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TITLE: Biomarkers and Brain Mechanisms of Gulf War Illness

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13. SUPPLEMENTARY NOTES

14. ABSTRACT: [**no new findings; original abstract provided**] Gulf War illness (GWI), a chronic and debilitating pain, headaches, impaired memory and thinking, fatigue, respiratory and gastrointestinal symptoms, and skin abnormalities. Exposure and sensitivity to chemical, pharmaceutical and/or environmental toxins in a combat theater of operations is believed to be causative of the illness. The pathobiological mechanisms of GWI are unknown; there are no validated diagnostic tests, nor are there effective treatments or cures. This is a case-control study consisting of 20 Gulf War veterans affected with GWI and 20 matched non-affected Gulf War veterans, who will serve as the normal control group. All subjects will undergo brain positron emission tomography and magnetic resonance imaging scans for assessments of metabolic or neurochemical disturbances that may be associated with GWI. In all consenting participants, a lumbar puncture will be performed to obtain cerebrospinal fluid (CSF), which will be analyzed for abnormalities in biochemical compounds that may be related to GWI. The derived neuroimaging and CSF metabolic or biochemical data will be compared between the groups to determine if there are abnormal changes in GWI veterans compared to controls, which may shed new light onto the pathophysiology of GWI, as well as serve as biomarkers of the disorder.

15. SUBJECT TERMS
Gulf War illness, neuroinflammation, oxidative stress, mitochondrial dysfunction, magnetic resonance imaging (MRI), Positron Emission Tomography (PET)

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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1. INTRODUCTION:

The overall objective of this study is to evaluate the suitability of a number of endogenous chemical compounds or metabolites to serve as sensitive brain imaging and cerebrospinal fluid (CSF) biomarkers of pathologic alterations in Gulf War illness (GWI) for use to facilitate early diagnosis, to assess disease progression and to monitor therapeutic response in future clinical trials of promising interventions. This is a case-control study that will enroll 20 Gulf War veterans affected with GWI and 20 matched non-affected Gulf War veterans, who will serve as the normal control group. All subjects will undergo brain positron emission tomography (PET) and magnetic resonance imaging (MRI) scans to assessment metabolic or neurochemical disturbances that may be associated with GWI. In all consenting participants, a lumbar puncture will be performed to obtain CSF samples, which will be analyzed for abnormalities in biochemical compounds that may be related to GWI. The derived neuroimaging and CSF metabolic or biochemical data will be compared between the groups to determine if there are abnormal changes in GWI veterans compared to controls, which may shed new light onto the pathophysiology of GWI, as well as serve as biomarkers of the disorder.

2. KEYWORDS:

Gulf War illness, Neuroinflammation, Oxidative Stress, Mitochondrial Dysfunction; Magnetic Resonance Imaging, Positron Emission Tomography

3. ACCOMPLISHMENTS:

What were the major goals of the project?

RESEARCH-SPECIFIC TASKS:		
For All Specific Aims: Recruitment & Regulatory Approvals	**Timeline**	**Site(s)**
Major Task 1: GWI and Non-GWI Subject Recruitment	Months	ALL
Subtask 1: Establish formal contact between Mount Sinai Beth Israel (MSBI) Medical Center and the New Jersey War Related Illness & Injury Study Center [NJ WRIISC] to discuss strategy for recruiting GWI and non-GWI veterans for the study.	1	Dr. Natelson & Dr. Helmer

Subtask 2: Develop complementary or cooperative IRB protocols, including study advertisement material that would enable seamless recruitment/characterization of subjects at NJ WRIISC/MSBI and referral to Weill Cornell Medicine [WCM].	1-3	Drs. Natelson, Helmer & Shungu
Subtask 3: Submit IRB protocols at each participating Institution. **Second-tier DoD human subjects regulatory review and approval conducted by the Office of Research Protections, Human Research Protections Office (HRPO)**.	**3-6**	Drs. Natelson, Helmer & Shungu; HRPO
Milestone(s) Achieved: All IRB protocols approved; recruitment starts in earnest by month 6 and will continue to end of project	6-30	Drs. Natelson, Helmer
Specific Aim 1: Neuroimaging Biomarkers Studies		
Major Task 2: Conduct *in vivo* brain ^{11}C-(R)-PK11195 PET to assess **neuroinflammation**		WCM
Subtask 1: Order supply for producing the radioligand and review chemistry and PET scanning protocol.	1-6	Dr. Babich
Subtask 2: Conduct PET scans in 10 GWI and 10 non-GWI veterans	6-30	Dr. Babich
Milestone(s) Achieved: Availability of radioligand on demand to end of study; clear ability to obtain good PET scans, reproducibly, in each subject using the PK11195 PET technique	30	
Major Task 3: To conduct ^{1}H and ^{31}P MRS studies for assessment of **oxidative stress** and **mitochondrial dysfunction** *in vivo*. Assess cerebral blood flow using ASL-MRI.		WCM
Subtask 1: Protocols for achievement of this Major Task are already fully developed and being used in an ongoing study in chronic fatigue syndrome that is identical to the one we are proposing in GWI. The protocol will be reviewed with the MR neuroimaging team to ensure its flawless implementation.	1-6	Dr. Shungu
Subtask 2: Conduct ^{1}H and ^{31}P MRS and scan in 20 GWI and 20 non-GWI veterans to assess oxidative stress and mitochondrial dysfunction; also measure CBF in all 40 subjects using ASL-MRI.	6-30	Dr. Shungu
Milestone(s) Achieved: Clear ability to obtain high-quality ^{1}H and ^{31}P MR spectra, as well as ASL-MRI CBF maps in each enrolled subject.	30	

