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TITLE: Prediction of Future Disability in MS Using Combined Novel MRI and Serological Markers

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14. ABSTRACT

In this annual report, we detailed our activities in the dates since initial IRB approval (10/26/2019). We have begun to contact and schedule patients for study visits, beginning chart review for patients who prefer to come later for their study visit. Three patients have completed study visits, 20 patients have been contacted. MRI data reanalysis has been completed for 48 subjects (88 total GEPCI MRIs). The REDCap database was developed and moved into production, ready for participant data entry. Training was completed for the study team members involved in extracting data through chart review, administering cognitive and functional assessments, and entering data into the REDCap database.

15. SUBJECT TERMS

None Listed

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1. INTRODUCTION:

We will study novel MRI and serological markers (analyzed on previously collected imaging and serum samples) as predictors of future disability in Multiple Sclerosis (MS). MS is heterogenous, patients display a wide spectrum of long-term disability levels that are not completely foreseen by early disease activity but may be explained, in part, by intrinsic patient-specific differences in central nervous system tissue susceptibility to damage and intrinsic ability to repair. Novel MRI and serological biomarkers, such as Gradient Echo Plural Contrast Imaging (GEPCI) and serum neurofilament light chains (NfL), may better reflect the early neurodegeneration in MS. We wish to determine if these novel biomarkers, separately and in combination, can be used as better predictors of disability in individual patients and can ultimately guide treatment choices. We identified 127 well-described subjects from four prior GEPCI studies that have at least one brain MRI with GEPCI data acquired at 3.0 Tesla. All 127 will be recruited to a single follow-up visit, in which each subject will undergo testing by a blinded examiner, with Expanded Disability Status Scale (EDSS), MS Functional Composite (MSFC) and Symbol Digit Modality Test (SDMT) and Montreal Cognitive Assessment (MoCA). MRI images will be re-analyzed for R2t* and Quantitative susceptibility maps (QSM). We will calculate average R2t* values from cortical and deep gray matter (GM), normal-appearing white matter and lesions, and determine individual regional patterns of CNS damage. We will also derive QSM from GEPCI to evaluate lesions and deep GM degeneration. In stored serum samples, we will measure serum NfL by single molecule array (Simoa). Associations of MRI changes and NfL levels with rate of disability accumulation will be determined. We all also build a multivariate model that best predicts future disability.

2. KEYWORDS:

Multiple Sclerosis, Biomarkers, Gradient Echo MRI, Neurofilament light chain, disability, prediction.

3. ACCOMPLISHMENTS:

What were the major goals of the project?

The major goals of the project were as follows:

Major Task 1: Preparation to begin the study (100% completion of this goal was met on 5/14/2020)

a) Prepare IRB and HRPO submission, including informed consent, completed 10/26/2019.
b) Finalize consent form and human subjects protocol, completed 5/14/2019
c) Prepare Data entry capture forms in REDCAP, completed 5/14/2020
d) Train investigators and research coordinator in REDCAP data entry, completed 5/14/2020
e) Milestone: Completion of IRB and HRPO review and consent process. Completion of RedCap data elements. Milestone met 5/14/2020

Major Task 2: Follow-up visits

a) Subtask 1: Ensure that clinical investigators are trained in Expanded Disability Status Scale (EDSS). 100% completed 10/26/2019
b) Subtask 2: Ensure that research coordinator is trained in administration of Multiple Sclerosis Functional Composite (MSFC), Symbol Digit Modalities Test (SDMT). 100% completed 10/26/2019
c) Subtask 3: Contact and evaluate study subjects (n=127, relapsing remitting MS (RRMS)=60, secondary progressive MS (SPMS)=43 and primary progressive MS (PPMS)=24). Subtask is at 7% completion
d) Milestone: Follow-up visits completed (n=127). 3% completed

Major Task 3: Image analysis

a) Download GEPCI data and prepare for analysis (n=127), one scan per MS patient. 100% completed
b) Re-analysis of GEPCI images to obtain R2t* values in all brain areas. 40% completed

c) Generating GEPCI-Barcode areas and values. 0% completed
d) Generating Quantitative Susceptibility maps (QSM) data. 0% completed
e) Milestone: Completion of MRI re-analysis. 0% completed

