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TITLE: Novel Neuroimaging Assessments of Glymphatic Disruption in Humans, a Plausible Key Pathophysiological Mechanism for CNS Lupus

PRINCIPAL INVESTIGATOR: Mark DiFrancesco, PhD

CONTRACTING ORGANIZATION: Children's Hospital Medical Center

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Fort Detrick, Maryland 21702-5012

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Novel Neuroimaging Assessments of Glymphatic Disruption in Humans, a Plausible Key Pathophysiological Mechanism for CNS Lupus

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6. AUTHOR(S)
Mark DiFrancesco, PhD & Hermine Brunner, MD

E-Mail: mark.difrancesco@cchmc.org & Hermine.brunner@cchmc.org

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Children's Hospital Medical Center
3333 Burnet Ave
Cincinnati, OH 45229-3026

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13. SUPPLEMENTARY NOTES

14. ABSTRACT: This project develops and applies novel brain imaging to probe a new route by which SLE can lead to neuronal degradation. It adds the glymphatic system to the array of neurovascular components that plausibly underlie the pathobiology of lupus, even before overt neuro-psychiatric syndromes. We have generated a combination of neuroimaging protocols allowing regional characterization of glymphatic structural and functional integrity. A vascular space occupancy (VASO) imaging sequence is being used to measure amplitude of fluctuations in vascular volume driven by respiratory and cardiac cycles, a purported mechanism for glymphatic flow. Protocol development led to a focus on detecting the glymphatic-specific microenvironment by adopting "microdynamic" imaging methods based on relaxation-diffusion correlation spectroscopy. In this way, we aim to detect a restricted diffusion microenvironment of CSF-like fluid (e.g. glymphatics) by employing wide regimes of relaxation and diffusion. To date, we have successfully imaged 7 lupus subjects using this protocol. We are also establishing advanced analysis pipelines for these novel datasets.

15. SUBJECT TERMS
Lupus, glymphatics, vasculature, brain, relaxation-diffusion correlation, VASO, MRI, tissue microenvironment

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	15	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. INTRODUCTION:

This project applies novel brain imaging to detect and characterize the glymphatic system, a neurovascular component that may underlie mechanisms of pathology in neuropsychiatric lupus. Methods detect glymphatic flow-inducing vascular fluctuations due to respiration and cardiac cycles and the unique microenvironment of fluid-bearing glymphatic channels surrounding blood vessels. With these new measures, we are comparing lupus patients to matched healthy controls.

2. KEYWORDS:

Lupus, glymphatics, vasculature, brain, relaxation-diffusion correlation, VASO, MRI, tissue microenvironment

3. ACCOMPLISHMENTS:

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Original goals for Aim 1: Develop and install three MRI approaches to characterize the glymphatic system: 1. CSF-spin labeling, 2. Ultra-high b-value DWI, 3. VASO correlated to respiration. Obtain local IRB and HRPO approval. (complete by end of November 2018)
Status of Aim 1: Development effort led to abandonment of CSF-spin labeling in favor of expanding the multiple b-value DWI approach to perform relaxation-diffusion correlation spectroscopy along dimensions of b-value, echo time (TE, for T2 relaxometry), and inversion time for T1 relaxometry. VASO was developed with respiration, cardiac, and CO2 monitoring. These are challenging approaches and they were developed and tested throughout the winter and installed in final form in May 2019. Local IRB approval was obtained in December 2018. HRPO approval was obtained mid-January 2019.
Original goals for Aim 2: Compare 16 lupus patients to 16 healthy controls using the new imaging protocol. Complete imaging acquisition by beginning of August 2019 and complete analysis by end of August 2019.
Status of Aim 2: As of September 27, 2019, 4 lupus patients successfully scanned. Recruitment of remaining lupus patients and matched controls ongoing. Streamlined analysis pipeline development underway.
No-Cost Extension requested May 2019 and granted June 2019 for one year: September 1, 2019 to August 31, 2020.
October 2019-January2020: recruitment efforts continued, with modifications for PedANAM cognitive test requirements, and expanded advertisement materials.
January 2020-February 2020: 3 more lupus patients enrolled and imaged.
March 2020: COVID-19 shutdown of research MRI.

