be related to the inflammation activity (increased perfusion and oedema), but our results are in consonance with the emerging evidence suggesting that eFBB may reflect cerebral hypometabolism associated with neurodegeneration and synaptic dysfunction, in a manner similar to [<sup>18</sup>F]FDG PET [30,31]. In a study including 103 patients with cognitive impairment due to Alzheimer's disease and 33 healthy controls, eFBB showed a strong correlation with [<sup>18</sup>F]FDG PET for the detection of regional hypometabolism in dementia patients [32]. Therefore, the lower SUVR<sub>mean</sub> and SUVR<sub>min</sub> in the eFBB in our study suggest that eFBB could be a useful approach for detecting cerebral hypometabolism associated with neurodegeneration in MS patients.

#### *Correlation with Clinical Variables Related to Disease Activity and Progression*

A higher number of relapses in the year prior to diagnosis was associated with greater demyelination and neurodegeneration in DWM and NAWM, reflected by lower % of change SUV<sub>mean</sub> in sFBB and SUVR<sub>mean</sub> in eFBB. These findings are consistent with those reported by Pietroboni et al., who identified greater NAWM demyelination in patients with higher acute inflammatory activity (defined by a higher relapse rate and the presence of new MRI lesions) [13].

Higher disability scores, as measured by the EDSS, were also associated with increased demyelination and neurodegeneration in DWM, reflected by lower % of change SUV<sub>max</sub> in sFBB and lower SUVR<sub>max</sub> in eFBB. These results support previous studies linking greater demyelination to higher EDSS scores and in progressive forms of MS compared to RRMS [9,12,15]. Moreover, our data show that reduced manual dexterity (9HPTD) was associated with increased demyelination and neurodegeneration (lower % of change SUV<sub>max</sub> in both eFBB and sFBB), while impaired gait function (T25FW) was linked to neurodegeneration (lower % of change SUV<sub>min</sub> in both eFBB and sFBB). These findings support the association between FBB PET-detected demyelination and neurodegeneration with higher scores on disability progression scales.

In terms of cognition, previous studies have reported an increased risk of cognitive impairment—particularly in visuospatial function and working memory—in patients with lower white matter tracer uptake [15]. In our study, we also found that reduced processing speed (SDMT), verbal memory, and learning (CVLT) were associated with increased neurodegeneration in DWM and NAWM, reflected by higher SUVR<sub>min</sub> in eFBB. Additionally, greater depressive symptoms, as measured by the BDI-II, were correlated with increased neurodegeneration in both DWM and

NAWM (higher SUVR<sub>min</sub> in eFBB). Finally, fatigue scores using the MFIS-5 were higher in patients with more demyelination and neurodegeneration in DWM (lower SUVR<sub>max</sub> in eFBB).

Study limitations include the interpretation of eFBB findings and the various SUV parameters, given the absence of prior studies including and analysing them. Nevertheless, prior research supports the use of eFBB for detecting cerebral hypometabolism associated with neurodegeneration in other neurological diseases [31,32]. We did not include a control group, although this was not the primary objective of our study, as previous work has already demonstrated differences compared to healthy controls [12,14,15]. The sample size is limited, but comparable to other studies ( $n < 30$ ), and this pilot study aims to evaluate the clinical utility of FBB PET in newly diagnosed MS patients, with the future goal of expanding the patient cohort. Patients with progressive forms of MS were not included, as all participants had RRMS at diagnosis. However, longitudinal follow-up will identify those who evolve to progressive forms and assess whether FBB PET can predict the risk of progression.

## 5. Conclusions

MS remains the leading cause of non-traumatic neurological disability in young adults, and there is a growing need for complementary biomarkers to improve risk stratification and guide personalized therapeutic strategies. This pilot study is the first to evaluate dual-phase FBB PET in newly diagnosed MS patients, including early-phase analysis and distinct SUV parameters.

Preliminary findings suggest that quantitative analysis of dual phase FBB PET can detect differential uptake between DWM and NAWM in newly diagnosed MS patients, likely reflecting demyelination and neurodegeneration. The correlation with clinical markers of disease activity, progression, cognitive dysfunction, and fatigue supports the potential role of FBB PET as a biomarker in MS. Further research is needed to confirm its prognostic value and clinical applicability for stratifying patients at higher risk of activity and progression, with relevant prognostic and therapeutic implications.

**Supplementary Materials:** The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

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**Institutional Review Board Statement:** This study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of the Biomedical Research Ethics Committee of the Province of Granada (Spain), under the code AMPET-MS22 (107-N-22).

**Informed Consent Statement:** Informed consent was obtained from all subjects involved in this study.

**Data Availability Statement:** All collected data were anonymized and stored in institutional databases and repositories to ensure the confidentiality and privacy of study participants, in accordance with the current legislation on personal data protection, specifically the General Data Protection Regulation (EU) 2016/679 (GDPR). Due to ethical and legal considerations regarding patient confidentiality and compliance with the General Data Protection Regulation (EU) 2016/679, the anonymized datasets generated and/or analyzed during the current study are not publicly available. For any clarification, the datasets are available from the corresponding author on reasonable request.

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## Abbreviations

The following abbreviations are used in this manuscript:

A $\beta$	amyloid- $\beta$ 1-42 oligomers	NAWM	Normal-appearing white matter
BDI-II	Beck Depression Inventory-II	PET	Positron emission tomography
CSF	Cerebrospinal fluid	RRMS	Relapsing–remitting multiple sclerosis
CVLT	California verbal learning test	sFBB	Standard acquisition $^{18}\text{F}$ -florbetaben
DWM	Damaged white matter	SMDT	Symbol Digit Modalities Test
EDSS	Expanded Disability Status Scale	SUV	Standardized Uptake Value
eFBB	Early acquisition $^{18}\text{F}$ -florbetaben	SUVmax	Maximum Standardized Uptake Value
EQ-5D	EuroQoL-5D	SUVmean	Mean Standardized Uptake Value
FBB	$^{18}\text{F}$ -florbetaben	SUVmin	Minimum Standardized Uptake Value
GM	Grey matter	SUVr	Standardized Uptake Value relative to the cerebellum
MFIS-5	Modified Fatigue Impact Scale 5-item version	T25FW	The Timed 25-Foot Walk
MRI	Magnetic resonance imaging	9HPT	The Nine-Hole Peg Test
MS	Multiple sclerosis	% of change SUV	Percentage of change between SUV of DWM and NAWM

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