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14. ABSTRACT
At least one in four of the nearly 700,000 U.S. veterans of the 1990-1991 Gulf War are affected by Gulf War illness (GWI), the chronic condition currently defined only by veterans' self-reported symptoms. Previous studies have identified neurological, inflammatory, endocrine, and hematological measures that significantly distinguish groups of GWI cases from controls. Using state-of-the-art biodiscovery techniques, the present study is designed to identify a biological signature for GWI that can be used clinically as a diagnostic blood test. A multiphase case-control design is used to canvas a broad spectrum of blood analytes in three independent samples of Gulf War veterans. The multiplex assay platform includes a diverse array of cytokines, chemokines, growth factors, hormones, hematological measures, and neurotrophic factors, and provides highly replicable and accurate quantitative values for each analyte. The pattern of analytes whose values most reliably distinguish veterans with GWI from healthy controls in the first two "development" samples will be assembled, and tested in the third "validation" sample, to determine the test's sensitivity and specificity for diagnosing GWI and/or identified GWI subgroups. If successful, the availability of an objective test for diagnosing GWI will be immensely beneficial to veterans and their healthcare providers, and provide an important tool for improving research to better understand and treat GWI.

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Biomarker Discovery in Gulf War Veterans:
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Introduction

Since their return from the 1990-1991 Gulf War, at least one in four of the nearly 700,000 U.S. veterans who served in that war have been affected by the condition known as Gulf War illness (GWI).[1-3] Gulf War illness is characterized by a complex of multiple chronic symptoms, typically some combination of persistent headache, widespread pain, cognitive difficulties, unexplained fatigue, gastrointestinal problems and diverse other abnormalities. Although individual symptoms can vary, studies report a generally consistent pattern of chronic symptoms across different Gulf War veteran populations. Longitudinal studies indicate that few veterans have recovered, or substantially improved over time.[4,5] Gulf War illness has, as a result, presented a difficult challenge for a large number of veterans for over 20 years.

For many ill veterans, the difficulty of living with chronic illness is accompanied by additional problems when seeking medical care. Physicians can be exceptionally challenged by veteran patients reporting this array of diverse symptoms—multiple, persistent symptoms not accounted for by established medical or psychiatric diagnoses and not explained by interpretable abnormalities on standard diagnostic tests.[6] Although some physicians are knowledgeable about problems of this nature, many veterans continue to report frustrating experiences with healthcare providers who are not familiar with GWI or similar problems, and may even be dismissive of veterans' illness as psychosomatic or malingering.

The pathobiology of GWI appears to be complex. Previous studies have identified neurological, immune, endocrine, and hematological measures that significantly distinguish groups of GWI cases from controls.[7-21] None of these measures, however, provides sufficient sensitivity and specificity to diagnose GWI. GWI is currently identified clinically and in research studies only on the basis of veterans' subjectively-reported symptoms. An objective test for use in diagnosing GWI would be immensely beneficial to veterans and their healthcare providers, and would also provide an important tool for improving research to better understand and treat GWI.

As has been described elsewhere,[1,22] there is considerable evidence to suggest that the diverse symptoms and biological alterations associated with GWI reflect a persistent disruption in central nervous system (CNS) proinflammatory and neuroendocrine parameters. These processes can precipitate, sustain, and respond to peripheral changes in immune, hypothalamic-pituitary-adrenal (HPA), autonomic, and hematological parameters. Reported differences in these systems are more subtle than the frank "abnormalities" identified with standard diagnostic tests (e.g., measures indicating adrenal failure, clotting pathology, or defective immune competence). These more subtle differences have been detectable only by comparing group values between sick and healthy Gulf War veterans. No single measure provides values that are distinct enough, or "abnormal" enough to serve as a suitable diagnostic marker on its own. The multisystem nature of GWI suggests that the diagnostic test that can best identify individual veterans with GWI, in the near term, may require evaluation of more than one measure, whose values are considered together.

The present study utilizes state-of-the-art biodiscovery techniques that canvas a broad spectrum of blood analytes to develop a limited panel of assays that can be combined onto a single

multiplex platform specific to GWI for use as a GWI diagnostic tool. Blood levels of 190 proteins associated with immune, inflammatory, endocrine, and neurological processes that potentially underlie the symptoms of GWI are analyzed using a two-phase, case-control design. In the initial "development" phase, multiplex assay results from two independent samples of 75 veterans (each with 45 GWI cases and 30 controls) are used to determine the biomarker signature pattern or patterns that best distinguish GWI cases from controls. In the second, "validation" phase, this biomarker signature will be evaluated in a third sample (90 GWI cases, 60 controls) to assess its sensitivity and specificity for identifying GWI cases and/or GWI subgroups of importance. An important aspect of the multiplex laboratory methods used in both phases is the provision of highly accurate and replicable quantitative values for each assay. This approach holds particular appeal, since the subgroup of analytes that most reliably distinguishes GWI cases from controls can readily be developed for use as a diagnostic tool in the clinic setting that uses a small blood sample, at a relatively low cost.