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

1) major activities: development and installation of new imaging protocols for assessment of glymphatic structure and function. Compare these assessments between lupus and healthy controls.
2) objectives: Be the first to use MRI to characterize the glymphatic system in humans and to gather the first evidence that glymphatic disruption may contribute to the pathology of neuropsychiatric lupus.
3) outcomes to date: while the general imaging approach did not change, our development efforts resulted in a change in strategy to detect glymphatic-specific microstructure: relaxation-diffusion correlation spectroscopy along T1, T2, and diffusion dimensions. This approach has been described in animals, but we adopted it for humans with challenging extensions of relaxation/diffusion regimes to capture glymphatic properties. We also developed the VASO method using a short TR and with concurrent monitoring of respiratory, cardiac, and CO2 variations. Seven lupus patients have been scanned to date and recruitment continues for both lupus patients and healthy controls. Extended advertisement materials have been approved by the IRB. We have accumulated over 140 names for recruitment of matched controls. Modification for more liberal lupus inclusion approved (allowing SLEDAI >4 with one clinical feature as well as SLEDAI >6). COVID-19 stopped or restricted recruitment for the last 4 months.

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

This project was not intended to provide training.
However, a University of Cincinnati medical physics graduate student, Steven Ewart, has taken an interest in the project and has been observing and participating in the development of the new imaging protocol. It is Steven's desire to take part in the analysis of the imaging data.
Several internal presentations have been given covering the objectives and strategy of the project. Most notably, these have been given to members of the Rheumatology and Radiology Departments at Cincinnati Children's Hospital. Mr. Ewart's participation has been interrupted by COVID-19 restrictions, but may resume in the Fall of 2020.

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?

1) With the protocol installed, we are striving to maintain recruitment to achieve our goal of completing image acquisition for 16 lupus and 16 healthy control subjects. Target completion by August 2021, with approval of a no-cost extension. As mentioned above, extended advertisement materials have been deployed. We have accumulated over 140 names for recruitment of controls, matched to the lupus patients. More liberal lupus inclusion is in place (allowing SLEDAI >4 with one clinical feature as well as SLEDAI >6)to increase lupus enrollment. If necessary, we will seek further modifications to widen inclusion criteria.
2) While we have prepared some of the analysis pipeline, we will need to work on finalizing the pipeline by the Winter 2020. (Target January 2021) This includes VASO analysis to extract both respiratory and cardiac contributions to blood vessel volume fluctuations. It will also include analysis to extract specific micro-environment properties adopted from the "MADCO" approach (e.g. Benjamini and Basser, Neuroimage (2017) 163: 183-196).

4. **IMPACT:**

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

The imaging methods developed for this project may be the first to detect and to provide quantitative measures of a system (glymphatics) in the brain that is purported to have a role in clearing debris and maintaining immunological function. Lupus is known to degrade vasculature and may lead to neurodegeneration by disruption of the glymphatic system. Detection of this process would have a large impact on the study and treatment of lupus by providing a new route for early pathogenesis and a promising brain biomarker for early disease.

What was the impact on other disciplines?

Other neurodegenerative disorders may have a pathological pathway that includes degradation of vascular integrity and the associated glymphatic system. These include small vessel disease, Alzheimer's Disease, Parkinson's Disease, diabetes, chronic hypoxia (sleep apnea), and traumatic brain injury.

What was the impact on technology transfer?

If this pilot work leads to larger studies, the methodology could eventually enter clinical radiology practice for lupus and other neurovascular disorders.

What was the impact on society beyond science and technology?

Nothing to Report

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change .

Though our imaging protocol development effort resulted in some changes in approach from our original proposal, these changes did not result in significant deviation from the scope or objectives of the study.

