Assessing Neuroinflammation in Gulf War Illness with Whole-Brain Magnetic Resonance Spectroscopy

Study Protocol & Statistical Analysis Plan

NCT04638998

December 20, 2023

Jarred Younger, PhD, Principal Investigator
University of Alabama at Birmingham
Birmingham, AL 35294

Study Protocol:

Overall design

This observational neuroimaging study uses a 3D MRSI sequence to measure several neuroinflammatory markers across the entire brain. A cross-sectional approach is used to contrast 20 veterans with GWI from 20 veterans who were deployed to the Persian Gulf but never presented with symptoms of GWI. Participants will attend a single neuroimaging session.

Participants

GWI group: GWI participants will be men who meet the Kansas GWI diagnostic criteria. CDC criteria will also be assessed, but not used for inclusion purposes. Our primary pool of participants will come from our existing database of individuals who have completed our online, phone, and in-person screening package for GWI eligibility.

Healthy control group: We have a database of individuals who served in Operation Desert Storm and do not present with symptoms of GWI. Individuals will be within the same age and body mass index range as the GWI group.

Screening

Individuals (GWI or healthy) will be excluded for any of the following reasons: Participants cannot be taking experimental medications, opioid analgesics, psychostimulants (except caffeine), or benzodiazepines. Participants cannot have major cardiovascular illness, cancer, or have any contraindications for MRI (including metallic implants or claustrophobia).

Laboratory visits

Participants will undergo venous blood draw at the UAB Clinical Research Unit. The neuroimaging visit will occur at the University of Alabama at Birmingham (UAB) Civitan International Neuroimaging Laboratory, in Highlands Hospital. Body temperature measurements will be taken using a Braun Pro 4000 ThermoScan aural thermometer. Self-report instruments will then be completed. Participants will then undergo scanning on a 3T Siemens Magnetom Prisma scanner with a 20-channel head coil, which will take approximately one hour. Last, participants will be debriefed and paid.

Neuroimaging sequences

T1-weighted high-resolution structural scan (5 minutes): For spatial registration, we will use a magnetization-prepared rapid gradient echo (MPRAGE) sequence with 224mm FOV, 320 x 320 matrix, 2400ms TR, 2.15ms TE, 0.9mm slice thickness, and no gap, yielding a 0.9 x 0.9 x 0.9mm 3D image of the brain. The MPRAGE sequence uses a longer TR than traditional ISPGR scans. Our sequences are based on the Human Connectome Project settings recommended for multi-site projects.

Whole-brain MRSI scan (20 min with shimming): The MRSI acquisition will use a whole brain 3D spectroscopic sequence, which acquires the metabolite and water signals in one TR using echo-planar readout. The acquisition will use the following parameters: FOV of 280mm in-plane and 180mm in the inferior-superior direction to yield 5.6x5.6x10mm voxels, 1710ms TR1, 591ms TR2, 17.6ms TE. Signals from subcutaneous lipids will be reduced using an inversion-recovery preparation with inversion time (TI) of 198ms and a saturation band placed across the lipid

region behind the eyes to suppress movement aliasing. A double echo steady state (DESS) pulse sequence will be applied prior to the scan in order to estimate the field. A dynamic shim using higher order coils will optimize the homogeneity of the B0 field and improve spectral resolution; data will be acquired using a full width at half maximum (FWHM) of the water peak at <25Hz.

*Arterial spin labeling cerebral perfusion scan (*7 min): This scan will be used to rule out the hypo-perfusion contribution to metabolite concentrations and temperature. A 2D ASL scan will be acquired using a Proximal Inversion with Control of Off-Resonance Effects (PICORE) labeling scheme for background suppression. 60 pairs of label/control ASL images will be collected in the axial direction at a single inversion time of 1800 ms, TR=2500 ms, TE=16.18 ms, 12 slices, 4×4×10 mm voxels. Data will be processed using ASLtbx, which works on the basis of SPM12 within MATLAB. Images will be motion-corrected then co-registered to the T1 image and smoothed with a 6mm full-width-at-half-maximum (FWHM) kernel to decrease noise. Each tag/control pair will be subtracted to create 60 perfusion-weighted images used to calculate cerebral blood flow (ml/100g/min).

Statistical Analysis Plan:

Univariate, independent samples t-tests will be used to test for group differences for each brain marker × ROI combination. The dependent variable will be each of the five neuroinflammatory outcomes and the main predictor of interest is group (GWI vs healthy). A total of 47 brain areas will be tested. Cortical and cerebellum averages will also be computed as a 'whole brain average'. To compute power, we used the preliminary results from the CFS versus healthy control data. The average significant differences between groups in that study had an effect size (Cohen's d) of 0.922. Power calculations were performed using G*Power 3.1.9.2. Using the means and standard deviations from that study, we would expect to have a power of 0.81 to detect GWI versus control differences with a statistical threshold of 0.05 and 20 individuals per group. Because this is a Discovery study with a limited sample size, we are not able to set a more restrictive statistical threshold to correct for multiple comparisons. We will report differences that are significant at $p < 0.05$; however, we will use a false-discovery-rate (FDR) adjusted p-value of < 0.0078 to identify differences that are most likely to be replicable. This process should provide an acceptable balance between false positives and negatives in an exploratory study.