AWARD NUMBER: W81XWH-15-1-0695

TITLE: Designing a Successful Acupuncture Treatment Program for Gulf War Illness

PRINCIPAL INVESTIGATOR: Dr. Lisa Conboy

CONTRACTING ORGANIZATION: MCPHS UNIVERSITY
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13. SUPPLEMENTARY NOTES

14. ABSTRACT

This project finalizes our work in the investigation of acupuncture as a treatment for the symptoms of Gulf War Illness. The goals of this application were:1) Gather follow up data from our veteran participants (from our completed parent study *The Effectiveness of Acupuncture in the Treatment Of Gulf War Illness* W81XWH-09-2-0064) on current symptom levels and use of services to explore the long-term effects of an acupuncture treatment program, 2) Develop suggestions for how an acupuncture program may be implemented using the viewpoints of multiple stakeholders.

15. SUBJECT TERMS
Gulf War Illness, Complex Medical Illness, Acupuncture, Treatment Trial, Secondary Date Analysis, Program Evaluation

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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Table of Contents

1. INTRODUCTION:

This project continues our work in the investigation of acupuncture as a treatment for the symptoms of Gulf War Illness. The goals of this current application are:1) Gather follow up data from our veteran participants (from our completed parent study *The Effectiveness of Acupuncture in the Treatment Of Gulf War Illness* W81XWH-09-2-0064) on current symptom levels and use of services to explore the long-term effects of an acupuncture treatment program, 2) Develop suggestions for how an acupuncture program may be implemented using the viewpoints of multiple stakeholders.

2. KEYWORDS: Gulf War Illness, Complex Medical Illness, Acupuncture, Treatment Trial, Secondary Data Analysis, Program Evaluation

3. ACCOMPLISHMENTS:

What were the major goals of the project?

The goals of this current application are:1) Gather follow up data from our veteran participants (from our completed parent study *The Effectiveness of Acupuncture in the Treatment Of Gulf War Illness*) on current symptom levels and use of services to explore the long-term effects of an acupuncture treatment program, 2) Develop suggestions for how an acupuncture program may be implemented using the viewpoints of multiple stakeholders, 3) Develop a treatment manual for acupuncture practitioners explaining the most effective methods of treating the symptoms of GWI.

What was accomplished under these goals?

As planned in the Statement of Work (see Appendix B), we have completed the tasks:

1. **Create program evaluation documents (Month 1):** Dr. Conboy finalized the survey instrument and focus group agenda. These materials were circulated to all other study staff for feedback. Suggestions and edits were made for submission to the IRB.
2. **Train research assistant, and review goals with consultants (Month 1-2**): Once funding was achieved the team had a face-to-face meeting (using Skype for long-distance members) to review program goals. Follow-up group e-mails will solidify our process.
3. **IRB Review (Month 1-3):** The protocol was submitted to the IRB as soon as funding was approved. This approval took longer than expected (6 months) but the project was approved by the New England IRB and HRPO April 12, 2016.

Task 4.1 Program evaluation with study subjects: All of the subjects who participated in the parent trial were contacted with the goal of obtaining current information on their health and requesting the subject's interest in participating in a focus group with multiple stakeholders to help design the best acupuncture protocols and program for veterans. We have contacted the original sample first with email (n=104). Those that did not respond were mailed an IRB approved letter requesting participation. We next sent invitation flyers to those that we have not heard from. Those that still have not responded were called. This process with multiple contact means was repeated twice. After extending the grant due to low participation, we again attempted to contact veterans to complete the survey. From the final push, we received two more completions of the study. Table 1 below lists the total number contacted subjects, the outcomes of those contacts, and number of additional contact attempts.

Table 1 Designing a Successful Acupuncture Treatment Program Recruitment Efforts	
Task	**Attempts**
Total sample of veterans to be surveyed	104
Number of Completed Surveys	52
Number of Veterans who Withdrew from Survey	12
Number of Phone Calls	316
Number of Mailings: Letters/flyers/Consent Forms	401
Number of Emails	113
Number of Veterans now Deceased	3

Providing further detail Table 1, 73 Informed Consents were sent out; sometimes a consent was sent more than once to unresponsive veterans. Fifty-five Informed Consents were completed and returned to us and these same subjects were sent surveys to fill out. Out of this 55 total, 52 completed the survey, 12 were entered manually into database, 3 never returned the surveys. Eight veterans no longer want to be contacted. Three veterans are now deceased. Fifteen veterans' addresses and/or phone numbers were no longer valid. We attempted further contact with the 25 remaining veterans that we have not had contact with to let them know of the end of the survey period; we received no additional responses.

Once contacted, subjects were mailed an Informed Consent and IRB approved survey which: (1) repeats the survey questions administered in the parent study, (2) asks additional questions about subjects' study experiences, use of health services since the study, and requesting feedback and suggestions for program and treatment improvement.

Subject contact was slow, thus we implemented some additional techniques to resurvey our sample. We started asking veterans we contacted what they would like as incentive. We were told that they wanted more acupuncture. Our host institution (New England School of Acupuncture at MCPHS University) independently provided 5 free treatment vouchers to our student clinic in Newton MA, and our Worcester MA student clinic is free. This information was shared in a mailing to subjects in June 2017. Two subject responses came from this. We repeated this with those that did not answer on Nov 1, 2017. Boston GWI researcher (Kim Sullivan at Boston University) has sent us leftover incentive materials (commemorative coins and Desert Storm tee shirts) and we sent those to our remaining sample November 1, 2017 as part of our next attempt to reach the subjects.

In summary, due to lack of participation, we extended our grant period to continue our effort in receiving veteran responses. We were able to receive 2 more completed surveys from veterans during that period.

Survey Results:
We are eager to share preliminary results of our follow up survey (see Appendix C). As noted earlier, we received a 50% response rate. More detailed analyses are necessary to uncover if there is a bias in our sample, related to acupuncture treatment response or other factors such as use of acupuncture outside the trial. In comparing clinical outcomes, we found only a slight

deterioration from the time of the study. Moreover, the veterans reported high usability and satisfaction with treatment; many continued treatment on their own after the trial was over.

Task 4.2 Program evaluation with multiple stakeholders (Month 11-22)
We executed the first set of meetings November 19th and 20th 2016. We decided to begin this process before the scheduled date (per the Statement of Work) as the re-surveying of the veteran-study subjects proved more time consuming than planned.

This Practitioner Meeting took please Saturday, November 19, 2017 – Sunday November 20, 2017 at the New England School of Acupuncture, 150 California St. Newton MA 02458. The purpose of the meeting was to connect with the practitioners from the parent grant to discuss the development of a Treatment Program for Gulf War Illness with the acupuncture consultants. This meeting followed a survey we conducted of acupuncture practitioners who participated in the parent grant. The group reviewed survey results from the practitioners and discussed strategies for development of program.

Attendance:

MCPHS University

Lisa Conboy, Director of Research

KaiYin Hsu, Research Assistant

Beth Ann Schmitt, Research Administration Specialist

Grant Consultants

Rosa Schnyer, Lic.Ac., School of Nursing, University of Texas at Austin

Lisa Taylor Swanson, Lic.Ac., School of Medicine, University of Utah

Joe Chang, Lic.Ac., Chang Acupuncture

Dr. Iris Bell, College of Medicine, Tucson, University of Arizona

Dr. Marc Goldstein, VA Central Western Massachusetts

Parent Study Acupuncturists

ZhenZhen Zhang

Diana Dia

Christine Lee

Brian Whidden

We were disappointed with the level of information found through this focus group and Delphi process. We were given general information, where we were looking for specifics on how to treat GWI from a Chinese Medicine perspective in order to create a document for acupuncturists. Thus, we asked (with informed consent) 4 study practitioners to participate in another interview. These four were chosen because they represent different levels of expertise, and training (some are Chinese trained and some US trained). All agreed, were consented, and we conducted interviews on how the practitioner thought through cases; termed "diagnostic reasoning," which is an established line of inquiry in medical education. These audio tapes were transcribed and Eric Jacobson searched for themes. We have identified distinct patterns of investigation that can inform practitioners who want to treat GWI. The manuscript is attached in Appendix A.

Task 4.2.2 Conduct focus groups (Month 11-15): On March 18, 2017 stakeholder teams were brought together discuss how to best design and implement an acupuncture treatment protocol for use within the VA system, as well as how to best utilize practitioners in the general community. The discussion was led by Dr. Conboy. The resulting document will condense these viewpoints into a plan to optimize program formation.

The New England School of Acupuncture at MCPHS hosted this meeting with GWI Veterans and Acupuncturists who were part of the part of the parent grant, as well as Veterans Advocates. The meeting was from 9am – 12pm. The purpose was to develop ideas for an Acupuncture Treatment Program for Gulf War Illness, as well as to explore how can we develop an Acupuncture GWI Program for the Veterans Administration.

The transcripts from these focus groups have been transcribed and summarized by one reader, and were double coded (by a second reader). Themes thus far include: (1) Reasons why a Veteran would participate if VA had GWI Acupuncture Program, (2) What practitioners need to be aware of in dealing with Veterans and GWI, (3) Issues for the VA to Consider with implementing acupuncture therapy, (4) Education on "What is Acupuncture and how it will benefit Veterans?", (5) Did Acupuncture treatments affect attitudes toward other forms of Complementary and Alternative Medicine or health behaviors?

Attendees:
MCPHS University
Lisa Conboy, Director of Research
KaiYin Hsu, Research Assistant

Grant Consultants
Lisa Taylor Swanson, Lic.Ac., School of Medicine, University of Utah
Joe Chang, Lic.Ac., Chang Acupuncture
Dr. Iris Bell, College of Medicine, Tuscon, University of Arizona
Dr. Marc Goldstein, VA Central Western Massachusetts

Study Veterans (veterans from our parent study)
Tara Batista

Jay Pinette
Pat Hoarty
Gareth Mannion

Study Acupuncturists (practitioners from our parent study)
Dinah Shatz
ZhenZhen Zhang
Christine Lee
Eva Lunetta
Diana Dia

Veteran Advocates
Dee Lane, Campaign for Military Families
Dr. David Chen, Edith Nourse Rogers Memorial *Veterans* Hospital, *Bedford, MA*

Task 4.2.3 Conduct data analysis: program evaluation (Month 15-40):
We have transcribed the audio recording of the focus groups and are currently working on a manuscript. One finding from these focus groups, that we have already been able to act on, involves educating veterans and VA officials about acupuncture. The focus group data suggests that many veterans want acupuncture, many VA officials are interested in incorporating acupuncture, and many acupuncturists are excited to treat veterans. The eventual transcript will help all three types of players. Acupuncture practitioners in particular often do not choose to work with VA patients because the paperwork necessary to be credentialed and/or reimbursed is believed to be insurmountable. To counter this, one of our acupuncturist colleagues shared written instructions on how he succeeded at reimbursement. We were able to share his instructions directly with approximately 70 practitioners who wrote us after reading the article about our work in ACUPUNCTURE TODAY, *Treating Gulf War Illness: The Lasting Effects of Desert Shield/Storm,* or contacted Dr. Conboy directly.

Dr. Conboy tabulated focus group responses by theme to be circulated to focus group participants, with a request for addition feedback and clarification.

Dr. Conboy, Beth Ann Schmitt, and graphic designer consultant, Annette Ivanisevic, completed work on two infographics based on the program evaluation. *Introduction to Gulf War Illness for Acupuncturists* utilized the materials from the Matrix Analysis (Taylor Swanson et. al 2018), and conducted focus groups; utilizing quotes from some of the Veterans. The purpose of this document is to help acupuncturists learn more about Gulf War Illness and the more common Traditional Chinese Medicine diagnoses to treat it. The other infographic, *Acupuncture Helps Gulf War Illness,* was based of the Matrix Analysis data, our parent study (Conboy et.al. 2016) and included quotes from the focus groups with veterans. The purpose of this infographic was to provide both veterans and providers an opportunity to learn about how acupuncture can help with this illness. This one page sheet can be shared from both patient to practitioner and vice versa. We have distributed the infographics to all of the New England Veterans Administrations including Community Based Outpatient Clinics, Vet Centers and Veteran Organizations. List provided in Appendix D. We have also distributed both infographics to all New England School of Acupuncture students and faculty, as well as schools of Acupuncture and Oriental Medicine

across the country. The infographics also reside online on the American Society of Acupuncturists website Resources/Publications page at the following links: http://www.asacu.org/wp-content/uploads/2019/04/Introduction-to-Gulf-War-Illness-for-Acupuncturists.pdf and http://www.asacu.org/wp-content/uploads/2019/04/Acupuncture-Helps-Gulf-War-Illness.pdf. List provided in Appendix D.

Toward continued outreach, we will continue with these projects to produce manuscripts and network with other researchers, within and outside the VA, to disseminate our results and strengthen a network of collaboration; for example, we are building a network of VA clinicians and researchers who are interested in Integrative Medicine including Cathy St Pierre MD (Bedford, MA VA), Stephanie Taylor PhD (Associate Director of Health Services Research and Development, Veterans Administration), Kristen Tillisch MD (Ronald Reagan UCLA Medical Center), Dee Lane (Founder of Campaign for Military Families, Burlington MA), Christine Cronin DAOM (Faculty member Pacific College of Oriental Medicine and Clinic Supervisor at the Veterans Free Clinic, American Legion Post #731), Dr. David Chen MD (Bedford, MA VA). Most exciting, this latter link with David Chen was utilized to start discussions about NESA collaborating with this local VA to establish a student clinic on site, which would offer treatment to veterans on site at no cost to the VA or the veteran patients. Ms. Felice Indindoli, Lic.Ac. (New England School of Acupuncture graduate) was hired in April 2019 at Edith Nourse Rogers Memorial Veterans Hospital in Bedford, MA.

We have continued to: 1) publish updates on social media, 2) Collaborate on projects with GWI collaborators, 3) continue outreach to our parent study sample, 4) complete the associated manuscripts/infographics (Appendix A).

Survey Results:
We are eager to share preliminary results of our follow up survey (see Appendix C). As noted earlier, we received a 50% response rate. More detailed analyses are necessary to uncover if there are biases in our sample, related to acupuncture treatment response or other factors such as use of acupuncture outside the trial. In comparing clinical outcomes, we found only a slight deterioration from the time of the study. Moreover, the veterans reported high usability and satisfaction with treatment; many continued treatment on their own after the trial was over.

Task 4.2.4 Conduct Delphi Process (Month 36-42):

The Delphi process began with drafting the initial treatment questions by collaborator Rosa Schnyer DAOM. The 5 (from a complete sample of 31) treating practitioners from the parent study with the best treatment responses and who agree to participate came together with the named members of our study team on the afternoon of November 20th, 2017 to review those questions. Rosa Schnyer determined that we were unable to complete a Delphi process because there was too much variability in the sample.

As a goal of this aim was to produce a document to help acupuncturists better treat GWI, we decided to harvest the wealth of clinical knowledge in our study practitioners by capturing their diagnostic reasoning. Diagnostic reasoning is a line of inquiry used in medical education concerning how medical decisions are made. There are standard models used in biomedical

medical education, but none about GWI concerning Chinese Medicine. Our manuscript describing found models (Erickson, Appendix A) is a product that we can share with acupuncture educators to develop more effective treatments for Gulf War Illness.

Operationally, we hired medical anthropologist Dr. Eric Jacobson, who with IRB approval interviewed acupuncturists who were a part of the parent grant to determine diagnostic reasoning with the practitioners. The interviews have been transcribed and reviewed for clarity. Dr. Eric Jacobson's draft manuscript can be found in Appendix A. These protocol changes were IRB approved then CDMRP approved August 15, 2017.

What opportunities for training and professional development has the project provided?
Not Applicable/Nothing to Report.

How were the results disseminated to communities of interest?

Dr. Conboy presented *Nonlinear Modeling of Acupuncture Treatment in Complex Medical Illness*, at the Ninth International Conference on Complex Systems, July 23, 2018 in Cambridge MA.

Dr. Conboy presented our results at the Military Health System Research Symposium in Kissimmee, FL on August 22nd, 2018. Dr. Schnyer presented our results at the 2nd International Symposium on Research in Acupuncture (October 20-21, 2018) in Bologna Italy.

Dr. Conboy and Ms. Schmitt met with collaborators at our local Bedford VA on Tuesday September 18, 2018 to discuss the study results and how we can help with CAM integration into their site. This meeting has turned into regular discussions and facilitated the start of a New England School of Acupuncture at MCPHS University acupuncture student clinic at the Bedford facility.

In addition to published articles and conference proceedings mentioned below, our parent paper results were posted to **five** Facebook social media groups that support veterans, Gulf War Illness specific information, or acupuncture: 1) *Acupuncturists on Facebook* (readership 11,958), 2) *Acupuncture researcher share group* (readership 8,125), 3) *Gulf War Illnesses Facebook Group* **(closed group/invitation only,** readership 11,253), 4) *Gulf War Illness-Save Our Service Members Facebook Group* **(closed group/invitation only,** readership 4,965), 5) *Gulf War Illness Veterans Support Group* **(closed group/invitation only,** readership 280).

Parent study results were also posted twice to the Gulf War Illness information cite *91 outcomes* 2015, 2017. (http://www.91outcomes.com/). 91outcomes.com is a health and news website for veterans of the 1991 Gulf War.

In addition, *our study team informally shared our results with Veteran Administration contacts (e.g. Stephanie Taylor PhD, Associate Director, VA HSR&D Center for the Study of Healthcare Innovation, Implementation & Policy, VA Greater Los Angeles Healthcare System*), other veteran researchers (e.g. *Kim Sullivan PhD at Boston University's CDMRP-funded Gulf War Illness Consortium*), and the veteran advocacy groups (e.g. *Campaign for Military Families).*

We also followed up an invitation that we published in our article *Treating Gulf War Illness: The Lasting Effects of Desert Shield/Storm.* Joe C. Chang, MAOM, Dipl. OM, LAc, Rosa N. Schyner, DAOM, CFMP, LAc and Lisa Conboy, ScD. Acupuncture Today. 18(8). This publication reaches acupuncturists nationally up to 26,436. We invited acupuncturists readers to contact Dr. Conboy for suggestions on how to succeed at reimbursement from the VA. One of our acupuncturist colleagues shared written instructions on how he succeeded at reimbursement. We were able to share his instructions with approximately 40 practitioners.

A list of where our two infographics were sent is in Appendix D. The two infographics can be found online on the American Society of Acupuncturists website in their Resources/Publications page: http://www.asacu.org/wp-content/uploads/2019/04/Introduction-to-Gulf-War-Illness-for-Acupuncturists.pdf and http://www.asacu.org/wp-content/uploads/2019/04/Acupuncture-Helps-Gulf-War-Illness.pdf.

What do you plan to do during the next reporting period to accomplish the goals?
Nothing to Report/Final Report

4. IMPACT:
What was the impact on the development of the principal discipline(s) of the project?
As outlined above we were able to educate and share information about our GWI science results with veterans, caregivers and their families, as a scientific audience, as well as acupuncturists and acupuncture students through our infographics, manuscripts, and presentations.
What was the impact on other disciplines?
Nothing to Report
What was the impact on technology transfer?
The results of this project will allow for a smooth implementation of an acupuncture treatment program for veterans, active military personnel, and the general citizen. This information will be most easily applied by, thus our results are supplied to, the Department of Veterans Affairs.
What was the impact on society beyond science and technology?
Our results have the potential to inform medical decision making in support of acupuncture as a viable treatment for veterans with GWI. The infographics will facilitate communication between veterans and their caregivers, as well as discussion between caregivers.

5. CHANGES/PROBLEMS:
Changes in approach and reasons for change:
As outlined in 4.2.4 above we did not attain adequate data to conduct a Delphi process, so toward the goal of producing materials that practitioners can use, we added a diagnostic reasoning investigation. Operationally, we hired medical anthropologist Dr. Eric Jacobson who with IRB approval interviewed acupuncturists who were a part of the parent grant to determine diagnostic reasoning with the practitioners. This process produced the attached manuscript (Appendix A). These protocol changes were IRB approved then CDMRP approved August 15, 2017.

Considering recruitment, we requested and received a 1-year no-cost extension to continue our recruitment efforts for the resurvey aspect of this project (Task 4.1). We implemented some additional techniques to resurvey our sample this past year. We started asking veterans we contacted what they would like as incentive. We were told that they wanted more acupuncture.

Our host institution (New England School of Acupuncture) agreed to provide 5 free treatment vouchers to our student clinic in Newton MA, and they agreed that we could tell subjects that treatments at our Worcester teaching clinic are free. This information was shared in a mailing to subjects in June 2017. Two subject responses came from this. We repeated this with those that did not answer on Nov 1, 2017. Boston GWI researcher (Kim Sullivan at Boston University) sent us leftover incentive materials (commemorative coins and Desert Storm tee shirts) and we sent those to our remaining sample November 1, 2017 as part of our next attempt to reach the subjects. In December 2017, we attempted a shortened phone survey to gather at least the main outcomes from the subject using questions over the phone and received 1 response by phone.

Actual or anticipated problems or delays and actions or plans to resolve them
N/A final report
Changes that had a significant impact on expenditures
None
Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
None

6. PRODUCTS:

Journal publications. *All listed show acknowledgement of federal support.*
Conboy L, Gerke T, Hsu K, StJohn M, Schnyer R. (2015). The Effectiveness of Acupuncture in the Treatment of Gulf War Illness, an Unblinded Phase II Randomized Clinical Trial. *Public Library of Science_One.* Mar 31;11(3):e0149161. doi: 10.1371/journal.pone.0149161.

Screening for novel central nervous system biomarkers in veterans with Gulf War Illness. Abou-Donia MB, Conboy LA, Kokkotou E, Jacobson E, Elmasry EM, Elkafrawy P, Neely M, Bass CR, Sullivan K. Neurotoxicol Teratol. 2017 May;61:36-46. doi: 10.1016/j.ntt.2017.03.002. Epub 2017 Mar 9. PMID:28286177. Published

Treating Gulf War Illness: The Lasting Effects of Desert Shield/Storm. Joe C. Chang, MAOM, Dipl. OM, LAc, Rosa N. Schyner, DAOM, CFMP, LAc and Lisa Conboy, ScD. Acupuncture Today. 18(8). Published

Matrix analysis of traditional Chinese medicine differential diagnoses in Gulf War Illness. Journal of Alternative and Complementary Medicine. Lisa Taylor-Swanson, Joe Chang, Rosa Schnyer, Kai-Yin Hsu, Beth Ann Schmitt, Lisa Conboy. JACM, 2018 Mar8 doi:10.189/acm.2017.0299.Published

Characteristics of Gulf War Illness participants in an acupuncture study. PLoS One. Lisa Taylor-Swanson, Kai-Yin Hsu, Joe Chang, Rosa Schnyer, Kai-Yin Hsu, Beth Ann Schmitt, Lisa Conboy. Submitted.

Effectiveness in Clinical Trial Recruitment: An Analysis of Methods used in a Novel Randomized Clinical Trial for the Treatment of Gulf War Illness. PlosOne. Matthew J. Hadfield, OMS-IV, Ann Barbetti MAc, Lisa Conboy, ScD. Submitted.

The Importance and Determination of Dosage in Acupuncture Treatment: The case of Gulf War Illness. Beth Sangree LicAc MAc, Lisa Conboy ScD. American Journal of Chinese Medicine. Under Review.

Development of Therapeutic Alliance in Acupuncture Treatments in a Veteran Population. Saadat Bagherigaleh, MD, Beth Ann Schmitt MEd, MAc, Lisa Conboy MA MS ScD. Social Science and Medicine. Under Review.*

Books or other non-periodical, one-time publications.
Nothing to Report

Other publications, conference papers, and presentations

Presentations marked with an * have an associated manuscript in the Appendix.

2016

Using Acupuncture to Treat Complex Veteran Illness. Joe Chang LicAc, Lisa Taylor-Swanson Lic Ac, Rosa Schnyer DAOM, Lisa Conboy MA MS ScD. Poster Presentation. Harvard Medical School/Osher Integrative Medicine Research Forum. November 18 & 19, 2016 Boston MA.* In addition to a poster presentation, our project was one of only 10 chosen to be presented as an oral presentation.

2017

April 24, 2017 scientific talk reviewing the results of the parent grant (*The Effectiveness of Acupuncture in the Treatment of Gulf War Illness* (W81XWH-09-2-0064) and secondary data analysis grant (*Bench to Bedside: Understanding Symptom Response to Acupuncture Treatment and Designing a Successful Acupuncture Treatment Program* (W81XWH-14-1-0533) at the University of California's medical campus (San Francisco campus).

How TCM Practitioners Treat Gulf War Illness; findings of an RCT with individualized treatments. Poster Presentation. Joe Chang LicAc, Lisa Taylor-Swanson LicAc, Rosa Schnyer DAOM, Lisa Conboy MA MS ScD. Society for Acupuncture Research International Symposium. April 27-29, 2017, San Francisco, CA.*

Treating Complex Veteran Illness with Acupuncture in the Community. Oral Presentation. Lisa Conboy MA MS ScD, Kai Yin Hsu LicAc, Joe Chang LicAc, Lisa Taylor-Swanson LicAc, Iris Bell MD, Marc Goldstein MD, Rosa Schnyer DAOM. Society for Acupuncture Research International Symposium April 27-29, 2017, San Francisco, CA.

Development of Therapeutic Alliance in Acupuncture Treatments in a Veteran Population Poster Presentation. Saadat Bagherigaleh, MD, Lisa Conboy MA MS ScD. Society for Acupuncture Research International Symposium April 27-29, 2017, San Francisco, CA.*

Management of Gulf War Syndrome Symptoms with Acupuncture: Findings of a Wait-list Controlled RCT. Lisa Conboy MA MS ScD. Oral Presentation to Center for Healthcare Organization and Implementation Research (CHOIR), June 9, 2017. Jamaica Plain VA Campus Boston, MA.

2018

Dr. Conboy presented *Nonlinear Modeling of Acupuncture Treatment in Complex Medical Illness*, at the Ninth International Conference on Complex Systems, July 23, 2018 in Cambridge MA.

Dr. Conboy presented *Pain Management - Using Acupuncture to Treat Complex Medical Illness*, at the Military Health System Research Symposium in Kissimmee, FL on August 22nd, 2018.

Dr. Schnyer presented *Acupuncture in the Treatment of Gulf War Illness in Veterans*, at the 2nd International Symposium on Research in Acupuncture (October 20-21, 2018) in Bologna Italy.

Dr. Conboy presented our results and a summary of our three CDMRP-funded grants at the *Integrative Medicine Network Forum, 2018,* Friday, November 16th, 2018, at Harvard Medical School, Boston, MA.

2019

Introduction to Gulf War Illness for Acupuncturists. Conboy LA, Schmitt, BA, Ivanisevic A. Infographic. March 26, 2019.*

Acupuncture Helps Gulf War Illness. Conboy LA, Schmitt, BA, Ivanisevic A. Infographic. March 26, 2019.*

Dr. Conboy presented the positive findings from the parent study, at the 1st Annual American Society of Acupuncturists Conference, 2019. Saturday, June 1, 2019 in Washington DC. (While this presentation is outside of the grant period. Dr. Conboy reported out on the status of Gulf War Illness Research and presented infographics to audience.)

Conboy L, Taylor-Swanson L, Prasad T. Complexity in Licensed Acupuncturist's Clinical Reasoning. Invited Oral Presentation. Society for Acupuncture Research international conference, 2019 Friday June 28th. Burlington, VT.

Conboy L, Saadat Bagherigaleh. The development of the Therapeutic Alliance in Acupuncture treatments of Gulf War Illness (GWI). Invited Oral Presentation. Society for Acupuncture Research international conference, 2019 Saturday June 29th. Burlington, VT.

Website(s) or other Internet site(s):

Posted to Facebook social media groups that support veterans, Gulf War Illness specific information, or acupuncture: 1) *Acupuncturists on Facebook* (readership 11,958), 2) *Acupuncture researcher share group* (readership 8,125), 3) *Gulf War Illnesses Facebook Group* (readership 11,253), 4) *Gulf War Illness-Save Our Service Members Facebook Group* (readership 4,965), 5) *Gulf War Illness Veterans Support Group* (readership 280).

Posted twice to the Gulf War Illness information cite *91 outcomes* 2015, 2017. (http://www.91outcomes.com/). 91outcomes.com is a health and news website for veterans of the 1991 Gulf War.

Spoke about study on Radio Program "Warrior Connection" with Patricia Axelrod, Wednesday Oct 4, 2017.

Integrative Health Research from Emerson Ecologics posted to SoundCloud on October 20, 2017 a broadcast of the PLoS One article The Effectiveness of Individualized Acupuncture Protocols in the Treatment of Gulf War Illness: A Pragmatic Randomized Clinical Trial. Conboy LA, Gerke T, Hsu KY, St John M. (https://soundcloud.com/user-895118142)

Infographic "Introduction to Gulf War Illness for Acupuncturists" is housed by the American Society for Acupuncturists online at http://www.asacu.org/wp-content/uploads/2019/04/Introduction-to-Gulf-War-Illness-for-Acupuncturists.pdf

Infographic "Acupuncture Helps Gulf War Illness" is housed by the American Society for Acupuncturists online at http://www.asacu.org/wp-content/uploads/2019/04/Acupuncture-Helps-Gulf-War-Illness.pdf

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:	*Lisa Conboy*
Project Role:	Principle Investigator
Researcher Identifier (e.g. ORCID ID):	ORCID # 0000-0003-2218-7841
Nearest person month worked:	2.4

Contribution to Project:	Dr. Lisa Conboy has acted as the Principal Investigator on this project. She is the main statistician, completing data cleaning and scale construction of the biomedical survey data. Dr. Conboy has conducted regular meeting with consultants, co-investigator and research assistants on the project.
Funding Support:	N/A

Name:	Meredith St. John
Project Role:	*Treatment Specialist*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*1.2*
Contribution to Project:	Meredith St. John has acted as Treatment Specialist for this project. She has reviewed acupuncture specific data from research assistant and consultants.
Funding Support:	*N/A*

Name:	*Kai-Yin Hsu*
Project Role:	*Research Assistant*
Researcher Identifier (e.g. ORCID ID):	ORCID # 0000-0002-5062-9953
Nearest person month worked:	*1.2*
Contribution to Project:	Kai-Yin Hsu is the Research Assistant for this project. She has coded and organized acupuncture specific data. She has participated in regular meetings with her PI and consultants. As of January 2018 Kai-Yin Hsu resigned her position at MCPHS University.
Funding Support:	N/A

Name:	Beth Ann Schmitt
Project Role:	Research Project Coordinator
Researcher Identifier (e.g. ORCID ID):	N/A

Nearest person month worked:	1.2
Contribution to Project:	Beth Ann Schmitt has assisted with recruitment. She has participated in regular meetings with her PI and consultants.
Funding Support:	N/A

Name:	Rosa Schnyer
Project Role:	Consultant
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1
Contribution to Project:	Rosa Schnyer is lead consultant on the project. She participated in hypothesis generation and manuscript preparation.
Funding Support:	N/A

Name:	Joe Chang
Project Role:	Consultant
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1
Contribution to Project:	Joe Chang assisted with categorizing acupuncture protocols and manuscript preparation.
Funding Support:	N/A

Name:	Dr. Marc Goldstein
Project Role:	Consultant
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1
Contribution to Project:	Dr. Goldstein used his skill as a medical doctor for secondary data analysis of the population and for manuscript preparation.

Funding Support:	N/A

Name:	Christine Cronin
Project Role:	Consultant
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	*1*
Contribution to Project:	Christine Cronin assisted with categorizing acupuncture protocols and manuscript preparation.
Funding Support:	N/A

Name:	Dr. Eric Jacobson
Project Role:	Consultant
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1
Contribution to Project:	Dr. Jacobson interviewed practitioners involved in the study for understanding diagnostic reasoning of practitioners.
Funding Support:	N/A

Name:	Annette Ivanisevic
Project Role:	Consultant
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1
Contribution to Project:	Annette Ivanisevic was the graphic designer on the Gulf War Illness infographics.
Funding Support:	N/A

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

As of January 2018 Kai-Yin Hsu resigned her position at MCPHS University.

What other organizations were involved as partners?

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

Not Applicable

9. APPENDICES

Appendix A: Publications
Appendix B: Statement of Work
Appendix C: Statistical Summary
Appendix D: Outreach/Impact Contact List

W81XWH-15-1-0695

Dr. Lisa Conboy

Appendix A

Publications

PLOS | ONE

RESEARCH ARTICLE

The Effectiveness of Individualized Acupuncture Protocols in the Treatment of Gulf War Illness: A Pragmatic Randomized Clinical Trial

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Data Availability Statement: All relevant data are within the paper and its Supporting Information files. Full trial protocol and data are available by request of the principal investigator (LC).

Funding: This work was supported by the Office of the Assistant Secretary of Defense for Health Affairs through the Gulf War Illness Research Program under Award No. W81XWH-09-2-0064. Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the Department of Defense.

Abstract

Background

Gulf War Illness is a Complex Medical Illness characterized by multiple symptoms, including fatigue, sleep and mood disturbances, cognitive dysfunction, and musculoskeletal pain affecting veterans of the first Gulf War. No standard of care treatment exists.

Methods

This pragmatic Randomized Clinical Trial tested the effects of individualized acupuncture treatments offered in extant acupuncture practices in the community; practitioners had at least 5 years of experience plus additional training provided by the study. Veterans with diagnosed symptoms of Gulf War Illness were randomized to either six months of biweekly acupuncture treatments (group 1, n = 52) or 2 months of waitlist followed by weekly acupuncture treatments (group 2, n = 52). Measurements were taken at baseline, 2, 4 and 6 months. The primary outcome is the SF-36 physical component scale score (SF-36P) and the secondary outcome is the McGill Pain scale.