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

Inadequate recruitment rate remains a concern, especially in light of ongoing potential disruption by COVID-19. Our institution is ahead of many with respect to reinstituting research activities, although with some restrictions.
We continue to be engaged in weekly meetings to discuss recruitment strategy and status. If recruitment rate should continue to fall short, we have the option to further consider modest adjustments to the inclusion/exclusion criteria. We can also continue to boost advertisement for the project and to disseminate materials in more clinical and community sites.
Our ambitious data collection and analysis plan may not produce the glymphatic-specific signature we seek. The limited analyses to date suggest we may be detecting a confined CSF-like component in the microstructure, but more complete analyses are still necessary. The data are nevertheless rich and offer the potential for a variety of analysis approaches.

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

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**
  Report only the major publication(s) resulting from the work under this award.

  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. Only internal presentations.

- **Website(s) or other Internet site(s)**

  Nothing to Report

  .

- **Technologies or techniques**

  Besides the new imaging protocol for MRI and potentially new analysis methods, nothing to report. Nothing shared.

- **Inventions, patent applications, and/or licenses**

  Nothing to Report

- **Other Products**

Nothing to report yet that has been disseminated.
Potential for new imaging sequences and tissue microstructure models.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Mark DiFrancesco, PhD. PI 1.5 months
Experiment design, development of imaging protocol, image acquisition, analysis development.

Hermine Brunner, MD, 1 month
Subject recruitment, neuropsychological testing

Gregory Lee, PhD, <1 month
Development of imaging protocol

Arjun Mather, (coordinator) 2.5 months
Study coordination, IRB communications, data acquisition, ushering of subjects through neuropsychological testing and imaging.

Research Associate and Biostatistian Mekibib Altaye: < 1 month.

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

Mark DiFrancesco

New

P30 AR076316 (Brunner/Kashikar-Zuck) **09/01/2019 - 08/31/2024** **0.6 calendar**
NIH/NIAMS $3,952,351
Pediatric musculOskeletal & RheumaTology Innovation COre center (PORTICO)
Role: Co-Investigator

The *primary goal* of the PORTICO's local Research Community is to boost investigations into personalized precision medicine for children with PMKSD to achieve optimal health outcomes.

R21DC017393 (Vannest) **04/01/2019 – 03/31/2021** **0.6 calendar**
NIH/NICHD $393,759
Multimodal Neuroimaging Distinguishes Developmental and Disordered Phenotypes in Speech Sound Disorders
Role: CCHMC Site PI (Co-Investigator)

The *major goal* of this project is to use multimodal neuroimaging (functional magnetic resonance imaging, structural analysis, and magnetoencephalography) to assess engagement of a priori auditory, somatosensory, speech motor and phonological networks in 5-year-old children with SSDs who make predominately "developmental" speech errors, children who make predominately "disordered" speech errors, age- gender-matched typically developing (TD) controls, and a cohort of younger TD controls.

Research Innovation & Pilot Fund (DiFrancesco) 09/01/2019 – 08/31/2020 **1.2 calendar**
Cincinnati Children's Hospital Med Ctr $70,000 (NCE)
Complementary Regional Lung Perfusion/Ventilation Imaging for New Insights in Cystic Fibrosis
Role: co-PI

The central hypothesis of this proposal is that pulmonary vascular disease precedes and contributes to the decline in lung function regionally and its progression can be monitored with specific inflammatory biomarkers.

Completed

None

Hermine Brunner

New

P30 AR076316 (Brunner/Kashikar-Zuck) **09/01/2019 - 08/31/2024** **0.6 calendar months**
NIH/NIAMS $3,952,351
Pediatric musculOskeletal & RheumaTology Innovation COre center (PORTICO)
Role: Co-Investigator

The *primary goal* of the PORTICO's local Research Community is to boost investigations into personalized precision medicine for children with PMKSD to achieve optimal health outcomes.