This interdisciplinary research study is led by a team of experienced GWI investigators and statisticians at Baylor University, working with clinical researchers at Scott & White Healthcare and laboratory scientists at Myriad-Rules Based Medicine (M-RBM), the company at which the biomarker discovery process used by the project was developed. The M-RBM multiplexed assay platforms have been widely used by the pharmaceutical, biotechnological, medical, and basic research communities for discovery and validation of biomarker patterns indicative of specific diseases, subgroup differences in clinical drug effects, and other purposes. The M-RBM process utilizes a platform that couples the precision of Luminex technology with the accuracy of automated liquid handling. This platform provides quantitative Multi-Analyte Profiles, or MAPs, of blood proteins using very small sample volumes (10-20 μL) over a dynamic range of fg/mL to mg/mL and intra-assay imprecision rates typically below 10 percent. In addition, all assays in M-RBM multiplex platforms are validated to Clinical and Laboratory Standards Institute (CLSI) guidelines and are run using a calibration/control strategy required for clinical laboratories. This approach therefore standardizes both the multiplex assay technology and the methodologies by which it is applied. These capabilities represent an important step forward for translating an identified biomarker profile into a clinically useful test.

In conjunction with the biomarker discovery process, the project involves a two-phase analytic effort to both develop and test algorithms for identifying GWI cases and, potentially, GWI subgroups. A number of bioinformatics and biostatistical techniques will be utilized by two independent analytic teams, at Baylor and M-RBM, to characterize assay patterns that distinguish GWI cases from controls. This dual analytic approach maximizes the potential for the project to provide the most informative and usable GWI case profiles from the collected data. In addition, the Baylor analytic team will determine whether unique patterns are associated with GWI subgroups of interest

If successful in developing a GWI-specific multiplex panel that identifies GWI with sufficient accuracy, the project will provide a major step forward for improving medical evaluation and care of veterans with GWI. It can also advance other aspects of GWI research, for example, by providing an objective measure for monitoring the effects of treatments evaluated in clinical trials.

Body

Task 1. Prepare and Submit Documents to Obtain Regulatory Approvals

This project requires human subjects review and approval by two Institutional Review Boards (Baylor and Scott & White IRBs) and by the USAMRMC's Office of Human Research Protections (HRPO). We also initially understood, based on information provided by DOD officials, that the project would require review and approval by the federal Office of Management and Budget (OMB), under the federal Paperwork Reduction Act (PRA). We were informed that the OMB approval process typically requires a minimum of eight months. We therefore designed the project timeline to allow ten months for obtaining regulatory approvals, as indicated in the Statement of Work.

Our initial strategy was to begin the process and document submissions required for OMB review and approval prior to HRPO and IRB submissions. This was because we understood that OMB approval would be needed to obtain our initial sampling data from the Defense Manpower Data Center (DMDC), and because the OMB approval process typically takes much longer than the IRB process. However, in a concurrent study, we were experiencing extended delays and considerable difficulties in connection with the DOD offices responsible for reviewing and forwarding our PRA documentation to OMB. These delays extended the timeline for OMB review to at least 15 months before we learned in 2013, after multiple requests, contacts, and discussions, that the DOD Information Management Office determined that our study would *not* be subject to the federal PRA and that OMB approval would not be required.

We then obtained initial Baylor IRB approval for the project in August 2013, and Scott & White IRB approval three months later. We submitted human subjects' documentation to the Army Office of Human Research Protections (HRPO) on November 26, 2013. Final Army HRPO approvals and authorization to proceed were provided on March 27, 2014. Since that time, we have continued to obtain continuing review approvals from both IRBs and from Army HRPO on schedule. However, subsequent project delays associated with obtaining DOD data required for developing our 3 study samples for the project are described below.

Our original timeline for this task anticipated that regulatory approvals could be obtained 10 months into the initial project year.

Task 2. Identify and screen three stratified random samples of Gulf War era veterans for study participation

This project was developed to establish a diagnostic tool for Gulf War illness, and so was designed to obtain blood samples from three independent "gold standard" population-based samples. Sampling and recruitment therefore requires that 1991 Gulf War veterans be contacted

proactively from random samples of veterans residing in a defined geographical area. As proposed and previously approved for this study, this is to be accomplished by obtaining data from DOD's Defense Manpower Data Center (DMDC) that includes names and contact information for veterans residing in our target regions.