Results

Of the 104 subjects who underwent randomization, 85 completed the protocol (82%). A clinically and statistically significant average improvement of 9.4 points ($p = 0.03$) in the SF-36P was observed for group 1 at month 6 compared to group 2, adjusting for baseline pain. The secondary outcome of McGill pain index produced similar results; at 6 months, group 1 was estimated to experience a reduction of approximately 3.6 points ($p = 0.04$) compared to group 2.

Competing Interests: The authors have declared that no competing interests exist.

Conclusions

Individualized acupuncture treatment of sufficient dose appears to offer significant relief of physical disability and pain for veterans with Gulf War Illness. This work was supported by the Office of the Assistant Secretary of Defense for Health Affairs through the Gulf War Illness Research Program under Award No. W81XWH-09-2-0064. Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the Department of Defense.

Trial Registration

ClinicalTrials.gov NCT01305811

Introduction

Clinical and registry programs indicate that 25% of the 700,000 veterans of the first Gulf War (Operation Desert Shield/Storm, years 1990–1991), are affected by clusters of symptoms and co-morbid medical diagnoses including chronic fatigue syndrome, fibromyalgia, irritable bowel syndrome, arthralgia, digestive complaints, and mood-related psychiatric disorders, including depression, posttraumatic stress disorder (PTSD), and other anxiety disorders [1,2]. Defined by the Centers for Disease Control and Prevention (CDC) [3], Gulf War Illness (GWI) is a complex, poorly understood illness, often with a highly individualistic presentation, and symptoms difficult for conventional medicine to treat effectively; GWI has been shown to be remarkably stable at 5- and 10- year follow-ups [1,4]. GWI is twice as prevalent in deployed veterans, and seen in 15% of non-deployed veterans [5]. There is no standard of care treatment for this syndrome at this time.

Although there are no published studies evaluating acupuncture's effectiveness in the treatment of GWI, acupuncture has been shown to successfully reduce many key symptoms of GWI including pain [5,6], musculoskeletal disorders [7,8,9], both acute and chronic pain after amputation in military contexts [10,11], fatigue [12], state, trait and situational anxiety [13], and depression [14,15,16]. Further, there is evidence that acupuncture may be effective in the treatment of other complex diseases such as irritable bowel syndrome [17], fibromyalgia [18], and post-traumatic stress disorder [19] and that acupuncture is well tolerated by patients, safe, and may be cost-effective compared to routine care [20].

Chinese Medicine, on which acupuncture is based, uses diagnostic and treatment procedures that are complex [21] and tailored to each individual's specific symptoms. Although this individualized treatment ideal is often replaced in clinical research with standardized protocols for the purposes of reliability and simplicity, the ability of Traditional Chinese Medicine (TCM) to be tailored to each patient is a core concept and strength that can be maintained successfully in a Randomized Controlled Trial (RCT) format [22]. This unblinded Phase II clinical trial utilized individualized treatment protocols, testing the effects of individualized acupuncture treatments offered in extant acupuncture practices.

Materials and Methods

Study design

Full trial design details have been published elsewhere [23]. Please see S1 File: Protocol. To maximize study compliance we employed acupuncturists to provide treatments in their own

offices in communities where veterans work and live. We began recruitment of subjects and practitioners with a catchment area of 30 miles from our research study offices, and widened our catchment area with increasing study duration.

Veterans with Gulf War Illness were randomized to either (1) acupuncture treatment twice per week for 6 months or (2) the wait-list comparison group consisting of usual care from baseline for 2 months, followed by weekly treatments for 4 months. We chose an active control group to maximize internal validity while allowing us to gather preliminary data on minimal effective treatment dose. The two month wait time allowed us to judge if GWI symptom presentations are stable in our sample, as has been shown in other GWI samples [1,5]. Our treatment schedule duration, dose, and specific Chinese Medicine techniques employed are based on the clinical experience of our expert practitioners, and informed by literature review. Details of the protocol and implementation were determined before the trial began via focus groups with senior acupuncture faculty at the New England School of Acupuncture.

The New England Institutional Review Board approved this research protocol on September 4, 2009. All human participants gave written informed consent. All of our study processes were approved and oversight is provided by: 1) The New England Institutional Review Board (http://www.neirb.com/), 2) United States Army Human Research Protection Office (https://mrmc-www.army.mil/rodorphrpo.asp). The study operated as planned between September 2009 and January 2013. Recruitment began immediately, and ran until July 2012. We initiated the clinical trial registration process before recruiting subjects, and registration was completed before we began to analyze the data. We confirm that all ongoing and related trials are registered. Please see S2 File. CONSORT Checklist.

Recruitment. We recruited via local advertisements and direct mailing to veterans of the first Gulf War drawn from the Defense Manpower Data Center (http://www.virec.research.va.gov/Non-VADataSources/DMDC.htm). Because the demographics of GWI veterans are unpublished, we did not know if there was a sufficient population near our study offices from which to draw our sample. Thus we designed the study to include treatment sites within a 100-mile radius of our study offices, and incorporated a mechanism to add treatment sites within that radius in areas where GWI veterans were found clustered. Thirty treatment sites were utilized. This design has the added benefit of allowing veterans to attain treatments near where they live and work, a technique that may have improved adherence.

Eligibility. All subjects met the illness definition of Gulf War Illness as determined by responses on the Gulf War Illness Symptom Checklist [5] and the inclusion/exclusion criteria set forth in the federal definition of Gulf War Illness as used for the Gulf War Registry (Please see Box 1). Subjects needed to pass through two eligibility screenings. First, a research assistant conducted a prescreening by phone, querying potential subjects about their illness and symptom experience. Second our study physician (MG) used the same criteria to complete an in-person medical screening. Our two-stage informed consent process included 1) verbal informed consent requested prior to the initial telephone screening, and 2) written informed consent administered in person, at the start of the screening visit. Please see Fig 1. An unblinded member of the study staff (LC) with no additional patient contact enrolled subjects. Study outcomes data were collected by electronic interface at our outpatient clinic. In fewer than 10% of cases, due to participants' time constraints, participants were allowed to take their surveys home to fill out, and then mail back. During this screening visit, participants chose their future treatment practitioner from a list of practitioners convenient for them.

Group assignment and interventions. Collaborator RD randomly assigned participants to the two study arms using permuted block randomization with variable block sizes and assignments provided in sequentially numbered opaque sealed envelopes. After baseline evaluation and consent, a member of the study staff without involvement in data collection with

Box 1: CDC Symptom Clusters for Gulf War Illness.

Inclusion:

1. deployed to the "*Gulf Theater of operations, as defined by 38 CFR 3.317, includes Iraq, Kuwait, Saudi Arabia, Bahrain, Qatar, the United Arab Emirates, Oman, the Gulf of Aden, the Gulf of Oman, the Persian Gulf, the Arabian Sea, the Red Sea, and the air-space above all of these locations*" in the years 1990–1,
2. have at least 2 of the following symptoms from the 3 CDC clusters of symptoms that have lasted for more than 6 months. Each symptom cluster must be characterized as "mild-moderate" or "severe" with at least one symptom in each cluster required to be severe.

A-Fatigability

- fatigue 24 hours or more after exertion

B-Mood and Cognition

- feeling depressed or
- feeling irritable or
- difficulty thinking or concentrating or
- feeling worried, tense, anxious or
- problems finding words or
- problems getting to sleep

C-Musculoskeletal

- joint pain or muscle pain

Exclusion:

Potential subjects will be excluded if they are:

- Currently enrolled in another clinical trial
- Have another disease that likely could account for the symptoms, as determined by our Medical Monitor
- Severe psychiatric illness (in the last 2 years psychiatric hospitalization, suicidal attempt, alcohol or substance abuse, use of antipsychotic medication) as measured by our primary screening instrument the Primary Care Evaluation of Mental Disorder (Prime MD).
- Unable to complete the protocol on based on the evaluation of the Medical Monitor
- Participants will not be excluded due to age, race, ethnicity, or gender limitations.
- Study is limited to United States veterans with Gulf War Syndrome, thus subjects could not be minors, illiterate, or unable to speak or understand English.

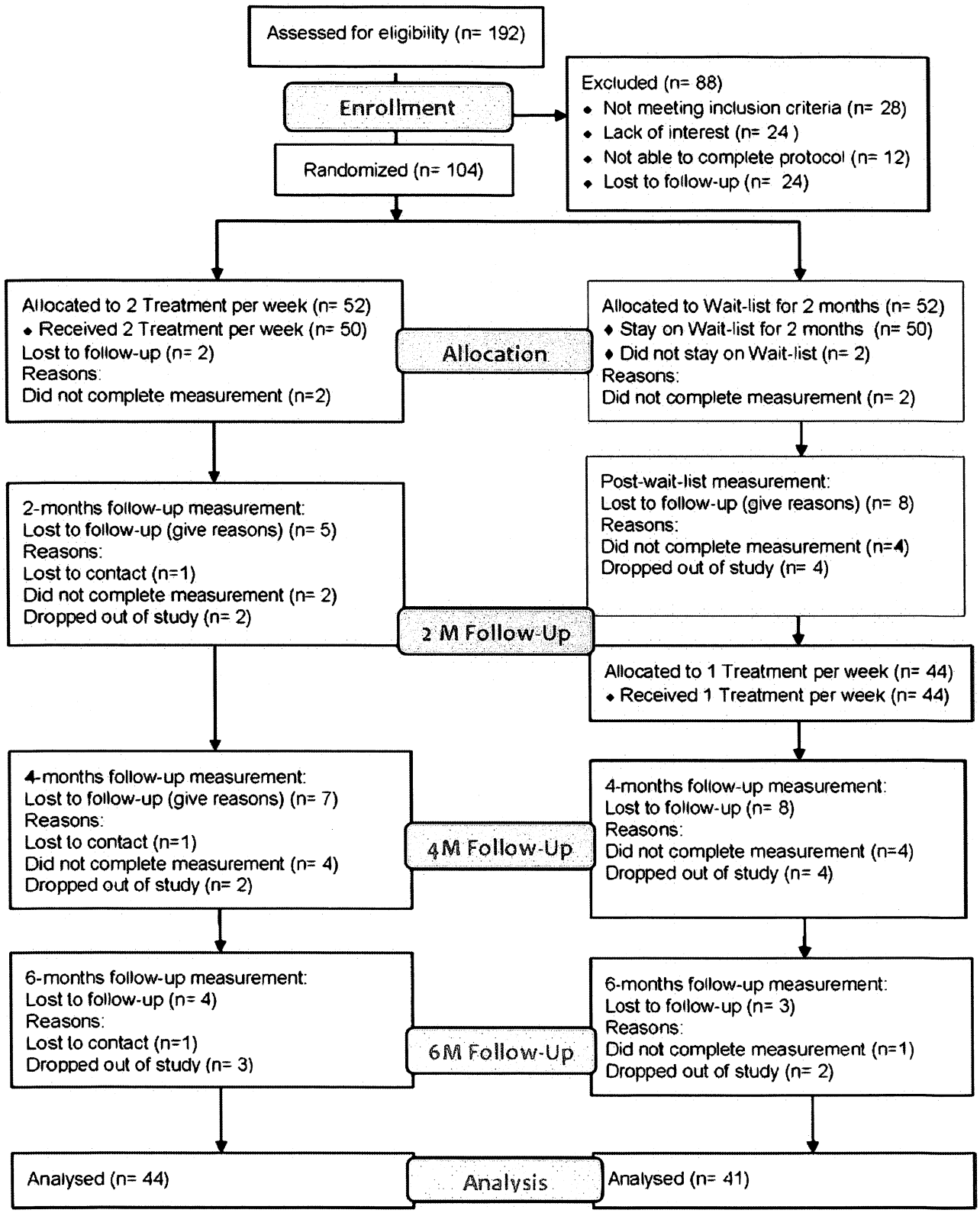

Fig 1. PLOS CONSORT Flow Diagram: Diagram of Screening, Randomization, and Follow-up.

doi:10.1371/journal.pone.0149161.g001

subjects (LC) opened the assignment envelopes and recorded the assignment of each participant in a confidential log. LC then reported to the subject's chosen practitioner the subject's contact information and study assignment. The chosen practitioner then took responsibility of contacting the subject to schedule and begin treatments. LC also called and/or emailed the subject to report if acupuncture treatments were to begin immediately (group 1), or in 2 months (group 2).

Licensed acupuncturists with at least 5 years of clinical experience, who received additional in-house training concerning GWI, provided the acupuncture treatments. Although there are many styles of acupuncture within Chinese Medicine, acupuncturists were chosen who self-reported use of the TCM model of diagnosis. During the first session, the acupuncturist conducted an interview reviewing the subject's medical history, symptoms and aspects of diagnosis from the perspective of TCM, including condition of the tongue, pulse, meridians, and acupoints. Each subject received an individualized diagnosis and treatment protocol addressing his or her unique pattern of symptoms. Brief interviews began each subsequent session, allowing patient and practitioner to prioritize symptoms, and identify any questions or concerns. Individualized treatment protocols allowed the practitioners to alter the treatment plan based on how the patient presented at the moment; including varying the selection of acupoints across treatments and adding particular co-interventions commonly used as part of TCM therapy to supplement manual needling (such as electroacupuncture for its efficacy in reducing pain and inflammation [24], heat therapies (e.g. heat lamp), Chinese massage, and press balls, tacks or magnets applied to points after needling). A sample treatment protocol is offered as S3 File.

Each session lasted approximately one hour. Acupoints were stimulated manually until "obtaining *de qi*," a technique characteristic of TCM to elicit a response felt by both the patient and the acupuncturist. This needling sensation, adjusted for the comfort and safety of each patient, may be experienced as a pinch that rapidly subsides, or a sense of spreading pressure, dull ache, or warmth. Needles were retained for 30–45 min (10–35 stainless steel, disposable needles per session). After needle insertion, subjects were left to rest or nap. The type of needle, including gauge [32–38] and length (15–50 mm) as well as the depth of insertion (subcutaneous to about 25 mm) varied according to the area of the body being treated (i.e. extremities vs. trunk). Choice of acupoints could vary during subsequent treatments to improve results. Herbs and supplements were not allowed. Subjects were encouraged (but not required) to remain with the same acupuncturist for the whole study period to allow for development of patient-practitioner rapport.

Outcomes. A single measure of severity that addresses all possible presentations of GWI does not exist. Thus we chose to use the SF-36, a 36-item, well-validated and reliable general measure of health [25]. Given the importance of function on quality of life, we focused the main outcome to the SF-36 physical component scale score (SF-36P). Similarly, as pain is a common to most GWI presentations and very relevant in veteran health, our secondary outcome is the McGill Pain scale, a 15- item measure recording participants' pain level and quality [26]. Outcomes were assessed with the assistance of a blinded study staff member at baseline, 2, 4, and 6 months. Raw data is offered as S4 File.

Subjects' confidence in and usability of acupuncture were measured in a few ways. Participants were asked (1) their confidence in recommending acupuncture to a friend or family member using a five point scale from "Very Confident" to" Not Confident", (2) the experience of the acupuncture using a five point scale from "Extremely Pleasant" to "Extremely Unpleasant", (3) the experience of their relationship with their practitioner on a five point scale from "Extremely Pleasant" to "Extremely Unpleasant", (4) how logical the acupuncture treatments were for them on a 6 point scale of "Very Logical" to "Not Logical at All".

Sample size

Our sample size was calculated to allow detection of clinically meaningful differences between treatment groups. Previous acupuncture research using our main outcome, the SF-36, in pain conditions [27,28] show a consistent standard deviation of 20 points in the SF-36 P for both baseline values and change scores. Sixty individuals per group (total n = 120) would offer us a power of 80% to detect the difference between groups of 7 points. Using Cohen's d estimation of effect size [29] a sample size of 60 would allow us to see a moderate effect. In further support of our main outcome, a 7.8-unit improvement has been estimated clinically relevant for patients with similarly serious conditions [30,31]. We estimated a dropout rate of 10%.

Statistical analyses

In our original proposal to the funder, to protect our main outcome from possibly large attrition, we proposed to initially test mean differences between groups following 2 months of treatment using Student's t-tests at an alpha = 0.05. Using this strategy, we observed a mean reduction in SF-36 for Group 1 of 0.32 versus a reduction 4.53 for Group 2 (p = 0.22).

Of more interest to the study team is what changes might be seen after the clinically informed 6 month treatment window. This 6 month analysis is done by author TG and investigates whether those subjects assigned to biweekly acupuncture experienced differences in the SF-36P score over follow-up compared to those subjects who received weekly acupuncture following a 2-month delay. To assess potential differences, generalized estimating equation (GEE) models were fit in order to account for the correlation induced by repeated measurements on each subject. Under the assumption that baseline McGill pain is prognostic for SF-36P over time, model adjustment was made for baseline pain to increase precision in the estimated parameters.32 Eight subjects did not report this baseline measurement and, under the assumption that missingness was completely at random, these subjects were not included in the analysis set. In summary, we estimated the GEE model

$$SF36 = \beta 0 + \beta 1 Itx + \beta 2 It^2 + \beta 3 It^4 + \beta 4 It^6 + \beta 5 \rho + \beta 6 Itx It^2 + \beta 7 It^4 + \beta 8 Itx It^6$$

where *Itx* denotes an indicator for biweekly acupuncture; It^2,It^4,*and* It^6, are indicators for months 2, 4, and 6; ρ denotes baseline pain; and time was coded categorically to reflect suspected nonlinearities.

As a secondary analysis, the McGill pain score was similarly assessed for differences over time by treatment status. The time trend for the GEE fit was modeled categorically to account for nonlinear trends. The pain model was not adjusted for additional covariates, since no strongly prognostic variables were assumed to have been measured. All GEE models were fit using the software package geepack [33] in R version 3.1.1 under an exchangeable working correlation structure.

Results and Discussion

Study Population

Recruitment began in July of 2010 and the final follow-up visit was completed in January 2013. Please see Table 1 for baseline demographics and Fig 1. for study flow.

Outcomes

Of the 104 subjects who underwent randomization 103 completed at least one measurement timepoint, yielding 99.0% of data for analysis. However, 8 subjects are missing baseline pain data, yielding 95/104 = 91.3% in the analysis set. General Estimating Equations were used to

Table 1. Baseline Characteristics of the Study Population. Baseline Characteristics of the Study Population. Standard Deviations (SD) are offered for age, baseline pain, and baseline Sf-36(P).

Characteristic	Biweekly Treatment (N = 52)	Waitlist to Weekly Treatment (N = 52)
Age-year +/- SD	48.2 +/-9.9	48.2 +/- 3.5
Female sex-N(%)	7 (13%)	7 (13%)
Self reported Race-N(% of total)		
White	43 (83%)	41 (79%)
Black or African-American	5 (0.1%)	5 (0.1)
Asian	1 (0.02%)	0
American Indian/Alaskan Native	0	1 (0.02%)
More than one race	0	2 (0.04%)
Other	3 (0.06%)	3 (0.06%)
Self reported Hispanic N(% of total)		
Yes	2 (0.04%)	4 (0.08%)
No	48 (0.92%)	46 (0.88%)
No answer	2 (0.04%)	2 (0.04%)
Baseline Pain N(group mean) +/- SD	50(29.5) +/- 8.5	45 (29.8) +/-8.9
Baseline Sf-36(P) N(group mean) +/- SD	51 (67.7) +/-24.6	49 (66.4)+/- 24.7

doi:10.1371/journal.pone.0149161.t001

compare the group 1 vs. group 2 acupuncture subjects' experienced differences in the SF-36P, and McGill Pain scale.

Our analysis comparing baseline symptom levels to those at 6 months showed a significant average increase of 9.4 points in the SF-36 physical component scale score (SF-36P) for the biweekly acupuncture group at month 6 in comparison to the weekly waitlisted group, adjusting for baseline pain. Within the biweekly group, scores were generally stable at months 0, 2, and 4, with a mean increase of 6.6 points in SF-36P observed at month 6. A nonlinear pattern was observed for the waitlisted group, though a lower SF-36P relative to baseline was estimated for all subsequent months. Table 2 offers mean and empirical 95% confidence limits estimated for both SF-36P and McGill from 5000 simulations of the fitted GEE models, and Fig 2 provides a graphical summary of this information for SF-36P [34]. Though overlapping confidence bands are observed at time points determined significantly different in the GEE fit, we note that the simulation-based approach, while useful for visual interpretation, relies on slightly different calculations than the GEE model itself.

The secondary outcome of McGill pain index produced similar results. A decreasing trend in McGill score indicating symptom improvement was observed for the biweekly group, with a significantly lower score compared to the waitlisted group appearing at month 6. At the end of follow-up at month 6, the biweekly group was estimated to experience a reduction of approximately 3.8 points on the McGill scale compared to the weekly waitlisted group. Fig 3 illustrates

Table 2. Mean and empirical 95% confidence limits estimated for both SF-36P and McGill from 5000 simulations of the fitted GEE models.

	Waitlist/Weekly SF-36P (95% CI)	Biweekly SF-36P (95% CI)	Waitlist/Weekly McGill (95% CI)	Biweekly McGill (95% CI)
Baseline	66.1 (60.6 to 71.6)	67.1 (61.2 to 72.9)	29.7 (27.1 to 32.2)	29.7 (27.4 to 32.0)
Month 2—Baseline	61.8 (54.8 to 68.6)	66.9 (61.1 to 72.5)	31.5 (28.9 to 34.1)	29.1 (27.3 to 31.0)
Month 4—Baseline	65.7 (58.4 to 72.8)	67.0 (60.1 to 72.8)	29.3 (26.7 to 31.9)	26.8 (24.6 to 29.1)
Month 6—Baseline	64.3 (57.1 to 71.4)	73.7 (67.7 to 79.8)	29.5 (26.8 to 32.0)	25.9 (23.5 to 29.3)

doi:10.1371/journal.pone.0149161.t002

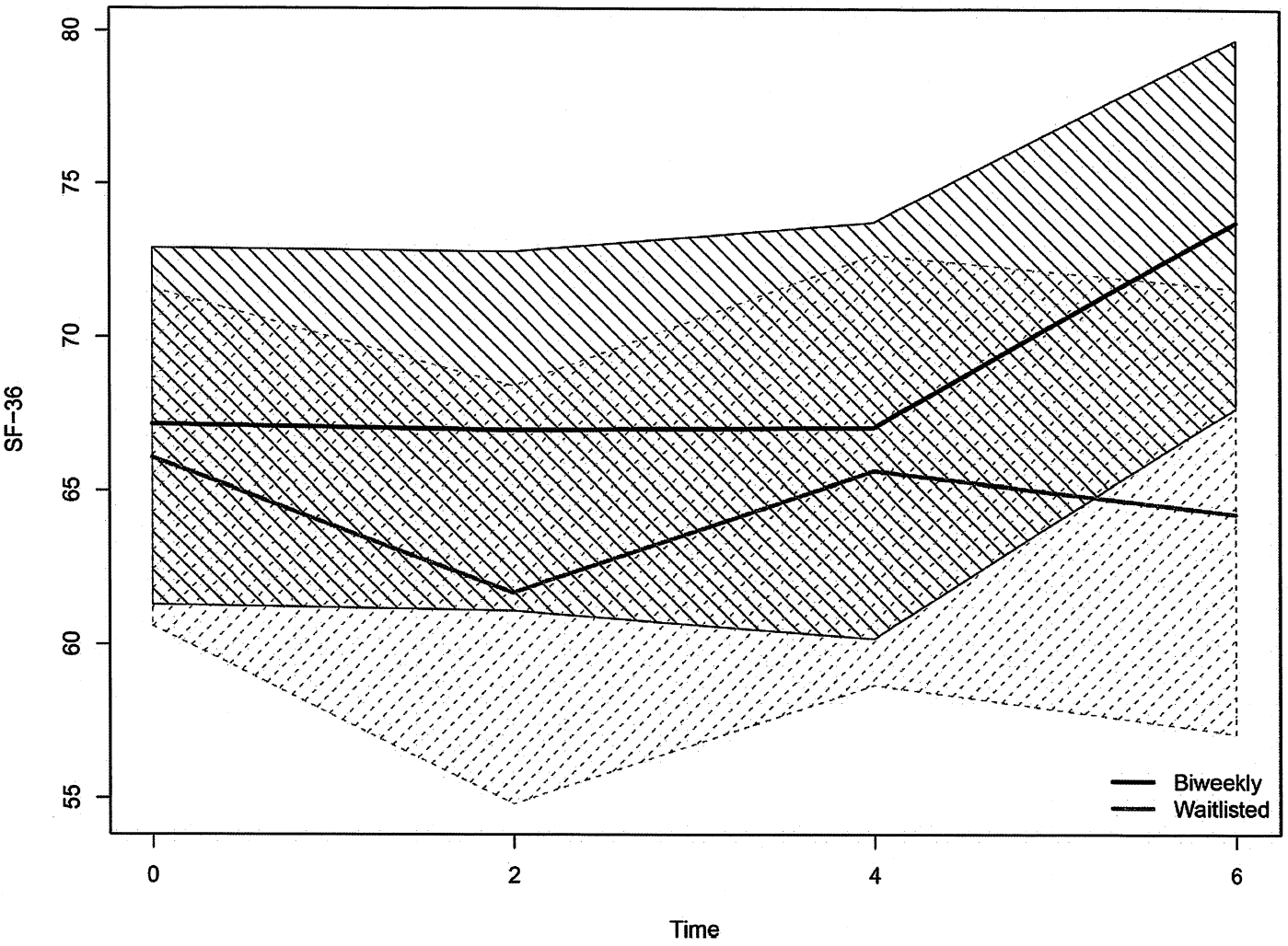

Fig 2. Summary of model-based simulations[xxxiii] of changes in mean SF-36P at 4 measurement timepoints. Scores moving in the positive direction indicate improvement. Scores moving in the positive direction indicate improvement.

doi:10.1371/journal.pone.0149161.g002

the estimated value and 95% confidence intervals. Tables 3 and 4 offer estimates and accompanying statistical values from the GEE modeling of the two outcomes.

Participants reported high usability of acupuncture with 96% of the veterans (averaged across both groups and over all time points) reporting confidence in recommending acupuncture to a friend or family member (or at least a 3 on a five point scale from "Very Confident" to" Not Confident"), 98% reporting that the acupuncture experience was at least pleasant (or at least a 3 on a five point scale from "Extremely Pleasant" to "Extremely Unpleasant"), 97% reporting that their relationship with their practitioner was pleasant (or at least a 3 on a five point scale from "Extremely Pleasant" to "Extremely Unpleasant") and 96% reported that the acupuncture treatments were logical for them (or at least a 3 on a 6 point scale of "Very Logical" to "Not Logical at All"). The trial had only two adverse events: (1) subject in biweekly treatment group reported pain on needling, (2) subject in weekly treatment group reported suicidal thoughts, which study staff followed up with additional medical oversight.

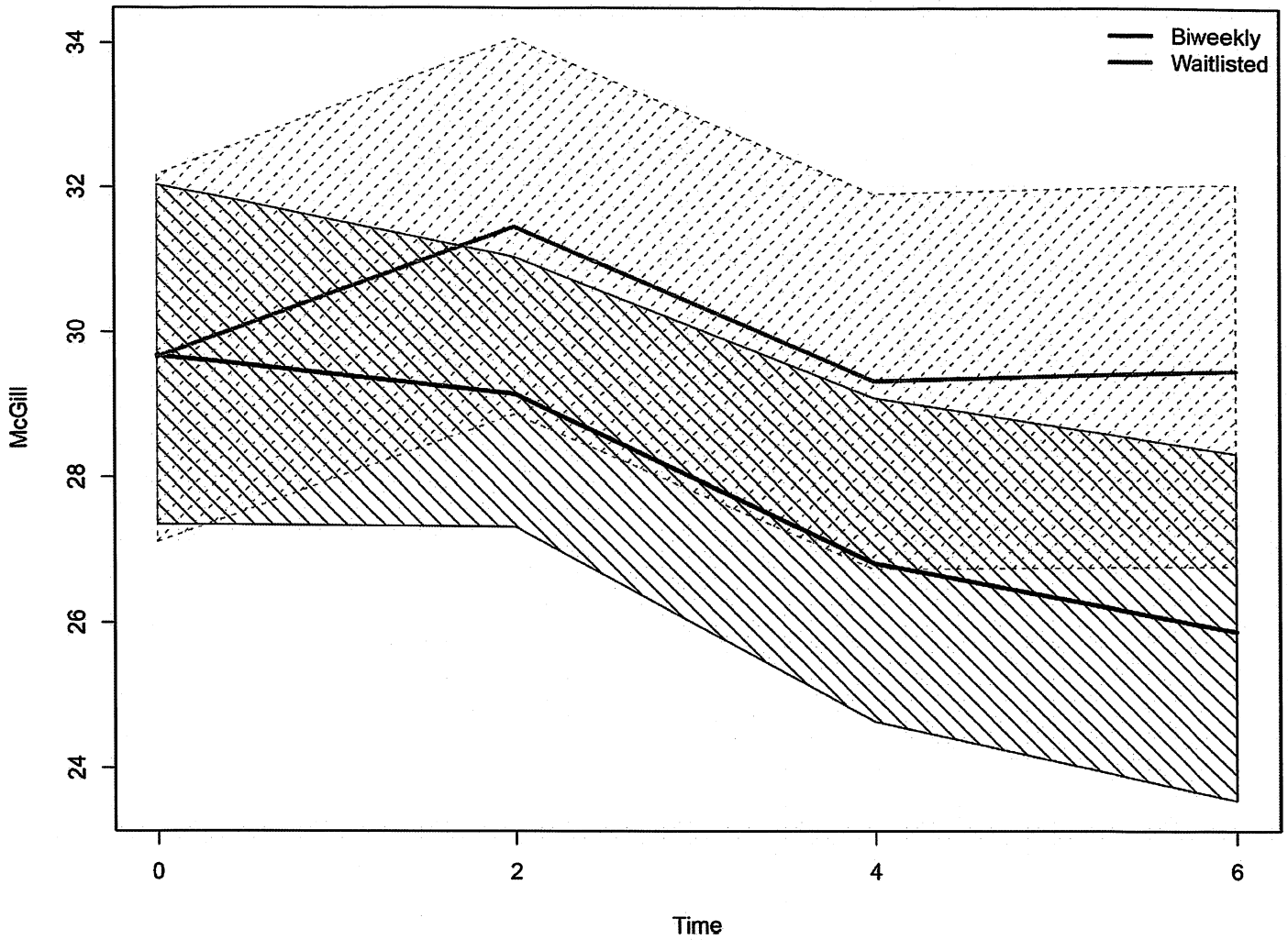

Fig 3. Summary of model-based simulations[xxxiii] of changes in mean McGill Pain Scale at 4 measurement timepoints. Scores moving in the negative direction indicate improvement. Scores moving in the negative direction indicate improvement.

doi:10.1371/journal.pone.0149161.g003

Table 3. Estimates and accompanying statistical values from the GEE modeling of the 6-month outcome SF-36P.

SF-36	Estimate	Std Err	95% CI	Wald X2	p-value
Intercept	106.91	6.96	(93.27, 120.56)	235.90	<0.001
Biweekly	0.98	4.07	(-7.00, 8.97)	0.06	0.81
Month 2	-4.38	2.52	(-9.32, 0.56)	3.02	0.08
Month 4	-0.42	2.97	(-6.24, 5.40)	0.02	0.89
Month 6	-1.78	3.27	(-8.19, 4.62)	0.30	0.59
Baseline pain	-1.37	0.21	(-1.79, -0.95)	41.12	<0.001
(Biweekly) X (Month 2)	4.18	3.56	(-2.81, 11.16)	1.37	0.24
Biweekly * Month 4	0.33	4.14	(-7.79, 8.46)	0.01	0.94
Biweekly * Month 6	8.39	3.76	(1.03, 15.76)	4.99	0.03

doi:10.1371/journal.pone.0149161.t003

Table 4. Estimates and accompanying statistical values from the GEE modeling of the outcome McGill Pain scale.

McGill	Estimate	Std Err	95% CI	Wald X2	p-value
Intercept	29.65	1.28	(27.15, 32.15)	539.82	<0.001
Biweekly	0.02	1.73	(-3.38, 3.42)	0.00	0.99
Month 2	1.82	1.06	(-0.26, 3.89)	2.93	0.09
Month 4	-0.31	1.41	(-3.07, 2.45)	0.05	0.82
Month 6	-0.20	1.35	(-2.84, 2.44)	0.02	0.88
Biweekly * Month 2	-2.35	1.29	(-4.88, 0.19)	3.29	0.07
Biweekly * Month 4	-2.56	1.81	(-6.11, 0.99)	2.00	0.16
Biweekly * Month 6	-3.59	1.71	(-6.94, -0.25)	4.43	0.04

doi:10.1371/journal.pone.0149161.t004

Discussion

This study supports the use of individualized acupuncture treatments for the management of GWI symptoms. Our results are in concordance with numerous other studies indicating that acupuncture is a widely available, safe, effective, and cost-effective option for the treatment of other diseases and syndromes with similar presentations to GWI [35] with high usability in veteran populations [36]. Given this research, it is likely that acupuncture treatment may be an effective, safe, low-cost treatment option for our returning military as well as civilian populations impacted by chronic multi-symptom illness and its co-morbidities.

The mechanisms of acupuncture in the treatment of GWI are unknown, which supports our choice of a low-constraint design. This naturalistic RCT includes individualized protocols, a clinically supported length and dose of treatment, and a wait list control. Data from our wait list arm (Table 2) indicates that symptoms are stable, as has been shown in published 5- and 10- year follow-ups [1,5]. The design aspect of the wait list group eventually receiving weekly acupuncture offers us data to begin to answer questions of minimal dose and satisfies ethical concerns allowing all subjects to receive treatment during the study. A sham acupuncture control arm was not used due to published indications that such sham interventions are effective and thus not appropriate controls; very high quality evidence of this is now available [37,38].

We chose a pragmatic design, and used practitioners in the community, to facilitate adherence and test the use of extant practitioners. Our positive results support the referral of GWI veterans to acupuncture treatments. Our low side effect rate mirrors that of the published literature that acupuncture is safe when provided by professionally trained practitioners [39]. Serious adverse events are extremely rare. In a systematic review of 12 prospective studies scrutinizing over a million treatments, the very low risk of serious adverse event, mostly trauma from needle puncture or infection, was estimated at 0.05 per 10,000 treatments, a risk below that of many common medical treatments [40]. Acupuncture is well tolerated, safe and effective in the management of Gulf War Syndrome. The inclusion of acupuncture in the routine management of this intractable condition is warranted.

Limitations and Future Directions

Our results suggest that 2 months of biweekly acupuncture is not sufficient to affect the outcome of physical function (as measured by the SF-36P), but pain scores (as measured by the McGill Pain Scale) did show group improvement as early as the first follow-up (2 months). These findings underscore the need for more dosage studies to determine the most therapeutic level of treatment for different illness presentations. Currently, we are conducting secondary data analyses exploring the effectiveness of acupuncture treatment on different subtypes of GWI to help treatment providers apply the best protocols for this complex illness. The team is

also categorizing the most effective treatment protocols from a Traditional Chinese Medicine point of view and matching these with different biomedical symptom presentations. Our low-constraint/non-standardized design allowed for collection of naturalistic clinical data, which may increase data validity and make such communications across medical systems more useful.

Other items related to observation window are also of interest. For example, the obvious yet non-statistically significant decrease in Sf-36P component scores which happened while the veterans were waiting for treatment could be natural history but probably is not as observations over longer periods show symptom stability over time [41]. Most likely, this symptom change is due to veterans' frustration in having to wait for treatment; a few of the veterans mentioned this frustration along with an acknowledgment that they were informed of the necessity for a wait list design and knowledge that they would receive treatment at 2 months. We will explore other changes which occurred during the wait list in later analyses as well as the symptom changes associated with different doses of acupuncture.

Although we did find support for our 6-month hypothesis we did not achieve the sample size determined to give us 80% power to see an effect. This leaves us more vulnerable to Type II (false negative) error. Future work should include larger sample sizes to protect against this. Larger sample sizes may also more easily support the use of standardized protocols, which become possible with the implementation of effectiveness trials such as ours that gather a range of clinically relevant treatment options

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Supporting Information

S1 File. Protocol.
(DOC)

S2 File. CONSORT Checklist.
(DOC)

S3 File. Example Treatment Protocol for Subject in Biweekly Treatment Condition.
(DOCX)

S4 File. Data Used in Calculations.
(XLSX)

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Author Contributions

Conceived and designed the experiments: LC MS RS. Performed the experiments: MG KH LC. Analyzed the data: LC TG. Contributed reagents/materials/analysis tools: LC TG. Wrote the paper: LC TG RS MS KH.

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Full length article

Screening for novel central nervous system biomarkers in veterans with Gulf War Illness

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ABSTRACT

Gulf War illness (GWI) is primarily diagnosed by symptom report; objective biomarkers are needed that distinguish those with GWI. Prior chemical exposures during deployment have been associated in epidemiologic studies with altered central nervous system functioning in veterans with GWI. Previous studies from our group have demonstrated the presence of autoantibodies to essential neuronal and glial proteins in patients with brain injury and autoantibodies have been identified as candidate objective markers that may distinguish GWI. Here, we screened the serum of 20 veterans with GWI and 10 non-veteran symptomatic (low back pain) controls for the presence of such autoantibodies using Western blot analysis against the following proteins: neurofilament triplet proteins (NFP), tubulin, microtubule associated tau proteins (Tau), microtubule associated protein-2 (MAP-2), myelin basic protein (MBP), myelin associated glycoprotein (MAG), glial fibrillary acidic protein (GFAP), calcium-calmodulin kinase II (CaMKII) and glial S-100B protein. Serum reactivity was measured as arbitrary chemiluminescence units. As a group, veterans with GWI had statistically significantly higher levels of autoantibody reactivity in all proteins examined except S-100B. Fold increase of the cases relative to controls in descending order were: CaMKII 9.27, GFAP 6.60, Tau 4.83, Tubulin 4.41, MAG 3.60, MBP 2.50, NFP 2.45, MAP-2 2.30, S-100B 1.03. These results confirm the continuing presence of neuronal injury/gliosis in these veterans and are in agreement with the recent reports indicating that 25 years after the war, the health of veterans with GWI is not improving and may be getting worse. Such serum autoantibodies may prove useful as biomarkers of GWI, upon validation of the findings using larger cohorts.

1. Introduction

Approximately one third of the 697,000 US military personnel who served in the Gulf War (GW) from August 1990 to June 1991, have reported persistent symptoms for many years after the war (RAC, 2008; IOM, 2012, RAC, 2016; White et al., 2016). This complex of symptoms, known as Gulf War Illness (GWI), include memory and attention problems, profound fatigue, chronic muscle and joint pain, severe headaches, persistent diarrhea, respiratory difficulties and skin rashes. GWI is primarily diagnosed by symptom report and no validated objective diagnostic biomarkers currently exist that fully segregate cases from controls. This study was designed to identify objective central nervous system (CNS) biomarkers of GWI using clues from prior clinical studies with GW veterans and from animal studies that modeled chemical exposures experienced by GW veterans.

Clinical studies have reported impaired cognitive functioning and reduced MRI volume and altered white matter microstructural integrity in organophosphate (OP) pesticide, sarin nerve agent and pyridogstigmine bromide (PB) anti-nerve gas pill-exposed GW veteran cohorts (White et al., 2016; Sullivan et al., 2013; Chao et al., 2010;

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Heaton et al., 2007; Proctor et al., 2006; Sullivan et al., 2003). Animal studies demonstrated that exposure to higher doses of the prophylaxis pill pyridostigmine bromide (PB), the insect repellent, DEET, and the insecticide permethrin and/or chlorpyrifos led to significant brain damage in animal models of GWI (Abou-Donia et al., 1996a,b). Further studies using 60 days of subchronic dermal exposure to DEET and permethrin, alone or in combination, at dose levels approximately equivalent to the exposures that occurred during the Gulf War in a rat-model of GWI, caused the following: (1) a diffuse neuronal cell death in the motor cortex, the different subfields of the hippocampal formation, and the Purkinje cell layer of the cerebellum, accompanied by sensorimotor deficits; (2) significant reduction of MAP-2-positive immunoreactive structures indicating atypical expression of MAP-2 in dendrites of surviving neurons, within the cerebral cortex and the hippocampus that was characterized by a beaded, disrupted, or wavy appearance; (3) a significant upregulation of GFAP-positive expression in structures in the CA3 subfield of the hippocampus, the motor cortex and the dentate gyrus (Abdel-Rahman et al., 2001, 2002a,b, 2004a,b; Abou-Donia et al., 2000, 2001, 2002, 2004; Terry et al., 2003). Similar results were exhibited in animals treated with sarin alone or accompanied by cited-above chemicals, with and without stress (Abdel-Rahman et al., 2004a).

The cytoarchitecture of the CNS is maintained by a complex cellular milieu that involves neuronal and glial cells that must maintain proper communication in order to function properly (Abou-Donia and Lapadula, 1990; McMurray, 2000). CaMKII phosphorylates cytoskeletal proteins, such as MAP-2, tau and tubulin. CaMKII accounts for 12% of all proteins in the brain. CaMKII has the ability to coordinate and transduce upstream Ca and reactive oxygen species (ROS) signals into physiological and pathophysiological downstream responses in the nervous system and cardiovascular biology and disease (Abou-Donia, 1995; Erickson et al., 2011). Tubulin, the major component of microtubules, is responsible for axonal migration and longitudinal growth and is involved in axonal transport. Although tubulin is present in virtually all eukaryotic cells, the most abundant source is the vertebrate brain, where it consists of approximately 10–20% of its total soluble protein (McMurray, 2000). Microtubule-Associated Protein-2 (MAP-2) is found in dendritic compartments of neurons. A loss of MAP-2, is a reliable indication of irreversible neuropathology and is a sensitive marker of seizure-related brain damage (Ballough et al., 1995). Tau Protein, a normal axonal protein, is involved in stabilization and assembly of axonal microtubules. Levels of tau proteins are elevated in the cerebrospinal fluid (CSF) and serum following TBI (Liliang et al., 2011) and has been used for diagnosis of Alzheimer's disease. Myelin basic protein (MBP) is an abundant myelin membrane proteolipid produced by oligodendroglia in the CNS and Schwann cells in PNS and may confirm the clinical assessment of neurodegenerative disorders such as multiple sclerosis and stroke (Jauch et al., 2006). Myelin Associated Glycoprotein (MAG) is selectively localized in periaxonal Schwann cell and oligodendroglial membranes of myelin sheaths, suggesting that it functions in glia–axon interactions in both the PNS and CNS (Schachner and Bartsch, 2000). Glial fibrillary acidic protein (GFAP) is expressed almost exclusively in astrocytes, where it is induced by neural injury and released upon disintegration of the astrocyte cytoskeleton (Rempe and Nedergaard, 2010). GFAP plays an essential role in maintaining shape and motility of astrocytic processes and contribute to white matter architecture, myelination and blood brain barrier (BBB) integrity (O'Callaghan et al., 2015). After traumatic brain injury (TBI), GFAP's serum concentration peaks at 2–6 h and has a half-life of <2 days (Diaz-Arrastia et al., 2014). S-100B exerts both detrimental and neurotrophic effects, depending on its concentration in brain tissues (Adami et al., 2001). After release, S-100B acts as a trophic factor for serotoninergic neurons, and plays a role in axonal growth and synaptogenesis during development. Thus, traumatic acute injury results in great destruction of astrocytes leading to massive release (50 to 100 fold) of S-100B into plasma, whereas S-100B levels in psychiatric disorders were only about 3 times higher in patients compared to controls (Uda et al., 1998; Arolt et al., 2003), correlating well with its neuroprotective action. Specifically, S-100B stabilizes tau and MAP-2. Its half-life in the serum is 2 h (Zurek and Fedora, 2012).

A recent study of airline pilots and other flight crew members chronically exposed to organophosphates through combustion of engine oil and hydraulic fluid that contain organophosphate esters resulted in symptoms similar to those reported by GW veterans (fatigue, headaches, confusion and memory problems). Interestingly, these crew members showed significantly elevated numbers of autoantibodies in their blood serum of CNS damage markers including those associated with axonal transport (microtubule associated protein-2 (MAP-2), tubulin, neurofilament triplet proteins (NFP) and microtubule associated protein-tau (tau protein)) and those exclusively associated with CNS glial activation and neuroinflammation (myelin basic protein (MBP), and glial fibrillary acidic protein (GFAP) (Abou-Donia et al., 2013). A follow-up histopathology autopsy study was performed on a deceased pilot with organophosphate exposure that confirmed CNS damage and demyelination (Abou-Donia et al., 2014). Specifically, the histopathology results showed axonal degeneration and demyelination and the post-mortem and pathological examination of the nervous system confirmed the autoantibody biomarker results.

Recent studies with GW veterans have shown persistent signs and symptoms characteristic of CNS injury including brain imaging and cognitive studies (White et al., 2016; Chao et al., 2010, 2011, 2014, 2016; Heaton et al., 2007; Sullivan et al., 2003). There are, however, no validated objective diagnostic tests to identify acute or chronic sequelae of brain injury in this veteran group. Diagnosis of brain injury using cranial computed tomography (CT) scan and magnetic resonance imaging (MRI) techniques such as diffusion tensor imaging (DTI), have not been able to clinically diagnose veterans with GWI because there have been no proven cutoff values for volumetric or other imaging parameters that have been able to provide the required near 100% accuracy in terms of sensitivity/specificity at the individual level to distinguish cases from controls needed for a diagnostic test. Imaging studies have been able to show differences and altered CNS functioning between veterans with GWI and healthy controls but have not yet been able to identify the groups diagnostically because of the significant overlap between the groups (Chao et al., 2010, 2011, 2014, 2016; Heaton et al., 2007). Hence, it is important to develop clinically available, simple and inexpensive biomarkers for detection of neuronal and glial injury essential in the diagnosis and understanding of the temporal progression of CNS damage in GWI. Recently, serum biomarkers such as cytoskeletal proteins, resulting from axonal degeneration, have been used in diagnosing brain injury (particularly traumatic brain injury). The use of these biomarkers is usually measured in serum shortly after brain injury, because they have short half-lives (Zurek and Fedora, 2011; Diaz-Arrastia et al., 2014).

However, many years have elapsed since the time that GW veterans returned from deployment and became ill therefore, this particular approach cannot apply to GWI. Based on results from both chronic and acute injury, we used our novel autoantibody biomarker panel described above for brain injury to test for the indication of CNS damage in veterans with chronic GWI (Abou-Donia et al., 2013, 2014). One prior study compared autoantibodies of myelin basic protein (MBP) and striated muscle antibodies in GW veterans and reported higher MBP and muscle antibodies in veterans with GWI (Vojdani and Thrasher, 2004). Autoantibodies have previously been recognized as potential objective biomarkers of GWI (Golomb, 2012). Therefore, we hypothesized that chemical exposure to pesticides, anti-nerve gas pills and/or sarin nerve gas during deployment in veterans with GWI caused an excitotoxic cascade (through potential glutamatergic, oxidative stress and proinflammatory cytokine signaling) resulting in neurodegeneration and apoptotic loss of brain cells, leading to blood brain barrier leakage of specific neuronal and glial proteins into circulation, with subsequent formation of autoantibodies (AB) against these

proteins (Abou-Donia et al., 2013; Banks and Lein, 2012; Golomb, 2008; Terry, 2012; Binukumar and Gill, 2010; Soltaninejad and Abdollahi, 2009). In this study, we determined circulating IgG- class autoantibodies in serum from 20 GWI cases and 10 symptomatic (low back pain) controls against the following 9 brain proteins: neurofilament triplet proteins (NFP), tubulin, microtubule associated protein-tau (tau proteins), microtubule associated protein-2 (MAP-2), calcium/calmodulin Kinase II (CaMKII), myelin basic protein (MBP), myelin associated glycoprotein (MAG), glial fibrillary acidic protein (GFAP) and S-100B.

2. Materials and methods

2.1. Materials

The sources of proteins were: NFP (bovine spinal cord), tau protein (human), MAP-2 (bovine serum), tubulin (bovine brain), and MBP (human brain), from Sigma-Aldrich (Saint Louis, Missouri); CaMKII (Human) recombinant Protein and MAG recombinant Protein from Novus Biologicals, Littleton, CO, GFAP (human) from Biotrend Chemikalien GmbH, (Cologne, Germany) and S-100B (human brain) from American Qualex International, Inc. (San Clemente, California). Horseradish peroxidase-conjugated goat anti-human IgG, and enhanced chemiluminescence reagent were obtained from Amersham Pharmacia Biotech (Piscataway, New Jersey). SDS gels, 2–20% gradient (8 × 8), and tris-glycine 15 mM were obtained from Invitrogen (Carlsbad, California). All other materials were purchased from Amersham.

2.2. Ethics statement

Approval for the use of stored blood samples for this study was obtained from the Duke University Medical Center Institutional Review Board.

2.3. Case and control samples

Serum samples from 20 GWI cases with GWI and 10 non-veteran symptomatic controls with lower back pain were tested in this pilot study. GW veteran serum samples were collected from a study of acupuncture treatment in veterans with GWI from 2010 to 2012 (Conboy et al., 2012). Control serum samples were derived from a separate study of non-veteran patients with chronic lower back pain who served as 'symptomatic low back pain' controls from 2011 to 2013 (Jacobson et al., 2015). Veterans with GWI will be referred to as 'cases' and low-back pain symptomatic controls will be referred to as 'controls'.

2.4. Description of the patient cohorts

2.4.1. GWI-case cohort

"The Effectiveness of Acupuncture in the Treatment of Gulf War Illness" PI: Conboy, (8/21/2010–12/26/2012) N = 104; Study Site: New England School of Acupuncture (NESA). Cases were recruited through the Defense Manpower Data Base (DMDC) personnel listings and advertisements. Cases were screened for GWI symptoms and were required to meet the CDC diagnostic criteria for chronic multi-symptom illness (CMI) in order for inclusion in the parent study and in the current study (Conboy et al., 2012; Fukuda et al., 1998). Inclusion in the current study also required that veterans were deployed to the 1990–1991 Gulf War. CMI is characterized by one or more symptoms of at least 6 months duration from at least two of three symptom categories: 1) fatigue; 2) mood-cognition; 3) musculoskeletal pain.

Symptoms were not necessarily required to have started during or after the Gulf War deployment. Exclusionary criteria included that the veteran was 1) currently enrolled in another clinical trial 2) Had another disease that likely could account for the symptoms, as determined by the Medical Monitor 3) Severe psychiatric illness (in the last 2 years psychiatric hospitalization, suicidal attempt, alcohol or substance abuse, use of antipsychotic medication) 4) Unable to complete the protocol based on the evaluation of the Medical Monitor.

2.4.2. cLBP-cohort

"Structural Integration for chronic low back pain" PI: Jacobson (3/4/2011–6/21/2013) N = 46. Study Site: Spaulding Rehabilitation Hospital (SRH). In this cohort, 46 outpatients from the Boston area with chronic nonspecific low back pain were randomized to parallel 20-week long treatment groups of structural integration (SI) plus outpatient rehabilitation (OR) versus OR alone. The details of the study are described in a recent publication (Jacobson et al., 2015). Inclusion criteria for the parent study included: (i) Men and women aged 18–65, (ii) cLBP of ≥6 months duration, not attributed to infection, neoplasm, severe radiculopathy (as indicated by frequent severe pain radiating down a leg), fracture, or inflammatory rheumatic process, (iii) bothersomeness of back pain self-rated on average over the preceding 6 months ≥3 on an 11-point ordinal scale (0 = none, 10 = worst imaginable), (iv) prior arrangement to enter a course of outpatient physical therapy for low back pain at a Boston area rehabilitation clinic, (v) English language fluency and mental capacity sufficient to provide informed consent and participate in the study. Exclusion Criteria for the study included: (i) Impaired hearing, speech, vision, and mobility sufficient to interfere with participation in the study, (ii) current or anticipated receipt of payments from Worker's Compensation or other insurance for disability attributed to low back pain, (iii) prior treatment with any SI therapy, (iv) plans to initiate additional treatment for back pain during the period of the study other than outpatient rehabilitation care, particularly massage or other manual therapies (e.g., chiropractic or osteopathic manipulation), (v) exclusions for safety: unresolved musculoskeletal pathology of the lower limbs, current pregnancy, any implanted medical device, osteoporosis, any hypercoagulation condition, eczema, skin infection, deep vein thrombosis, burns or other acute trauma including unhealed bone fractures or open wounds, psoriasis, psychiatric illness not well controlled, or current episode of exacerbated major depressive disorder.

2.5. Collection and storage of samples

Samples from the GWI-cohort and the cLBP-cohort were all collected from the Boston area at the same time period at two different sites from 2010 to 2013. All sites followed exactly the same protocol for venipuncture, blood handling, serum separation, aliquoting and storage at −80 °C. The same phlebotomy and sample protocol was distributed in writing to all sites. All samples analyzed were baseline blood samples collected pre-intervention therapy. Samples used for this study have not been previously thawed and are free of hemolysis by visual inspection (Tuck et al., 2009).

2.6. Participant demographics

The participant demographics indicate that a total of 20 veterans with GWI, 18 males and 2 females, compared to 6 females out of 10 cLBP controls participated in the study. The age of the GWI cases ranged from 38 to 61 (mean ± SD 46.0 ± 6.8) compared to 25 to 64 (mean ± SD 50 ± 11.4) years for controls; all study participants were white (Table 1). Seventy percent of veterans with GWI reported taking PB

Table 1
Study participant demographics.[a]

Demographics	Cases	Controls
Age (mean ± SD)	46 (6.4)	50 (11.4)
Gender (% female)*	10	60
Race (% Caucasian)	100	100

Age range of Cases = 38–61 years and Controls = 25–64 years in 2010–2013 when the blood was collected.

[a] A total of 20 cases and 10 controls participated in the study.

* Cases were significantly different from controls for gender $p < 0.05$ but not for age.

pills during the war (n = 14). The groups differed with respect to gender (X2 = 8.5; $p < 0.05$) with significantly more women in the control group but did not differ with respect to age (t-value = −1.3; $p > 0.05$).

2.7. Western blot assay

To screen for the presence of autoantibodies against a battery of proteins, we applied a Western blot approach as previously reported (Abou-Donia et al., 2013). Each serum sample was analyzed in triplicate. Each protein was loaded as 10 ng/lane except for IgG that was loaded as100 ng/lane. Proteins were denatured and electrophoresed in SDS-PAGE (4% to 20% gradient) purchased from Invitrogen (Carlsbad, CA). One gel was used for each serum sample. The proteins were transferred into polyvinylidene fluoride (PVDF) membranes (Amersham Pharmacia Biotech Piscataway, New Jersey). Nonspecific binding sites were blocked with Tris-buffered Saline-Tween (TBST) (40 mM Tris [pH 7.6], 300 mM NaCl, and 0.1% Tween 20) containing 5% non-fat dry milk for 1 h at 22 °C. Membranes were incubated with serum samples at 1:100 dilutions in TBST with 3% non-fat dry milk overnight at 4 °C. After five washes in TBST, the membranes were incubated in a 1:2000 dilution of horseradish peroxidase-conjugated goat anti-human IgG (Amersham Pharmacia Biotech (Piscataway, New Jersey). The dot blots were probed with anti-human IgG (H + L) HRP conjugate antibody (Cat. No. 31410, Thermo Fisher Scientific Inc., Pittsburgh, PA, USA) for 1 h at RT, incubated with ECL reagent (Cat. No. 34096). The membranes were developed by enhanced chemiluminescence using the manufacturer's (Amersham Pharmacia Biotech) protocol and a Typhoon 8600 variable mode imager. The signal intensity was quantified using Bio-Rad image analysis software (Hercules, California). All tests were performed with the investigators blinded to participant diagnosis.

2.8. Specificity of serum autoantibodies

Previously we checked the specificity of the serum autoantibody by performing peptide/antigen competition assay, in which the serum was spiked with the target protein or peptide (Abou-Donia et al., 2013). The serum from random healthy controls was mixed with or without tau, MAP or MBP. The serum/protein mix was centrifuged at 15,000 rpm to pellet any immune complexes. The supernatants were then carefully removed and used in Western blotting.

2.9. Calculations

The mean value of the optical density measurement from the triplicate testing was used for each serum sample tested and normalized by total IgG. Thus, the results are expressed as mean values of triplicate assays of optical density arbitrary units normalized to total serum IgG.

2.10. Power analysis

A total of 20 GWI cases were available for testing in this convenience sample. Effect size calculations were based on two-sample t-test assuming a common standard deviation between groups. The power analysis assumes that cases and controls are not matched. In a t-test of difference between two independent means, selecting power of 80%, 2-sided alpha 0.05, and size of 20 vs 10, the study was powered to detect an effect only if at least 1.12 SD.

2.11. Statistics

Grouped data are reported as mean ± SD. The values from cases were compared to the control group using t-tests and Pearson correlation analyses (SigmaStat, Systat Software) and p-values were

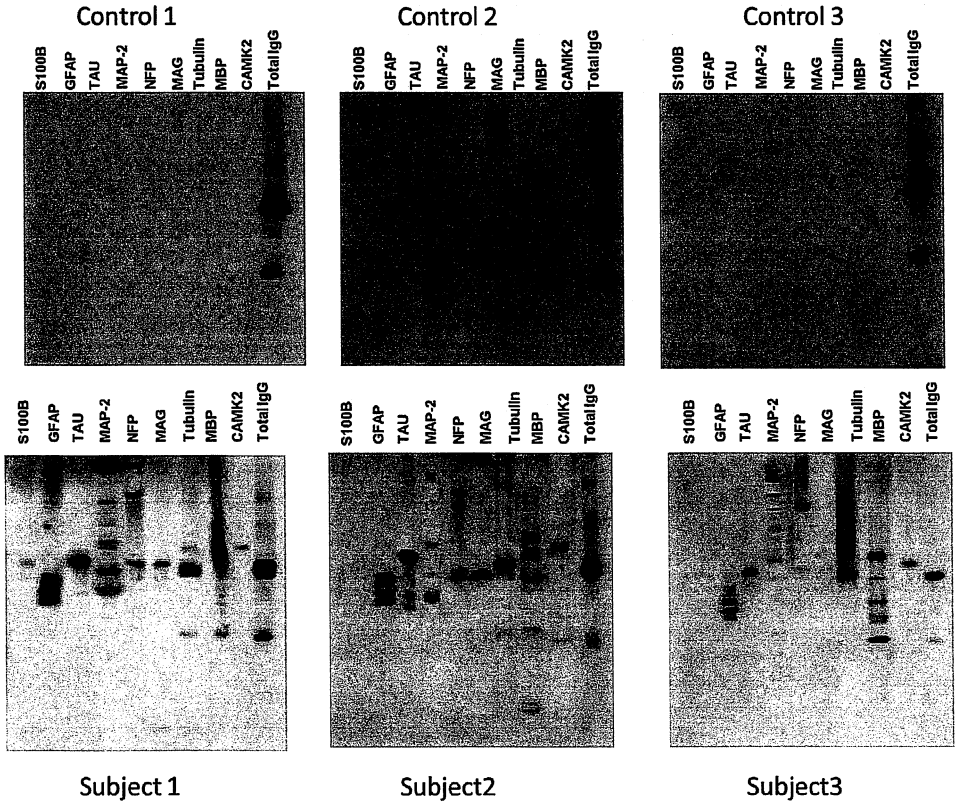

Fig. 1. A representative sample of Western blot gels from three cases showing that the majority of GWI serum reacted intensely to neural proteins, while most control serum showed a weak or no reaction.

Table 2
Chemicals, environmental and other exposures of cases during the Gulf War.[a]

Chemical exposures	Exposed	%	Environmental and other exposures	Exposed	%
Pyridostigmine bromide (PB)	14	70	Khamisiyah notification letter	8	40
Organophosphorus pesticides (OP)	7	35	Contaminated food/water	18	90
Carbamates	7	35	Vaccines	18	90
Pyrethroids	4	20	Malaria	12	60
DEET	11	55	Sand	18	90
Sarin	9	45	Tent heater	11	55
Depleted uranium (DU)	6	30	Jet fuel	14	70
Solvents	10	50	Oil fires	18	90

[a] A total of 20 veterans with GWI participated in the study.

calculated. Pairwise correlations among the nine biomarkers were assessed. A 2-sided p value <0.05 was considered significant. Due to the exploratory nature of this pilot study, analyses were not adjusted for multiple comparisons.

3. Results

As previously described, we assessed the specificity of the serum autoantibody by performing peptide/antigen competition assay, in which the serum was spiked with the target protein or peptide. The serum bound to tau eliminated the tau band in the Western blot (see Fig. 1) while the band of MAP-2 or MBP were present and not affected. The serum bound to MAP-2 eliminated the MAP-2 band in the Western blot while the band of tau or MBP was present. The serum bound to MBP eliminated the MBP band in the Western blot while the bands of tau and MAP-2 were present. These results indicate that each autoantibody in the serum was specifically neutralized by its target protein in serum sample and was no longer available to bind to the epitope present in the protein on the Western blot. This confirmed that the assay used in this study, was specific and accurately determined autoantibodies against tested proteins in serum samples.

To detect autoantibodies in serum, we probed Western blots with individual serum samples. A total of 30 human serum samples (20 veterans with GWI and 10 non-veteran symptomatic low-back-pain controls) underwent measurement of the levels of the serum circulating IgG-class autoantibodies against nine neuronal- and glial- specific proteins. Table 2 lists the number of GWI cases who were exposed to chemical and environmental exposures. It shows that 14 cases (70%) used PB as a prophylaxis against possible exposure to nerve agents and nine cases reported being exposed to the nerve agent sarin. In addition, a total of eight cases reported receiving notification from the Department of Defense (DOD) that they were potentially exposed to sarin and other chemicals due to their proximity to the Khamisiyah, Iraq underground weapons depot where a chemical weapons cache was destroyed in March 1991 (US DOD, 2002). Eight cases reported exposure to depleted uranium. All of the cases reported exposure to one or more insecticides or a mixture of pesticides including organophosphates, carbamates, pyrethroids and organochlorines. Eleven cases used the insect repellant DEET. All cases underwent environmental and other exposures listed in Table 2. Other chemicals that the cases reported exposure to included oil well fires, sand, tent heaters, jet fuel, and solvents. Some veterans reported exposure to malaria and 18 reported being vaccinated. Serum from GWI cases showed significantly increased levels of autoantibodies against all cytoskeletal proteins except those against S-100B compared to non-veteran symptomatic (low back pain) controls (Table 3). Due to the gender differences between the cases and controls, analyses were also run with just the males in the groups. Although there was only a small number of males ($n = 4$) in the control group which could be problematic in this type of analysis, results of this comparison showed a very similar pattern of significant differences in all autoantibodies (GFAP $p < 0.001$; Tau $p < 0.001$; MAP $p < 0.002$; MAG $p < 0.001$; PNF $p < 0.006$; Tubulin $p < 0.003$; MBP $p < 0.01$; S-100B $p = 0.31$). The majority of GWI serum reacted intensely to neural proteins, while most control serum showed a weak or no reaction. Fig. 1a and b present Western blots results from three representative GWI cases and three controls. The levels of serum autoantibodies in GWI cases and controls to neural-specific proteins expressed as mean values ± SD of triplicate assays of optical density arbitrary units normalized to total serum IgG optical density ranged from 0.30 for S-100B and 4.09 for GFAP for the cases compared to 0.30 and 0.62, respectively for controls are listed in Table 3 and shown in Fig. 2. The percentage of autoantibodies against neural proteins of cases compared to controls (in descending order) were: CaMKII, 927, GFAP 660, Tau 483,Tubulin 441, MAG 360, MBP 250, NFP 245, MAP-2 230, S-100B 103. Fig. 3 presents the mean values ± SD ($p < 0.001$) of fold increase of autoantibodies against neural proteins for the cases compared with the controls. Serum from controls had no or low levels of circulating autoantibodies to nervous system-specific biomarkers. Autoantibodies against CaMKII were more predominant in the cases' serum than in controls' serum (Fig. 3).

Fig. 4 shows that Tubulin and GFAP had the highest values in the GWI cases compared with the controls. Pairwise correlations among the nine autoimmune biomarkers were significant only for the pair Tau and MBP. When comparing the correlation between each pair, only tau and MBP were significantly linearly correlated to each other (Fig. 5). Fig. 5 shows that the control values of those two biomarkers were <1 optical density unit, whereas GWI cases had values strongly linearly correlated with each other such that on average tau was elevated up to 10 times higher than controls in some GWI cases, and MBP was also elevated up to 5 times higher for the same cases vs the controls.

Finally, when each biomarker was compared separately between individual cases and controls for potential fold-increase cut-points to discriminate the groups, results indicated that tubulin values had some of the highest-fold increased values in the individual GWI cases compared with the individual control values although only 60% of the individual cases ($n = 12$) showed that effect (Fig. 6a). However, in 9 (out of the 20) cases tubulin values were elevated by a factor of 3 to 9-fold higher than the controls. In Fig. 6b, GFAP was elevated the most in cases compared to controls. In fact, GFAP was higher in all of the cases compared with all of the controls with 20 out of 20 cases having 2 to 7 fold higher

Table 3
Statistical analysis of the levels[a] of serum autoantibodies (AA) in controls[b] and GWI cases[b] to neural-specific proteins.

	NFP	Tau	Tubulin	MBP	MAG	MAP2	GFAP	S-100B	CaMKII
Cases Mean ± SD	1.42±0.24	2.52±0.31	3.48±0.78	1.75±0.30	1.44±0.28	2.18±0.29	4.09±0.33	0.30±0.03	1.02±0.20
Controls Mean ± SD	0.58±0.09	0.60±0.09	0.79±0.11	0.70±0.11	0.40±0.04	0.086±0.09	0.62±0.11	0.29±0.04	0.11±0.03
p values	0.02	0.0001	0.001	0.001	0.007	0.002	0.00001	0.40	0.015

[a] The results are expressed as mean values of ± triplicate assays of optical density arbitrary units normalized to IgG optical density as fold of healthy controls.
[b] Values from cases were compared to the control group using *t*-tests; most were highly significant $p < 0.001$ (2-sided), except for S-100B that was not significantly different from controls. Cases were significantly different from controls with respect to gender $p < 0.05$ but not with respect to age.

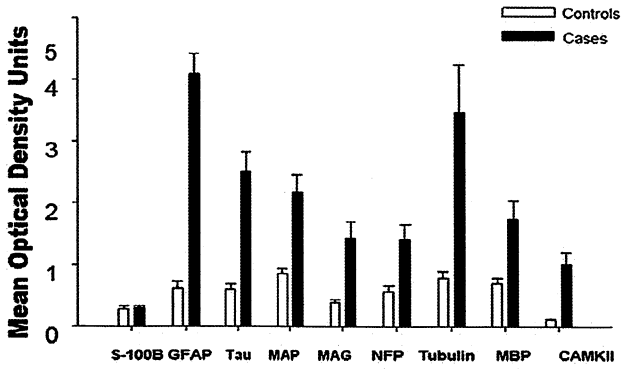

Fig. 2. Mean autoantibodies against neural proteins from cases and controls expressed in mean optical density units.

value than the control mean. Thus GFAP values completely distinguished the cases from the controls. GFAP values did not overlap in cases vs controls in this small sample; however, the separation in the ranges was small relative to the substantial standard deviations. In Fig. 6c, tau was higher than controls in 18 cases and 50% of the cases had double the value of tau compared with the controls. In Fig. 6d, MAP was higher than the controls in 15 cases and 75% of the cases had a 0.5 to 11-fold higher value than the controls. In Fig. 6e MAG was higher than controls in 15 cases and 75% of the cases had up to a 10-fold higher value than the controls. In Fig. 6f NFP was higher than controls in only 50% of the cases (n = 10) and they showed 0.5 to 11-fold higher values than controls. MBP was higher than controls in 12 cases and 60% of the cases were higher than controls with 2 to 5-fold higher values than controls. CAMKII was higher than controls in 16 cases and 50% of the cases had a 3 to 30-fold higher value than the controls. S100B values were not statistically significant as the values overlapped with cases and controls.

4. Discussion

This pilot study reports significantly elevated levels of autoantibodies against neurotypic- and gliotypic-specific proteins in serum from a sample of 20 veterans with GWI and 10 non-veteran symptomatic (low back pain) controls with musculoskeletal symptoms rather than CNS symptoms. The increased levels in GWI cases compared to controls ranged from 9.27 fold for CaMKII to 6.6 fold for GFAP to 2.45 fold for neurofilaments. Autoantibody levels against S-100B were not different in GWI cases than controls (1.03 fold) consistent with its neural protective role and in agreement with presence of chronic injury and

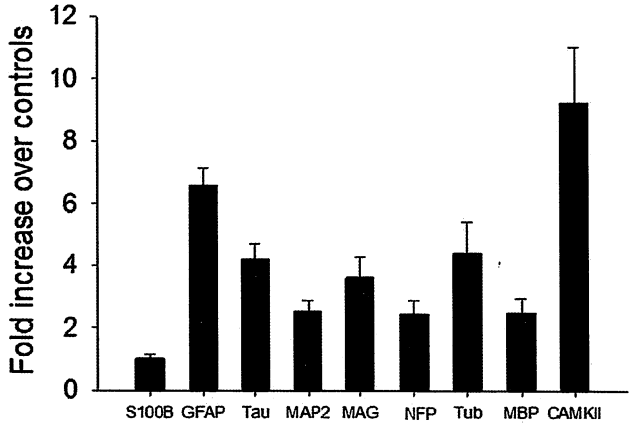

Fig. 3. Folds increase of autoantibodies against neural proteins from cases relative to controls.

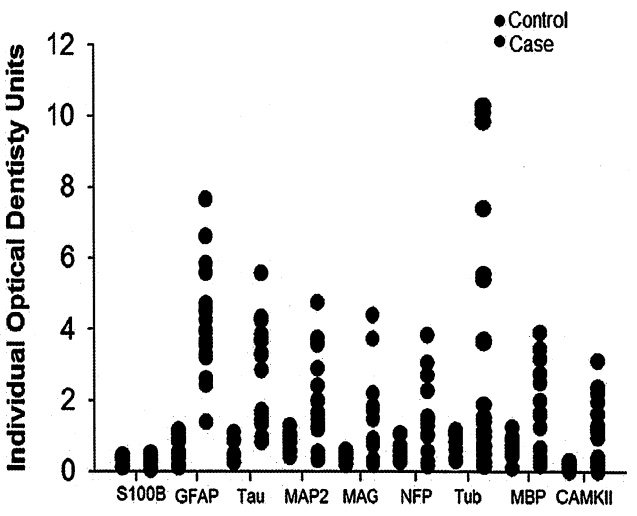

Fig. 4. The levels of autoantibodies of neural proteins of GW cases and of controls expressed as optical density units.

absence of acute brain injury in veterans with GWI (Zurek and Fedora, 2011; Diaz-Arrastia et al., 2014; Stalnacke et al., 2006, 2004; Coch and Leube, 2016). Previous studies, using animal models of GWI, showed that exposure to the neurotoxicants that were present in the GW environment, caused deficits in behavioral outcomes that were accompanied by neuronal and glial degeneration (Abdel-Rahman et al., 2001, 2002a,b, 2004a,b; Abou-Donia et al., 2000, 2001, 2004). Following neurodegeneration, there is accumulation of cellular neurological waste products or debris such as misfolded or hyper-phosphorylated proteins that form toxic stable aggregates (Nedergaard, 2013; Edgar et al., 2004). This extracellular debris send damage signals that cause the CNS immune cells - microglia to become activated and act as profound antigen presenting cells that secrete pro-inflammatory cytokines (IL-1b, TNF-a and IL-6) and mediators (reactive oxygen species, ROS) resulting in the recruitment of T-lymphocytes (Milligan and Watkins, 2009; Banks and Lein, 2012). Multiple exposures to these waste proteins can cause microglia and astrocytes to become primed to react more strongly after each subsequent exposure (Watkins and Maier, 2003). This can result in a persistent neuroimmune response and chronic neuroinflammation contributing to chronic health symptoms, such as those seen in GW veterans (Johnson et al., 2016; Milligan and Watkins, 2009; Maier and Watkins, 1998; Watkins and Maier, 2003). These waste proteins are eventually released into circulation due to defects in the

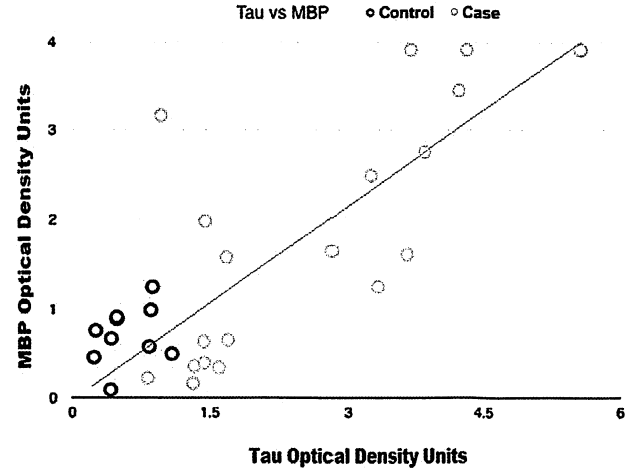

Fig. 5. Paired correlations of Tau and MBP optical density levels in cases relative to controls.

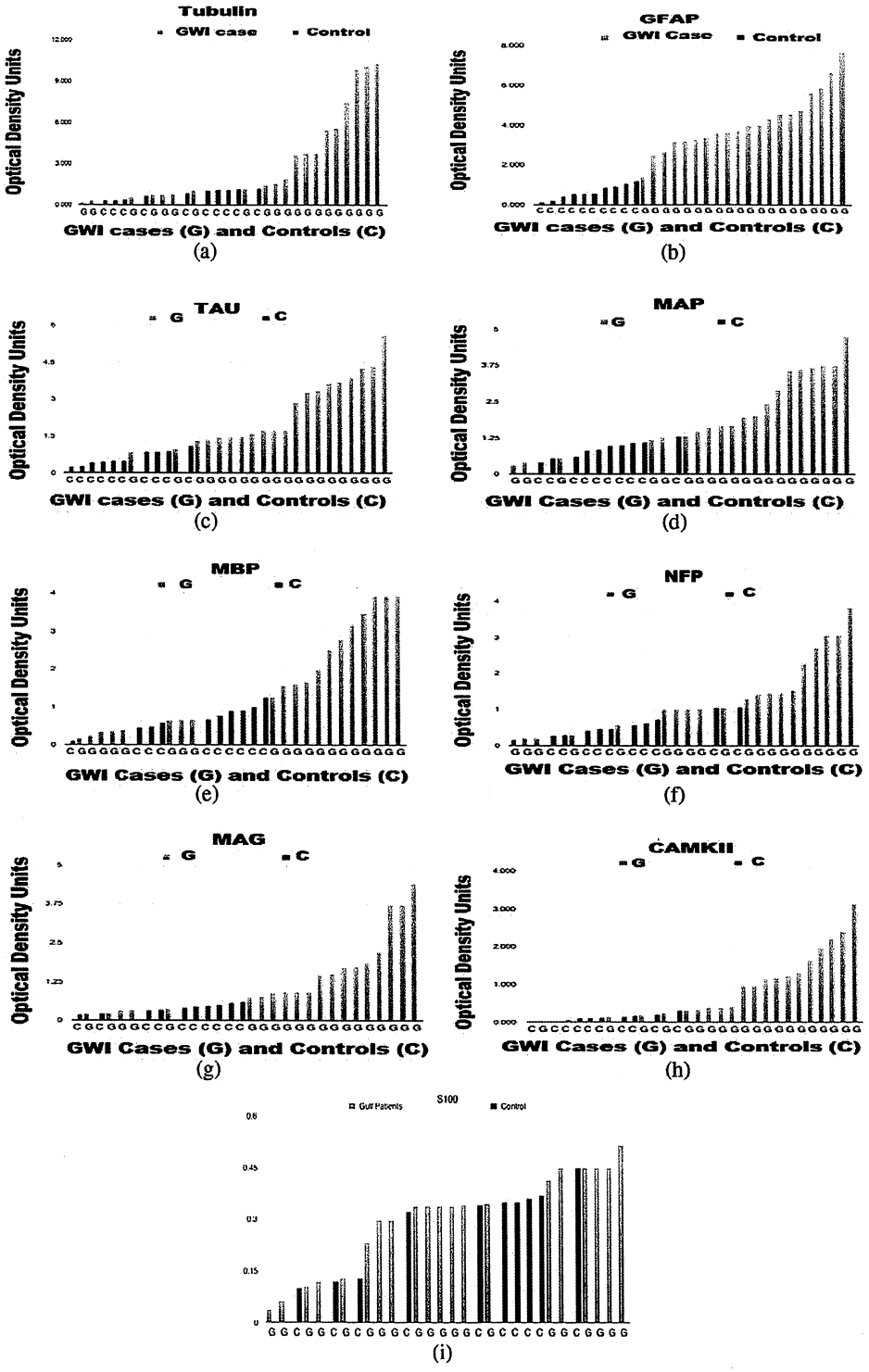

Fig. 6. a) Tubulin levels were higher than all controls in 12/20 cases. b) GFAP levels were higher than all controls in 20/20 cases. c) Tau levels were higher than all controls in 17/20 cases. d) MAP levels were higher than all controls in 15/20 cases. e) MBP levels were higher than all controls in 12/20 cases. f) NFP levels were higher than all controls in 10/20 cases. g) MAG levels were higher than all controls in 15/20 cases. h) CAMKII levels were higher than all controls in 16/20 cases. i) S100B levels overlap with cases and controls.

brain-blood barrier induced by astrocyte alterations. Waste proteins in the brain ultimately reach the liver through a mechanism known as the "glymphatic system" where they are degraded (Nedergaard, 2013). However, the released proteins that could serve as markers of injury are present in the short-term and cannot be used as biomarkers in the case of chronic GWI (Zurek and Fedora, 2011; Diaz-Arrastia et al., 2014). Thus detection of autoantibodies can serve as surrogate markers for these circulating waste proteins as described in this study.

The highest increase in autoantibodies was against CaMKII which was 9.27 times higher than that of controls followed by GFAP which was 6 times higher than controls. This result is consistent with the veterans' exposure during their deployment to the Gulf War to

organophosphorus compounds such as pesticides, and the nerve agent sarin that have been shown to increase the activity and mRNA expression of CaMKII (Patton et al., 1983, 1985, 1986; Gupta et al., 1998; Barbier et al., 2009) as well as enhanced CaMKII-induced phosphorylation of NFP, tubulin (Serrano et al., 1986) and tau activity leading to the aggregation, deregulation and accumulation of NFP (Abou-Donia et al., 1993; Norgren et al., 2003) and tubulin in the axon (Abou-Donia, 1993; Jensen et al., 1992, Gupta et al., 2000; Grigoryan and Lockridge, 2009). Aggregated neurofilaments result in slowing of axonal transport as has been illustrated in GW-relevant animal and cell neurotoxicant models (Gupta et al., 1997; Reagan et al., 1994; Terry et al., 2012; Gao et al., 2016; Edgar et al., 2004). GW-relevant exposure models have also been associated with astrocyte activation (Zakirova et al., 2015; Ojo et al., 2014).

Neuronal proteins studied in this pilot analysis represented various anatomical regions of the neuron with distinct functions which can be instructive with regard to the pathobiology of GWI (Lapadula and Abou-Donia, 1992). All of the proteins used are involved in axonal structure and function and are released as products of neural degeneration of various regions of the neuron. MAP-2 is present in the dendrites; CaMKII, tau, tubulin, and neurofilament proteins are located in the axon; myelin basic protein (MBP) and myelin associated glycoprotein (MAG) are an integral part of myelin (McMurray, 2000). Furthermore, the central nervous system-specific glial protein, GFAP and S-100B are secreted by astrocytes after neuronal injury (McMurray, 2000). Following axonal and myelin degeneration, neuronal and glial proteins are released and once in circulation, activated lymphocytes, B and T cells lead to the formation of autoantibodies against these proteins (Schwartz and Shechter, 2010a,b).

Increased autoantibodies against nervous system-specific proteins leads to structural consequences in various regions as follows: increased autoantibodies against neurofilaments proteins, tau, CaMKII and tubulin are indicative of axonal degeneration; increased autoantibodies against MAG and/or MBP suggest demyelination, increased autoantibodies against MAP-2 suggest dendritic degeneration, increased autoantibodies against GFAP suggest astrogliosis, and the low or no-increased levels of autoantibodies against S-100B is consistent with chemical-induced brain injury (Zurek and Fedora, 2011, Diaz-Arrastia et al., 2014; Stalnacke et al., 2006, 2004). The linear correlation pattern of tau and MBP in this study suggests an important potential effect of axonal degeneration followed by demyelination that would correspond with prior neuroimaging studies in neurotoxicant exposed GW veterans (Heaton et al., 2007; Chao et al., 2010).Furthermore, these structural changes of the nervous system lead to functional alterations. Hence axonal degeneration in the cerebral cortex leads to: motor and sensory abnormalities, ataxia, deficit in posture, locomotion, and skilled fine motor movements (fingers, speech, facial expression) and weakness; degeneration of the limbic system including the hippocampus leads to: learning and memory deficits, and neurobehavioral (mood, emotion and judgment) abnormalities; increased autoantibodies against MAP-2 suggests damage to the dendrite-rich Purkinje cells in the cerebellum resulting in: gait and coordination abnormalities, staggering gate and ataxia (McMurray, 2000; Abou-Donia, 2015). Increased autoantibodies against GFAP indicate astrogliosis and potential neuroinflammation and/or glial scarring. GFAP contributes to white matter architecture, myelination and blood brain barrier (BBB) integrity (O'Callaghan and Sriram, 2005; Amourette et al., 2009; Lamproglou et al., 2009). Consequently, blood levels of GFAP in healthy individuals are very low. GFAP levels were higher in GWI cases and completely discriminated between the cases and controls in this study. This is particularly relevant because disorders with higher levels of GFAP include memory disorders such as Alzheimer's and vascular dementia that have significant axonal neurodegeneration and neuroinflammation (Mecocci et al., 1995). Increased autoantibodies against S-100B suggest traumatic brain damage and can help to differentiate between acute and chronic brain injury (Stroick et al., 2006; Stalnacke et al., 2006, 2004; Zurek and Fedora, 2011; Diaz-Arrastia et al., 2014; Coch and Leube, 2016). Their lack of increase in this study suggests against acute traumatic brain injury in veterans with GWI.

Important mechanistic clues from animal and cell studies of these GW-relevant neurotoxicants have shown deficits in axonal transport, as well as aberrations in neurofilaments and microtubules, which are the structural railways for axonal transport (Gupta and Abou-Donia, 1995a, b; Gearhart et al., 2007; Grigoryan and Lockridge, 2009; Prendergast et al., 2007, Jiang et al., 2010). Mitochondria are also delivered by axonal transport to provide the energy required to power the biochemical reactions necessary for the functioning of the axon and have shown altered functioning in GW-relevant neurotoxicant models (Middlemore-Risher et al., 2011). GW-relevant chronic low-level organophosphate exposure has also been associated with mitochondrial compromise from oxidative stress induction and with neuroinflammation resulting in cell damage or cell death resulting in debris of waste proteins in the extracellular spaces (Laetz et al., 2009; Kaur et al., 2007; Banks and Lein, 2012). In fact, one hypothesis of GWI suggests that mitochondrial damage and oxidative stress in the brain and the periphery have caused the chronic symptoms of GWI; notably, increased autoantibodies were expressly cited among objective markers and mediators in this model (Golomb et al., 2014; Golomb, 2012; Koslik et al., 2014).

Another hypothesis of GWI suggests that the neurotoxicants acted synergistically to create a self-perpetuating neuroinflammatory state, which in turn has an ongoing negative impact on brain cells including neurons (microtubules, motor proteins, mitochondria) and glia (microglia, astrocytes, oligodendrocytes) and blood-brain barrier function (O'Callaghan and Sriram, 2005). Clinical studies have also found consistent results with GW veteran cohorts who showed impaired cognitive functioning and reduced volume and altered white matter microstructural integrity on MRI in OP pesticide, sarin nerve agent and PB pill exposed cohorts (White et al., 2016; Sullivan et al., 2013; Chao et al., 2010; Heaton et al., 2007; Proctor et al., 2006; Sullivan et al., 2003). These prior results suggest clear CNS alterations in neurotoxicant-exposed GW veterans which correlated with behavioral outcomes that are related to neurodegeneration and perhaps with both a chronic neuroinflammatory and Mitochondrial/OS hypothesis.

The only other study that we are aware of that compared CNS autoantibodies in GW veterans compared MBP and striated and smooth muscle antibodies and reported higher MBP and muscle antibodies in veterans with GWI when compared with controls (Vojdani and Thrasher, 2004). The current study validates the prior MBP findings and expands on those findings with a larger panel of 8 additional CNS autoantibody markers. Collectively, these findings suggest that alterations in white matter as evidenced by circulating autoantibodies to MBP appear to be associated with GWI. This finding corresponds with both leading hypotheses for GWI given that white matter alterations can be associated with oxidative stress and neuroinflammation as a result of glial activation and signaling of both proinflammatory cytokines and oxidative stress (Milligan and Watkins, 2009). The additional finding of this study that higher Tau autoantibody levels were significantly linearly correlated with higher MBP autoantibody levels in GWI cases suggests that axonal degeneration may be occurring before demyelination in veterans with GWI and warrants further more conclusive study to distinguish it from the more myelin-specific toxic leukoencephalopathies (Schmahmann et al., 2008; Filley, 2013). These findings also correspond with MRI findings of differences on both white and gray matter brain volumes in neurotoxicant-exposed GW veterans (Heaton et al., 2007; Chao et al., 2010, 2011, 2014, 2016). These findings also clearly suggest that glia and astrocytes in particular should be further studied in GWI given significantly higher levels of GFAP in the GWI cases that correspond with prior animal models of GWI (Abdel-Rahman et al., 2001, 2002a, 2002b, 2004a, 2004b; Abou-Donia et al., 2000, 2001, 2002, 2004; Zakirova et al., 2015; Ojo et al., 2014) and with recent studies illustrating the ability of astrocytes to donate mitochondria to damaged neurons (Hayakawa et al., 2016).

4.1. Limitations and future directions

This study, like all studies has important limitations. Although the present pilot study can serve as a proof-of-concept it has a small sample size and non-matched subject groups for age, gender and for CNS symptoms. This is particularly important as it has also been shown that CNS autoantibodies have been reported to be age-related in animal models (Lal and Forster, 1988). In addition, the convenience comparison group utilized in this study had musculoskeletal symptoms and not CNS symptoms therefore, it remains to be shown that these CNS autoantibody markers can clearly distinguish between GWI cases and additional groups with CNS specific symptoms. However, the strong results including 9-fold higher levels of CAMKII, 6-fold higher levels of GFAP and 4-fold higher levels of tau and tubulin that were presented in this study warrant further research for a blood-based objective marker of GWI in larger, well-characterized veteran cohorts. These results suggest a possible new avenue for further development of an objective biomarker of GWI. The identification of this small panel of neural-specific autoantibody biomarkers in GWI shows promise for further validation in larger study samples that are more carefully matched for subject demographics (particularly age), different types of control groups (i.e. healthy and CNS symptomatic groups) and that classify cases by both the CDC and the more specific Kansas GWI criteria which also specifies the time period of deployment which may be relevant to particular OP and other deployment-related exposures (Steele, 2000; Fukuda et al., 1998). Future directions will be to compare these CNS autoantibody markers with specific behavioral outcomes including cognitive performance and brain imaging of gray and white matter volume and microstructural integrity to further validate these suspected brain-immune-behavioral outcomes.

5. Conclusions

In conclusion, in this pilot study GWI was significantly associated with 2–9 fold increased serum autoantibodies against 8 neuronal- and glial-specific proteins (CaMKII, GFAP, Tau, Tubulin, MAG, MBP, NFP, MAP-2) and not with a marker of more acute damage (S-100B). The autoantibodies that were found here to be elevated in GWI, targeted proteins/ antigens that play critical roles in the structure and function of the neuron including axonal transport and myelination. Many of them are explicit markers for neurodegenerative disorders, consistent with axonal and myelin degeneration of myelinated neurons and with astrogliosis, cell signaling and neuroinflammation. These same proteins have been shown to be affected in other clinical groups and animal models with similar organophosphate and carbamate exposures (Abou-Donia et al., 2013, 2014). These results validate prior reports of increased MBP autoantibodies in GWI cases and suggest that oligodendrocyte signaling, glia and white matter alterations should continue to be further studied in GWI and validated with health symptom and behavioral outcomes (Vojdani and Thrasher, 2004). The results also indicate that veterans with GWI may be continuing to show brain neuronal degeneration and glial activation that would be consistent with recent reports of chronically persistent and in some cases worsening health of these veterans (Smith et al., 2013; Ozakinci et al., 2006; Li et al., 2011; Kang et al., 2009; Dursa et al., 2016; White et al., 2016). These results suggest a possible avenue for further development of a panel of objective biomarkers of GWI upon further validation in larger study samples that are more carefully matched for subject demographics.

Conflict of interest statement

The authors report no relationships that could be construed as a conflict of interest.

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Treating Gulf War Illness: The Lasting Effects of Desert Shield/Storm

By Joe C. Chang, MAOM, Dipl. OM, LAc, Rosa N. Schyner, DAOM, CFMP, LAc and Lisa Conboy, ScD

Clinical and registry programs indicate that 25 percent of the 700,000 veterans of the first Gulf War (Operation Desert Shield/Storm, years 1990-1991), have been affected by clusters of symptoms, and co-morbid medical diagnoses. Symptoms include chronic fatigue syndrome, fibromyalgia, irritable bowel syndrome, arthralgia, digestive complaints, and mood-related psychiatric disorders, including depression, post traumatic stress disorder (PTSD), and other anxiety disorders.[1,2]

Gulf War Illness (GWI) is a complex and difficult medical condition to treat, with highly individualistic symptom presentations, including fatigue, sleep and mood disturbances, cognitive dysfunction, and musculoskeletal pain. The Centers for Disease Control and Prevention (CDC) has defined Gulf War Illness as three symptom clusters that includes: fatigability (fatigue 24 hours or more after exertion), mood and cognition (feeling depressed, irritable, anxious, difficulty in concentrating, problems getting to sleep), and musculoskeletal (joint or muscle pain).

GWI & Acupuncture

The complex diagnostic and treatment process of Chinese medicine, which is tailored to each individual's clinical presentation, can provide an effective framework for evaluating and addressing the complex constellation of symptoms presented in GWI. Currently, no biomedical standard of treatment care exists. One study, completed by the *New England School of Acupuncture,* included an unblinded phase II Randomized Controlled Trial (RCT), which offered individualized acupuncture treatments, using the available community resources.

The treatment schedule duration, dose, and specific Chinese medicine techniques employed were based on the clinical experience of the expert practitioners, and informed by literature review. Details of the protocol and implementation were determined before the trial began via focus groups with senior acupuncture faculty.

Case Study Team

Licensed acupuncturists with at least five years of clinical experience, who received additional in-house training concerning GWI, provided the acupuncture treatments. Although there are many styles of acupuncture within Chinese medicine, acupuncturists were chosen who self-reported use of the TCM model of diagnosis.

During the first session, the acupuncturists conducted an interview reviewing the subject's medical history, symptoms and aspects of diagnosis from the perspective of TCM, including condition of the tongue, pulse, meridians, and acupoints.

Each subject received an individualized diagnosis and treatment protocol addressing his or her unique pattern of symptoms. Brief interviews began each subsequent session, allowing patient and practitioner to prioritize symptoms, and identify any questions or concerns.

Individualized treatment protocols allowed the practitioners to alter the treatment plan based on how the patient presented at the moment; including varying the selection of acupoints across treatments and adding particular co-interventions commonly used as part of TCM therapy to supplement manual needling.

For example, electroacupuncture for its efficacy in reducing pain and inflammation24, heat therapies (e.g. heat lamp), Chinese massage, and press balls, tacks or magnets applied to points after needling. Each session lasted approximately one hour. Acupoints were stimulated manually until "obtaining de qi," a technique characteristic of TCM to elicit a response felt by both the patient and the acupuncturist.

This needling sensation, adjusted for the comfort and safety of each patient, may be experienced as a pinch that rapidly subsides, or a sense of spreading pressure, dull ache, or warmth. Needles were retained for 30-45 min (10-35 stainless steel, disposable needles per session).

After needle insertion, subjects were left to rest or nap. The type of needle, including gauge (32-38) and length (15-50 mm) as well as the depth of insertion (subcutaneous to about 25 mm) varied according to the area of the body being treated (i.e. extremities vs. trunk). Choice of acupoints could vary during subsequent treatments to improve results. Herbs and supplements were not allowed. Subjects were encouraged (but not required) to remain with the same acupuncturist for the whole study period to allow for development of patient-practitioner rapport.[3]

Local advertisements and direct mailings to veterans of the first Gulf War, drawn from the Defense Manpower Data Center, recruited participants for the case study. Since the demographics of GWI veterans are unpublished, it was not known if there would be a sufficient population near the study offices to draw a fair sample. Therefore, the study was designed to include treatment sites within a 100-mile radius of the study offices, and incorporated a mechanism to add treatment sites within that radius in areas where GWI veterans were found clustered.

Thirty treatment sites were utilized. This design had the added benefit of allowing veterans to receive treatments near where they lived and worked, a technique that may have improved adherence. The resulting study provided treatments at extant acupuncturist offices to 104 veterans with GWI. The results of the study were overwhelmingly positive, achieving both clinically and statistically significant levels of improvement.

Randomized to six months of either bi-weekly acupuncture treatments or two months of waitlist followed by weekly acupuncture treatments, 82 percent of the veterans completed the protocol. Measurements were taken at baseline, two, four and six months to evaluate physical function (SF-36 physical component, SF 36P) and pain (McGill Pain scale).

Veterans who received twice per week treatments, experienced a clinically and statistically significant improvement in both pain and function at sixth month, compared to veterans receiving treatment just once per week, who also experienced a benefit in some scales such as severity of main and secondary complaint Levels of satisfaction with treatment and confidence with acupuncture and the acupuncturist were also very high, all at least 95 percent.[4]

Please note that in 2012 Congress appropriated funds to VAs for veteran treatments received in the community. For information on how to receive reimbursement for treating veterans in your clinic, please email Lisa Conboy at: **lisa_conboy@hms.harvard.edu** .

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ORIGINAL ARTICLE

Matrix Analysis of Traditional Chinese Medicine Differential Diagnoses in Gulf War Illness

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Abstract

Objective: To qualitatively categorize Traditional Chinese Medicine (TCM) differential diagnoses in a sample of veterans with Gulf War Illness (GWI) pre- and postacupuncture treatment.

Subjects and methods: The authors randomized 104 veterans diagnosed with GWI to a 6-month acupuncture intervention that consisted of either weekly or biweekly individualized acupuncture treatments. TCM differential diagnoses were recorded at baseline and at 6 months. These TCM diagnoses were evaluated using Matrix Analysis to determine co-occurring patterns of excess, deficiency, and channel imbalances. These diagnoses were examined within and between participants to determine patterns of change and to assess stability of TCM diagnoses over time.

Results: Frequencies of diagnoses of excess, deficiency, and channel patterns were tabulated. Diagnoses of excess combined with deficiency decreased from 43% at baseline to 39% of the sample at 6 months. Excess+deficiency+channel imbalances decreased from 26% to 17%, while deficiency+channel imbalances decreased from 11% to 4% over the study duration. The authors observed a trend over time of decreased numbers of individuals presenting with all three types of differential diagnosis combinations. This may suggest that fewer people were diagnosed with concurrent excess, deficiency, and channel imbalances and perhaps a lessening in the complexity of their presentation.

Conclusion: This is the first published article that organizes and defines TCM differential diagnoses using Matrix Analysis; currently, there are no TCM frameworks for GWI. These findings are preliminary given the sample size and the amount of missing data at 6 months. Characterization of the TCM clinical presentation of veterans suffering from GWI may help us better understand the potential role that East Asian medicine may play in managing veterans with GWI and the design of effective acupuncture treatments based on TCM. The development of a TCM manual for treating GWI is merited.

Keywords: Gulf War Illness, acupuncture, matrix analysis, Traditional Chinese Medicine

Introduction

ACCORDING TO CLINICAL and registry programs, 25% of the 700,000 veterans of the first Gulf War are affected by multiple symptoms and comorbid medical diagnoses that include: chronic fatigue syndrome, fibromyalgia, irritable bowel syndrome, arthralgia, digestive complaints, and mood-related psychiatric disorders, including depression, post-traumatic stress disorder (PTSD), and other anxiety disorders. Approximately 200,000 Veterans of the Gulf War are experiencing these symptoms even 20 years after the war.[1,2] First Gulf War veterans have been studied in comparison to

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Gulf Era veterans (those who served elsewhere in the world but at the same time). Gulf War veterans report higher prevalence of physical and mental health conditions and likewise have a significant burden of disease, including multiple comorbidities and higher body mass index.[1] People diagnosed with Gulf War Illness (GWI) have difficult symptom presentations, often with complex comorbid symptoms that consist of fatigue, sleep and mood disturbances, cognitive dysfunction, and musculoskeletal pain. These symptoms are grouped into three symptom clusters by the Centers for Disease Control and Prevention (CDC) as follows: fatigability (fatigue 24 h or more after exertion), mood and cognition (feeling depressed, irritable, anxious, difficulty in concentrating, problems getting to sleep), and musculoskeletal (joint or muscle pain). Veterans must experience symptoms from each of the three clusters to be diagnosed with GWI. Overall, veterans diagnosed with GWI are stable (not improving) at 5- and 10-year follow-ups[3] and no standard of care presently exists.[4] There is a need for innovative and effective care of GWI.

Acupuncture has been shown to be effective in treating some of the symptoms of GWI as evident in published research studies, specifically for pain,[5] anxiety, and depression.[6] The parent study to this project demonstrated clinically and statistically significant improvement in SF-36 physical and McGill Pain Index scores in the group randomized to receive individualized Traditional Chinese Medicine (TCM) acupuncture treatments twice per week for 6 months.[4] Preliminary research indicates that acupuncture may be effective in the management of complex conditions that share a similar cluster of symptoms of pain, sleep, and mood problems such as GWI[4] and fibromyalgia.[7] The complex diagnostic and treatment process of TCM, which is tailored to each individual's clinical presentation, may provide an effective framework for evaluating and addressing the complex symptoms presented in GWI. Currently, there are no theoretical TCM frameworks for GWI. Their team set out to evaluate the theoretical TCM framework used by TCM practitioners evaluating participants with GWI through a matrix analysis of TCM differential diagnoses. Their main purpose was to characterize ways in which TCM patterns combine in clinically significant ways in this chronic and complex condition.

Methods

Quantitative parent study

Detailed methods are described elsewhere,[4] but a brief summary of the parent study is provided in this study for context. The authors randomized 104 veteran participants after affirming that each did meet diagnostic criteria of GWI. The authors provided individualized acupuncture care in private practices by experienced Licensed Acupuncturists. Participants provided informed consent and were randomized either to biweekly acupuncture for 6 months or a 2-month wait-list, after which they received weekly acupuncture for 4 months. This research was approved by New England Institutional Review Board and U.S. Army Human Research Protection Office. All participants continued to receive standard care as needed.

The acupuncture intervention lasted ~1 h and consisted of consultation with a Licensed Acupuncturist, assessment of the participant, and the development of an individualized treatment plan. Acupuncture points were chosen according to TCM differential diagnosis; acupuncture needles were inserted and retained for 30–45 min. Acupuncture and additional therapies were allowed, including electroacupuncture, heat therapies (e.g., heat lamp), Chinese massage (*tui na*), cupping, and press balls, tacks, or magnets applied to acupoints after needles were removed. Herbal medicine and supplements were not allowed.

Qualitative substudy

Descriptive statistics were drawn from the parent study, and all participants were included in this study. Frequency counts were tabulated for each differential diagnosis. Matrix analysis was then applied to categorize differential diagnoses according to categorizations of excess, deficient, and channel disorders for baseline and 6-month visits. This allowed the detection of patterns of consistency and patterns of change in TCM differential diagnoses over time.

Matrix analysis

Matrix analysis is a qualitative data analysis that involves "the crossing of two or more main dimensions ... to see how they interact."[8] In this study, the authors have two dimensions: individual differential diagnoses and categories of excess, deficiency, and/or channel diagnoses. These categories are not mutually exclusive and commonly co-occur in clinical practice. Matrices can be descriptive (depicting conditions), outcome oriented (depicting results or consequences), or process oriented (depicting dynamics of change).[9] In this study, the authors develop both descriptive and process-oriented matrices. Each author examined 10 participants' data to develop the coding scheme. Once this was agreed upon by the team, two licensed experienced acupuncturists (L.T.-S. and J.C.) acted as coders. They first developed fidelity of the coding scheme by categorizing a sample of veteran participants' data ($n=20$) to find consensus. After this training, all participants' data were coded first according to all differential diagnoses and then according to excess, deficiency, and/or channel imbalances. Cohen's kappa was calculated, and the two coders compared any differences and discussed their interpretations of the coding scheme until agreement was obtained.

Results

Demographics

The study participants' demographics were as follows: the mean age was 48.2, 14% were female, and 81% were self-reported as white. Please refer to Table 1.

Reliability

Data were double coded by two Licensed Acupuncturists (J.C. and L.T.-S.) with master's degrees in TCM and a combined 23 years' clinical experience. Reliability was calculated with Cohen's kappa. The reliability coefficient obtained after coders were trained was 0.93.

Matrix analysis

Participants' differential diagnoses were noted at the baseline and 6-month visit. Participants' differential diagnosis consisted of one to six diagnoses (e.g., concurrent

TABLE 1. BASELINE CHARACTERISTICS OF THE STUDY POPULATION

Characteristics	
Age-year ± SD (N=103)	48.2±7.5
Female sex, N (%) (N=104)	14 (13.5)
Self-reported race, N (% of total) (N=104)	
White	84 (80.8)
Black or African American	10 (9.6)
Asian	1 (1.0)
American Indian/Alaskan Native	1 (1.0)
More than one race	2 (1.9)
Unknown	1 (1.0)
Other	5 (4.8)
Self-reported Hispanic, N (% of total) (N=104)	
Yes	6 (5.8)
No	94 (90.4)
No answer	4 (3.8)
Baseline pain, N (group mean) ± SD	92 (29.0)±9.7
Baseline SF-36 Physical N (group mean) ± SD	87 (67.7)±23.5

SDs are offered for age, baseline pain, and baseline SF-36 Physical.
SD, standard deviation.

spleen (SP) *qi* deficiency and LV *qi* constraint and stagnation in the channels). Frequencies of each differential diagnosis component at baseline are displayed below in Table 2. Next, each diagnosis was organized according to TCM theory into one of three categories. These categories are general or overarching theoretical constructs and encompass deficiency (e.g., insufficient *qi*), excess (e.g., overabundance of *qi*, which then may become stagnant), and imbalance of the acupuncture channels or meridians (e.g., *qi* is stagnant in a channel, causing local pain). The categories and the associated differential diagnoses are as follows: Deficiency (of *qi*, Blood, *yin*, or *yang*); Excess (of Damp, Heat, Cold, Phlegm, Fire, Yang rising, Wind, *qi* stagnation, or Blood stagnation); and Channel pathology (*qi* and Blood stagnation in the channels, Deficiency of Blood or *yin* in the channels, eight Extraordinary Meridian imbalance or Damp *Bi* Syndrome).

TABLE 2. DIFFERENTIAL DIAGNOSIS CATEGORIES AT BASELINE

Category	N
Deficiency	
Qi	67
Blood	24
Yin	33
Yang	7
Excess	
Damp	25
Heat	23
Cold	2
Phlegm	11
Fire	1
Yang rising	13
Wind	8
Qi stagnation	51
Blood stagnation	10
Channel	
Qi and blood stagnation	30
Deficiency	2
Eight extraordinary meridians	4
Damp *Bi* syndrome	13

The authors further analyzed 10 of 17 most frequent categories with Matrix Analysis, according to frequency. The 10 highest frequency (cutoff is 11 out of a maximum possible of 104) categories were as follows: *qi* deficiency, Blood deficiency, *yin* deficiency, *qi* stagnation excess, Dampness excess, Heat excess, Yang rising excess, Phlegm excess, *qi* and Blood Stagnation channel imbalance, and Damp *Bi* syndrome channel imbalance. Please refer to Appendix Table 1 for descriptions and Table 2 for frequencies. Total number of subjects exceeds N=104 due to the possibility of multiple TCM diagnoses per person: these categories are not mutually exclusive.

Each participant's differential diagnosis was then categorized as to whether there was a single type of pathology (e.g., only deficiency, or only excess, or only channel imbalance—no combination of the categories) or two co-occurring differential diagnoses categories (e.g., combination of excess and deficiency, or excess and channel issues, or deficiency and channel issues; or all three, with excess and deficiency and channel issues all co-occurring). These co-occurrences of diagnosis categories at baseline are noted in Table 3.

Matrix Analysis at Baseline

Single TCM differential diagnosis

Twelve individuals had a single category of differential diagnosis. Of the participants with a single category of diagnosis, *qi* deficiency was the most common (five instances), followed by *yin* deficiency, Yang rising, Damp *Bi* syndrome, *yang* deficiency, Blood deficiency, *qi* stagnation, and channel *qi* stagnation (one instance each).

Dual TCM differential diagnoses

Forty participants presented with a combined diagnosis of excess and deficiency differential diagnosis. The most frequent differential diagnosis combination was *qi* deficiency and *qi* stagnation (n=12 cases), followed by *qi* and *yin* deficiency with *qi* stagnation (n=3 cases), *qi* deficiency with Dampness and Heat (2 cases), Dampness and Cold (2 cases), Dampness and Heat (2 cases), *qi* and Blood deficiency with *qi* stagnation, Dampness and Heat (2 cases), *qi*, Blood, and *yin* deficiency with *qi* stagnation and Yang rising (2 cases).

TABLE 3. CO-OCCURRENCE OF DIFFERENTIAL DIAGNOSES AT BASELINE

Category	N
Only one category (e.g., excess or deficiency or channel)	12
Two categories: excess+deficiency	40
Three categories: excess+deficiency+channel	24
Two categories: deficiency+channel or excess+channel (not excess and deficiency)	16
Data missing	12
Total	104

Fourteen (14) other combinations were each seen in one case only. One case could not be summarized in the table.

Sixteen participants presented with mixed excess and channel imbalances or deficiency concurrent with channel imbalances. Of these, six were diagnosed with excess and channel imbalances and each person's were unique (e.g., phlegm/dampness obstructing the lungs and stagnation in the channels and LV *qi* stagnation). Ten participants were diagnosed with deficiency concurrent with channel imbalances, and each person's differential diagnosis combination was unique as well (e.g., SP *qi* deficiency, *Bi* syndrome).

Triple TCM differential diagnoses

Twenty-four participants presented with differential diagnoses at baseline categorized by co-occurring excess and deficiency and channel imbalance. These participants were the most complex in their differential diagnoses, with multiple co-occurring categories of disharmony.

Matrix Analysis at 6 Months

Differential diagnoses were evaluated at 6 months and classified according to excess, deficiency, and channel problems. Baseline and 6 months' differential diagnoses were compared (please refer to Table 4). A within-person analysis was also performed, 25 participants' diagnoses changed from one category to another (e.g., two co-occurring categories of excess and deficiency at baseline and one category only at 6 months), 51 participants' category was the same at baseline and at 6 months, and 28 participants could not be categorized due to missing data.

Discussion

The results of the matrix analyses indicate that the clinical presentation of people GWI can be characterized by a broad constellation of TCM differential diagnoses or Chinese Medicine patterns. GWI presents in participants of this study with a combination of excess and deficiency patterns both at baseline (38% of the sample) and at 6 months (35% of the sample). The next most frequent category was that of three co-occurring differential diagnoses (excess, deficiency, and channel imbalances all co-occurring), which was the next most frequent category both at baseline (23%) and 6 months (15%).

Regarding the single category of diagnosis

Qi deficiency was the most frequent type of deficiency, and this can progress to include concurrent *yin* or Blood deficiency. Chronic stress and/or environmental exposures may lead to Yang rising, Dampness, or *qi* stagnation. These are plausible differential diagnoses in fatigue, cognitive, and mood disorders and musculoskeletal pain.

Regarding two categories of diagnosis

Qi deficiency and *qi* stagnation co-occur frequently, and this is commonly seen in clinic. *Qi* deficiency can lead to *qi* stagnation and the formation of Dampness and generation of internal Heat and Phlegm. *Yang* can become unrooted without the *yin* and Blood to anchor it, and this leads to Yang rising (the authors see that it co-occurs with *qi* and Blood deficiency and *yin* deficiency).

Regarding three categories of diagnosis with co-occurring excess, deficiency, and channel imbalances, the authors observed TCM patterns described by TCM theory that are also common in practice. Clinically, the authors would interpret long-standing *qi* deficiency and *qi* stagnation to engender Dampness, Phlegm, internal Heat, and eventually *qi*/Blood stagnation in the channels and Damp *Bi* syndrome. In this study, participants exhibited a superficial excess condition, with underlying deficient symptoms, which leads to symptoms within the channels of pain (*qi* and blood stagnation and damp-*Bi* syndrome).

It is interesting to note that participants in the biweekly acupuncture treatment group demonstrated clinically and statistically significant improvement in SF-36 Physical and McGill Pain scores,[4] and yet the overall trend seen in the present study is stability in differential diagnoses. While this finding may be counterintuitive from a biomedical point of view, it may be understood from the view of TCM theory. TCM diagnostic strategy allows for the description of the state of health of multiple systems in the body simultaneously. This holism can be described with the metaphor of root (constitutional disharmony) and branch (acute or emergent disharmony). The concept of a constitutional disharmony is not a concept shared with biomedicine. Yet, it is certainly intuitive in a biomedical context to consider that some people have weak digestion from a young age or a susceptibility to colds and asthma. It is plausible in this sample that the differential diagnoses were reflecting root presentations, meaning very stable individual constitutions. This root can be compared to branch diagnoses, which are reflective of emergent or acute conditions. For this reason, it is plausible that while participants' symptoms improved, their differential diagnoses were rather stable, as these reflected root constitutions and not acute or emergent branch symptoms. This same finding, of clinical improvement tracking with

TABLE 4. BASELINE AND 6-MONTH DIFFERENTIAL DIAGNOSES

Category	*Baseline frequency,* N *(%)*	*6-Month frequency,* N *(%)*	*Descriptive changes*
Only one category (e.g., excess or deficiency or channel)	12 (12)	15 (14)	Slight increase
Excess+deficiency	40 (38)	36 (35)	Slight decrease
Excess+deficiency+channel	24 (23)	16 (15)	Decrease
Deficiency+channel	10 (10)	4 (4)	Decrease
Excess+channel	6 (6)	5 (5)	Slight decrease
Data missing	12 (11)	28 (27)	Increase
Total	104	104	

branch, but not root diagnosis, has been found in other studies of Asian Medicine treatments.[10]

Limitations of this study include the small sample size, particularly given that there are 28 cases of missing data at 6 months. It is important to note that not all participants with missing data dropped out of the study. Missing data made it impossible to categorize 28 participants' differential diagnoses at 6 months. In addition, while the parent study was adequately powered to detect clinically and statistically significant change in primary and secondary outcomes, the analyses in this study demonstrated very small *N* in several classification categories. Due to the sample size, the matrix analysis needs to be replicated with a larger GWI acupuncture study to further validate the differential diagnosis co-occurrences for GWI. Another potential limitation of this study is that the authors have not organized their sample according to more detail regarding symptoms, pulse, and tongue characteristics. This would lend additional levels of detail that might help us better discover who best responded to acupuncture in the trial and who might be more likely to transition from, say, three or two to one type of differential diagnosis (possibly indicating a lessening in complexity of clinical presentation with fewer differential diagnoses). Finally, an important limitation to this work is that stability of TCM differential diagnoses cannot be verified across TCM practitioners. Meaning that if a patient was seen by two TCM practitioners, they may receive differential diagnoses that do not align. The present work aims to characterize patterns of excess, deficiency, and channel imbalances and does not aim to confirm or refute validity of differential diagnoses across TCM practitioners.

Since GWI is a recently defined illness and treatment with acupuncture has only initially been evaluated (their parent study), the development of a TCM treatment manual is warranted. The treatment manual could utilize the differential diagnosis framework developed in this study (deficiency, excess, channel concerns) as a basis for symptom presentations that are most likely to occur in patients with GWI. A theoretical TCM pathologic foundation could also be explored as to the causes of *qi, yin, yang*, and blood deficiency or excess in GWI patients.

Future research with this matrix analysis technique could help explain common symptom clusters seen across different complex medical illnesses. For example, patients with GWI and fibromyalgia often present similar symptom clusters (chronic fatigue, pain, anxiety, and depression). The rich diagnostic framework of TCM theory—and all of traditional East Asian medicine theory and the myriad of approaches that encompasses—presents novel patient classification into subgroups, which may respond differentially to variations in treatment. The present analysis aims to support a beginning discussion with TCM and biomedical scientists toward a fully integrated science of Integrative Evidence Based Practice, one that theoretically supports the participating disciplines to harness the healing potential available to all patients.

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Authors' Contributions

Conceptualization of study: L.A.C. and L.T.-S. Data coding: J.C., R.S., K.-Y.H., B.A.S., L.A.C., L.T.-S. Data analysis: L.T.-S. and J.C. Article: L.T.-S., J.C., L.A.C., R.S., K.-Y.H., B.A.S.

Author Disclosure Statement

The authors have nothing to disclose.

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(*Appendix follows* →)

Appendix

Appendix Table 1. Definitions of Deficiency, Excess, and Channel Pathology

	Definition	*Symptom examples*
Deficiency		
Qi	When *qi* is deficient, or weak, it will not have the energy to provide some or all the basic functions of lifting, warming, transforming, transporting, holding, and defending.	Low energy, shortness of breath, bloating, catch colds easily
Blood	Blood deficiency occurs when food we eat is not transformed into the energy needed to create Blood. Blood deficiency is also created from too much blood loss.	Pale complexion, dizziness, dry skin/hair, palpitations, anxiety, poor memory, weak limbs
Yin	*Yin* deficiency occurs when the body is too weak and is unable to cool.	Insomnia, flushing, night sweats, irritable
Yang	*Yang* deficiency is a weakness where the body is unable to warm itself.	Fatigue, cold hands and feet, water retention, sweating easily
Excess		
Dampness	Improper regulation of fluids within the body, which can become stuck or thickened.	Diarrhea, epigastric fullness, swelling, difficult or painful urination
Heat	Also if *qi* becomes too blocked it can start to form heat.	Irritability, red face, scanty dark urine, emotional disturbances, yellow mucus, sweating
Cold	An internal pathogen that can affect the organs.	Low temperature, pale, profuse urination, loose bowels
Phlegm	If *qi* doesn't move, it can cause phlegm. If dampness accumulates it can turn to phlegm.	Cough, asthma, nausea
Fire	Caused by a pathogenic invasion of heat in the body.	High fever, red face, constipation, urinary difficulties with dark urine
Yang rising	The body is too deficient to keep this energy subdued and excess heat signs will surface.	Flash anger, headache on one side, tinnitus
Wind	A pathogenic factor can cause wind in the body. Also deficiency of blood can allow wind to flow in the channels.	Twitching, tics, spasms
Qi stagnation	*Qi* becomes stagnant when it is unable to flow freely.	Depression, mood swings, sighing, sensation of a lump in the throat, ache in muscles
Blood stagnation	Blood stagnation is when the blood is not moving freely and becomes stuck.	Sharp pain, traumatic injury, menstrual pain
Channel pathology		
Qi and blood stagnation	When the *Qi* and Blood become stagnated within a specific channel or area of a limb.	Pain that is both sharp and aches. Spasmodic pain
Deficiency	When a channel is deficient, *qi* cannot continuously flow.	Catch colds easily
Eight extraordinary vessels	The eight extraordinary vessels are deeper energetic reservoirs through which *qi* flows.	*Yang Qiao/Du Mai:* psychosis, insomnia, low back pain *Yang Wei/Dai Mai*: lethargy, pain at sides/ribs *Yin Qiao/Ren Mai:* unable to be joyful; Neurosis, gynecologic issues *Yin Wei/Chong Mai:* sad, anxious, overthink
Damp *Bi* syndrome	Obstruction of *qi* and Blood in the channels.	Pain in the joints and muscles, swelling

Title: Characteristics of Gulf War Illness participants in an acupuncture study

Running Title: Characteristics of GWI participants

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Abstract

BACKGROUND: Gulf War veterans reported significantly more often nonspecific multiple complex medical symptoms, including fatigue, sleep and mood disturbances, cognitive dysfunction, and musculoskeletal pain.

METHODS: We analyzed baseline characteristics reported by Gulf War Illness (GWI) study participants. The data was from a pragmatic randomized clinical trial to test the effects of individualized acupuncture treatments offered in extant acupuncture practices in the community. Veterans with diagnosed symptoms of GWI were included in the trial. This report focuses on sociodemographic characteristics, the SF-36 physical component scale score (SF-36P), and the McGill Pain scale at baseline.

RESULTS: Of the 104 subjects who underwent randomization.

DISCUSSION: Gulf War Veterans diagnosed with Gulf War Illness report many similar symptoms and diagnoses to GW veterans studied elsewhere. Specifically, increased mental health conditions such as depression, anxiety, and PTSD were identified in this sample, as well as physical conditions of Chronic Fatigue Syndrome

and unidentified symptoms. Safe and effective interventions for these symptoms and condition need to be identified and studied for this population.

CONCLUSIONS: Further research is needed to identify and test safe and effective interventions for symptoms and conditions experienced by Gulf War veterans.

Introduction:

Gulf War veterans have experienced poorer health than Gulf Era veterans (veterans who served during the same time-period as the Gulf War but not deployed to the Gulf War itself). Gulf War veterans' health was examined in 1995 (1) to study veterans' health 5 years after the war, and again in 2012-13 (2) to examine veterans' health 20 years after the war. Five years after the war, Gulf War veterans reported a multitude of health issues with higher prevalence than Gulf Era veterans, including unexplained symptoms and medical conditions, increased healthcare utilization and functional impairment. Gulf War veterans also reported poorer general health than non-deployed veterans. (1) Twenty years after the war, GW veterans reported a significantly higher prevalence of physical and mental health conditions compared to Gulf Era veterans. (2) These conditions included Gulf War Illness, Chronic Fatigue Syndrome, neuralgia, gastritis, chronic obstructive pulmonary disease, fibromyalgia, tachycardia, dermatitis, rheumatoid arthritis, seizures, coronary heart disease, migraine headaches, hypertension and asthma. Statistically significantly higher rates of mental health conditions in Gulf War Veterans compared to Gulf War era veterans included Post Traumatic Stress Disorder, major depressive disorder, anxiety disorder,

and high somatic symptom severity. Gulf War veterans that rated their health as excellent (3.6%) or very good (10.3%) was significantly lower ($p<0.001$) than the proportion of Gulf Era veterans who reported their health as excellent (6.1%) or very good (16.2%).

The CDC ("CDC - Veterans Health - Gulf War Studies - Air Force Study," 2010) defined GWI as having at least one chronic symptom from two of the following three areas: mood/cognition, fatigue, and musculoskeletal. GWI is also a chronic multisystem condition that is significantly associated with deployment to the Gulf War (Fukuda et al., 1998).

Although no specific disorder has been identified in GWI veterans, and the etiologic basis and clinical significance of their symptoms remain unclear (Fukuda et al., 1998), recent GWI research studies have elucidated the mechanisms of GWI. Our primary objectives were to characterize self-reported survey findings among GWI veterans who met our case definition and participated in this study to add additional information to the literature on the topic of Gulf War veteran health and disease.

Methods:

89 We analyzed baseline characteristics reported by GWI study participants. The
90 data was from a pragmatic randomized clinical trial (NCT01305811) to test the effects
91 of individualized acupuncture treatments offered in extant acupuncture practices in the
92 community (Conboy et al., 2016). Veterans with diagnosed symptoms of Gulf War
93 Illness were included in the trial. Full trial design details have been previously
94 published (Conboy et al., 2012). This report focuses on baseline sociodemographic
95 characteristics, secondary diagnoses, types of diseases, the SF-36 physical
96 component scale score, SF-36 Fatigue scale (McHorney et al., 1994), McGill Pain
97 scale (Melzack, 1987), Measure Yourself Medical Outcome Profile (MYMOP),
98 Optimism, Loss of control-Internal, Locus on control-Chance, Locus of
99 control-Powerful, People Social Support (SOC), Body Consciousness,
100 Catastrophization, Whitely Depression, Beck Anxiety Inventory, Depression Score
101 (CDEP sum), Factor of Depression (Guilt, Work, Retardation, Agitation and Anxiety
102 subscales).

103 Missing data were coded as missing completely at random (MCAR), under the
104 assumption that omissions happened completely at random. All statistical analyses
105 were performed with SAS software (SAS Institute Inc, 2010), version 9.4 (SAS

106 Institute, Cary, NC).

107 **Results**:

108 Of the 192 participants assessed for eligibility, 104 subjects who underwent

109 randomization. Fifty-two were randomized to biweekly treatment and 52 were

110 randomized to waitlist to weekly treatment. [Include description of mean age, % female

111 and male, self-reported race.] Baseline characteristics are summarized in Table 1.

112 [Please insert Table 1 here]

113

114 Table 1. Demographic and characteristics of all participants

Table 1. Baseline Characteristics of the Study Population. Baseline Characteristics of the Study Population. Standard Deviations (SD) are offered for age, baseline pain, and baseline SF-36(P).

Characteristic	Biweekly Treatment (N = 52)	Waitlist to Weekly Treatment (N = 52)
Age-year +/- SD	48.2 +/-9.9	48.2 +/- 3.5
Female sex-N(%)	7 (13%)	7 (13%)
Self reported Race-N(% of total)		
White	43 (83%)	41 (79%)
Black or African-American	5 (0.1%)	5 (0.1)
Asian	1 (0.02%)	0
American Indian/Alaskan Native	0	1 (0.02%)
More than one race	0	2 (0.04%)
Other	3 (0.06%)	3 (0.06%)
Self reported Hispanic N(% of total)		
Yes	2 (0.04%)	4 (0.08%)
No	48 (0.92%)	46 (0.88%)
No answer	2 (0.04%)	2 (0.04%)
Baseline Pain N(group mean) +/- SD	50(29.5) +/- 8.5	45 (29.8) +/-8.9
Baseline SF-36(P) N(group mean) +/- SD	51 (67.7) +/-24.6	49 (66.4)+/- 24.7

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115

116

Participants were asked about their concurrent secondary diagnoses. Over 1/3 of participants indicated that they were currently diagnosed with anxiety (36% of the sample), depression (35%) and Post Traumatic Stress Disorder (33%). Other concurrent diagnoses included sleep apnea (28%), Chronic Fatigue Syndrome (28%), Gastroesophageal reflux (19%), Irritable Bowel Syndrome (13%), Fibromyalgia (9%), Other diagnoses (7%) and Interstitial Cystitis (1%).

Table 2. Frequency of secondary diagnosis (Q6 of GWI survey)

Secondary Diagnosis	Total N	Yes – I have it currently N (%)	Yes – I had it in the past N (%)	No – I have never had it N (%)	I'm not sure N (%)	Missing N (%)
Fibromyalgia	103	9 (8.74%)	2 (1.94%)	63 (61.17%)	13 (12.62%)	16 (15.53%)
Interstitial Cystitis	103	1 (0.97%)	0 (0%)	73 (70.87%)	10 (9.71%)	19 (18.45%)
Chronic Fatigue	103	28 (27.18%)	8 (7.77%)	40 (38.83%)	14 (13.59%)	13 (12.62%)
Gastroesophageal reflux	103	20 (19.42%)	13 (12.62%)	43 (41.75%)	9 (8.74%)	18 (17.48%)
Sleep apnea	103	29 (28.16%)	3 (2.91%)	44 (42.72%)	10 (9.71%)	17 (16.50%)
Depression	103	36 (34.95%)	18 (17.48%)	36 (34.95%)	6 (5.83%)	7 (6.80%)

Anxiety	103	37 (35.92%)	15 (14.56%)	33 (32.04%)	10 (9.71%)	8 (7.77%)
IBS	103	13 (12.62%)	5 (4.85%)	49 (47.57%)	14 (13.59%)	22 (21.36%)
PTSD	103	34 (33.01%)	9 (8.74%)	31 (30.10%)	18 (17.48%)	11 (10.68%)
Other	103	7 (6.80%)	0 (0%)	23 (22.33%)	7 (6.80%)	66 (64.08%)

126

127 Major types of diseases experienced by participants were queried by listing

128 ICD-10 diagnoses. Diagnoses were then bundled and labeled by type of disease

129 (listed as either symptom 1 or symptom 2, combined here) and include Diseases of the

130 musculoskeletal system and connective tissue (75% of the sample reported

131 experiencing these diseases), Symptoms, signs and abnormal clinical and laboratory

132 findings, not elsewhere classified (51%) and Mental, Behavioral and

133 Neurodevelopmental disorders (31%).

134 Table 3. Major symptoms (Q29 of GQI survey)

135 SX 1 and 2 Combined by LJTS

2016 ICD-10-CM Codes	Sx 1 Percent (%)	Sx 2 %	Total %
Mental, Behavioral and Neurodevelopmental disorders	**16.5**	**14.56**	**31.06**
Diseases of the nervous system	**2.91**	**4.85**	**7.76**
Diseases of the eye and adnexa	**0.97**	**0**	**0.97**
Diseases of the ear and mastoid process	**0.97**	**0**	**0.97**
Diseases of the digestive system	**1.94**	**8.74**	**10.68**

Diseases of the musculoskeletal system and connective tissue	**43.69**	**32.04**	**75.73**
Symptoms, signs and abnormal clinical and laboratory findings, not elsewhere classified	**26.21**	**25.24**	**51.45**
Injury, poisoning and certain other consquences of external causes	**0.97**	**0**	**0.97**
Unknown	**5.83**	**14.56**	**20.39**
Total	**100**	**100**	**200**

136

137 Baseline scores for measures of physical function, fatigue, mood states,

138 interpersonal support, optimism, locus of control, social support, body consciousness,

139 catastrophizing, depression and anxiety are noted in Table 4.

140 [Please insert Table 4 here]

141 Table 4. Baseline measures

Measure	Average Score both groups Mean (SD); N
Physical Functioning (SF-36 Physical Component)	67.73 (23.48) N=87
Summary Fatigue Score	29.01 (7.47) N=84
Mood states (POMS sum)	112.73 (22.88) N=63
Interpersonal social support (ISEL sum)	35.46 (7.72) N=91
MYMOP Score	4.84 (0.88)

	N=86
Optimism	27.13 (7.60) N=91
Locus of control-Internal	24.51 (4.98) N=96
Locus of control -Chance	16.76 (5.13) N=95
Locus of control-Powerful	15.54 (4.93) N=94
People social support (SOC)	2.26 (0.53) N=94
Body Consciousness	16.46 (4.08) N=94
Catastrophizing	17.79 (7.68) N=92
Whitely Depression	9.90 (1.91) N=88
Beck Anxiety	36.90 (10.97) N=83
Depression Score (CDEP sum)	7.15 (1.10) N=94
Factor of Depression -Guilt	6.78 (0.75) N=93
Factor of Depression –Work Interests	6.15 (1.02) N=92
Factor of Depression -Retardation	6.77 (0.92) N=91
Factor of Depression - Agitation	6.71 (1.08) N=91
Factor of Depression –Psychological Anxiety	6.29 (1.19) N=90

Discussion:

Participants in this study were predominately male, white, not Hispanic and the average age was 48 years. Over a third of this sample reported current diagnoses of anxiety, depression, PTSD. Over a quarter of the sample reported concurrent diagnoses of sleep apnea and Chronic Fatigue Syndrome. Diseases of the musculoskeletal system and connective tissue were reported by 75% of the sample, symptoms, signs and abnormal clinical and laboratory findings, not elsewhere classified were experienced by 51% of the sample, and Mental, Behavioral and Neurodevelopmental disorders were reported by 31% of the sample. Ratings on standardized instruments were higher than population norms for scales.

This study adds an additional perspective of Gulf War veterans who are diagnosed with Gulf War Illness. Data were collected 2009-2012, roughly twenty years after the Gulf War. Data reported here parallel prior studies of Gulf War veterans, specifically that this sample all experienced Gulf War Illness, and both studies report Gulf War veterans with higher levels of mental health conditions including PTSD, anxiety and depression. Neuralgia, fibromyalgia and migraine headaches were

reported in another study (ref 2) and in this sample, over 75% of participants reported musculoskeletal pain. We cannot draw exact comparisons due to the heterogeneity of questions being asked, but it is clear that the present study parallels other findings regarding pain experienced by Gulf War veterans. Chronic Fatigue Syndrome is another parallel between the study by Dursa EK, Barth SK, Schneiderman AI, Bossarte RM. et al (2016) and the present study.

There are limitations to the present study. All participants were drawn from the New England area, and these data are not necessarily transferrable to other regions of the USA. Second, we sampled only Gulf War veterans diagnosed with Gulf War Illness. It is plausible that our sample was 'more sick' than the general Gul War veteran population. Last, we sampled people with GWI who were interested in participating in a trial of acupuncture for GWI. It is plausible we have a skewed sample, as well.

It is clear that veterans of the Gulf War are more likely to experience mental illness such as depression, anxiety and PTSD, as well as physical illnesses and symptoms, as compared to veterans of the same era who did not serve int eh Gulf

War. Further research is needed to identify safe and effective interventions for veterans of the Gulf War.

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Effectiveness in Clinical Trial Recruitment: An Analysis of Methods used in a Novel Randomized Clinical Trial for the Treatment of Gulf War Illness

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The authors would like to report that no conflicts of interest exist with regards to this manuscript.

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Introduction:

Available data indicates that following the first Persian Gulf War (Operating Desert Shield/Storm, occurring 1990-1991) a quarter of veterans returning were found to experience a symptom cluster, that later became known as Gulf War Illness [1,2]. There are an estimated 100,000 veterans that have GWI. This symptom cluster included chronic fatigue syndrome, fibromyalgia, irritable bowel syndrome and digestive complaints. Additionally, these veterans have reported mood-related psychiatric disorders including depression, anxiety, and post-traumatic stress disorder (PTSD) [3]. GWI illness has been poorly understood with no clearly delineated pathophysiologic process since it was first defined by the Centers for Disease Control (CDC) in 1995. Due to the nature of the disease, the presentation from one patient to the next is often highly variable making successful treatment with conventional pharmacologic strategies.

Methods:

A Department of Defense (DoD) funded randomized-controlled clinical trial was conducted evaluating the effectiveness of acupuncture in the treatment of Gulf War Illness. This was carried out through the utilization of private practice acupuncture offices dispersed throughout

southern New Hampshire and Massachusetts. Further details on the design of the trial [4], and positive results of the trial are published elsewhere.

Recruitment Strategies and Goals:

In the original design and planning of the study, recruitment would rely heavily on the Gulf War Illness Registry, a registry constructed by the Department of Veterans Affairs to track GWI cases. Unfortunately, access to this registry was revoked just prior to the onset of patient recruitment. This created a large roadblock in recruitment as the study team would no longer have direct access to the patient population in question. Several challenges arose from losing access to the GWI registry. First and foremost, the study was geographically restricted to New England. With no database to refer to, it was difficult to determine how many Gulf War veterans were in this region of the United States. It was also impossible to assess what percentage of Gulf War veterans located in New England were suffering from Gulf War Illness.

Recruitment methods were quickly redirected towards mass media efforts to reach as many Gulf War veterans as possible. Initially, mass mailings were conducted and were aimed at organizations that frequently had contact with veterans. The purpose of these mailings was to introduce these organizations to the study and to ask for assistance in veteran outreach.

The goal of recruitment was 120 subjects. At the nine-month mark, the study team had managed to consent and enroll only 16 study participants. Re-evaluation of recruitment tactics at that point led to the application for veteran information from the Defense Manpower Data Center (DMDC), a database containing all past and present military employees. DMDC provided very basic metrics that proved invaluable in improving veteran recruitment into the study. Because of this newly acquired information, we could apply for a grant extension to continue enrollment for six additional months.

Study recruitment lasted 22 months from August 2010 to June 2012. The total number of veterans enrolled was 104. Results of the clinical Trial were published in 2016 by authors Conboy, et al. (2016).

Methods:

Media – Due to the novel nature of the trial, the principle investigator (PI) of the study, Lisa Conboy ScD, was able to gain interviews with publications such as the Boston Herald, Boston Globe, Newton Tab, Springfield Republican and Stars and Stripes. These interviews occurred between 2010 and 2012, and garnered attention about the work being done and assisted with patient recruitment. The study PI was also interviewed on Rt. 9 Veterans Forum Cable Access television program in July of 2011. In addition, advertisements were placed in the Boston Metro, a free newspaper distributed at metro stations and various locations around the greater Boston area. Advertisements also appeared on Somerville, Massachusetts's local cable access station.

Radio advertisements were placed on WRKO/WEEI (local talk and sports radio programming) in 2011. Each advertisement was 30 seconds in duration and a total of 17 were run. The study PI interviewed on WRKO AM talk radio in Boston, MA and WADK in Newport, RI. Both interviews occurred in 2011.

Mass Transit – A full color advertisement measuring 11' x 27' was placed on Boston Metro trains running on the 'red' line train service. This line was strategically chosen based on the demographics of the areas the line serviced: Cambridge and downtown Boston. The line ran

from Mattapan, Ma to Braintree Ma. The advertisement ran from 12/21/2010-01/14/2011. The dates were chosen in a fashion that would allow veterans who may have been traveling during the holidays to still be exposed to the advertisement and inquire about the study.

Internet – Brief, text only, advertisements were listed on several websites that were strategically chosen for a high likelihood of exposure to Gulf War veterans. These web pages included clinicaltrials.gov, craigslist, clinicalconnections.com, and gulfweb.org. The study was also promoted on the New England School of Acupuncture website.

Veteran specific websites including 91outcomes.com and veteransnewsnow.com also featured study related information on their web pages.

Events:
The study team attended a total of five Yellow Ribbon Post Mobilization seminars to reach veterans returning from deployments. The goal was to reach out to veterans that may have served in the first Gulf War and to spread awareness about the clinical trial. Flyers were also handed out for display in National Guard barracks and the offices of several veteran centered agencies attending these events.

A Disabled American Veterans (DAV) conference was attended in 2011 to increase study awareness in the disabled veteran population.

Flyers, Letters and Post Cards:
The study team utilized 8.5 x 11' flyers printed in color for distribution. These posters featured pertinent information for potential study participants including symptoms of Gulf War Illness, requirements pertaining to deployments (dates, times, locations), and contacts for inquiring about more study information. The flyers were printed in two different formats. The first format was in the form of a letter containing all information for the study. The second format was printed with information and pull-away tabs at the bottom of the flyer that an interested person could take with them. These tabs had contact information for the study.

Multiple methods were employed to disseminate the flyers. A mass mailing sent to personnel at employment and career centers throughout the Commonwealth (Department of Employment and Training, Commonwealth of Massachusetts.) These centers were chosen to hopefully get information to veterans that may have been seeking employment. It is unknown if these flyers were posted or distributed in any way.

Flyers were also posted in areas felt by the study team to have a high likelihood of being seen by a veteran or the family member/friend of a veteran. These sites included Massachusetts Bay Transportation Authority (MBTA) stations, Veterans of Foreign Wars (VFW) posts, veteran centered agencies and private businesses. These flyers were placed by paid work study students. Flyers were also placed in the registration bags that were distributed at a Healthy Living Expo occurring in the greater Boston area.

Flyers were distributed to all acupuncturists affiliated with the study to display in their offices and to distribute to any individuals who inquired. As the study progressed, flyers were also given to study participants to distribute to their acquaintances and to post in their local communities.

Letters were sent to pain management clinics in the greater Boston area but no responses were ever received.

Letters and postcards were also sent directly to veterans using address information we had acquired. This was anticipated to be the most effective way to reach veterans and to recruit them for the study. Nine separate mass mailings were conducted with 2,481 total letters being distributed. Mailings were grouped based on towns/cities and their distance from Newton, Massachusetts (where the main study would be conducted) and their distance to participating acupuncture offices. Cities and towns closest to Newton were targeted first. Mailings were spaced out over 2-4 weeks to allow adequate time for potential participants to be appropriately screened and processed without overwhelming the study team.

After the initial letters were sent, the study team utilized the mailing of 4,000 postcards that were mailed in batches of 200-400 every 3-4 weeks. We continued to expand our network of affiliated acupuncturists and our subject recruitment mailing areas to reach more potential participants. Whenever a post-card or letter failed to reach an address and was returned to sender, this was noted in a Microsoft Excel spreadsheet. Participants that went through a screening process were also noted in the Excel spreadsheet. This database was utilized to ensure no one would be receiving multiple mailings.

Of the 3,244 contacts utilized from the DMDC, 984 (29%) were addresses that yielded undeliverable mail. Five hundred names from the DMDC were called to increase exposure to the study; of these only 3 study subjects were acquired.

Data Collection:

Follow up to inquiries began with an initial screening call, conducted by trained study staff members. These study members followed a script of open ended questions to learn how the potential subject learned about the study. A database was constructed in Filmmaker pro to track information provided by callers. This database tracked how the potential subject learned about the study, results of their initial screening for the study, the results of their medical screening for the study and if they were ultimately enrolled or not. The data regarding how participants learned about the study is listed in table 1.0. The categories were developed from respondents' initial response to the standardized question of "how did you learn about this study." No category was created for participants who learned about the study from multiple, overlapping sources.

Advertising Costs:

Advertising costs for the Boston Metro totaled $2,224 and included seven advertisements that each ran for six weeks. This form of advertising generated 4 total study participants (cost per participant being $561.00.) Advertisements run on clinicalconnection.com totaled $207 and lead to three study participants (cost per participant $207.) Sports radio advertisements on WRKO Boston/WEEI Worcester cost a total of $833 dollars and generated three study participants (278$ per participant.) Many participants had difficulty remembering how they were initially exposed to the study, making tracking which method was most effective more difficult.

Results:

The recruitment effort with the highest yield for generating study participants was recruitment aimed directly at veterans from the DMDC. We were privileged to contact information for 3,390

veterans residing in the New England area. Of that number, 3,244 veterans fit our criteria in terms of location and being able to participate in the study. These 3,244 veterans received postcards and flyers. The second most successful source of study participants came from flyers/posters distributed at veteran's associations and agencies.

We also asked study participants to tell other veterans in their social network. A total of six study participants were recruited from these word of mouth referrals.

Through all of our recruitment efforts employed we generated sixty e-mail inquiries about the study and 225 phone calls. One hundred and sixty-three of these inquires made it through to an initial study screening interview (the others were disqualified upon initial contact screening). This initial screening process was conducted over the phone and consisted of a checklist that was based on the original GWI diagnostic criteria set forth by the Centers for Disease Control. These criteria included: 1. Deployed to the "Gulf Theater of Operations" as defined by 38 CFR 3.317 which included Iraq, Kuwait, Saudi Arabia, Oman, the Persian Gulf, the Arabian Sea and the Red Sea as well as the airspace above these regions in the years 1990-1992. 2. Subject has at least two of the symptoms from the three CDC clusters of symptoms. 3) Symptoms have lasted longer than six months in duration. Each symptom cluster must be characterized as "mild-moderate" or "severe" with a least one symptom in each cluster having the designation as "severe."

Information from the phone screening was logged into the Filemaker Database. This database automatically determined the eligibility of each veteran to enter the study based on the inputted information. Eligible veterans were asked if they would like to participate in the study and those who wished to move forward were scheduled for an in person medical screening performed by the study physician. Medical screenings were conducted on one to two Saturdays each month depending on the physician's availability.

Of the 225 initial calls that were placed, 29 veterans declined to participate in the study. The reasons for these included being too far from the study location, lack of reimbursement for travel, and inability to commit the time. Of the 163 veterans who went through the pre-screening, 22 were found to be ineligible for the study. Five eligible veterans chose not to move forward with the medical screening. The remaining 136 participants moved on to the medical screening stage of the recruitment process.

Fifteen medical screening appointments that were arranged resulted in a "no-show" by the potential study participant. In these cases, the veteran was contacted by the study team but contact could not be established. Forty-nine veterans partially completed the screening but were lost to follow up. Ultimately, we successfully recruited a sample of 104 subjects (90 men and 14 women.) The average study participant age was 48 years old. Seventeen people removed consent from the study for personal reasons.

Discussion:

The most successful recruitment strategy for the study was directed posters/flyers/phone calls. These efforts proved to be far more effective than undirected advertising in public areas. It is also likely (although difficult to quantify) that exposure to study advertising through multiple channels increased the likelihood of veteran inquires.

Several unexpected hurdles were encountered throughout the study. The internet proved to be a very effective medium for garnering inquiries about the study. However, due to the global

nature of web searches, the study team received inquiries from all over the country. In most of these instances, it was not possible for these veterans to participate in the study.

Other road blocks stemmed from staffing issues related to the study. Since there was only one study physician it was necessary to work around his schedule to conduct medical screens. In some instances, veterans, would need to wait 4-6 weeks to get a medical screen conducted. This wait time decreased the likelihood of the veteran showing up to the appointment.

The study also had difficulties with veterans not showing up to appointments for medical screenings. The study team would call the week prior to the appointment but this did not always ensure the veteran's arrival. This resulted in more phone calls to reschedule and an increased need for manpower to logistically handle the rescheduling.

As well, the price of gas rose above $4.00/per gallon twice during the study. This was an issue raised by the participants and impacted study recruitment and compliance. Future projects may benefit from budgeting sufficient transportation costs as this proved to be a large hurdle for veteran participation in the study.

As our understanding of GWI and the veterans effected increases, our ability to find research participants in this difficult to target population improves. Our study team found success using multiple recruitment strategies to reach out to this dedicated participant population.

Tables/Figures:

Source of Inquiry	No. of Responses
Boston Herald	5
Boston Metro	4
Clinical Connection.com	1
Friend	10
Internet Search	1
Manpower Database	103
MBTA	2
National Gulf War Resource Center	1
NESA	1
Other	19
Other Veteran Agency	12

Radio (WRKO)	3
Springfield Republican	5
Stand Down Event	1
Stars & Stripes	1
Unknown	3

a. General Inquiries

Source of Inquiry	**No. of Responses**
Boston Herald	4
Boston Metro	1
Friend	6
Internet Search	1
Manpower Database	65
MBTA	2
NESA	1
Other Veteran Agency	12
Radio (WRKO)	3
Springfield Republican	4
Stand Down Event	1
Stars & Stripes	1
Unknown	3

b. Sources by Study Subject

Number of Subjects	Race	Percentage	
83	Caucasian	79.8	
10	African American	9.6	
4	Hispanic/Latino	3.8	
1	Asian	1.0	
1	Native American	1.0	
4	Other	3.8	

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The Importance and Determination of Dosage in Acupuncture Treatment (Final draft?)

Acupuncture is a widely used and increasingly studied treatment modality, yet substantial disagreement remains about its effectiveness and how it should best be utilized. Great progress has been made in elucidating the physical processes involved in Acupuncture treatment, but there is still much that is only poorly understood. (Zhuang, Xing, Li, Zeng, & Liang, 2013) There are also a very wide range of treatment modalities and protocols that are used in Acupuncture treatments, (O'Connor & Bensky, 1981)(Maciocia, 2008) adding to the complexity of evaluating treatment effectiveness. For example, there are different styles of treatment (Chinese, Japanese, Korean etc.) as well as different treatment methodologies (5 elements, 8 principals, etc.) within each style. (O'Connor & Bensky, 1981)(Maciocia, 2008) These approaches vary greatly in terms of specific Acupuncture points used, and amount and type of stimulation. These differences need to be taken into account when comparing treatment effectiveness.

One of the important aspects of any medical procedure is dosage. Dosage is typically defined as quantity of treatment over time; i.e. how much, how often, and for how long is a treatment to be administered. Defining dosage is straightforward in the case of standardized medications based on patient's body weight or very clearly defined procedures, however the definition becomes more complex in the case of therapies such as Acupuncture which by nature may be non-standardized. Traditional Chinese Medicine (TCM), on which acupuncture is based, uses diagnostic and treatment procedures that are complex and tailored to each patient's specific symptoms. The dosage for each individual patient may vary based on initial presentations and patients' body reactions to the received acupuncture treatments which makes determining a suggested optimal dose difficult. Optimal dose in pharmacotherapy is defined as a dose that maximizes improvements in psychological and/or physical outcomes ("efficacy") and minimizes adverse side effects. (Voils, Corrine I., et al.,2014) However, TCM treatment system as a complex adaptive system treats whole organism and by nature maximize clinical outcomes and minimize side effects in any individual patient. In other words, the dose might be determined in the process of treatment not prior.

Attempts have been made to define Acupuncture dosage, but to date there has been no universally adopted definition. In their discussion of this issue White et.al. defined a dose of Acupuncture treatment as "The physical procedures applied in each session, using one or more needles, taking account of the patients resulting perception (sensory, affective, and cognitive) and other responses (including motor). The dose may be affected by the state of the patient (e.g. nervous, immune and endocrine systems); different doses may be required for different conditions." (White et al., 2008) While this definition is comprehensive, it is too general and descriptive to result in anything more than defining a dose of Acupuncture as a single Acupuncture treatment.

In their paper on Acupuncture dosage and dysmenorrhea Armor and Smith (Armour & Smith, 2016) make a distinction between "neurophysiological dose" and "cumulative dose". Neurophysiological dose variables include number of needles, Acupuncture points used, retention time, and mode of stimulation (including needle manipulation and Electro-

acupuncture, as well as adjunctive techniques such as cupping, and press tacks.) Cumulative dose refers to the total number and frequency of treatment sessions. The authors also mention the potential importance of treatment timing; i.e. when in the time course of an illness should Acupuncture best be used. Armour and Smith's second set of definitions are more specific and therefore more clinically useful, and it is this definition that we will use to frame the definition for Acupuncture dosage in this paper.

In an effort to simplify meaningful data collection, many Acupuncture studies have deliberately chosen to treat conditions and/or use treatment paradigms which administer the same neurophysiological dose for all patients. Unfortunately, this type of study design may often interfere with the clinical effectiveness of Acupuncture treatment and may be of limited value since it does not reflect how Acupuncture is usually practiced. (Liu et al., 2015, MacPherson, et. al.2007)

Acupuncture as it is actually practiced clinically is an individualized treatment process, with neurophysiological dosage determined by the patient's condition on the day of treatment. (O'Connor & Bensky, 1981)(Maciocia, 2008)(Langevin & Schnyer, 2017). Since the neurophysiological dose will vary between patients, and from treatment to treatment, even patients who have been treated for the same condition using the same cumulative dosage of treatments, are unlikely to have received the same neurophysiological dosage of Acupuncture. This situation can lead to confusion as to how total Acupuncture dosage over time should be compared across patients.

However, if a consistent diagnostic and treatment process is followed in determining optimal neurophysiological dosages for each patient, meaningful comparisons about cumulative dosage and treatment effectiveness can be made. This approach has been followed in a number of recent studies. (Schnyer, Iuliano, Kay, Shields, & Wayne, 2008) (Conboy, St John, & Schnyer, 2012) (Conboy et al., 2016) In all of these studies the participating Acupuncture practitioners were trained and experienced in using the same treatment protocols, and they agreed to use the same specifically outlined diagnostic procedures in deciding how to treat their patients. Within this constraint they were free to choose whatever specific neurophysiological dose that they felt was appropriate for a patient on a given day. This process reflects the way that Acupuncture is actually practiced in most clinics. Studies designed in this way are both naturalistic and rigorous, conforming to the STRICTA recommendations for Acupuncture controlled trials. (MacPherson et al., 2002)

Once the issue of neurophysiological dosage is addressed, it becomes meaningful to compare cumulative dosages. Currently there is surprisingly little data about the importance of cumulative dosage in determining Acupuncture treatment effectiveness. A literature review was done by this author searching Medline, Google scholar, and Cochrane databases using the keywords 'Acupuncture therapy' (MeSH term) AND 'frequency,' 'dosage,' and 'schedule'. Give dates of search. No comprehensive studies or reviews of the significance of cumulative dosage across all types of Acupuncture treatment were found.

However, there are numerous studies containing some cumulative dosage data, and some of these studies include data comparing the effectiveness of different cumulative dosages for specific conditions. A 2005 study investigating Acupuncture treatment for fibromyalgia indicated that cumulative dosage was more important than correct Acupuncture point needle placement in determining treatment success, and that 3 treatments weekly provided greater pain relief than 1 treatment weekly.(Harris et al., 2005). A second large study investigating Acupuncture treatment of chronic pain indicated that both number of needles used, and total cumulative dosage were significant; with more needles and more treatments associated with greater pain relief.(MacPherson et al., 2013) A 2016 study investigating the treatment of Gulf War Illness with Acupuncture found that treatment twice weekly was more effective than treatment once weekly. (Conboy et al., 2016)

Armor and Smith did a literature review to specifically investigate the relationship between Acupuncture treatment dosages and pain relief outcomes in treating dysmenorrhea. Summarizing the results of 11 trials, they concluded that treatment timing, needle stimulation, number of needles, and frequency of treatment were all significant factors in influencing pain reduction during menses, one needle appeared to be more effective than many for pain reduction during menses, but conversely more needles used before menses was most effective. Regarding cumulative dosage, comparisons between studies with varying cumulative dosage amounts and treatment frequencies did not indicate any clear dosage responses, however unfortunately none of the studies undertook direct comparisons of treatment frequency or total number of treatments. The authors concluded that future studies should include these comparisons in the study design. (Armour & Smith, 2016)

Baxter suggested laser acupuncture may represent a portion of successful management of chronic LBP, when employed at appropriate dosages. He didn't find any significant differences between either of the groups studied (laser acupuncture vs sham laser acupuncture) with lower than recommended dosage of 8 J -40 times higher than their dosage (0.2) J- for (regular or non-acupuncture) laser treatment of low back pain (LBP). However, his systematic review of laser acupuncture trials for the treatment of low back pain (n = 9 studies) with higher dosages varied from 0.57 J to 5 J per point indicated significant results. (Baxter & Bleakley & McDonough, 2008)

A 2012 Chinese review article on Acupuncture cumulative dosage summarized a number of small un-blinded trials examining varying cumulative dosages for different conditions. These preliminary studies indicated that the optimal cumulative dosages are different for different conditions; varying from 2 treatments per day for stroke patients, to 1 treatment every 3 days for neurologic pain, to 1 treatment weekly for some chronic conditions.("Research on the Effect that Frequency Do to the Curative Effect of Acupuncture--《Journal of Liaoning University of Traditional Chinese Medicine》2012年10期," n.d.)

Traditional Acupuncture conventions regarding ideal cumulative dosage amounts differ considerably. In China, patients are typically treated 3 or more times weekly, for at least a month, often more. (O'Connor & Bensky, 1981) In the West the norm is more likely to be 1 or perhaps 2 treatments weekly, typically for 6 to 10 weeks, although this will vary considerably depending upon the practitioner and the economic circumstances of the patient. In China, patients often get both a larger and a more concentrated cumulative dose than in the West. Frequently, in both Western and Chinese Acupuncture texts, no justifications other than historical convention or specific clinical case studies are given for choosing specific cumulative dosage amounts.(O'Connor & Bensky, 1981)("Research on the Effect that Frequency Do to the Curative Effect of Acupuncture--《Journal of Liaoning University of Traditional Chinese Medicine》2012年10期," n.d.)

The studies cited above indicate that cumulative dosage appears to be a significant factor in determining treatment success, and that this relationship is not necessarily a straightforward or linear one. However, many of these studies did not include the parameters discussed above for addressing the issue of individualized neurophysiological dosages. In addition, the primary goal of many Acupuncture studies is to compare Acupuncture treatment effectiveness to sham Acupuncture treatment or no treatment. Therefore, cumulative dosage data is usually not as comprehensive as it might be if the studies were designed with this goal in mind. In addition, many studies of Acupuncture are small and preliminary in nature, and many of them utilize low cumulative dosages across all subjects.(Liu et al., 2015)

In order for Acupuncture to be both as effective and as economical as possible, it is important to determine the ideal cumulative dosage and dosage schedules that should be used for a given condition or range of conditions. Future Acupuncture studies should include more comprehensive cumulative dosage comparisons in their study designs.

This analysis considers the influence of dosage using data from our 2016 study investigating the treatment of Gulf War Illness with Acupuncture. We designed the study to prospectively compare the effectiveness of twice weekly vs once weekly treatment. We found that the group assigned to twice weekly treatment received more relief in pain and physical function.(Conboy et al., 2016)

This current secondary data analysis considers the influence of actual dose, adjunctive treatments, ?? Alliance, belief, ??

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Introduction:

The Therapeutic Alliance (TA) is the relationship created between patient and practitioner. Also known as therapeutic relationship, therapeutic bond, treatment alliance, helping alliance, or working alliance, TA encompasses three major elements: (i) tasks, the collaborative endorsement of the intra therapy activities (includes an understanding of what is required of each of the parties in the performance of these tasks, and an appreciation of the relevancy of the tasks to the therapy process); (ii) goals, the mutual agreement and valuing of the outcomes of the therapy; and (iii) bonds, that encompass the complex elements of attachment between the patient and clinician such as trust, empathy, personal liking and valuing [1].

TA is vital to treatment effectiveness in general psychology [2-4] and is one of the most studied subjects within concurrent clinical psychology [5-7]. Interaction and communication between doctor and patient is essential in creating TA. It is rooted in brain coupling of patient and therapist. This is the Interpersonal Synchrony (In-Synch) model in Psychotherapy [2]. Activation of mirror neuron system (MNS) areas, including insula and STG (superior temporal gyrus) has been detected in synchrony [8]. The patient and the therapist synchronize through sharing, and the development of trust. This synchrony is purported to create the necessary climate and conditions in which other intervention contents can be successfully delivered by the therapist and absorbed by the patient [4, 9-12].

Similarly, in Traditional Chinese Medicine (TCM), the taking of the pulse and clinical observation establishes a contact and relationship that is a main active mechanism of change. TCM is a form of Eastern Medicine that is over 2000 years old. TCM practitioners utilize various techniques like acupuncture, tui na, nutrition, moxabustion, and tai qi to treat the mind and body of patients; of this toolkit the technique of acupuncture is most commonly used in America. In TCM, TA is a form of artistry, called the "Penetrating Divine Illumination" and is "the refinement of the physician's art." It is a transformative healing state between the Qi of physician and patient, which resonates at a spiritual level. Empathetic dialogue and assessment create the Penetrating Divine Illumination, which becomes part of the treatment or intervention [13].

The practitioner gives signs to the patient through his blood and qi (xueqi) indicating quality of his vitality without having any control or awareness over them. This quality cannot be faked and

ideally nothing should interfere the contact made between spirits, beyond clear consciousness and is constructed day after day [14].

Meta- analyses of allopathic clinical data support that the practitioner patient relationship has a significant effect on multiple healthcare outcomes including quality of life, psychological problems (e.g. anxiety, depression), re-consultation rate, smoking quit rate, pain relief, blood pressure, weight loss, etc [15-28]. TA consistently predicts outcomes in psychotherapy [1, 29-32] but has been studied to a lesser extent in other health fields such as Traditional Chinese Medicine (TCM) [33].

Acupuncture, similar to other forms of health care areas, occurs in a specific setting that can affect clinical outcomes; the patient-acupuncturist relationship is one variable of this setting [34]. Studies conducted in this area indicate TA status predicts therapeutic outcomes following TCM treatment [33]. TA in acupuncture develops through the interaction between practitioner and patient in the initial health history intake and individualized treatment [35]. This interaction involves the Acupuncturist asking a series of ten questions, which make up the diagnostic for TCM. These ten questions pertain to the patient's overall health and lifestyle. The answers to these questions, in addition to taking of the pulse and looking at the patient's tongue comprise most TCM intakes. This relationship building then continues via an individualized treatment that is based on the TCM diagnosis. This process is then repeated each time the patient visits the acupuncturist. Further work has demonstrated that positive clinical changes following acupuncture treatment are not placebo effects or due to patient expectations but instead to patient-practitioner relationship quality [36].

The degree of patient-practitioner agreement on the quality of the patient-provider relationship, termed *concordance*, may be related to better clinical outcome [32, 37]. In 2013, our study team completed a Phase II Randomized Controlled Trial (n=104) testing the effects of individualized acupuncture treatments offered to veterans in extant acupuncture practices [38]. The current study investigated the impact of concordance on therapeutic outcome of patients who received TCM treatment for Gulf War Illness. Gulf War Illness is a complex illness found among veterans of the first Gulf War, and characterized by multiple symptoms, including fatigue, sleep and mood disturbances, cognitive dysfunction and musculoskeletal pain. For this study, GWI patients were able to see an acupuncturist in their community rather than at a Veteran Administration Hospital.

Methods:

Detailed methods are described elsewhere [39], but a brief summary of the parent study is provided here for context in this study. The authors randomized 104 veteran participants after affirming that each did meet diagnostic criteria of GWI. The authors provided individualized acupuncture care in private practices by experienced Licensed Acupuncturists. Participants provided informed consent and were randomized either to biweekly acupuncture for 6 months or a 2-month wait-list, after which they received weekly acupuncture for 4 months. This research was approved by New England Institutional Review Board and U.S. Army Human Research Protection Office. All participants continued to receive standard care as needed. The acupuncture intervention lasted ~1 hour and consisted of consultation with a Licensed Acupuncturist, assessment of the participant, and the development of an individualized treatment plan. The Licensed acupuncturists who provided the acupuncture treatments had at least 5 years of clinical experience and received additional in-house training concerning GWI. Acupuncture points were chosen according to TCM differential diagnosis; acupuncture needles were inserted and retained for 30–45 min. Acupuncture and additional therapies were allowed, including electroacupuncture, heat therapies (e.g., heat lamp), Chinese massage (tui na), cupping, and press balls, tacks, or magnets applied to acupoints after needles were removed. Herbal medicine and supplements were not allowed.

Horvath's Working Alliance Inventory (WAI) was used to measure TA [40-43] or the therapeutic relationship between the client and therapist. Concordance and also predicts clinical outcomes [32, 44], so for the purpose of this study, we followed Miller's study, and tailored the WAI questions to be relevant to *Acupuncture Practitioner or Acupuncturist* [33]. Some studies evaluated TA from either the practitioner's or the patient's standpoint. However, we measured acupuncture-patient relationship from the standpoints of acupuncturists and patients concurrently [45], since, TA is clearly a two-sided interactive process [46].The WAI contains 36 items, with three subscales (Task, Goal, and Bond). Each item is scored on a 7-point scale ranging from 1 (never) to 7 (always) [29]. The WAI has strong published support for reliability and validity [47]. The WAI was administered to both subjects and clinicians at 2, 4, and 6 months of study exposure.

Sample size:

The sample size per treatment group varied due to missing data. For group 1, where acupuncture treatment was administered without delay and continued twice per week for a 6 month period, N=36 for Affective Social Support *(ISEL_Sum)*; N=37 for Total Beck Depression score (*CDEP_Depression_Sum)*; Total Beck Anxiety score (*CDEP_Psychological_Anxiety_Sum*). For treatment group 2, where treatment was delayed by 2 months and then continued weekly for the remaining 4 months, N=32 for ISEL_Sum, CDEP_Depression_Sum and CDEP_Psychological_Anxiety_Sum.

Statistical Analysis

Average WAI scores were calculated for each participant and practitioner and results for each factor were graphed by patient-practitioner dyad over time. As the study progressed, Working Alliance Inventory-Short Revised (WAI-SR) scores were compared using Paired sample Student's t-tests. We next calculated change in concordance for each dyad from baseline to 6 months endpoint. Linear regression models are used to measure the influence of degree of change in concordance by the change in outcomes of pain and physical function.

To assess whether a more congruent patient and practitioner relationship was indicative of superior treatment outcomes, **WAI-SR** difference scores between patients and practitioners were utilized and compared to clinical outcomes for ISEL_Sum, CDEP_Depression_Sum and CDEP_Psychological_Anxiety_Sum. WAI-SR difference scores were reviewed for similarity of agreement in tasks, goal and the formation of an affective bond during treatment.

Outcomes

Demographics

The study participants' demographics were as follows: the mean age was 48.2, 14% were female, and 81% were self-reported as white. Please refer to Table 1.

TABLE 1. BASELINE CHARACTERISTICS OF THE STUDY POPULATION

Characteristics	
Age-year ± SD (N = 103)	48.2 ± 7.5
Female sex, N (%) (N= 104)	14 (13.5)
Self-reported race, N (% of total) (N = 104)	
White	84 (80.8)
Black or African American	10 (9.6)
Asian	1 (1.0)
American Indian/Alaskan native	1 (1.0)
More than one race	2 (1.9)
Unknown	1 (1.0)
Other	5 (4.8)
Self-reported Hispanic, N (% of total) (N = 104)	
Yes	6 (5.8)
No	94 (90.4)
No answer	4 (3.8)

SD, standard deviation

As the study progressed over time, dyads reported increasingly more positive scores on the all WAI-SR factors (Task, Bond and Goal) (figure 1), and dyads moved toward higher levels of concordance (Figure 2). Although, both the weekly dose and biweekly dose groups approximately had same WAI-SR scores at the endpoint (6 months), WAI-SR initial scores on all factors (Task, Bond and Goal) were lower in the weekly dose group compared to the biweekly group and the scores of all WAI-SR factors (Task, Bond and Goal) in the weekly treatment group increased more sharply than the biweekly group (figure 1). Once again, the weekly treatment group experienced 2 months of waitlist before starting treatment.

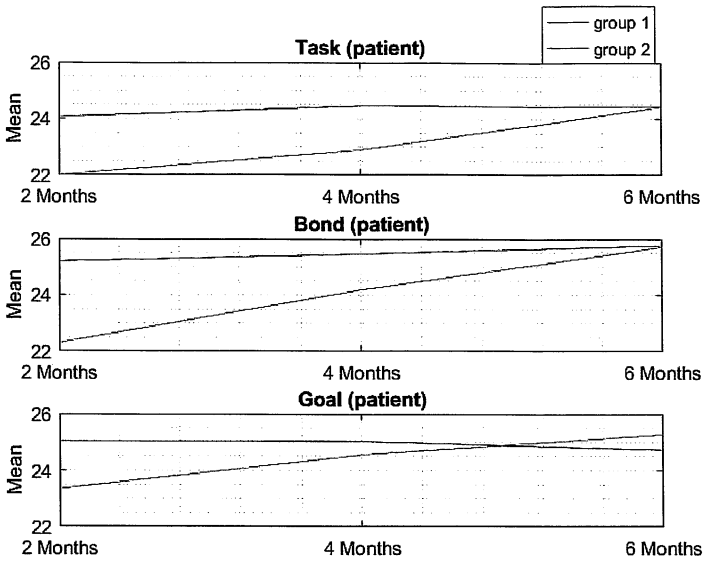

Figure 1. Patients' WAI-SR scores versus times for both group 1 (weekly waitlisted) & 2 (biweekly).

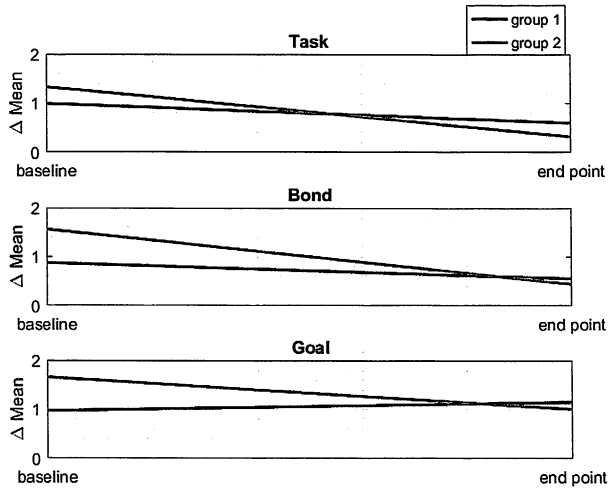

Figure 2. Δ Patient Practitioner WAI-SR scores versus times for both group 1 (weekly waitlisted) & 2 (biweekly).

There was a significant improvement in WAI-SR scores for baseline (2 months) and endpoint (6 months) in all factors (task, bond and goal) in the weekly group using paired-samples t-test (Table 2-4).

Table 2. WAI -SR scores in Task factor

Task

Pair	Time Point	Treatment	Mean	SD	N	p-Value	Diff. Means
Pair 1	2 months	BiWeekly	24.07	3.08	45	0.5858	0.4
	4 months	BiWeekly	24.46	3.54	39		
	2 months	Weekly	22	6.20	37	0.5186	0.9
	4 months	Weekly	22.9	5.75	38		
Pair 2	2 months	BiWeekly	24.07	3.08	45	0.5945	0.36
	6 months	BiWeekly	24.43	3.24	42		
	2 months	Weekly	22	6.20	37	0.0504	2.42
	6 months	Weekly	24.42	4.18	38		
Pair 3	4 months	BiWeekly	24.46	3.54	39	0.9652	0.03
	6 months	BiWeekly	24.43	3.24	42		
	4 months	Weekly	22.9	5.75	38	0.1894	1.52
	6 months	Weekly	24.42	4.18	38		

Table 3. WAI -SR scores in Bond factor

Bond

Pair	Time Point	Treatment	Mean	SD	N	p-Value	Diff. Means
Pair 1	2 months	BiWeekly	25.2	3.84	46	0.733	0.26
	4 months	BiWeekly	25.46	3.22	39		
	2 months	Weekly	22.3	6.42	36	0.1585	1.89
	4 months	Weekly	24.19	4.8	37		
Pair 2	2 months	BiWeekly	25.2	3.84	46	0.4821	0.56
	6 months	BiWeekly	25.76	3.67	42		
	2 months	Weekly	22.3	6.42	36	0.0048	3.41
	6 months	Weekly	25.71	3.2	38		
Pair 3	4 months	BiWeekly	25.46	3.22	39	0.6972	0.3
	6 months	BiWeekly	25.76	3.67	42		
	4 months	Weekly	24.19	4.8	37	0.1082	1.52
	6 months	Weekly	25.71	3.2	38		

Table 4. WAI -SR scores in Goal factor

Goal

Pair	Time Point	Treatment	Mean	SD	N	p-Value	Diff. Means
Pair 1	2 months	BiWeekly	25.04	3	45	0.98	0.01
	4 months	BiWeekly	25.03	3.57	38		
	2 months	Weekly	23.34	3.69	35	0.1657	1.21
	4 months	Weekly	24.55	3.69	38		
Pair 2	2 months	BiWeekly	25.04	3	45	0.6706	0.31
	6 months	BiWeekly	24.73	3.92	42		
	2 months	Weekly	23.34	3.69	35	0.0228	1.93
	6 months	Weekly	25.27	3.34	37		
Pair 3	4 months	BiWeekly	25.03	3.57	38	0.7221	0.3
	6 months	BiWeekly	24.73	3.92	42		
	4 months	Weekly	24.55	3.69	38	0.3803	0.72
	6 months	Weekly	25.27	3.34	37		

Regression analysis suggest that concordance across subscales is associated with improved clinical outcomes. In particular, the factor of GOAL (shared treatment goals) was significantly related to improvements in physical functioning as measured by the SF-36 physical function subscale to $p<=0.05$) in both groups (figure 3).

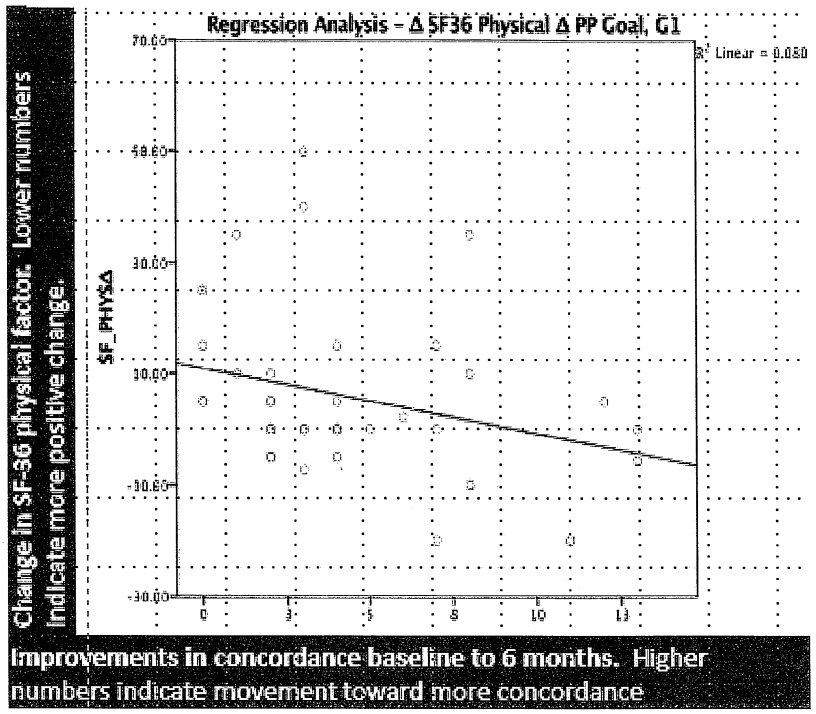

Figure 3. Clinical improvement on the SF-36 physical factor by increasing patient-practitioner concordance

The correlation coefficients that were statistically significant ($p<0.05$) included:

Treatment group 2 (weekly): CDEP_Psychological_Anxiety_1Sum Δ with PP_ Δ_Bond ($p=0.009$).

Correlation coefficient analysis for ISEL_Sum and CDEP_Psychological_Anxiety_Sum varied; for this trend no statistical significance was detected (Table 5).

Table 5. Pearson correlation coefficient per treatment group

Treatment Group 1						
Clinical Outcome	**PP_Δ_Task**	**Sig. (1-tailed)**	**PP_Δ_Bond**	**Sig. (1-tailed)**	**PP_Δ_Goal**	**Sig. (1-tailed)**
ISEL_1Sum (N=36)	-0.099	0.282	0.105	0.272	0.06	0.364
ΔISEL_Sum (N=36)	-0.051	0.385	-0.012	0.472	0.149	0.193
CDEP_Depression_1Sum (N=37)	-0.16	0.173	-0.21	0.106	-0.071	0.338
ΔCDEP_Depression_Sum (N=37)	0.029	0.432	0.035	0.418	0.085	0.309
CDEP_Psychological_Anxiety_1Sum (N=37)	-0.08	0.318	-0.205	0.112	-0.107	0.265
ΔCDEP_Psychological_Anxiety_Sum (N=37)	0.147	0.193	-0.02	0.453	0.117	0.246

Treatment Group 2						
Clinical Outcome	**PP_Δ_Task**	**Sig. (1-tailed)**	**PP_Δ_Bond**	**Sig. (1-tailed)**	**PP_Δ_Goal**	**Sig. (1-tailed)**
ISEL_1Sum (N=32)	-0.21	0.125	-0.081	0.33	-0.196	0.141
ΔISEL_Sum (N=32)	0.092	0.308	0.114	0.267	-0.037	0.421
CDEP_Depression_1Sum (N=32)	-0.044	0.406	-0.062	0.367	0.107	0.281
ΔCDEP_Depression_Sum (N=32)	0.114	0.267	-0.142	0.22	0.009	0.481
CDEP_Psychological_Anxiety_1Sum (N=32)	-0.214	0.12	-0.418	0.009	-0.103	0.287
ΔCDEP_Psychological_Anxiety_Sum (N=32)	0.117	0.262	-0.042	0.41	0.128	0.243

The correlation coefficients that were statistically significant ($p<0.05$) where:

Treatment group 1: Δ SF-36 Goal ($p=0.046$)

Treatment group 2: Δ SF-6 Task ($p=0.042$) and Δ SF-36 Goal ($p=0.027$) (Table 6).

Table 6. Pearson correlation coefficient per treatment group

Treatment Group 1						
Clinical Outcome	**Task**	**Sig. (1-tailed)**	**Bond**	**Sig. (1-tailed)**	**Goal**	**Sig. (1-tailed)**
Δ SF-36 (N=37)	-0.166	0.162	-0.269	0.054	-0.281	0.046
Δ McGill (N=37)	0.000	0.499	-0.221	0.095	0.044	0.399

Treatment Group 2						
Clinical Outcome	**Task**	**Sig. (1-tailed)**	**Bond**	**Sig. (1-tailed)**	**Goal**	**Sig. (1-tailed)**
Δ SF-36 (N=32)	-0.309	0.042	-0.158	0.195	-0.345	0.027
Δ McGill (N=31)	0.126	0.250	0.144	0.220	0.062	0.371

Discussion

Dyads reported increasingly positive scores on the all WAI-SR factors **(Task, Bond and Goal)** as the study advanced. This underscores the importance of having sufficient clinical time to allow the patient-practitioner relationship to grow stronger. More time with patients, and more treatments improve therapeutic alliance.

As our research progressed, we observed more concordance between practitioner and patient. This increase in synchrony is related to better therapeutic outcome [48] and supports the results of other experiments [49]. The concordance across WAI-SR factors, especially the factor Goal (shared treatment goals) was remarkably related to improved outcomes, specifically physical function in both groups, which can be indicative of importance of agreement on treatment objectives for clinical outcomes. Also, the concordance across the factor Bond was notably related to reductions in anxiety; perhaps agreement on the BOND quality of therapeutic relationship is stress relieving for the patients.

Interestingly, newer information suggests that the level of sensitivity to the impact of the relationship with the practitioner is different based on the patients' genotype. Hall et al. examined this hypothesis with the gene Catechol-O-methyltransferase (or COMT), which is implicated in dopamine levels in the body. One variant of rs4680 (a snippet of the genome) provides low level of COMT, while another provides high levels. In Hall's study patients were randomized to either a rich/naturalistic or pared down/scientific, acupuncture treatment series. Patients with Low-COMT or less available dopamine, has worse clinical outcomes in the rich/naturalistic condition. Patients that had best clinical outcomes were those with more available dopamine and the a rich/naturalistic treatment condition. These results point to the complexity inherent in health care, and that our patients may vary on their needs, wants, or what they are able to use [50]. In this study, we didn't analyzed genotype of the patients. However, future research that evaluate the genotype might find an interesting correlation between the genotype and the level of improvement in clinical outcomes due to therapeutic relationship.

Despite the fact that both the weekly waitlisted and biweekly groups had roughly the same quality of therapeutic relationship at the end of the study, the weekly waitlisted group had much lower

values of therapeutic alliance in the beginning of the study that could be due to being in waiting list for two months. These results suggest adverse effects of being on a waiting list on initial therapeutic alliance, and importance of receiving treatments for improving the relationship. We postulated that a two-month waiting list is superior to the use of sham acupuncture, as a systematic review demonstrated that sham acupuncture might be as effective as true acupuncture [50]. In our study, participants were randomly allocated to receive either biweekly acupuncture or weekly after two months waiting.

Limitations and future directions

A limitation to our study was that it was not probably reflective of the real-world effect of therapeutic alliance on therapeutic outcome. Patients were veterans with Gulf War illness who are different from general population. However, our study does provide evidence to support therapeutic alliance as an important influence on clinical outcomes.

Future research should investigate the relationship of therapeutic alliance and clinical outcomes in more representative samples.

Conclusions

These findings suggest that acupuncturists are skilled at gaining and improving therapeutic alliance. Although much medical research considers alliance to be a *non-specific* factor or one to be controlled, as in psychotherapy, nonspecific aspects of therapy can be active [49]. Our findings are in line with other research showing more concordance is related to better clinical outcome. More research on how acupuncturists create and maintain alliance in the clinical encounter is needed.

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The structures of retrospective clinical reasoning in Traditional Chinese medicine practitioners: a pilot study

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Retrospective accounts of the clinical reasoning of Traditional Chinese Medicine practitioners: a pilot study

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INTRODUCTION

The rise of individualized treatment clinical trials of Traditional Chinese Medicine (TCM) acupuncture has raised concerns as to the degree of inter-rater agreement among practitioners. Studies of agreement on findings of the four traditional methods of examination, initial diagnoses, and treatment planning – both acupoint selection and herbal prescriptions – have appeared. According to the few reviews that have appeared, those studies have generally found low rates of agreement among groups of acupuncturists staffing clinical trials or hospitals.[1,2,3] In this connection several paper and pencil instruments have been developed as guides to that the reasoning, with the intention of increasing rates of inter-practitioner agreement, or at least of consistency in reasoning. Although information on the methods used to develop these is sparse, (the work of Schnyer and associates is an exception[4]), the generally attempt to channel the practitioner's reasoning through one idealized process. As a whole this area of research has assumed that practitioners' agreement across practitioners' at the initial encounter is necessary and sufficient to enable statistical assessments of the extent to which those correlated with patient outcomes. At the conclusion of an earlier review, however, the present authors briefly raised the possibility that diagnoses and treatment plans across a number of clinical encounters might be more significant predictors of treatment outcome than those of the first encounter.[1]

In reading this literature one finds sparse reference to the long and robust history of clinical reasoning studies in biomedicine, apart from frequent references to evidence of low rates of diagnostic agreement in various fields of biomedicine. This is curious because the quest to achieve high inter-rater agreement on initial diagnoses is clearly an attempt to conform to the epistemology of biomedicine, and more particularly to that of clinical trials. Although much of the literature on clinical reasoning in biomedicine is concerned with normative pedagogies intended to inculcate reasoning and conclusions that conform to some norm, a smaller fraction explores and attempts to typologize the various ways in which novice physicians or nurses actually do reason. While a review of these is beyond the scope of this article, we do report on our application of one such framework to the retrospective accounts of reasoning by acupuncturists who worked on a clinical trial of TCM acupuncture for Gulf War Illness.

Many methods of analysis of biomedical reasoning have been tried, and, again, a comprehensive review is beyond our scope. The primary author (EJ) has been particularly impressed with a method developed by the psychologist Vimla Patel and her associates.[5,6] It involves a detailed analysis of transcripts of "think-aloud" accounts of reasoning in response to written case presentation. While there is some variation across their publications, the general scheme was to identify four categories of statements a) identifications of specific symptoms or signs as of interest, b) indications of the possible significance of a symptom or sign for a physiological mechanism or medical condition, c) hypothesized diagnoses, and d) diagnostic conclusions. The networks of reasoning revealed by such analyses were reported to reveal one particularly significant variation in reasoning. Medical students and novice physicians typically generated numerous diagnostic hypotheses, and then tried to back check each one by asking additional questions or conducting additional examination. This type of reasoning which proceeds by considering every logical possibility for a given set of symptoms and signs, is known

as "hypothetico-deductive." Their eventual conclusions – which were assessed relative to a normative "correct" conclusion - were more frequently in error than those of more experienced physicians. The reasoning of the latter typical proceeded from noting a small number of symptoms and signs as significant directly to a single hypothesized diagnosis, which they then confirmed and differentiated from others by asking additional questions. Their conclusions were more often assessed as correct, and their type of reasoning conforms in very general terms to the notion of "pattern recognition." The success of this mode of analysis in documenting two different styles of clinical reasoning encouraged us to attempt its application to similar data in the realm of TCM, although we did not expect to find the same two styles, especially given that TCM practitioners usually reason their way to specific treatment within a framework of "pattern identification" or *bien zhang.* Which differs in important ways from the biomedical paradigm of mutually exclusive diagnostic categories.

METHOD

Sample Data was obtained from clinical records produced in the course of a randomized clinical trail The Effectiveness of Acupuncture in the Treatment of Gulf War Illness (DOD W81XWH-09-2-0064) that was conducted at the New England School of Acupuncture, Watertown, Massachusetts, between July 2009 and January 2013. That study employed 32 practitioners, who treated a total of 104 patients, all of whom were veterans living in the Boston area, who had been diagnosed with Gulf War Illness (GWI). The study provided each subject with twice-weekly acupuncture treatments for eight weeks. Each practitioner produced a detailed record of their observations, examination findings, diagnoses, and treatment plans for each session. Under a grant for secondary data analysis (DOD W81XWH-15-1-0695) we examined the records of the first two to four sessions of each patient, and preferentially selected those which indicated some difference in diagnostic conclusion across those first sessions. With the hope of obtaining more variety in styles of reasoning we also preferentially selected records produced by two of the more senior acupuncturists and two of more limited clinical experience. The sample size was dictated by the limited funding that could be devoted to this pilot study.

Interviews The clinical notes for each selected case were divided into four sections roughly corresponding to the type of information requested on the clinical notes forms used in the parent study. Each acupuncturist was interviewed separately, and encouraged to think aloud their responses to each of the four sections of notes which were presented sequentially, with a request for them to summarize their thinking after completing each section. Audio recordings of the interviews were then transcribed, and those were the basis for our analysis of reasoning styles.

Analysis The practitioner's speech as recorded in each transcripts was parsed into the following categories, which are an expansion of Patel's framework: a) symptoms or signs appearing in the clinical notes which are identified as of interest, b) statements as to the possible significance of type a) items as indicators of specific pathomechanical processes of diagnostic patterns, c) questions and descriptions of their own cognitive strategies, d) statements of general

knowledge of TCM pathomechanics and diagnosis, e) hypothesized pattern differential diagnoses, f) pattern differentiation conclusions, and g) treatment plans

RESULTS

We present in Appendix A our analysis (EJ) of the reasoning of one of the four acupuncturists as they responded to the notes written by one of the other study acupuncturists on their initial meeting with one of the GWI patients. This will provide a general introduction to the type of analysis that we intend to complete for all 16 transcripts of study acupuncturists think aloud in response to clinical notes from the parent study. Each such analysis will then be summarized for the numbers of identified signs and symptoms, pathomechanics or diagnostic patterns that are implied by those signs and symptoms, questions and descriptions of cognitive strategies, general TCM knowledge, hypothesized pattern diagnoses, conclusive pattern diagnoses and treatment plans. This is an expansion of the analytic categories used by Patel and associates to incorporate additional types of statements that are found in our transcripts. A final level of analysis will identify features of the time course of reasoning, including a) how many signs or symptoms are noted before the initial pathomechanic or diagnostic hypotheses, how many before the pathomechanic(s) or diagnoses that were eventually conclusive,. And how many links were "dead ends" not followed up on as suggesting any hypothesis.

CONCLUSION

This general type of analysis, which seeks to uncover structural variants of clinical reasoning in TCM practitioners, would provide a more substantial basis for attempts to explore the relationship between their diagnostic conclusions and treatment plans on one hand, and clinical outcomes on the other. Some structures of reasoning might be found to be associated with better outcomes than others, and the detailed picture of the features of the less effective courses of thinking might then provide a guide for interventions to improve outcomes,

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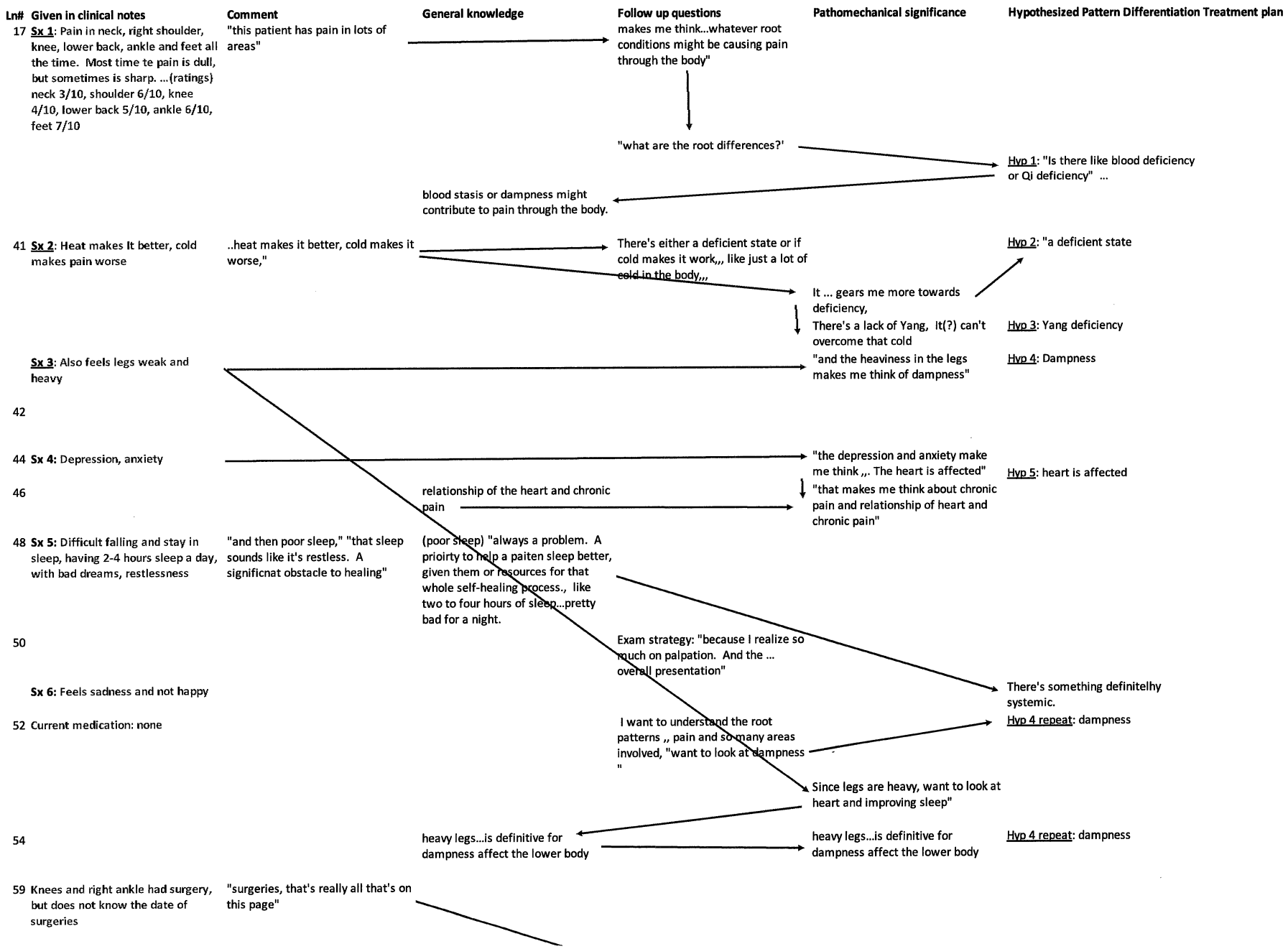
TCM Clinical Reasoning: Appendix A - Practitioner A Responding to Case 762, 1st meeting



TCM Clinical Reasoning: Appendix A - Practitioner A Responding to Case 762, 1st meeting

Ln#	Given in clinical notes	Comment	General knowledge	Follow up questions	Pathomechanical significance	Hypothesized Pattern Differentiation Treatment plan
61				"so I want to check out the scars,,since they had surgeries"		
63			"the scars can disrupt channel flow, if the scars didn't heal well, there can be ..knots in the fascia and they can impede circulation through the area. Scars.... Can be disruption to the channel system. ..."			
				Exam strategy: do basic... physical exam on what's the range (of) emotion like"		
71	**Sx 7:** Feel chills and cold all the time. Having cold extremities	"so ... chills and fever and sweating .,,,this person is cold all the time,	I don't call them... pathogenic factors, that's like thinking of the eight principles like this picture is cold" and likely a lock of more of a Yang deficiency:			
73			When its more of like systemic cold, not just cold extremities(then) theyr'e chilled and ... cold all the time,	I might ask how they feel about summertime, if they like summer and hot waeather." That wil help confirm... the depth of their coldness. But if they don't mind winter or they're wearing shorts." Cognitive strategy: it's really important to know how Yang deficient they are"		Hyp 3 repeat: Yang deficiency
74	**Sx 8**: Under stress will have rashes	"... so under stress they have rashes.			like the liver is affecting their skin	
			...liver Qi stagnation, that can affect their skin	whether they're ... liver bood deficient or they're... liver Qi stangnation that can affect their skin.		Hyp 5: Liver qi stagnation
	Sx 9: If hungrey will have headache	and if the'r hungery they have headaches		that makes me curious about their eating habilts. Do they have low blood sugar, do they need to eat every few hours or get a headache, ..if they don't eat for 10 hours do they get a headache?		
	Sx 10: Somnetimes having blurry vision	Sometimes they have blurry vision	That doesn't tell much			
	Sx 11: Chest always having a tightness feeling	and the chest always has a tight feeling				
75			..that is interesting from an acupncture point of view, what's a significant area of blocked, if its always feeling tight, what's going to help free the flow through that area, So Qi can flow in the right direction out of the extremeties"			
76			That could be just about any channel involved in that one, just being a major area of the torso			

TCM Clinical Reasoning: Appendix A - Practitioner A Responding to Case 762, 1st meeting

Ln#	Given in clinical notes	Comment	General knowledge	Follow up questions	Pathomechanical significance	Hypothesized Pattern Differentiation Treatment plan
	Sx 12: Appette is good, like drink water, 20-30 oz a day Having gas, but digestion is "OK"	..the appetite is good and digestion, there' some gassiness	Cognitive strategy: I want to keep focused on the main complaint (so) I'm not giving that one (tightness in chest) big credence or much curiosity now,			
	Bowel movement once a day Urination no problem	and bowels are fine, once a day, that sounds fine, not urinary problems				
	Sx 13: Hard to faling and hard to stay in sleep, Have 2-4 hours sleep a day, with bad dreams	it's hard to fall asleep , hard to stay....not getting much sleep			So this person looks like they're Yang deficient.,,but then their spritis are not rooting, (because) they're having sleep problems"	Hyp 3 repeat: Yang deficient
80	**Sx 14:** Energy is low, 2/10				the Yang is not storing, thus able to store the spirit, or there's some kind of factor that's keeping the spirit from being able to descend.	
				there's some kind of factor that's keeping the spirit from being able to descend.		
					It brings me back to the chest being really tight for acupuncture purposes like ther's tension there, There's not free flow and the spirit has to descend down through the chest	Tacit hyp 6: Spirit not descend through chest
84					And the energy is low	
				So how much is Yang deficiency, how much is just another factor that might make that energy unavailable?		Hyp 3 repeat: Yang deficiency
		And then poor sleep	that's a huge factor for low energy			
	Sx 15: Neck, right hsoulder, lower back, knee and ankle and knee having pain, ...neck 3/10, shulder 6/10, lower back 5/10, knee 4/10, ankle 6/10, feet 7/10 (low arch)	shoulder, feet and ankles have the worst pain				so my priorities, the pain and the sleep seem like the biggest treatment priorite and that ... reinstates the musculoskeletal pain,
85	**Sx 15:** feeling cold in lower back and knee	And then the feeling of cold in the lower back and the knees,				So I probably would want to use heat during
					And someone who seems like they're Hyp 3 repeat yang deficient	I"ll use heat lamps, the table warmer
87				[So I't sYang deficiency?] I'm thinking ?? Or cold is a major fractor.?		
				Without looking at pulse and tongue yet, I'm thinking like I"ll be really curious about Yang deficfiency. Yes		Hyp 3 repeat: Yang deficient
89	**Summary of that page**					
90					Summary of page: The potential for Yang deficiecy the patient being cold all the time, the chest being tight, the sleep and ... the pain, those are the areas that I'm concerned about	
	Summary Sxs of interest: cold all the time, chest ~~tight, sleep (poor), pain~~					Hyp 3 repeat: Yang deficient

TCM Clinical Reasoning: Appendix A - Practitioner A Responding to Case 762, 1st meeting

Ln#	Given in clinical notes Last page	Comment	General knowledge	Follow up questions	Pathomechanical significance	Hypothesized Pattern Differentiation	Treatment plan
93	**Sx 16:** Face lusterless, looks tired	The.,,physical presentation is ., a lusterless face and tired kook, everything else... really disliking cold			That's matching more of like a deficiency kind of presentation	Hyp 2 repeat: deficiency	
	Sx 17: [one item earlier] Distrust, anxeity and angry	an the emotion,,sounds significant		Or just the heart is affected[?]			So managing the patient's pain ...want to make sure the spirit is addressed, as wer'e
94	**[So the emotions makes you think of the heart too?]**						
95			The emotions make me think of the heart yes, That's in the way the patient preceives the world if he or she has anxiety and distress, There's not a sense of safety and groundedness, So (they are) going to be more reactive to body sensations and is going to pereicive their pain, or need to be more vigilant which usually makes pain worse				
96	Pulse qualities, checked Taut*wiry} Thin, Note: Thjin and wiry; pluse low on kidney position	And pulse,, I'm not a big pulse person			Ther's nothing in the pulse that pulls me way from my curiousy about Yang deficiniecy and sort of the deficient,,,underlying deficient aspects,	Hyp 3 repeat: Yang deficiency, and [Hyp 2 repeat:] (other) underlying deficient aspects	
	Tonque: checked pale, swollen, scalloped, think, white	This persons' tongue helps confirm that there's				Hyp 2 repeat: there's a significant deficient quality	
98					The pale and scalloped qualities of the tongue are definitely deficiency signs	Hyp 2 repeat: Deficiency	
	Note: Tongue large and swollen, pale color, deep teeth mark, thin white coating	The "thin" ...I don't know if this acupunctursit means the body [of tongue] "thin" must mean "thin white coat" because tongue can't be swollen and thin at the same time,,,,must have been referring to the coating					
102		... the tongue can't be swollen and thin, So it's swollen			If the body [of tongue] was thin, like pancake thin, that's another deficiency sign. But that doesn't appear to be what this is		
105		So the additional tongue description clarifies the thin part, as "thin white"			"swollen" tells me there's dampness, but that's because of deficiency... that's defnintely a **factor**	Hyp 2 repeat: deficiency	
106	**[That could be from defiency?]**						
107					Yes the dampness could be from deficiencey		

TCM Clinical Reasoning: Appendix A - Practitioner A Responding to Case 762, 1st meeting

Ln#	Given in clinical notes	Comment	General knowledge	Follow up questions	Pathomechanical significance	Hypothesized Pattern Differentiation Treatment plan
108						So..a[Hyp 3 repeat] Yang deficient patient definitely with [Hyp 4] damp and cold in the lower body. And then the unsettled spirit [Hyp 6][is the last + Hyp: heart?]
	[In summary how would you understand this case?]					
110						There's a body of pain, ...so there may be local areas of stagnation, but the whole system is not able to circulate Dosen't have so much Qi avaiable and isn't really able to circulate it Hyp 7: Qi deficiency
					So you get this cold and this damp	So I would definitely do more about root
112						The root treatment would be addressing the Yang deficiency and cold damp, I probably would
						Hyp 3 repeat: Yang deficency Hyp 4 repeat & ?:Cold damp
					Cognitive strategy: I"m going to look at my palpatory reference and use those to help me decide, ... is that the way to go?	
113	**[My impressoin is your'e talking about two kinds of patomechanics: Your'e talking about Qi deficiency and blood and shen not being able to descend]**					
114			Yes. But then the Shen can be ... not feeling nourised and supported, You think of shen like a baby, If the baby is being held (with) weak arms, the baby is going to get restless, because the baby doesn't feel supported and is worried, like, I'm not going to be dropped, So think of it that way, The arms holding them need to be nice and firm ... provide a good container			Hyp 6 repeat: Weak Shen
115	**[do you literaly mean the patient's arms, or do you mean?]**					
116			I mean ...the whole system. You feel contained and void(?) up and so if there's Qi available too for the spiritual aspects to be housed. Not necessarily the arms per se			
121			Like the whole system's Yang, It needs to be able to contain that spirit, so (it) can just rest and this being, this form, that has integrity			
123			So this also... so much beatury. It's beautiful to think in those			
124	**[It seems like that way of thining will go a long road at spiritual pradtice]**					
125			Yes.,.and observing nature			

INTRODUCTION TO

GULF WAR ILLNESS *for Acupuncturists*

GULF WAR ILLNESS (GWI)

During Operation Desert Storm/Desert Shield (1990-91), deployed troops suffered multiple chemical exposures from neurotoxic insecticides, burning oil fields and other environmental contaminants. Of the 700,000 servicemen and women who were deployed in the Persian Gulf, one fourth are affected by a cluster of symptoms and co-morbid medical conditions: Complex Medical Illness.

SYMPTOMS OF GWI

1. Fatigability - Persistent fatigue 24 hours or more after exertion.
2. Mood and Cognition - Depressed, anxious, worried, trouble concentrating, difficulty thinking, trouble finding words, problems thinking.
3. Pain - Headaches, joint pain, muscle pain.
4. Other symptoms include - Respiratory issues & skin disorders.

No standard of treatment currently exists in Western Medicine.

ACUPUNCTURE AND GWI

A Congressionally Directed Medical Research Programs (CDMRP) funded 2010 Randomized Control Trial of Gulf War Illness from the New England School of acupuncture demonstrated that Acupuncture is an effective treatment. Researchers randomized 104 Veterans diagnosed with GWI into 6 month weekly or biweekly acupuncture treatments. TCM differential diagnoses were given at baseline and 6 months.

Preliminary research indicates that acupuncture may be effective in the management of complex conditions that share a similar cluster of symptoms of pain, sleep disorders, and mood problems such as GWI and fibromyalgia.

TRADITIONAL CHINESE MEDICINE DIFFERENTIAL DIAGNOSIS OF GWI VETERANS AT BASELINE

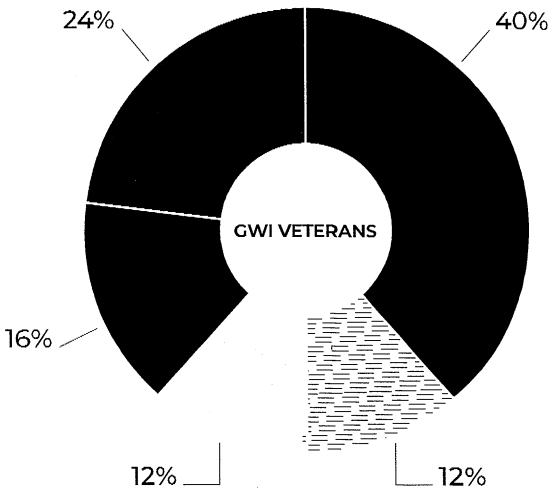
DEFICIENCY		EXCESS	
QI	67	DAMP	25
BLOOD	24	HEAT	23
YIN	33	COLD	2
YANG	7	PHLEGM	11
CHANNEL		FIRE	1
QI & BLOOD STAGNATION	30	YANG RISING	13
DEFICIENCY	2	WIND	8
8 EXTRAORDINARY MERIDIANS	4	QI STAGNATION	51
DAMP BI SYNDROME	13	BLOOD STAGNATION	10

The most common patterns for deficiency were Qi and Yin. The most common Excess patterns were Qi Stagnation, Damp and Heat. The most common pattern for Channel was Qi and Blood Stagnation and Damp Bi.

CO-OCCURRENCE OF DIFFERENTIAL DIAGNOSES AT BASELINE

Complex medical illnesses may present with two to three mixed TCM patterns.

- Two categories: excess+deficiency - 40%
- Three categories: excess+deficiency+channel - 24%
- Two categories: deficiency+channel or excess+channel (not excess and deficiency) - 16%
- Only one category (e.g., excess or deficiency or channel) - 12%
- Data missing - 12%



TIPS FOR TREATING GULF WAR VETERANS COMPILED BY PRACTITIONERS AND VETERANS IN STUDY

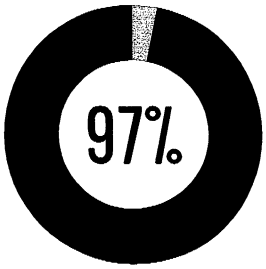
Given the level of different types of trauma in this population, carefully craft the treatment for their needs. You may need to remain in room with needles in place. Assess if they would want a face down treatment.

Make sure that there are no signs of them being triggered-make sure that they are oriented and comfortable.[5]

Community Acupuncture styles of treatment may not work for all veterans as the open setting can make some veterans feel vulnerable. Offer eyepillows and earbuds.

Be aware some veterans will have Chemical Sensitivities and may not be able to handle Moxa or scents.

Have music or white noise machines as some loud sounds can trigger veterans.



reported that their relationship with their practitioner was pleasant.

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ACUPUNCTURE
Helps Gulf War Illness!

WHAT IS ACUPUNCTURE? Acupuncture is the ancient East Asian medicine of inserting needles into the body to help the body heal and function better.

WHO RECOMMENDS IT? The VA and the Army are actively adding acupuncture to the services provided to veterans and active military.[1] Acupuncture is supported by: National Institutes of Health, American Academy of Pain Medicine and the Joint Commission.

WHAT CAN ACUPUNCTURE TREAT: Acupuncture can reduce symptoms of complex medical illnesses, such as Gulf War Illness.

The Centers for Disease Control defined Gulf War Illness as a Complex Medical Illness[2] with clusters of symptoms and medical diagnoses such as:

- **chronic fatigue syndrome**
- **fibromyalgia**
- **irritable bowel syndrome**
- **joint pain (arthralgia)**
- **digestive complaints**
- **mood-related psychiatric disorders:**
  - **-including depression**
  - **-PTSD**
  - **-other anxiety disorders[3, 4]**

A recent Gulf War Illness study showed that biweekly acupuncture significantly helped veterans' mood and cognition, fatigability, and pain.[5, 6, 7] No biomedical standard of care treatment exists at this time.

PATIENT SATISFACTION:

of the veterans reported confidence in recommending acupuncture to a friend or family member.

reported that the acupuncture experience was at least pleasant.

"I've participated in this program (Gulf War Illness Acupuncture Study), I thought it was absolutely outstanding. I was wary of acupuncture when I first started but by the time the program ended, I was a full supporter."
-Quote from Veteran

HOW CAN I FIND AN ACUPUNCTURIST AND HOW DO I CHOOSE ONE?
Contact the following:

- **Your local VA** https://www.va.gov
- **American Society of Acupuncturists** http://www.asacu.org/find-a-practitioner/
- **National Certification Commission for Acupuncture and Oriental Medicine** http://www.nccaom.org/find-a-practitioner-directory/

Acupuncture treatments are covered by some national insurance programs. Please contact your insurer to see if you have coverage. Medicare does not cover acupuncture currently.[8] Medicaid coverage varies by state.[9]

OTHER RESOURCES/HELPFUL LINKS

Veteran Administration

https://www.va.gov/PATIENTCENTEREDCARE/docs/2017-AR-Vet-Facing_FNL-W508.pdf

NIH

https://nccih.nih.gov/health/acupuncture

Pain Medicine Journal

https://academic.oup.com/painmedicine/article/16/9/1806/1876743#28945703

Evidence-Based Acupuncture

https://www.evidencebasedacupuncture.org/

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W81XWH-15-1-0695

Dr. Lisa Conboy

Appendix B

Statement of Work

Statement of Work 2018

This project is a program evaluation of the Congressionally Directed Medical Research Program funded project "The Effectiveness of Acupuncture in the Treatment of Gulf War Illness" (W81XWH-09-02-0064). This single-blind randomized controlled clinical trial evaluated the effectiveness of individualized acupuncture treatment on subjects' overall health and disease burden.

Objectives: This current application has two objectives: 1) Gather follow up data from our veteran participants on current symptom levels and use of services to explore the long-term effects of an acupuncture treatment program, 2) Develop suggestions for how an acupuncture program may be implemented using the viewpoints of multiple stakeholders.

Tasks:

1. **Create program evaluation documents (Month 1):** Dr Conboy will finalize the survey instrument and focus group agenda. These materials will be circulated to all other study staff for feedback. Suggestions and edits will be made for submission to the IRB.
2. **Train research assistant, and review goals with consultants (Month 1-2)**: Once funding is achieved the team will have a face-to-face meeting (using Skype for long-distance members) to review program goals. Follow-up group e-mails will solidify our process.
3. **IRB Review (Month 1-3):** The protocol will be submitted to the IRB as soon as funding is approved. This approval should take no more than a month. Review will take place at our contracted IRB, the New England IRB (www.neirb.com). Review by the HRPO will also be submitted and completed.
4. **Program Evaluation (Month 4-42):**

Task 4.1 Program evaluation with study subjects: Collect (Month 4-42)

All of the subjects who participated in the parent trial will be mailed an IRB approved survey which: (1) repeats the survey questions administered in the parent study, (2) asks additional questions about subjects' study experiences, use of health services since the study, and requesting feedback and suggestions for program and treatment improvement. Subjects' participation will be requested up to three times (by email, mail, and phone) and subjects can opt out of participation at any time. The mailing will also ask for the subject's interest in participating in a focus group with multiple stakeholders to help design the best acupuncture protocols and program for veterans. Extension update: due to our poor response rate, our institution (MCPHS) has agreed to offer all veterans coupons for 5 free treatments in our student treatment center as an incentive for completing the survey. This should increase our response rate, as 'more acupuncture' is the one thing that the subjects consistently requested more of.

Task 4.1.2 Enter subject surveys into electronic database format. Data will be double entered with appropriate accuracy checks (see Attachment 8: Data Management).

Task 4.1.3 Tabulate subject surveys for use in program evaluation meetings (below Task 4.2) Subjects' responses to survey questions of symptoms, reported beliefs about acupuncture, study experiences, and use of acupuncture since trial completion will be tabulated.

Task 4.1.4 Analyze responses to subject symptom surveys. Paired Student's t-tests will be used to compare subjects' symptom reports since trial completion to the present day to initially determine the long-term effectiveness of acupuncture. The eventual manuscript for publication will utilize regression equations and consider control variables such as age, time since study completion, and baseline symptom levels.

Task 4.2 Program evaluation with multiple stakeholders (Month 11-42)
Assemble team members. Named members include: (1) Joe Chang Lic Ac, an acupuncturist with experience working in military settings; (2) Marc Goldstein MD, a physician at the VA in Boston MA who was the medical screener for the parent project; (3) Meredith St John Lic Ac, an acupuncturist and designer of the protocol for the parent project; (4) Dr. Christine Chronin DAOM, a former Marine and clinical supervisor Pacific College of Oriental Medicine's Veteran Clinic of San Diego, (5) Rosa Schnyer Lic Ac, DAOM, an acupuncturist and researcher expert on the design of scientific acupuncture protocols and complex conditions who was the lead acupuncture consultant on the parent grant, (6) John Coville Lic Ac, the first acupuncturist to work in the Massachusetts VA hospital in Beverly, MA.

Task 4.2.2 Conduct focus groups (Month 11-15): Led by Dr Conboy, stakeholder teams will discuss how to best design and implement an acupuncture treatment protocol for use within the VA system, as well as how to best utilize practitioners in the general community. The resulting document will condense these viewpoints into a plan to optimize program formation.

Task 4.2.3 Conduct data analysis: program evaluation (Month 18-40): Dr Conboy will tabulate focus group responses by theme to create a highly usable document of treatment program recommendations. A late-stage manuscript will be circulated to focus group participants, with a request for addition feedback and clarification. These final comments and edits will be incorporated into the final document.

Task 4.2.4 Conduct Delphi process (Month 23-40). The 5 (from a complete sample of 31) treating practitioners from the parent study with the best treatment responses and who agree to participate will complete a Delphi process including a review of the protocols used to determine the most effective protocols from a TCM point of view.

5. Final Manuscript write ups (Month 36-42)
The last 6 months of the project will be used to complete manuscripts and any remaining analyses.

Figure 1. Timeline: Designing a Successful Acupuncture Treatment Program for Gulf War Illness

	Year 1		**Year 2**		**Year 3**		**Year 4**
Study Month-→	6	12	18	24	30	36	42
Create program evaluation documents	X						
Train research assistants, and review goals with consultants	X						
IRB review	X						
Collect and tabulate subject surveys		X	X	X	X	X	X
Collect and analyze focus group data			X	X	X	X	X
Data analysis: program evaluation			X	X	X	X	X
Conduct Delphi process and analysis				X	X	X	X
Annual reports submission		X		X		X	X
Final manuscript writing				X	X	X	X

STATEMENT OF
WORK – February 13, 2018
PROPOSED START DATE June 1, 2015

Site 1: New England School of
Acupuncture
150 California Street/Newton, MA
PI: Lisa Conboy

Specific Aim 1(specified in proposal)	Timeline	Site 1
Aim 1 Survey veteran participants for their current symptom levels, and use of acupuncture and other services	Months	
Create program evaluation documents	1	Dr Conboy
Train research assistant, and review goals with consultants	1-2	
IRB Review/ HRPO/ACURO Approval	1-3	
Survey participants of parent trial	4-36	
Aim 2 Conduct effectiveness research/program evaluation of our acupuncture treatments and study design from the viewpoint of multiple stakeholders:		
Survey participants of parent trial as described in Aim 1	4-36	Dr Conboy
Conduct Focus groups	11-15	
Conduct Delphi process	23-40	
The product of Delphi analysis is a document of concrete recommendations for implementing an acupuncture treatment program in both VA and community settings.	30-42	

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

	Year 1				Year 2				Year 3
Target Enrollment (per quarter)	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1
New England School of Acupuncture	50	54							
Target Enrollment (cumulative)	50	54							

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

W81XWH-15-1-0695

Dr. Lisa Conboy

Appendix C

Statistical Summary

Preliminary Statistical Summary

We are eager to share results of our follow up survey. As noted earlier, we received a 50% response rate. More detailed analyses are necessary to uncover if there is a bias in our sample, related to acupuncture treatment response or other factors.

Descriptively, most (53%) of the sample continued with acupuncture; note that the parent study ended in 2013 at least 5 years before this resurvey.

Figure 1: Below find the frequencies of veteran's reported acupuncture use less than 1 year ago, 1 to 3 years ago, more than or equal to 4 years ago.

When was the last time that you had acupuncture?

Answered: 51 Skipped: 1

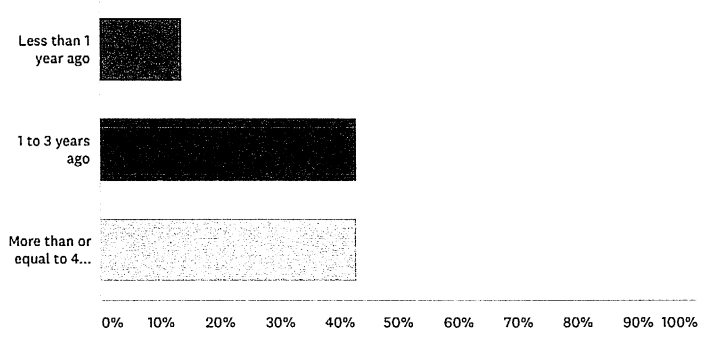


Figure 2: Below find the frequencies of reported number of acupuncture treatments, less than 5, 5 to 10, 10 or more treatments.

How many acupuncture treatments did you receive at that time?

Answered: 51 Skipped: 1

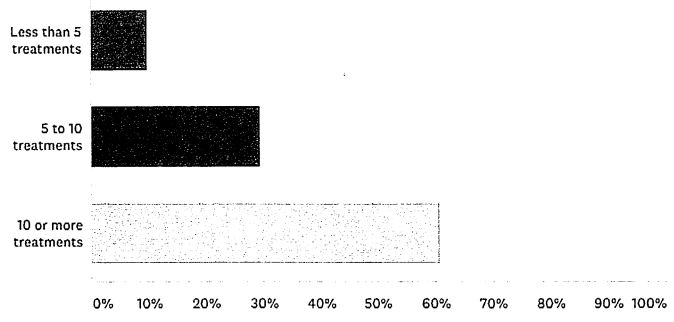


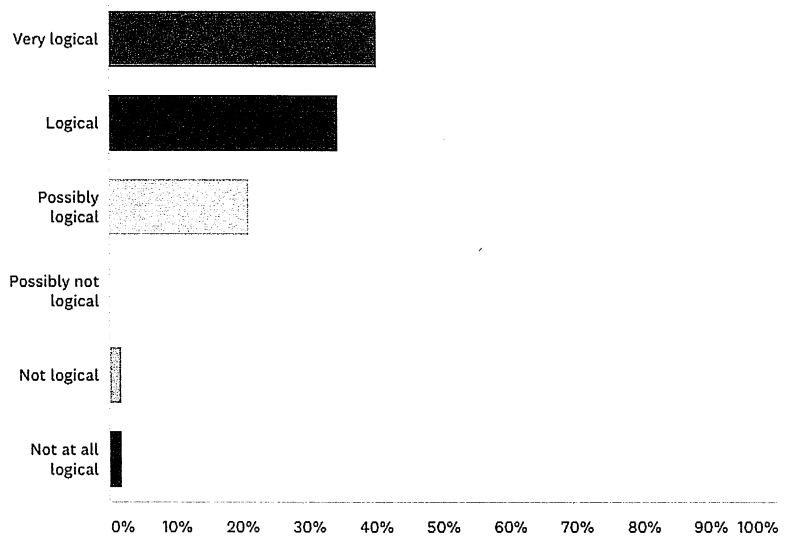
Figure 2 above suggests that of those that continued with acupuncture, 90% had more than 5 treatments. This indicates a dedication to treatment and treatment satisfaction.

Figure 3 displays that participants found the treatments to be logical, an important determinant of adherence.

Figure 3: Below find the frequencies of how logical acupuncture seemed to the patient.

Please check the option that best describes your impression of acupuncture. How logical do the treatments seem to you?

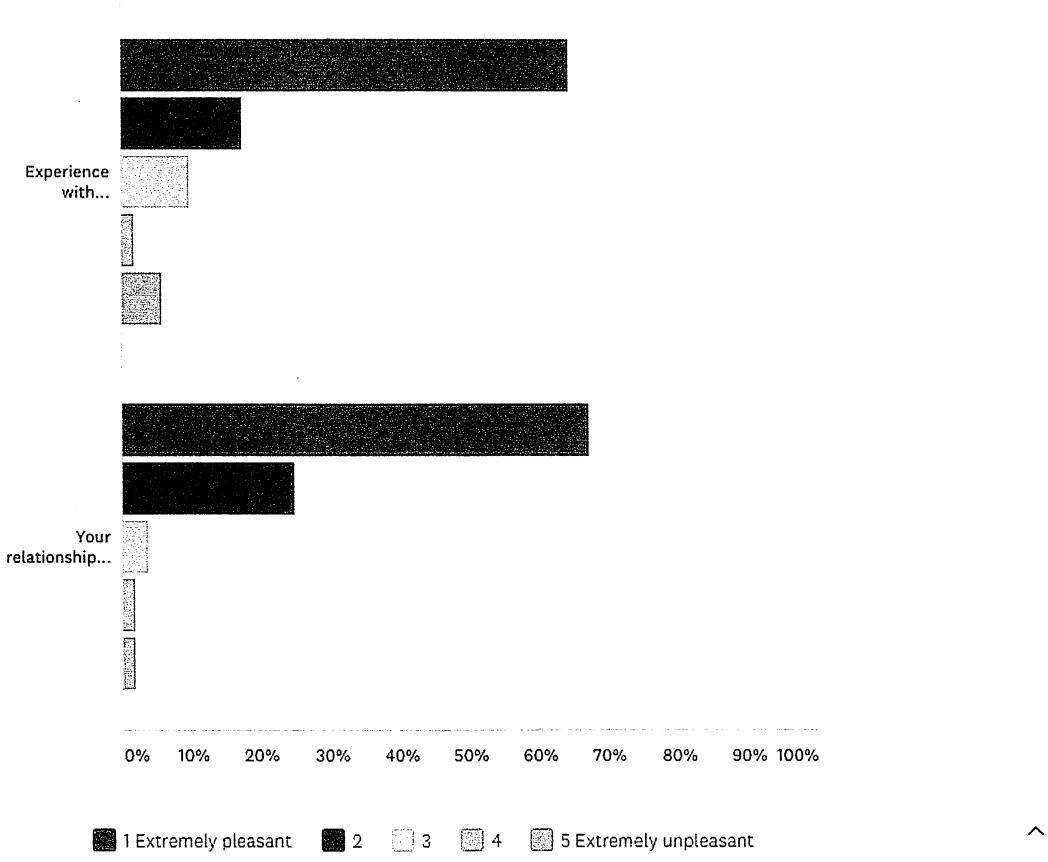
Answered: 52 Skipped: 0



Below in Figure 4, veterans gave high satisfaction ratings for their acupuncture treatments and relationship with practitioner.

Figure 4: Below find the frequencies of how pleasant the veteran found the experience of acupuncture treatments (top figure), and second their relationship with their practitioner (bottom figure). Ratings range from 1 (extremely pleasant) to 5 (extremely unpleasant.

Answered: 52 Skipped: 0



In comparing **clinical outcomes,** we found only a slight differences comparing results of the follow-up survey to those found at the time of the study. This could be because our resample is biased towards those that did well in the acupuncture trial and these same veterans are the ones that maintain positive clinical effects. Future work will use regression modeling and consider the existence of subgroups, such as controlling for amount of relief found in the parent trial. From a Chinese Medicine theoretical viewpoint, regular treatments are recommended to maintain symptom relief in a condition this severe. Yet there is little western science done on persistence of acupuncture treatment effects; such an analysis of our data will add to this persistence literature and inform how to best treat our veterans and service members with acupuncture.

Table 1. The table below shows responses to the general health question of the Sf-36, "*In general you would say your health is" 1.Excellent, 2. Very good, 3. Good, 4. Fair, (5) Poor*

Percentage reporting During parent trial	**Percentage reporting on resurvey**
Excellent (2.5%)	*Excellent (3.9%)*
Very good (18.2%)	*Very good (15.7%)*
Good (35.3%)	*Good (39.2%)*
Fair(35.5)	*Fair (37.2%)*
Poor (8.5%)	*Poor (3.9%)*

Table 2 shows the percentage of the sample reporting various severity levels of personal main and secondary complaints. These items are part of the standardized scale *Measure your medical outcomes profile MYMOP).* Subjects were asked to choose two symptoms and rate their severity on a scale of 0 (as good as it can be) to 6 (as bad as it can be).

Response set	Percentage reporting During parent trial Sx1	Percentage reporting During parent trial Sx2	Percentage reporting on resurveySx1	Percentage reporting on resurvey Sx2
(0) As good as can be	3.1%	3.0%	5.8%	2.3%
(1)	5.1%	3.9%	7.7%	13.9%
(2)	10.9%	12.5%	7.7%	16.3%
(3)	18.8%	27.9%	26.9%	18.6%
(4)	33.7%	24.9%	28.8%	37.2%
(5)	21.4%	19.8%	21.2%	16.3%
(6) As bad as it can be	7.0%	7.9%	1.9%	0

In summary, the veterans reported high usability and satisfaction with treatment; most continuing treatment on their own after the trial was over. Clinically, our low response rate makes conclusion difficult due to the potential bias. In aggregate, the sample of veterans overall did not change much since the end of the study. More powerful analyses will follow to determine the existence of subgroups.

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Dr. Lisa Conboy

Appendix D

Outreach/Impact Contact List

List of Organizations sent "Acupuncture Helps Gulf War Illness" Infographic:

VISN 1: VA New England Healthcare System

VISN
VISN 1: VA New England Healthcare System (Bedford, MA)
VA Health Care System
VA Boston Healthcare System (Jamaica Plain, MA)
VA Connecticut Healthcare System (West Haven, CT)
VA Medical Center
Edith Nourse Rogers Memorial Veterans Hospital (Bedford VA) (Bedford, MA)
Manchester VA Medical Center (Manchester, NH)
Providence VA Medical Center (Providence, RI)
VA Boston Healthcare System, Brockton Campus (Brockton, MA)
VA Boston Healthcare System, Jamaica Plain Campus (Jamaica Plain, MA)
VA Boston Healthcare System, West Roxbury Campus (West Roxbury, MA)
VA Central Western Massachusetts Healthcare System (Leeds, MA)
VA Connecticut Healthcare System, Newington Campus (Newington, CT)
VA Connecticut Healthcare System, West Haven Campus (West Haven, CT)
VA Maine Healthcare System - Togus (Augusta, ME)
White River Junction VA Medical Center (White River Junction, VT)
Outpatient Clinic
Causeway OPC (Boston, MA)
Fort Kent Access Point Clinic (Fort Kent, ME)
Houlton Satellite Clinic (Houlton, ME)
Mobile Medical Unit (Bingham, ME)
Community Service Programs
Lowell Veterans Community Care Center (Lowell, MA)
Community Based Outpatient Clinic
Aroostook County (Caribou) Community Based Outpatient Clinic (Caribou, ME)
Bangor Community Based Outpatient Clinic (Bangor, ME)
Bennington Outpatient Clinic (Bennington, VT)
Brattleboro Community Based Outpatient Clinic (Brattleboro, VT)
Burlington Outpatient Lakeside Clinic (Burlington, VT)
Calais Outpatient Clinic (Calais, ME)
Conway Outpatient Clinic (Conway, NH)
Danbury Outpatient Clinic (Danbury, CT)
Fitchburg Outpatient Clinic (Fitchburg, MA)
Framingham Outpatient Clinic (Framingham, MA)
Gloucester Community Based Outpatient Clinic (CBOC) (Gloucester, MA)
Greenfield Outpatient Clinic (Greenfield, MA)
Haverhill Community Based Outpatient Clinic (CBOC) (Haverhill, MA)
Hyannis Outpatient Clinic (Hyannis, MA)
John J. McGuirk (New London) VA Outpatient Clinic (New London, CT)
Keene Outpatient Clinic (Keene, NH)
Lewiston/ Auburn Community Based Outpatient Clinic (Lewiston, ME)

Lincoln Community Based Outpatient Clinic (Lincoln, ME)
Littleton Community Based Outpatient Clinic (Littleton, NH)
Lowell Outpatient Clinic (Lowell, MA)
Lynn Community Based Outpatient Clinic (CBOC) (Lynn, MA)
Middletown Outpatient Clinic (Middletown, RI)
New Bedford Outpatient Clinic (New Bedford, MA)
Newport Community Based Outpatient Clinic (Newport, VT)
Pittsfield Outpatient Clinic (Pittsfield, MA)
Plymouth Outreach Clinic (Plymouth, MA)
Portland Community Based Outpatient Clinic (Portland, ME)
Portsmouth Outpatient Clinic (Portsmouth, NH)
Quincy Outpatient Clinic (Quincy, MA)
Rumford Community Based Outpatient Clinic (Rumford, ME)
Rutland Community Based Outpatient Clinic (Rutland, VT)
Saco Community Based Outpatient Clinic (Saco, ME)
Somersworth Outpatient Clinic (Somersworth, NH)
Springfield Outpatient Clinic (Springfield, MA)
Stamford Outpatient Clinic (Stamford, CT)
Tilton Outpatient Clinic (Tilton, NH)
Waterbury Outpatient Clinic (Waterbury, CT)
Willimantic Outpatient Clinic (Willimantic, CT)
Winsted Outpatient Clinic (Winsted, CT)
Worcester Outpatient Clinic (Worcester, MA)

Vet Center

Bangor Vet Center (Bangor, ME)
Berlin Vet Center (Gorham, NH)
Boston Vet Center (Boston, MA)
Brockton Vet Center (Brockton, MA)
Cape Cod Vet Center (Hyannis, MA)
Danbury Vet Center (Danbury, CT)
Hartford Vet Center (Rocky Hill, CT)
Keene Outstation (Keene, NH)
Lewiston Vet Center (Lewiston, ME)
Lowell Vet Center (Lowell, MA)
Manchester Vet Center (Hooksett, NH)
New Bedford Vet Center (Fairhaven, MA)
New Haven Vet Center (Orange, CT)
Newington Outstation (Newington, NH)
Northern Maine Vet Center (Caribou, ME)
Norwich Vet Center (Norwich, CT)
Portland Vet Center (Portland, ME)
Providence Vet Center (Warwick, RI)
RCS North Atlantic District 1, Zone 1 District Office (Auburn, NH)
Sanford Vet Center (Springvale, ME)
South Burlington Vet Center (South Burlington, VT)
Springfield Vet Center (West Springfield, MA)

White River Junction Vet Center (White River Junction, VT)
Worcester Vet Center (Worcester, MA)

New York: VA Albany area

VA Hudson Valley Healthcare System
Montrose Campus (Montrose, NY)
Castle Point Campus (Wappingers Falls, NY)
VA Medical Center
Albany VA Medical Center: Samuel S. Stratton (Albany, NY)
Vet Center
Albany Vet Center (Albany, NY)

Veteran Organizations

New York
New York State Division of Veterans Affairs, Albany NY
Veterans Employment and Training Service U.S. Department of Labor, Albany NY
Disabled American Veterans, Albany NY
Homeless Program C/O Veterans Industries, Albany NY
Community Mental Health Initiatives U.S. Department of Veterans Affairs, Albany NY
Military Order of the Purple Heart, Binghamton, NY
Veterans Employment and Training Service U.S. Department of Labor, Albany NY
American Legion of New York, Albany NY
Albany Housing Coalition, Inc, Albany NY
Connecticut
Labor--Veterans Program State of Connecticut, Wethersfield, CT
Blinded Veterans Association Connecticut, Old Saybrook, CT
Connecticut Department of Veterans Affairs, Rocky Hill, CT
Military Order of the Purple Heart, Derby, CT
Disabled American Veterans Department of Connecticut, Rocky Hill, CT
American Legion, Milford, CT
American Legion, Plantsville, CT
Veterans Employment & Training Service U.S. Department of Labor, Wethersfield, CT
Homes for the Brave, Bridgeport, CT
American Legion, Rocky Hill, CT
Health Care for Homeless Veterans Program, West Haven, CT
American Legion, Newington, CT
Veterans of Foreign Wars, Newington, CT
Hartford Regional Office U.S. Department of Veterans Affairs, Hartford, CT
Connecticut Veterans Legal Center, West Haven, CT

Massachusetts
Veterans Transitional House, New Bedford, MA
Paralyzed Veterans of America, Boston, MA
Veterans' Employment and Training Service U.S. Department of Labor, Boston, MA
Veterans Association of Bristol County, Fall River, MA
Veterans of Foreign Wars Massachusetts, Boston, MA
Veterans Benefits Clearinghouse, Roxbury, MA
Vietnam Veterans of America, Massachusetts State Council, Turners Falls, MA
New England Shelter for Homeless Veterans, Boston, MA
Montachusett Veterans Outreach Center, Inc. Garner, MA
Bi-lingual Veterans Outreach Centers of Mass Inc. Springfield, MA
USS BOSTON Shipmates, Inc. Brockton, MA
Department of Veterans' Services, Commonwealth of Massachusetts Boston, MA
American Legion Department of Massachusetts, Boston MA
Women Veterans' Network, Boston, MA
New England Shelter for Homeless Veterans Boston, MA
Massachusetts Veterans, Inc. Boston, MA
Veterans Hospice Homestead, Inc. Fitchburg, MA
Blinded Veterans Association Volunteer Office, Granby, MA
Chelsea Soldiers Home, Chelsea, MA
American Legion of Massachusetts, Boston, MA
Paralyzed Veterans of America, Walpole, MA
Soldiers' Home in Holyoke, Holyoke, MA
Disabled American Veterans, Boston, MA
Jeremiah's Inn, Worcester, MA
North Adams Housing Authority, North Adams, MA
Maine
Veterans Employment and Training Service, U.S. Department of Labor Lewiston, ME
Veterans of Foreign Wars, Augusta, ME
Maine Veterans Home, Scarborough, ME
Maine Veterans Home, Augusta, ME
Maine Veterans Home, Caribou, ME
Bureau of Employment Services, Maine Department of Labor, Augusta, ME
Disabled American Veterans, Augusta, ME
Maine Bureau of Veterans Services, Augusta, ME
Division of Veterans Services, Augusta, ME
American Legion Service Office, Augusta, ME
American Legion of Maine State Headquarters, Winslow, ME
Maine Veterans Home, Machias, ME
Blinded Veterans Association Volunteer Office, Augusta, ME
Health Care for Homeless Veterans U.S. Department of Veterans Affairs, Augusta, ME

Maine Veterans' Home, Bangor, ME
Vietnam Veterans of America, Limestone, ME
Maine Veterans Home, South Paris, ME

New Hampshire

Veterans Count, Manchester, NH
New Hampshire Veterans of Foreign Wars, Concord, NH
Homeless Veterans Programs, U.S. Department of Veterans Affairs, Manchester NH
Disabled American Veterans, Manchester, NH
New Hampshire Veterans Home, Tilton, NH
New Hampshire State Veterans Council, Manchester, NH
Veterans Employment and Training Service U.S. Department of Labor, Concord, NH
American Legion of New Hampshire, Manchester, NH
American Legion of New Hampshire, Concord, NH

Rhode Island

Veterans Employment and Training Service U.S. Department of Labor, Wakefield, RI
American Legion, North Providence, RI
American Legion of Rhode Island, Providence, RI
Operation Stand Down, Johnston, RI
Blinded Veterans Association Volunteer Office, Providence, RI
Military Order of the Purple Heart, Providence, RI
Workforce Development Services Rhode Island Department of Labor and Training, Cranston, RI
Ocean State Center for Independent Living, Warwick, RI
Disabled American Veterans, Providence, RI
Vietnam Veterans of America, Providence, RI
Operation Stand Down Rhode Island, West Warwick, RI
Rhode Island Veterans Action Center, Providence, RI
Operation Stand Down Rhode Island, Newport, RI

Vermont

Vermont Office of Veterans Affairs, Montpelier, VT
American Legion, Chester, VT
Veterans of Foreign Wars, White River Junction, VT
Veterans Employment and Training Service U.S. Department of Labor, Montpelier, VT
American Legion, Montpelier, VT
Disabled American Veterans, White River Junction, VT
Friends of Veterans, Inc. White River Junction, VT
Vermont Veterans Home, Bennington, VT
Vietnam Veterans of America Rutland, VT

List of Acupuncture Schools Sent "Introduction to Gulf War Illness for Acupuncturists" and "Acupuncture Helps Gulf War Illness" Infographics:

New England School of Acupuncture at MCPHS University, Worcester/Newton, MA
Southwest Acupuncture College, Boulder, CO
Southwest Acupuncture College, Santa Fe, NM
South Baylo University, Los Angeles, CA
American College of Oriental Medicine, Houston, TX
Midwest College of Oriental Medicine – Racine, WI
Yo San University of Traditional Chinese Medicine, Los Angeles, CA
AOMA Graduate School fo Integrative Medicine, Austin, TX
Pacific College of Oriental Medicine , Chicago IL
Pacific College of Oriental Medicine, San Diego, CA
Institute of Taoist Education and Acupuncture, Louisville, CO
Midwest College of Oriental Medicine, Evanston, IL
American College of Traditional Chinese Medicine, San Francisco, CA 94107
Texas Health and Science University, Austin, TX
Texas College of Traditional Chinese Medicine, Austin, TX
Five Branches University, Santa Cruz, CA
Phoenix Institute of Herbal Medicine & Acupuncture, Phoenix, AZ
Arizona School of Acupuncture and Oriental Medicine, Tucson, AZ
University of East-West Medicine, Sunnyvale, CA
Acupuncture & Integrative Medicine College, Berkeley, CA
South Baylo University, Anaheim, CA
Colorado School of Tradtional Chinese Medicine, Denver, CO
Dongguk University Los Angeles, CA
Five Branches University, San Jose, CA
Atlantic Institute of Oriental Medicine, Ft. Lauderdale, FL
Academy of Chinese Culture and Health Sciences, Oakland, CA
Emperor's College, Santa Monica, CA
Dragon Rises College of Oriental Medicine, Gainesville, FL
Pacific College of Oriental Medicine, New York, NY
Southern California University of Health Sciences College of Eastern Medicine, Whittier, CA
Academy for Five Element Acupuncture, Gainesville, FL
Acupuncture & Massage College, Miami, FL
Florida College of Integrative Medicine, Orlando, FL
Won Institute of Graduate Studies, Glenside, PA
East West College of Natural Medicine, Sarasota, FL
Institute of Clinical Acupuncture and Oriental Medicine, Honolulu, HI
Texas Health & Science University, Austin, TX
Maryland University of Integrative Health, Laurel, MD
Minnesota College of Acupuncture and Oriental Medicine, Bloomington, MN

Bastyr University, Kenmore, WA
American Academy of Acupuncture and Oriental Medicine, Roseville, MN
Eastern School of Acupuncture and Traditional Medicine, Bloomfield, NJ
AOMA Graduate School of Integrative Medicine, Austin, TX
New York College of Traditional Chinese Medicine, Mineola, NY
Daoist Traditions College, Asheville, NC
Texas Health & Science University, San Antonio, TX
Jung Tao School of Classical, Sugar Grove, NC
Oregon College of Oriental Medicine, Portland, OR
Seattle Institute of Oriental Medicine, Seattle, WA