WI211648 (Brunner) **11/28/2019-11/27/2022** **0.0 Calendar**
Pfizer
A 3-part open-label study assessing safety, tolerability, pharmacokinetic and -dynamic profiles, and efficacy of tofacitinib in young adults from age 18 to 30 with moderate to severe skin involvement due to lupus
Role: Site PI

Completed

None

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

Gregory Lee

New

1R01AR074795-01A1 (Coghill) **08/01/19 – 07/31/23** **0.6 calendar**
NIH/NIAMS $263,369
Identifying neural pathophysiology in juvenile fibromyalgia
The *major goal* of this proposal is to identify multivariate patterns of brain activity during core symptom-provocation tasks that, together, are sensitive and specific for JFM (vs. healthy adolescents) and can be tested in future adolescents and research studies for validation.
Role: Co-Investigator

Completed

None

Mekibib Altaye

New

P30 AR076316 (Brunner/Kashikar-Zuck) **09/16/2019-06/30/2024** **1.2 calendar**
NIH/NIAMS $3,409,545
Pediatric musculOskeletal & RheumaTology Innovation COre center (PORTICO)
The primary goal of the PORTICO's local Research Community is to boost investigations into personalized precision medicine for children with PMKSD to achieve optimal health outcomes.
Role: Biostatistician

R01 EY030521 (Angeles-Han) **09/30/2019 – 06/30/2024** **0.48 calendar**
NIH $3,258,472
Predicting uveitis onset in children with juvenile idiopathic arthritis
Goal: Our long-term goal is to prevent sight-threatening ocular damage in children with JIA-U by improving uveitis detection and treatment.
Role: Biostatistician

R01 DC018734-01 (**Hunter)** **04/01/2020 – 03/31/2025** **0.48 calendar**
NIH $3,949,394
Earliest predictors of language outcomes in high-risk infants
Goal: The goal is to develop a robust prediction model using innovative measures to enable early identification of children at high risk for SLD.
Role: Biostatistician

R01AR076153-01A1 (Myer) **04/01/2020 – 03/31/2025** **0.48 calendar**
NIH $1,436,619
Neuroplastic Mechanisms for Acquisition and Transfer of Injury-Resistant Movement Patterns Assessed in VR Simulated Sport
Our proposal to discover the neural mechanisms for injury-resistant movement pattern acquisition and transfer will lead to enhanced interventions that allow more children' and adults to lead a healthy and active lifestyle.
Role: Biostatistician

R01DC018550-01 (Meinzen-Derr) **04/01/2020 – 03/31/2025** **0.48 calendar**
NIH/NIDCD $3,063,297
Technology-assisted language intervention for children who are deaf/hard-of-hearing (TALI)
The study will test the effectiveness of a technology-assisted language intervention using augmentative and alternative communication software as a tool to enhance language development in children who are DHH.
Role: Biostatistician

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

Mekibib Altaye (continued)

1R01MH123831-01 (Tamm/Epstein) **07/01/2020 -- 06/30/2025** **0.12 calendar**
NIH/NIMH $1,999,291
Parsing Neurobiological Bases of Heterogeneity in ADHD
The goal of the study is to explore attention in an attempt to parse heterogeneity within children with ADHD. Specifically, we plan to explore the neurobiological basis of well-validated reaction time computational indicators. We will then use those reaction time parameters to characterize unique attentional subgroups and their neurobiological signature within the ADHD population.

Completed
UL1 TR001425 (Heubi) **08/14/2015 – 03/31/2019** **2.4 calendar**
NIH/NCATS $2,911,225
National Center for Advancing Translational Sciences, National Institutes of Health
Cincinnati Center for Clinical and Translational Science and Training
This is the national consortium of medical research institutions, funded through Clinical and Translational Science Awards (CTSAs), which is working together to:
- Improve the way biomedical research is conducted across the country
- Reduce the time it takes for laboratory discoveries to become treatments for patients
- Engage communities in clinical research efforts
- Train the next generation of clinical and translational researchers

90IF0122-01-00 (Meinzen-Derr) **09/30/2016 – 09/29/2019** **0.6 calendar**
NIH $128,115
Improving Outcomes Using Aided Augmentative and Alternative Communication for Children who are Deaf or Hard of Hearing
Goal: The objective of this proposal is to test the efficacy of a technology-assisted language intervention (TALI) program designed to incorporate aided AAC using iPads for improving language in children who are DHH.

What other organizations were involved as partners?

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: Not applicable.