Our research team, as well as other CDMRP GWIRP-funded investigators had previously worked with DMDC to obtain this type of data for sampling and recruitment purposes. We had been in contact with the DMDC data management team prior to submitting our data request for the current project, which was identical to an earlier request submitted for a previously-funded GWIRP project. However, in March 2014, we became aware of serious challenges that have emerged for research institutions applying to DMDC to obtain data for research studies. These difficulties, associated with recently established DMDC Privacy Office requirements for IT credentialing and authorization of data release, have resulted in our still being unable to obtain the population-based information on Gulf War veterans residing in the geographical areas targeted for inclusion in the study.

None of the multiple and varied attempts to put together the required partnerships and IT assurances through August of 2014 were acceptable to DMDC. In the Fall of 2014, Dr. Lidie, our CDMRP Program Manager, assisted us in working with the Research Facilitation Team (RFT) of the Army Analytics Group (AAG) to identify potential solutions. The RFT/AAG team indicated they would work with us on a plan under which they would attempt to establish a Memorandum of Understanding (MOU) with DMDC to obtain and share the requested data. Draft language for the MOU, including Baylor IT security documentation, was provided to the AAG RFT in March 2015. Subsequent communications with RFT personnel resulted in their developing the data sharing plan, which was presented to DMDC. We were informed in May, 2015, however, that after discussions with DMDC senior leadership, AAG determined that they could not assist with our data request. The Principal Investigator subsequently contacted DMDC senior leadership to request additional information re: requirements that would need to be met to obtain the requested DMDC data. No additional information has yet been provided.

Parallel to the data efforts involving the RFT/AAG, the PI identified additional avenues that might provide options for acquiring the data. This includes working with an Army officer and faculty colleague in the joint Baylor-Army academic program in San Antonio who has offered to work with us to obtain the DMDC data. Repeated attempts to obtain DOD or DMDC-specific guidelines for data security provisions to be followed by this partner for obtaining and sharing the DMDC data have not been successful.

Alternate Strategies for Sample Development and Recruitment

While continuing to work to identify solutions to address challenges in obtaining DMDC data, we have developed two alternate approaches for identifying, screening and recruiting Gulf War veterans who may be eligible to participate in the study. Study design amendments reflecting the alternate recruitment strategies described below were approved by Baylor's IRB in January, 2015.

Alternate Recruitment Strategy 1. Recruitment of veterans identified through VA's Gulf War Registry. The VA Gulf War Registry rolls include all 1991 Gulf War veterans in the region who came forward to obtain free medical exams and testing at VA between 1994 and the present. Accessing veterans through the registry is preferred to other recruitment strategies, since the sample of veterans recruited in this way is more similar to a population-based sample, and more representative of Gulf War veterans overall, than veterans identified through other methods.

Alternate Recruitment Strategy 2. This more standard recruitment strategy involves a combination of media outreach, working with veterans' organizations, and scheduled events to inform area Gulf War veterans about our program and invite their participation in the study. It is the least desirable recruitment option from a scientific perspective, but appears to be the primary avenue that we can implement independently.

Additional Research Planning

We previously prepared for study startup with Baylor's Center for Community Research and Development (CCRD), which is responsible for programming and formatting the Computer Assisted Telephone Interview (CATI) software for the screening interviews to be used in conjunction with subject recruitment. The screening program and protocol have been tested and can be fielded once final recruitment details are in place.

Note that no subject recruitment or data collection activities have been initiated and no research results are yet available.

Task 3. Collect and freeze blood samples from 300 Gulf War veterans comprising 3 independent population samples

As previously described, our inability to obtain study sampling information in order to recruit and screen veterans for the study has meant that no start date has yet been established for beginning subject intake and blood collection. We have, however, held planning meetings to discuss details of study start up with our clinical partners at Scott & White. However, no activities related to blood collection have been initiated at this time.

Tasks 4 – 6.

No activities completed or underway at this time. No blood samples have been obtained, no analyses have been initiated, and no research results are yet available.

Project Timeline and Location

As previously described, extensive delays resulting from DOD's initial misdirection concerning OMB approvals, followed by our inability to obtain DMDC data have caused considerable concerns regarding the timeline required for completing the project. Due to these delays as well as institutional difficulties that have slowed implementation of the current and other federally-funded research projects, we have concluded it is unlikely that the project can be completed in an acceptable timeframe under current circumstances. We therefore requested a 12 month extension without funds (EWOF) for the project on September 8, 2015. The Principal Investigator has also initiated the process of seeking an alternate location for implementing the current project and other studies in her program. She has prioritized locating at a research institution with enhanced capabilities and resources for implementing clinical studies at an accelerated pace, without the need for clinical site subawards. Additional priorities include enhanced IT security capabilities that provide FISMA-certified data protections, in accordance with DMDC requirements.

Key Research Accomplishments

Only regulatory submissions and finalizing study protocol, instruments, and clinical site planning have been accomplished to date. Data collection has not yet been initiated.

Reportable Outcomes

There are no manuscripts or other reportable outcomes at this time.

Conclusion

No research results are yet available; no conclusions can be drawn at this time.