Specific Aim 2: CSF Biomarkers		
Major Task 4: Collect CSF samples from all consenting subjects for validation of neuroimaging biomarkers.		WCM
Subtask 1: Collect and cryo-freeze CSF samples using lumbar puncture	6-30	Dr. Mangat
*Milestone(s) Achieved: Clear ability to collect and freeze CSF samples for later analyses to determine markers of oxidative stress and neuroinflammation (cytokines, **including IL-17**).*	30	
For Specific Aims 1 & 2: Data Analysis and Hypothesis Testing		
Major Task 5: **Data Analysis, Reduction, Statistical Analyses**.		WCM
Subtask 1: Analyze/process and reduce the data from all the active tasks, combine with the clinical data in a **master** database and perform statistical analyses and hypothesis testing.	30-36	All investigators with Dr. Shungu supervising
*Milestone(s) Achieved: Determination of whether: (a) **neuroinflammation**, **oxidative stress** and **mitochondrial dysfunction** play a role in GWI pathobiology; and (b) the outcome measures either individually or in concert can serve as **biomarkers** for GWI and point to potential **brain mechanisms** for the illness.* *Submission of at least 3 manuscripts for publication.*	36	

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.

The primary activities for the second year of this research were to carry out neuroimaging biomarkers studies on the enrolled GWI and non-GWI subjects, consisting of:

1) brain ^{11}C-(R)-PK11195 PET scan to assess neuroinflammation

2) ^{1}H and ^{31}P MRS scans to assess oxidative stress and mitochondrial dysfunction in vivo; and ASL-MRI to measure regional cerebral blood flow.

3) to collect blood plasma/urine, and in consenting subjects, CSF samples from for corroboration with and validation of neuroimaging biomarkers.

Accomplishment goals to date:

This study obtained the final approval by the DoD HRPO to begin both the recruitment and assessment phases of the project on 12 July 2016. Since the last annual progress report, we screened 9 subjects from support groups and social media. Overall, 3 subjects have completed all the proposed clinical and neuroimaging assessments.

Despite having enrolled only 3 subjects who met eligibility, our efforts to reach Gulf War veterans have considerably increased the interest, response and number of subjects we have screened to date for eligibility to participate. As part of those efforts, we recently attended monthly meetings of a Gulf War Veterans support group at the Brooklyn VA Hospital Center, where we presented information on our study, provided our study recruitment flyer for inclusion in the Newsletter of the VA Advisory Board, as well as for posting on bulletin boards on the premises. We have worked on a similar outreach effort involving the Manhattan VA Hospital Center and the Bronx VA Hospital Center. We remain hopeful that these outreach efforts will prove fruitful and enhance what has been an extremely challenging study to recruit.

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

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to report

How were the results disseminated to communities of interest?

As part of our recruitment efforts, we have attended a monthly meeting of Gulf War Veterans support group at the Brooklyn VA Hospital Center, where we presented information on our study and have undertaken similar outreach efforts to involve potential participants from the Manhattan and Bronx VA Hospital Centers.

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

Our plan for the next reporting period is to continue our outreach efforts to ensure that they pan out. The study protocols and procedures have been shown to yield the desired data. Therefore, for the next reporting period we'll describe our progress in advancing the study toward fulfilling its objectives based on our latest outreach efforts.

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?

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report.

What was the impact on society beyond science and technology?

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report.

5. **CHANGES/PROBLEMS:** The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Problem: Continued recruitment challenge.

Remedial Actions:

1. Attended monthly meetings of the VA support group at the Brooklyn VA Center
   - Presented study in-person to potential participants
   - Provided recruitment flyer for dissemination.

2. Contacting Manhattan & Bronx VA Centers to similarly present our study in-person to potential participants at these additional VA Centers.

3. Continue tapping into existing websites and social media outlets
   - institutional clinical trials site
   - recruitment specific sites (ClinicalConnection, Craiglist)
   - social media (Facebook)

Changes that had a significant impact on expenditures

None to date.

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

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

None

Significant changes in use or care of vertebrate animals.

Not applicable

Significant changes in use of biohazards and/or select agents

Not applicable

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.

- **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**

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *biospecimen collections;*
- *audio or video products;*
- *software;*

- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*

Funding Support: *The Ford Foundation (Complete only if the funding support is provided from other than this award).*

Name:	Dikoma C. Shungu, Ph.D.
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	orcid.org/0000-0001-9452-2245
Nearest person month worked:	1 calendar Month
Contribution to Project:	Dr. Shungu oversees all the MR Neuroimaging aspects the proposed research, as well as its day-to-day coordination of the study.
Name:	Xiangling Mao, M.S.
Project Role:	Co-I
Researcher Identifier (e.g. ORCID ID):	orcid.org/0000-0003-2274-8282
Nearest person month worked:	1 Calendar Month
Contribution to Project:	Ms. Mao perform work in the area of regulatory activity, MR Scan and data processing.
Name:	Benjamin H. Natelson, M.D.
Project Role:	Sub-site PI at BIMC
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1 Calendar Month
Contribution to Project:	Dr. Natelson will perform work in the area of subject recruitment and characterization.
Name:	Diana Vu
Project Role:	Study coordinator at BIMC
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1 Calendar Month
Contribution to Project:	Ms. Vu will perform work in the area of subject recruitment.
Name:	Michelle Blate
Project Role:	Nurse-practitioner at MSBI
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1 Calendar Month
Contribution to Project:	Ms. Blate performs work in the area of subject recruitment.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what

has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to Report.

What other organizations were involved as partners?
N/A

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.
Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: N/A

QUAD CHARTS: N/A

9. APPENDICES: N/A