Major Task 4: Serum analysis

a) Subtask 1: Purchase NfL assay, prepare for Single molecule array (Simoa) analysis. 0% completed.
b) Subtask 2: Coordinate timing of equipment use with Holtzman laboratory and ensure NfL assay is working accurately. 0% completed
c) Subtask 3: Analysis of samples from study subjects (n=88). One serum sample for each patient for a total of 88 samples. 0% completed
d) Milestone: Completion of serum analysis (n=88). 0% completed

Major Task 5: Data organization and analysis

a) Subtask 1: Obtain and organize previously collected data on study subjects (and enter in REDCAP). 50% completed
b) Subtask 2: Descriptive and summary statistics. 0% completed
c) Subtask 3: Study the association between MRI variables and future disability. 0% completed
d) Subtask 4: Study the association between serum NfL levels and future disability. 0% completed
e) Milestone: Complete initial data analysis. 0% completed

Major Task 6: Develop and test a multivariate model for the prediction of disability in MS.

a) Subtask 1: Construct a multivariate linear model for disability prediction (that incorporates MRI, serum makers and other important disease and patient variables). 0% completed
b) Subtask 2: Validate the multivariate model using a cross-validation technique. 0% completed
c) Subtask 3: Review study results, statistical analysis output and discuss results with study team. 0% completed
d) Milestone: Complete data analysis and study. 0% completed

What was accomplished under these goals?

For the first Major Task, 100% completion of this goal was achieved 5/14/2020. Under this goal initial materials, including the finalized consent form, were submitted to the Washington University IRB on 5/14/2019 and received approval on 10/26/2019. Data capture forms were developed for the project REDCAP database, which was moved from development into production on 5/14/2020. Investigators and study coordinators were trained on the entry of study data into the REDCAP database.

For the second major task, the training of clinical investigators and coordinators for the administration of their allocation study assessments was confirmed to be complete when IRB approval was received for this project. Participants with the oldest GEPCI MRIs were prioritized when participant contact began. For patients who are unable to be seen for a study visit in conjunction with a clinical appointment, with their permission via phone consent the chart review process is started with plans to complete an in-person study visit at a later date. As of 10/5/2020, 20 patients have been contacted by study coordinators, three participants have completed an in-person study visit and five (with the earliest GEPCI MRIs) have upcoming visits in the next three months. Two additional patients, currently participating in other clinical trials, will plan to complete a study visit during their next visit to the University for other study appointments.

For the third major task, Drs. Yablonskiy and Xiang have downloaded the MRI data for all 127 study participants onto local computers and servers. The MRI data have been processed and are ready for re-analysis. Those working with the imaging data are blinded to the clinical outcomes of the subjects. Dates from clinical MRIs completed around the same time as the research MRIs are currently being collected to supplement imaging data, if certain research MRI anatomical sequences (such as FLAIR images and contrast-enhanced images) are missing or inadequate in the research images. To date, scans from the initial GEPCI MRI study and CombiRX study have

been reanalyzed. For both cohorts, CombiRx (62 scans; 22 patients) and GEPCI (26 scans; 26 patients), reconstructed GEPCI scans were imported into MATLAB for further processing. We used an in-house MATLAB script developed by the Dr. Yablonskiy group to carry out further processing. For processing, first a Hanning filter was applied to correct for image artifacts. Then using a FSL Brain extraction tool we stripped the skull and extracted the brain from the images. Calculations for frequency mapping were run, then frequency unwrapping and frequency extension were performed. To complete processing, the first BOLD procedure calculation was completed. After completing the processing pipeline, we obtain the primary GPCI images, including: 't1w', 'R2t', 'fre', 'dfre', 'Kesai', 'Ksi'. The resulting R2t maps were converted to NIFTI format and visually inspected for image artifacts.

All components for the fourth major task (laboratory assays) were suspended for almost 6 months due to the COVID 19 pandemic. Now that the lab based research is resuming at our institution, we plan to purchase the assays and run the serum analysis in this second year of the study.

For the fifth major task, data is being collected and organized through study review and chart review for input into the redcap database.

The sixth major task is dependent on the completion of the previous five tasks and is not yet started.

What opportunities for training and professional development has the project provided?

Nothing to Report.

How were the results disseminated to communities of interest?

Nothing to Report.

What do you plan to do during the next reporting period to accomplish the goals?