References

1. Research Advisory Committee on Gulf War Veterans' Illnesses. *Gulf War Illness and the Health of Gulf War Veterans: Scientific Findings and Recommendations.* Washington, D.C. U.S. Government Printing Office, Nov 2008.

2. Institute of Medicine. *Gulf War and Health: Volume 8 - Health Effects of Serving in the Gulf War*. Washington, DC: National Academy Press; 2010.

3. Steele L. Prevalence and patterns of Gulf War illness in Kansas veterans: association of symptoms with characteristics of person, place, and time of military service. *Am J Epidemiol.* 2000;152:992-1002

4. Hotopf M, David AS, Hull L, Nikalaou V, Unwin C, Wessely S. Gulf war illness--better, worse, or just the same? A cohort study. *BMJ.* 2003;327:1370.

5. Kang HK. Longitudinal health study of Gulf War era veterans. Presentation at: Meeting of the Research Advisory Committee on Gulf War Veterans' Illnesses; Sep 16, 2008; Washington, D.C.

6. Richardson RD, Engel CC, Jr., McFall M, McKnight K, Boehnlein JK, Hunt SC. Clinician attributions for symptoms and treatment of Gulf War-related health concerns. *Arch Intern Med.* 2001;161:1289-1294.

7. Zhang Q, Zhou XD, Denny T, et al. Changes in immune parameters seen in Gulf War veterans but not in civilians with chronic fatigue syndrome. *Clin Diagn Lab Immunol.* 1999;6:6-13.

8. Vojdani A, Thrasher, JD. Cellular and humoral immune abnormalities in Gulf War veterans. *Environ Health Perspect.* 2004;112:840-846.

9. Skowera A, Hotopf M, Sawicka E, et al. Cellular immune activation in Gulf War veterans. *J Clin Immunol.* 2004;24:66-73.

10. Whistler T, Fletcher MA, Lonergan W, et al. Impaired immune function in Gulf War Illness. *BMC Med Genomics.* 2009;2:12.

11. Sullivan K, Krengel M, Proctor SP,et al. Cognitive functioning in treatment-seeking Gulf War veterans: pyridostigmine bromide use and PTSD. *J Psychopathology and Behavioral Assessment.* 2003;25:95-103.

12. Toomey R, Alpern R, Vasterling JJ, et al. Neuropsychological functioning of U.S. Gulf War veterans 10 years after the war. *J Int Neuropsychol Soc.* 2009;15:717-729.

13. Haley RW, Spence JS, Carmack PS, et al. Abnormal brain response to cholinergic challenge in chronic encephalopathy from the 1991 Gulf War. *Psychiatry Res.* 2009;171:207-220.

14. Chao LL, Rothlind JC, Cardenas VA,et al. Effects of low-level exposure to sarin and cyclosarin during the 1991 Gulf War on brain function and brain structure in US veterans. *Neurotoxicology.* 2010.

15. Heaton KJ, Palumbo CL, Proctor SP,et al. Quantitative magnetic resonance brain imaging in U.S. Army veterans of the 1991 Gulf War potentially exposed to sarin and cyclosarin. *Neurotoxicology.* 2007

16. Golier JA, Schmeidler J, Legge J, Yehuda R. Enhanced cortisol suppression to dexamethasone associated with Gulf War deployment. *Psychoneuroendocrinology.* 2006;31:1181-1189.

17. Golier JA, Schmeidler J, Legge J, Yehuda R. Twenty-four hour plasma cortisol and adrenocorticotropic hormone in Gulf War veterans: relationships to posttraumatic stress disorder and health symptoms. *Biol Psychiatry*. 2007;62:1175-1178.

18. Sastre A, Cook MR. Autonomic Dysfunction in Gulf War Veterans. Fort Detrick, MD: U.S. Army Medical Research and Materiel Command; November, 2004. DAMD17-00-C-0018.

19. Haley RW, Vongpatanasin W, Wolfe GI, et al. Blunted circadian variation in autonomic regulation of sinus node function in veterans with Gulf War syndrome. *Am J Med.* 2004;117:469-478.

20. Bach RR, Slater B. Tissue Factor and Gulf War-Associated Chronic Coagulopathies. Presentation at: Meeting of the Research Advisory Committee on Gulf War Veterans' Illnesses; June 29, 2009; Boston, MA.

21. Hannan KL, Berg DE, Baumzweiger W, et al. Activation of the coagulation system in Gulf War Illness: a potential pathophysiologic link with chronic fatigue syndrome. A laboratory approach to diagnosis. *Blood Coagul Fibrinolysis*. 2000;11:673-678.

22. Fields D. New Suspect in Gulf War Syndrome. Huffington Post. Available at: http://www.huffingtonpost.com/dr-douglas-fields/new-suspect-in-gulf-war-s_b_483875.html