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

9. APPENDICES: Updated Award Chart

LR170169: Novel Neuroimaging Assessments of Glymphatic Disruption in Humans, a Plausible Key Pathophysiological Mechanism for CNS Lupus

PI: Mark DiFrancesco, Cincinnati Children's Hospital Medical Center

Budget: $149,951.00 **Topic Area:** CNS Lupus **Mechanism:** FY17, Lupus Research Program, Concept Award

Research Area: 1405, 1407 **Award Status:** 01-SEP-2018 – 31-AUG-2020 under NCE: second NCE requested ending 31 AUG 2021

Study Goals: We aim to develop a combination of neuroimaging approaches that will allow regional characterization of glymphatic integrity. Our plan includes comparison of these imaging measures in patients with SLE and matched healthy controls (HC). The objectives are motivated by our central hypothesis that impairment of the glymphatic tissue-clearing process contributes to neurodegeneration in SLE.

Specific Aims: We propose three features of the glymphatic system that may be impacted by SLE: a) Sufficient flow of CSF from the subarachnoid space into perivascular channels; b) Maintenance of patent perivascular channel morphology; c) Respiratory modulation of vascular size to drive glymphatic flow.

Aim 1: Assess key glymphatic features by developing targeted imaging approaches: a) Detecting the glymphatic-specific microenvironment by adopting "microdynamic" imaging methods based on relaxation-diffusion correlation spectroscopy. In this way, we aim to detect a restricted diffusion microenvironment of CSF-like fluid (e.g. glymphatics) by employing wide regimes of relaxation and diffusion, and b) Vascular space occupancy (VASO) imaging geared toward vascular volume changes with respiration.

Aim 2: Assess the impact of SLE on imaging measures of glymphatic integrity: a) Compare glymphatic imaging features between homogeneously phenotyped SLE patients and matched HC, focused on brain regions previously observed to suffer functional and/or structural MRI changes in SLE; b) Explore regional correspondence, among SLE patients, between each glymphatic measure and tissue volume, a localized indicator of brain health.

Key Accomplishments:

Publications:

Patents:

Funding Obtained:

This generic Statement of Work document is intended to assist applicants with the format preferred by CDMRP. This particular SOW does not contain any specific scientific information and is intended to be easily modifiable for any project. Not all components will be applicable for every project; please consult your Program Announcement for specific award requirements.

STATEMENT OF WORK – 07/20/2020
PROPOSED START DATE Sept 01, 2020

Site 1: Cincinnati Children's Hospital
3333 Burnet Ave.
Cincinnati, OH 45229
PI: Mark W. DiFrancesco, PhD

Site 2: N/A

Specific Aims (specified in proposal)	Timeline	CCHMC
Aim 1: Development/optimization of imaging protocols for glymphatic features	Months	
Aim completed	-	Drs. DiFrancesco and Lee
Aim 2: Comparison of SLE and healthy controls with respect to new glymphatic measures.		
Complete recruitment and data acquisition of lupus and control subjects. 7 lupus acquired. remaining N = 25 9 SLE/16 controls	1-10	Drs. Brunner and DiFrancesco
Optimize analysis pipeline	1-6	Dr. DiFrancesco
Test for correspondence between glymphatic measures and local tissue volume: Analysis	7-11	Drs. DiFrancesco, Lee, and Altaye
Compare SLE to healthy controls for each imaging measure: Analysis.	7-11	Drs. DiFrancesco, Lee, and Altaye
Image acquisition completed	10	Drs. Brunner and DiFrancesco
Analyses completed	11-12	Drs. DiFrancesco, Altaye
Results interpretation and preparation of publication(s)	11-12	Drs. DiFrancesco, Lee, Altaye, and Brunner

If human subjects are involved in the proposed study, please provide the projected quarterly enrollment in the following table.

	Year 1			
Target Enrollment (per quarter)	**Q1**	**Q2**	**Q3**	**Q4**
CCHMC	6	8	8	3
Target Enrollment (cumulative)	**6**	**14**	**22**	**25**

Note: The Government reserves the right to request a revised SOW format and/or additional information.