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14. ABSTRACT

Our study proposes to translate a novel fibrin-specific molecular imaging approach based on ^{64}Cu-FBP8 brain positron emission tomography (PET), developed by investigators at Massachusetts General Hospital for detecting in vivo fibrin deposition in brain of patients with relapsing-remitting multiple sclerosis (RRMS). The overall hypothesis is that, using this method, we will be able to non-invasively measure in vivo persistent, deposition of fibrin in the brain of people with RRMS including both the white matter (WM) and the gray matter (GM), where it will be closely associated with the presence of local structural damage. Our initial data show in RRMS, relative to a control population, an increase in ^{64}Cu-FBP8 PET signal that is particularly evident in acute WM lesions, but also present in the normal appearing WM and cortex. Specifically, fibrin deposition as measured by ^{64}Cu-FBP8 uptake can be detected in patients at the site of the acute brain WM plaque that shows contrast enhancement on post-gadolinium MRI scans. We found that in these areas ^{64}Cu-FBP8 uptake persisted at late time points following the radiotracer injection, indicating that the abnormal ^{64}Cu-FBP8 uptake reflects local brain deposition of fibrin and not simply increased brain delivery of the tracer due to blood-brain barrier disruption.

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None listed.

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1. Introduction

In multiple sclerosis (MS), a growing body of evidence indicates that fibrin and the pathways that control the formation and degradation of fibrin could represent early triggers that contribute to the initiation of neuroinflammation and the promotion of demyelination and axonal loss[1]. Fibrinogen is a 340 kDa glycoprotein generally considered a good surrogate marker of blood brain barrier (BBB) disruption because of its abundance, restriction to the intravascular compartment and lack of expression in the healthy CNS. Upon activation of the coagulation cascade, fibrinogen is converted into insoluble fibrin by thrombin. Converting fibrinogen into fibrin exposes cryptic epitopes that may promote inflammation[2, 3]. Plasmin, in turn, mediates fibrin dissolution in a process tightly regulated by plasminogen activator inhibitors.

Fibrin deposition is not only an early feature of acute MS pathology, but it also persists throughout the course of the disease, as it can be found either in acute active white matter (WM) lesions and early experimental autoimmune encephalitis models, or in some chronic WM plaques despite the lack of contrast enhancement on magnetic resonance imaging (MRI) scans[4-7]. Experimental MS data have also shown that fibrin deposition can induce rapid and progressive microglial activation, which, in turn, contributes to neuroinflammation and axonal damage, before the onset of clinical signs[7-9]. It has been further demonstrated that dysregulation and disturbances in fibrin clearance might be implicated in disease pathogenesis and inhibition of tissue repair[10, 11]. Finally, neuropathological examinations of ex vivo MS brains have provided evidence that fibrin(ogen) deposition can be also extensive and frequent in the MS cortex, where it correlates with neuronal loss[12].

Despite all the above-mentioned evidence, diagnostic and clinical use of fibrin(ogen) deposition in MS beyond rudimentary immunohistochemical detection on post-mortem samples has not been achieved yet. Contemporary evidence regarding fibrin(ogen) as a biomarker in human studies has thus far mainly been limited to peripheral blood and/or CSF examination. Fibrin(ogen), however, is an acute phase reactant, meaning peripheral levels can be elevated due to several other factors/diseases, and not necessarily be linked to MS-specific disease events. Hence, the *in vivo* study and monitoring of the early processes that lead the cascade of the events that promote demyelination and neurodegeneration in the MS brain is limited.

In this study, we propose to bridge this gap by translating a novel fibrin-specific molecular imaging approach based on ^{64}Cu-FBP8 brain positron emission tomography (PET), developed by investigators at Massachusetts General Hospital for detecting *in vivo* fibrin deposition in brain of patients with relapsing-remtting MS (RRMS).

Our overall hypothesis is that, in 20 RRMS patients with evidence of active WM lesions and cortical plaques, we will be able to non-invasively measure *in vivo* persistent, pathological deposition of fibrin in the brain, including both the WM and the gray matter (GM), specifically the cortex, where it will be closely associated with the presence of local structural damage. To test our hypothesis, we will use a multimodal imaging approach by combining ^{64}Cu-FBP8 imaging on an integrated 3 Tesla (T) magnetic resonance-PET (MR-PET) system with the Rapid Estimation of Myelin for Diagnostic Imaging (REMyDI) a novel MRI-based myelin quantification technique (which can be acquired during the MR-PET session) and with 7 Tesla (7T) MRI to assess cortical lesion load.

Specifically, the study proposes the following aims:

AIM 1. To assess, using simultaneous ^{64}Cu-FBP8 MR-PET, fibrin deposition in patients with relapsing-remitting MS (RRMS) relative to matched healthy individuals.

Hypothesis 1a. Fibrin deposition as measured by ^{64}Cu-FBP8 uptake can be detected in patients at the site of the acute brain WM plaque that shows contrast enhancement on post-gadolinium MRI scans. In these regions ^{64}Cu-FBP8 uptake will persists at late time points following the radiotracer injection, indicating that the abnormal ^{64}Cu-FBP8 uptake reflects local brain deposition of fibrin and not simply increased brain delivery of the tracer due to BBB disruption.

Hypothesis 1b. Fibrin deposition as measured by ^{64}Cu-FBP8 uptake can be detected in the cortex of MS patients with prior evidence of cortical lesions on 7T scans. This would suggest that local fibrin-tracer deposition can be detected also in the absence of macroscopic disruption to the BBB.

AIM 2. To assess in RRMS, by combining ^{64}Cu-FBP8 MR-PET with morphometric MRI at ultra-high field (7T) and REMyDI the relation between fibrin deposition and brain structural pathology.

Hypothesis 2a. Abnormal ^{64}Cu-FBP8 uptake at MR-PET, indicating local fibrin deposition, colocalizes with cortical demyelinating lesions and cortical thinning.

Hypothesis 2b. Cortical sulci rather than cortical gyri are preferential site of ^{64}Cu-FBP8 SUVR abnormal increase, indirectly linking fibrin deposition to meningeal and cortical pathological processes as emerged from neuropathology and previous *in vivo* 7T studies.

Hypothesis 2c. ^{64}Cu-FBP8 uptake at MR-PET in NAWM is inversely associated with myelin content as measured by REMyDI.

AIM 3. To assess in RRMS, by combining ^{64}Cu-FBP8 MR-PET with longitudinal morphometric MRI at ultra-high field (7T) and REMyDI, whether fibrin deposition can predict progression of structural brain damage at 1-year follow-up.

Hypothesis 3a ^{64}Cu-FBP8 uptake at MR-PET at baseline in active WM lesions is associated with progressive local decrease in lesion myelin content as measured by REMyDI.

Hypothesis 3b. In NAWM ^{64}Cu-FBP8 uptake at MR-PET at baseline is associated with decrease in myelin content, as measured by REMyDI, at follow up.

Hypothesis 3c. Cortical ^{64}Cu-FBP8 uptake at baseline predicts accumulation of cortical lesions and cortical thinning at follow-up.

Finally, we will explore whether brain fibrin deposition, either globally or in different WM and gray matter regions of interest is independently associated with standard clinical measures including EDSS, and Symbol Digit Modalities Test (SDMT).

2. Keywords

BBB: Blood brain barrier

CNS: Central nervous system

Cu: Copper

EDSS: Expanded Disability Status Scale

EPI: Echo-planar imaging

FLAIR: Fluid attenuated inversion recovery

FBP: Fibrin binding probe

GM: gray matter

HC: healthy control

ME-MPRAGE: Multiple gradient echoes-3D-magnetization-prepared rapid acquisition

MS: Multiple sclerosis

MR-PET: Magnetic resonance imaging-positron emission tomography

MRI: Magnetic resonance imaging

NAWM: Normal appearing white matter

PET: Positron emission tomography

REMyDI: Rapid Estimation of Myelin for Diagnostic Imaging

RRMS: Relapsing-remitting multiple sclerosis

SDMT: Symbol Digit Modalities Test

SUV: Standardized uptake values

SUVR: Standardized uptake values normalized by a pseudoreference region

SyMRI: Synthetic magnetic resonance imaging

T: Tesla

3T: 3 Tesla

7T: 7 Tesla

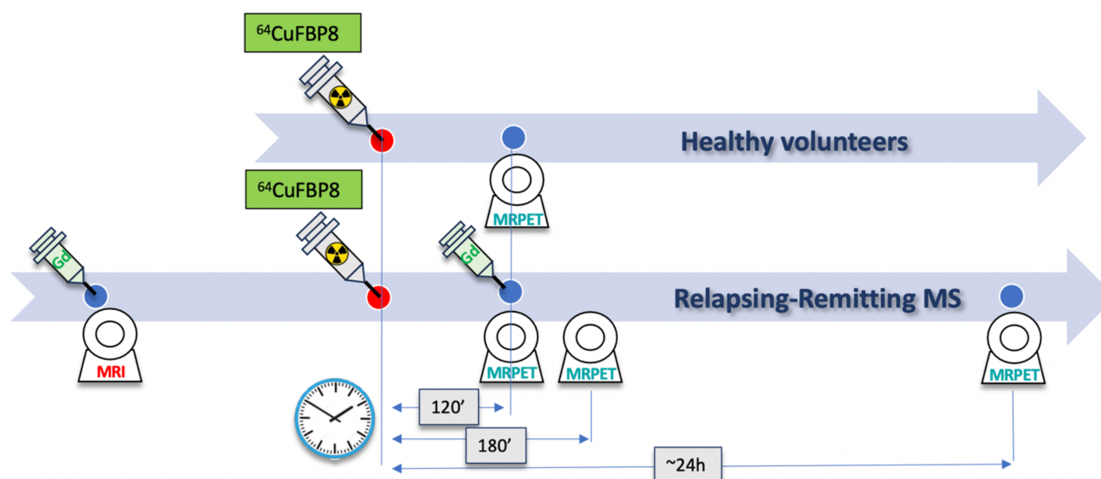
TA: Acquisition time

TE: echo time

WM: white matter

3. Accomplishments

To date we have screened 13 RRMS patients for eligibility criteria, and have consented and enrolled 5 patients (4 females, 1 male; mean±SD age= 37±9 years; mean±SD disease duration= 70±118 months) fulfilling the inclusion criteria. None of the patients had any acute and/or chronic medical conditions, including cardiac and cerebro-vascular disease and/or related risk factors, hypertension, diabetes, smoking or history of smoking. Neurological disability was assessed using the Expanded Disability Status Scale (EDSS, median, min-max= 2.5, 1.5-3.5). One subject dropped off the study.
In patients, fibrin imaging was performed in an integrated 3T MRI PET system (MR-PET) after an intravenous bolus injection of ^{64}Cu-FBP8 (up to max ~15 mCi). PET data were acquired in list-mode format starting ~120-140 minutes post-injection (the blood is cleared of the radiotracer at ~140 minutes post-injection) with the following timeline (the 24 h time point was obtained only in one RRMS):



MR-PET data in RRMS were compared to PET data in 3 HC acquired using the same protocol.
Additionally, the following MRI scans simultaneous to PET were obtained: 1) multiple gradient echoes-3D-magnetization-prepared rapid acquisition (ME-MPRAGE) scans (1 mm isotropic) before and after the administration of Gadolinium (0.1mm/kg), which was used for generation of attenuation correction maps, cortical reconstruction, and identification of possible areas of lesion enhancement in the WM and cortex; 2) conventional 3D fluid-attenuated inversion recovery (FLAIR) images (1 mm isotropic) for WM lesion segmentation; 3) a quantitative multi-dynamic multi-echo MRI sequence (axial field of view of 230×187 mm, voxel size of 0.7×0.7×4 mm^3 with a 25% distance factor, 32 slices, repetition time of 4,720 ms, echo times of 23/104 ms, inversion time 25 ms, flip angle of 130°) to generate the Rapid Estimation of Myelin for Diagnostic Imaging (REMyDI_ myelin content maps, as well as synthetic T1- and T2-weighted images; 4) resting state functional MRI (fMRI) data, which will be acquired using an echo-planar imaging (EPI)-based sequence (echo time [TE] 5 30 msec, repetition time [TR] 5 2000 msec, slice thickness 3 mm) during most of PET data acquisition to be used for motion correction. Two out of the RRMS subjects underwent 7T imaging for cortical lesion identification; in the remaining subjects where 7T was not possible due to contraindications at ultra high field, cortical lesions were identified on 3T.

Data processing. In-house software was used to compute voxel-wise, for each subject, standardized uptake values (SUV: mean radioactivity/injected dose/weight) from the 30 minutes PET data at each time point at 1.2 mm isotropic voxel size. SUV were then normalized by a pseudo-reference region (SUVR). Because MS is a diffuse disease that lacks an anatomically consistent reference region, we will use a clustering-based approach to identify a pseudo-reference region in the normal appearing WM. Motion correction of PET data was performed by incorporating temporally correlated fMRI images to account for intraframe motion. In each subject, mean SUVR was extracted from masks of WM lesions (acute lesions on post gadolinium scans and chronic inactive lesions respectively), normal appearing WM (global WM in HC). In each subject, mean SUVR was extracted from whole cortex and/or cortical regions of interest registered to PET data including cortical gyri and sulci.

Main results. Overall, we found an increase in mean ^{64}Cu-FBP8 SUVR in RRMS patients relative to HC, though a statistical comparison was not possible yet due to the low sample size (**Figure 1**). This was observed for all the brain tissue compartments examined, as shown in the boxplot below and including both WM and GM regions of interest.

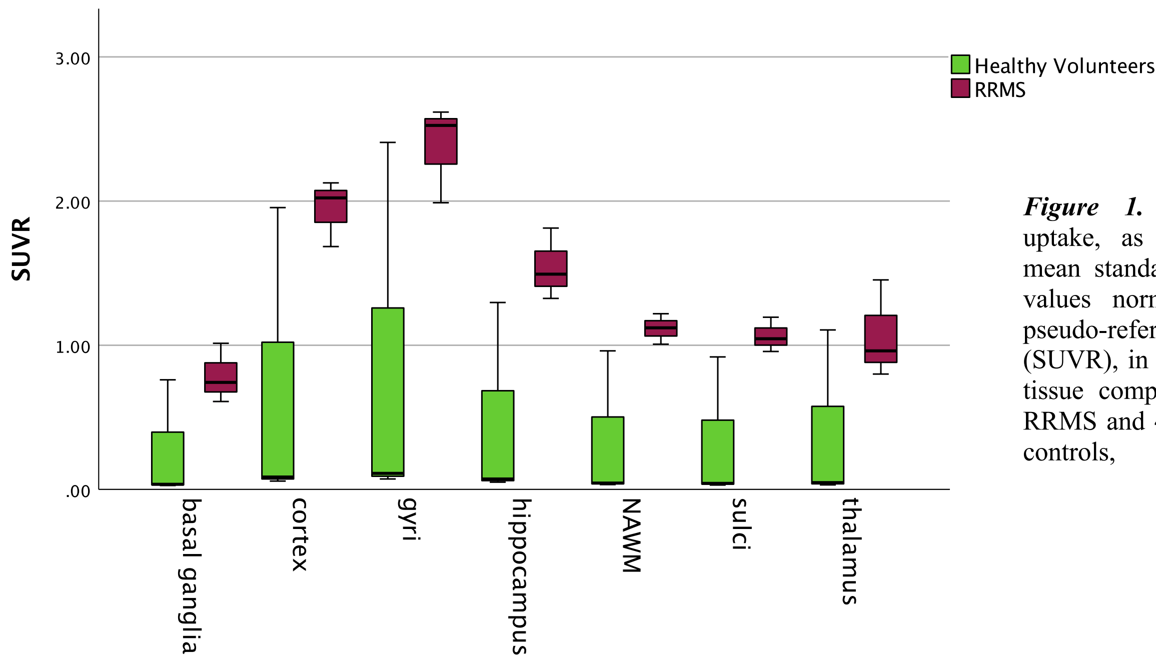


Figure 1. ^{64}Cu-FBP8 uptake, as measured by mean standardized uptake values normalized by a pseudo-reference region (SUVR), in different brain tissue compartments in 4 RRMS and 4 age-matched controls,

Mean±SD ^{64}Cu-FBP8 SUVR in RRMS and controls in are reported in **Table 1** below.

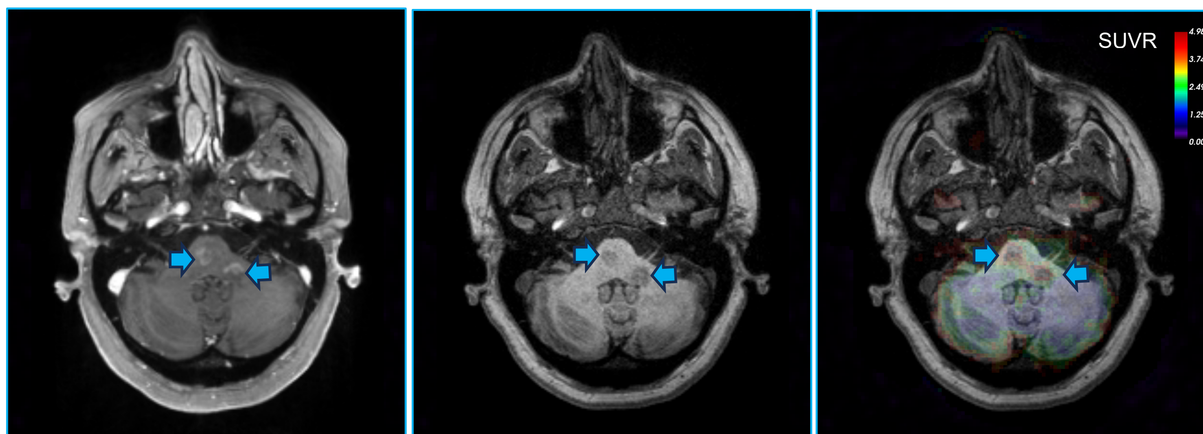
Region	RRMS	HC	% increase in RRMS vs HC
Cortex	1.9±0.2	0.7±1.0	178%
Cortical Gyri	2.4±0.3	0.9±1.3	175%
Cortical Sulci	1.1±0.1	0.3±0.5	222%
Thalamus	1.1±0.3	0.4±0.6	171%
Basal Ganglia	0.8±0.2	0.3±0.4	178%
NAWM	1.1±0.1	0.3±0.5	222%
Active WM lesions (N=8 lesions)	2.1±1.3	0.3±0.5	493%
Chronic inactive WM lesions	0.9±0.1	0.3±0.5	168%

Relative to controls, the increase in ^{64}Cu-FBP8 SUVR in RRMS was diffuse. In one RRMS patients, we were able to acquire MR-PET data at 3 different time points (as shown in the MR-PET scheme) and to measure the corresponding SUVR in active WM lesions.

In this patient, we found that ^{64}Cu-FBP8 SUVR in active WM lesions were greater relative to inactive WM lesions and NAWM at all time points, **Table 2** below.

	% difference SUVR in active vs. inactive WM lesions	**% difference SUVR in active WM lesions vs. NAWM**
120’	321	175
180’	534	188
~24h	328	121

The **Figure 2**. below shows (Left panel) ME-MPRAGE images acquired ~5 min post Gadolinium administration demonstrating 2 active MS lesions in the pons and left middle cerebellar peduncle; (Middle panel) ME-MPRAGE images showing lack of enhancement ~24 post Gadolinium administration; (Right Panel) PET ^{64}Cu-FBP8 SUVR maps showing radiotracer uptake in the active MS lesions, which was detectable at all time points (~120'; ~180' and ~24h post radiotracer injection).



Overall, these data show that fibrin deposition as measured by ^{64}Cu-FBP8 uptake could be detected in patients at the site of the acute brain WM plaque that showed contrast enhancement on post-gadolinium MRI scans. In these lesions ^{64}Cu-FBP8 uptake persisted at late time points following the radiotracer injection, indicating that the abnormal ^{64}Cu-FBP8 uptake reflects local brain deposition of fibrin and not simply increased brain delivery of the tracer due to BBB disruption.

We have combined PET data from RRMS acquired under this study with PET data from patients with progressive MS acquired under another study on fibrin imaging in advanced progressive disease. The same imaging protocol for acquisition and data analysis was used for the two MS populations. ^{64}Cu-FBP8 SUVR in MS patients (N=11) were compared to those from age-matched HC. Differences between groups in each tissue compartment were assessed by pairwise comparisons adjusted for age and multiple comparisons (Sidak). The analysis disclosed a significant increase in tracer uptake in MS relative to HC in different regions as shown in the boxplot below (**Figure 3**) In this analysis, active WM lesions were excluded as they were only present in RRMS (the WM lesion compartment included only chronic inactive WM lesions, in which ^{64}Cu-FBP8 SUVR did not significantly differ from ^{64}Cu-FBP8 SUVR from global WM of HC).

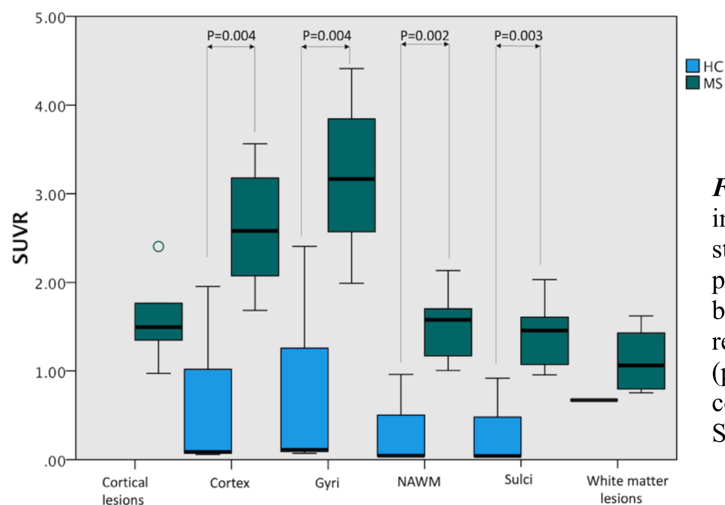


Figure 3. Boxplots showing significantly increased ^{64}Cu-FBP8 uptake, as measured by standardized uptake values normalized by a pseudo-reference region (SUVR), in different brain tissue compartments in 11 MS patients and relative to 4 age-matched controls, by Ancova (pairwise comparisons), adjusting for age and correcting for multiple comparisons using the Sidak method.

4. Impact

Our initial findings demonstrate a way to visualize and quantify in vivo in MS patients direct brain involvement of the fibrin(ogen) pathways. Specifically, they provide *in vivo* evidence of fibrin deposition at the site of the acute, inflammatory plaque in WM, which evidence from experimental and histopathological studies indicates as one of the earliest event and trigger of neuroinflammatory demyelination.They also demonstrate a way to visualize and quantify in vivo fibrin deposition in the cortex but also other brain regions including subcortical GM and NAWM.

Quantification of fibrin deposition by ^{64}Cu-FBP8 uptake will then be used to track the evolution of MS tissue damage in acute WM lesions, NAWM and cortex by investigating whether, and to which extent, the amount of fibrin deposition relates or can even predict changes in myelin content and progressive cortical damage. Once more subjects and longitudinal data will be acquired, allowing to establish the existence of an in vivo link between fibrin deposition and detrimental brain structural pathology.

5. Changes/Problems

We had some challenges in finding patients with active WM lesions, given that they are not so frequent and that the majority of patients are receiving MS-related treatments per standard medical care. This might cause some delay in patient recruitment. We have considered as an additional inclusion criteria, as also suggested by reviewers of the original application, including paramagnetic rim lesions, which are chronic active WM lesions that can be readily visible using our 7T protocols and are considered a subset of WM lesions associated with disease progression and remyelination failure.

6. Products

N/A

7. Participants & Other Collaborating Organizations

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8. Special Reporting Requirements

N/A

9. Appendices

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