In the next reporting period we plan to continue to contact participants, beginning our chart review process. Previously collected data that has been organized and chart review data for enrolled participants will be added into the study's redcap database. Image analysis tasks will proceed with the reanalysis of the remaining GEPCI MRIs. Further processing of the GEPCI images will be conducted once the high-resolution structure images are available which will include GEPCI-structural image co-registration using FSL FLIRT, the second BOLD procedure calculation and the extraction of average CRPCI metrics in different fray and white matter regions of interest. We will follow the University's COVID-19 guidance for research labs and begin serum analysis tasks soon now that labs are open at the "Yellow" level (working with physical distancing and masks required).

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Nothing to Report.

What was the impact on other disciplines?

Nothing to Report.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

Nothing to Report.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

We have experienced delay in our ability to schedule and perform on-site visits because of University-wide restrictions on in-person visits and laboratory research due to the COVID-19 pandemic. While we have started to increase the number of face-to-face visits in the clinic, the University is not yet fully operational. To work around this, we have added the ability to start the chart review process prior to patients' visits, if applicable. We anticipate a continued increase in the number of in person visits and study visits in the next year.

Actual or anticipated problems or delays and actions or plans to resolve them

In order to address the delays experienced due to the COVID-19 pandemic, modified our chart review procedures to be able to collect data prior to the completion of an in-person study visit and prioritizing participants who would likely only complete assessments by phone due to mobility or disease related limitations. For patients who scheduled for in-person clinic visit, we offering to complete their study visit on the same day to minimize the need for additional trips to our site.

Changes that had a significant impact on expenditures

Nothing to Report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to Report.

Significant changes in use or care of human subjects

Nothing to Report.

Significant changes in use or care of vertebrate animals

Nothing to Report.

Significant changes in use of biohazards and/or select agents

Nothing to Report.

6. PRODUCTS:

Publications, conference papers, and presentations

Nothing to Report.

Journal publications

Nothing to Report.

Books or other non-periodical, one-time publications.

Nothing to Report.

Other publications, conference papers and presentations.
Nothing to Report.

Website(s) or other Internet site(s)

Nothing to Report.

Technologies or techniques

Nothing to Report.

Inventions, patent applications, and/or licenses

Nothing to Report.

Other Products

Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name	Dorothy Anne Cross
Project Role	Principal Investigator
Nearest person month worked	0.96
Contribution to Project	Dr. Cross has overseen the overall study, the build of the REDCap database, and performed study visits.
Funding Support	(effort supported by this grant)

Name	Amber Salter
Project Role	Co-Investigator
Nearest person month worked	1.2
Contribution to Project	Dr. Salter has contributed to the build of the REDCap database.
Funding Support	(effort supported by this grant)

Name	Dmitriy Yablonskiy
Project Role	Co-Investigator
Nearest person month worked	1.08
Contribution to Project	Dr. Yablonskiy has managed the MRI analysis and image quality control.
Funding Support	(effort supported by this grant)

Name	Biao Xiang
Project Role	Co-Investigator
Nearest person month worked	4
Contribution to Project	Dr. Xiang has contributed the analysis and procurement of MRI images for the study.
Funding Support	Effort supported from this grant through 6/30/2020, supported 100% from the National Multiple Sclerosis (MS) Society **beginning 7/1/2020**

Name	Sayan Kahali
Project Role	Co-Investigator
Nearest person month worked	1
Contribution to Project	Dr. Kahali has contributed the analysis and procurement of MRI images for the study.
Funding Support	Work (effort) on this project supported by this grant (Dr. Kahali started on 7/1/2020)

Name	Salim Chahin
Project Role	Co-Investigator
Nearest person month worked	4.8
Contribution to Project	Dr. Chahin has overseen the overall study and the build of the REDCap database. He has performed study visits and trained study team members on data extraction during chart review, REDCap data entry and the cognitive and functional assessments for this study.
Funding Support	(effort supported by this grant)

Name	Amjad Samara
Project Role	Research Analyst
Nearest person month worked	1.08
Contribution to Project	Dr. Samara has contributed to the MRI analysis
Funding Support	National Institute on Drug Abuse

Name	Alyssa Spurling
Project Role	Research Coordinator
Nearest person month worked	2.4
Contribution to Project	Mrs. Spurling has organized the subjects list, identified clinical MRIs proximal to GEPCI MRI, organized additional patient data, and contacted study subjects.
Funding Support	(effort supported by this grant)

Name	Courtney Dula
Project Role	Research Coordinator
Nearest person month worked	2.4
Contribution to Project	Mrs. Dula has organized the subjects list, organized additional patient data, contacted study subjects, performed the study visits and help with the quarterly and annual reports.
Funding Support	(effort supported by this grant)

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

See below and see attached for updated support documents.
One change in personnel occurred in July 2020. Dr. Biao Xiang received an NMSS fellowship award with support beginning 7/1/2020; he will remain in an advisory capacity but will not receive any further effort support. Dr. Sayan Kahali has taken over Dr. Xiang's tasks and will work under the supervision of Dr. Yablonskiy and advice of Dr. Xiang, he will receive effort support from this grant at the same level as Dr. Xiang. See updated budget justification and SOW.

Anne Cross (Principal Investigator)

Project(s) Ended:

"Biomarkers and pathogenesis of MS: From Mouse to Human" NIH/NINDS P01 NS059560. **Ended 4/30/2020**

Project(s) Started:

"Cladribine Tablets: Collaborative Study to Evaluate Impact On Central Nervous System Biomarkers in Multiple Sclerosis" EMD-Serono Award #: NCT03963375. **Started 6/1/2019**

Dmitriy Yablonskiy (Co-Investigator)

Project(s) Ended:

"In Vivo Human Lung Morphometry with Hyperpolarized 3He MRI and CT: Effects of Aging, Smoking, and COPD" NIH. R56AG052582. **Ended 8/31/2019**

"Determinants of change in lung function during pulmonary exacerbation & recovery in Cystic Fibrosis" Children's Discovery Institute. PDII2015472. **Ended 6/30/2019**

Salim Chahin (Co-Investigator)

Project(s) Ended:

"Accelerometry and activity diaries in understanding mobility and fatigue in MS" The Barnes-Jewish Foundation. **Ended 10/1/2019**

Project(s) Started:

"Cerebrospinal fluid-biomarkers-based diagnostic and prognostic models for Multiple Sclerosis" National MS Society. **Started 7/1/2019**

"COVID-19 Risk in Multiple Sclerosis (Survey)" Biogen. **Started 9/1/2020**

Biao Xiang (Co-Investigator)

Project(s) Ended:

Role Ended for "Prediction of Future Disability in MS Using Combined Novel MRI and Serological Markers" U.S. Army Medical Research and Development Command. **Role ended 6/30/2020**

Project(s) Started:

"Using a Novel MRI technique - Simultaneous Multi-Angular Relaxometry of Tissue - to Measure Evolution of tissue damage in Progressive Multiple Sclerosis" National MS Society FG-1908-34882. **Started 7/1/2020**

Amber Salter (Co-Investigator)

Project(s) Ended:

"First-in-human trial of a Receptor-Targeted Nanoparticle PET Tracer for Atherosclerosis" NIH R01HL132600. **Ended 4/30/2020**

"Marilyn Hilton Award for Innovation in MS Research" Conrad H. Hilton Foundation. **Ended 12/31/2019**

"Molecular Mechanisms of Chronic Airway and Alveolar Disease in HIV" NIH R01HL144478. **Ended 7/31/2019**

"Longitudinal Study to Evaluate the Prognostic Value of Cerebrospinal Fluid in Multiple Sclerosis" Conrad H. Hilton Foundation. **Ended 6/30/2019**

"Understanding SOD1 Kinetics in Amyotrophic Lateral Sclerosis" NIH R01NS097816. **Ended 5/31/2019**

"Development of the Disease-specific PedsQL for Pediatric Patients with MS" National MS Society PP-1712-29484. **Ended 5/31/2019**

Project(s) Started:

"Metadata Catalogue Project" National MS Society SI-1907-34399. **Started 9/1/2019**

"Identification of Predictors for Clinical Outcomes in Femoroacetabular Impingement Surgery" DOD W81XWH-19-2-0042. **Started 10/1/2019**

"PET Detection of CCR2 in Human Atherosclerosis" NIH R01HL150891. **Started 12/1/2019**

"MR measured regional hypoxia as an early biomarker in cerebral small vessel disease" NIH/NINDS RF1NS116565. **Started 4/1/2020**

"Exploring CD38 Molecular Biology and Imaging in Multiple Myeloma Pathogenesis" NIH/NCI R01CA248493. **Started 7/1/2020**

What other organizations were involved as partners?

Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

Nothing to Report.

QUAD CHARTS:

Nothing to Report.

9. APPENDICES: