Defining Neurobiological Signatures for Chronic Traumatic Brain Injury Using PET-MRI Technology

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1.0 INTRODUCTION

1.1 Introduction

Over 1.5 million Americans suffer traumatic brain injuries annually resulting in over 50,000 deaths, 300,000 hospitalizations, and 80-90 thousand individuals with long term disability referred to as "Chronic Traumatic Brain Injury (cTBI)", with estimated costs at greater than 60 billion dollars (1). However, there is a lack of studies using comprehensive diagnostic imaging tools to better understand physiological ramifications of the injury that may help guide therapy.

The goal of the proposed study is to utilize a unique and comprehensive diagnostic imaging battery focusing on PET-MRI in patients with traumatic brain injury and chronic symptoms. With this level of diagnostic detail, we can secondarily assess an integrative medicine approach that prioritizes beneficial dietary behaviors and a natural supplement that improves antioxidant status in patients with cTBI.

A key integrative approach for cTBI developed by our team is to distinguish inflammation from injury using the PET-MRI technology. Inflammation is typically associated with immunological activity that results in the release of a variety of inflammatory mediators and, in the brain, excitotoxicity. Injury is associated with reduced function in damaged areas. Utilizing fluorodeoxyglucose (FDG) PET imaging, we have the ability to observe increased metabolic activity in the brain associated with inflammation, or decreased activity associated with injury. Further, we plan to utilized fMRI to evaluate functional and structural changes associated with response to an integrative medicine approach.

The purpose of this project will be to create the first comprehensive, and extensive longitudinal diagnostic evaluation of cTBI patients using a battery of neurocognitive tests, plasma levels of specific inflammatory compounds, and the use of functional MRI, PET, and quantitative EEG. Patients would be evaluated with this overall test battery initially, and then at 3 and 6 months in order to determine the time course of changes within the brain associated with an integrative medicine approach for improving brain function.

1.2 Inflammation and Injury in TBI

Traumatic brain injury consists of the immediate damage produced from the insult, and the ensuing chronic phase which may extend for years. The chronic phase is associated with inflammation, cell death, and ultimately neural dysfunction or damage (1,2). The inflammatory component is caused in large part by pro-inflammatory molecules, reactive oxygen species, and other damaging byproducts from the primary injury site. Pro inflammatory molecules include tumor necrosis factor (TNF-α) and interleukin (IL-1β) and anti-inflammatory molecules include IL-10 and TGF-β (3). There is also the primary site of injury as well as a surrounding or penumbra area (4).

Once in the chronic phase of TBI there is a neuroinflammatory aspect which, if it persists, can trigger the persistent symptoms associated with the chronic phase, eventually perpetuating cell death. Reducing chronic inflammation in these TBI patients has been proven to be neuroprotective (34). The neurodegenerative inflammation after TBI is also linked to dysfunction of the blood-brain barrier (BBB) (5,6). Disruption of the BBB allows a variety of proteins such as albumin, fibrinogen, and thrombin, in addition to immune cells, to enter the brain's parenchyma. These molecules and cells activate the brain's microglia resulting in the immune response associated with chronic TBI that persists for as long as the BBB remains

compromised (56,67). It has been suggested that possible therapeutic techniques would target the chronic inflammatory process (7).

A number of studies have suggested the importance of oxidative stress in the inflammatory pathophysiology of TBI. Oxidative stress itself is classically defined as a redox imbalance in which there is an excess formation of oxidants or a decrease in amount of function of antioxidants (8). The brain has difficulty withstanding substantial amounts of oxidative stress because of the presence of high amounts of polyunsaturated fatty acids and low levels of antioxidants such as glutathione (9). Finally, since neurons are in a post-mitotic state, they are unlikely to recover from an oxidative stress insult.

The mechanism by which oxidative stress occurs begins with the production of reactive oxygen species such as superoxide anion (O2−U), hydrogen peroxide (H2O2) and hydroxyl radical (UOH), which are byproducts of metabolism of molecular oxygen by the mitochondria. Excessive formation of reactive oxygen and nitrogen species in TBI may damage key cellular components such as lipids, proteins, and DNA. Reactive nitrogen species such as nitric oxide (NO) and its metabolite peroxynitrite (PN) may also play a major role in TBI. Evidence for oxidative damage in the brain of TBI patients includes the finding of an increase in the amount of lipid peroxidation products such as malondialdehyde and 4-hydroxynonenal, an increase in protein oxidation as evidenced by protein cross-linking and fragmentation, and an increase in the concentration of 8- hydroxy-2′-deoxyguanosine, a product of DNA oxidation (910).

Glutathione is an important reducing agent in the neurons, which is found to be depleted in the brain of TBI patients. Glutathione depletion may result from an accelerated loss of glutathione or diminished production. Glutathione is not the only antioxidant molecule reported to be altered in TBI, but the magnitude of glutathione depletion may parallel the severity of the disease. Neuronal injury associated with cTBI appears to be mediated by a variety of mechanisms including apoptosis, planned necrosis, neuronal cross talk mechanisms, neuroinflammation, and oxidative damage (10). Human and animal studies suggest that therapy with antioxidants as well as compounds that might stimulate brain function could potentially be useful in helping patients resolve neuronal injury (11,12).

Overall, there appears to be substantial evidence that oxidative stress, and associated nitrosative stress, likely play a prominent role in the pathophysiology of TBI. The normal homeostasis health of a neuron requires adequate maintenance of the redox potential in the cell. When oxidative stress occurs, the cell can no longer protect itself resulting in dysfunction and ultimately cell death. The question is whether interventions designed to restore the redox potential will be effective in attenuating the disease process.

1.3 Utilizing Neuroimaging in TBI patients

A number of studies using PET for the evaluation of patients with head trauma have been reported in the literature. Lesions associated with neuronal damage such as cortical contusions, intracranial hematoma, and resulting encephalomalacia have reduced metabolism that are confined primarily to the site of injury. Subdural and epidural hematomas often cause widespread hypometabolism and may even affect the contralateral hemisphere(13). Diffuse axonal injury (DAI) has been found to cause diffuse cortical hypometabolism as well as a marked decrease in metabolism in the parieto-occipital cortex. This is particularly true in the visual cortex of patients with DAI when compared to normal subjects and patients with other types of head injuries. The cause of this parieto-occipital hypometabolism is unknown. However, it is believed to result from the disruption of callosal input into the visual cortex or by

disruption of primary visual input due to the shearing axonal injuries of the optic and geniculo-calcarine tracts.

Of particular relevance to the use of PET imaging in brain injury is that ischemic cell damage occurs in over 90% of patients (14). This ischemia is probably mediated by the release of various toxins in response to the molecular events associated with brain injury. This also leads to an ischemia--reperfusion type of injury (15,16). Most ischemic changes in brain injury are observed in the fronto-parietal watershed regions and have been noted particularly in patients with severe injuries (17).

Functional brain imaging, in addition to physiological measures of inflammation and oxidative damage, aids in understanding the cTBI patients' symptomatology and can assist clinicians in developing targeted treatment strategies (18,19). For example, evidence of inflammation may be more treatable by anti-inflammatory medications while evidence of oxidative damage might be helped by anti-oxidants or programs to enhance brain function (20). It is also frequently the case that cTBI patients have normal findings in some tests (for example: EEG, CT, and/or MRI scans) while still complaining of headaches, memory loss, concentration difficulties, dizziness, perceptual sensitivities, and emotional lability. In such cases, PET imaging may observe a number of subtle metabolic abnormalities, particularly those associated with neuronal damage (21) Newer MRI techniques such as diffusion tensor imaging and functional connectivity may also be useful in this setting (22,23). The question is to determine which diagnostic studies are the most effective for detecting persistent abnormalities in cTBI patients in addition to determining the best techniques for detecting those abnormalities. Specifically, some neuroimaging studies are read clinically as "normal" even though quantitatively there are significant abnormal findings. Thus, the purpose of this project will be to better assess the diagnostic studies of QEEG, fMRI, PET, and physiological measures to determine the best techniques for evaluating brain function in patients with persistent symptoms from cTBI.

1.4 Integrative Approaches in TBI Patients

The integrative approach that is part of this study focuses on developing anti-inflammatory dietary behaviors and specific natural supplements. The goal of the integrative approach is to augment anti-inflammatory or anti-oxidant processes in the body in a more effective way. We will have two intervention arms of the study, both of which have been developed as part of our related studies on ways of reducing inflammation and oxidative damage in the body.

One arm will focus on adjusting dietary practices to eat foods that have a lower amount of inflammatory molecules that might help reduce overall inflammation in the brain and body. Animal models suggest that a diet high in saturated fats and pro-inflammatory foods might lead to higher levels of inflammation and neuronal damage (24,25). Reducing caloric intake and constructing a diet of anti-inflammatory foods (natural foods and foods high in omega-3 fats) might have neuroprotective effects (26,27). Thus, this arm will introduce patients to an integrative diet that reduces saturated fats and carbohydrates and emphasizes proteins and omega-3 fats that help reduce inflammation and oxidative damage.

The second approach to reducing oxidative damage is to provide patients with a natural supplement that supports antioxidants in the body. Thus, based on the research literature and also our recent research projects in Parkinson's disease and multiple sclerosis, we will utilize n-acetyl cysteine (NAC) which is the N-acetyl derivative of the naturally occurring amino acid, L-

cysteine. It is a common over-the-counter supplement and also is available as an injectable pharmaceutical that protects the liver in cases of acetaminophen overdose. In the exercise physiology literature, both oral and injectable NAC have been shown to reduce fatigue and improve recovery from exertion (28,29), which has interesting implications for exploring fatigue related to TBI. Laboratory studies have suggested how NAC might have a beneficial effect in neurodegenerative disorders such as TBI. For example, one study showed that NAC may reduce misfolded protein levels and ameliorate proteotoxicity through heat shock proteins (30). The authors suggested that their findings broaden the potential mechanisms of action for NAC in neurodegenerative proteinopathies. Another study tested the hypotheses that a combined exposure of nerve cells to oxidative stress caused by hydrogen peroxide and paraquat would elicit synergistic neurodegeneration and that this toxicity would be prevented by NAC (31). The findings revealed that when neuronal N2a cells received two hits of hydrogen peroxide the result was a severe loss of glutathione which was attenuated by NAC.

In humans, an MRS study of 3 neurological patients showed that blood glutathione increased after the start of an NAC infusion and reached a maximum at approximately 60 to 75 minutes (32). Brain glutathione also increased with maximal values observed at approximately 90 to 110 minutes. Subjects who had the greatest percent change in blood glutathione after NAC infusion also had the greatest percent change in brain glutathione. Interestingly, none of the subjects returned to their baseline brain glutathione levels even at 120 minutes after NAC infusion. The results suggest that NAC might be useful in reducing oxidative damage in TBI patients.

2.0 OBJECTIVES

AIM #1. Utilize the most comprehensive and accurate neuroimaging and physiological test battery in patients presenting with cTBI to determine how the injury affects brain function.

AIM #2. To evaluate the physiological effects and potential symptom improvement of integrative medicine approaches in cTBI patients.

3.0 STUDY PLAN

3.1 Subject Recruitment

Subjects may be pre-screened by telephone using a standardized script and screening form. Verbal consent and HIPAA Authorization to obtain the prescreening information will be obtained from subjects prior to the prescreening interview. If subjects are prescreened in person, a signed consent and HIPAA Authorization to obtain prescreening information will be obtained. Information collected during pre-screening will be incorporated into the research records as source documentation for subjects included in the study. If subjects are not eligible to participate in the study they will be asked if the information provided in during prescreening maybe retained for consideration in other studies. Prescreening information will be retained for an indefinite period on an official screening form that will be kept in a secure locked area that will only be used by persons involved with research with this research study.

We plan on recruiting 120 subjects over a three year period and will include the coordination of the Marcus Institute of Integrative Health along with the Department of Neurology and the Rothman Institute of Orthopedics at Thomas Jefferson University. One hundred and twenty patients will initially undergo the following:

1. Quantitative EEG
2. FDG PET (fluorodeoxyglucose positron emission tomography)
3. Functional MRI to assess functional connectivity, tractography, brain volumes
4. Physiological measures (neopterin, 2-hydroxyguanosine, etc.)
5. Neurocognitive and psychological testing (see below)

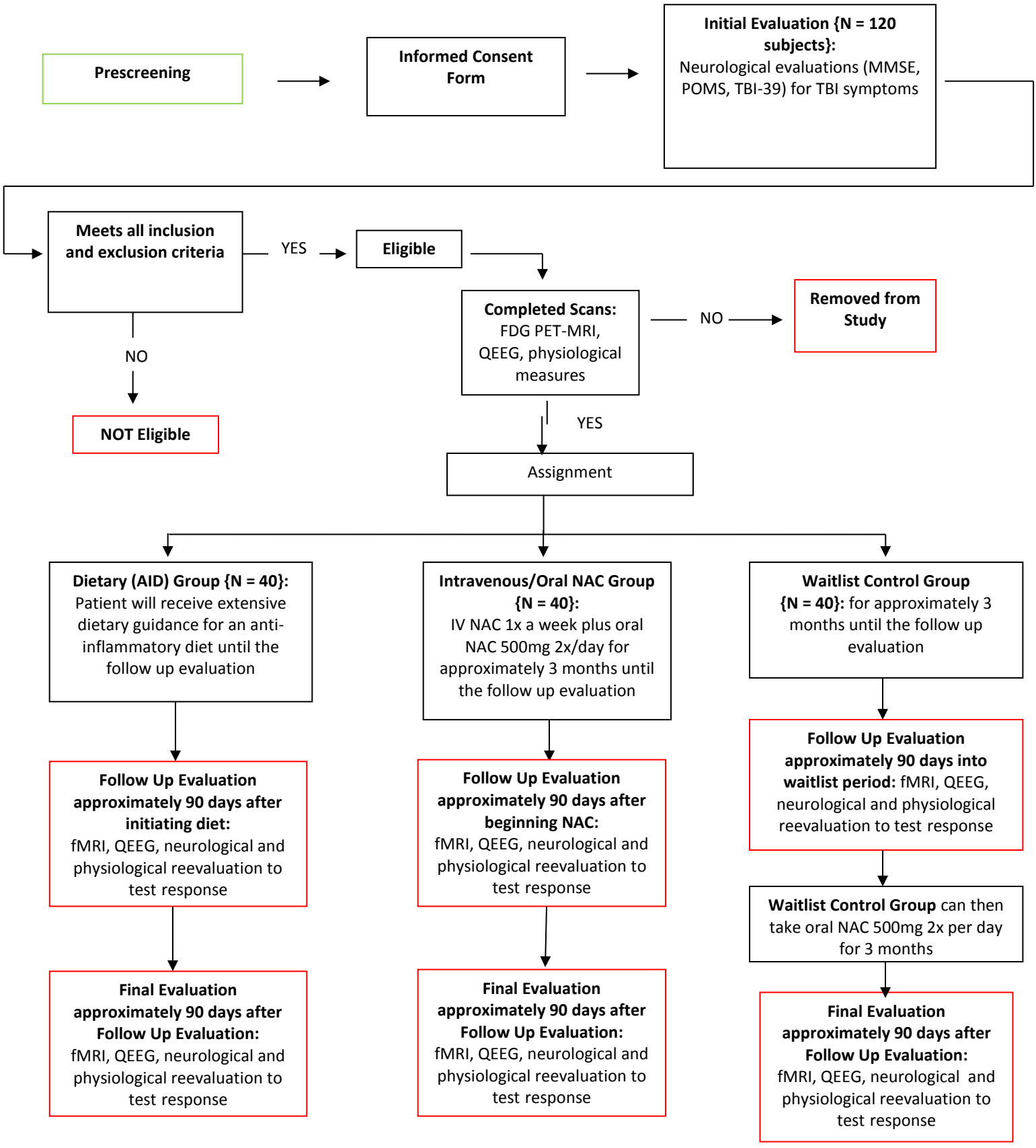
Patients would then undergo the following tests at 3 months ± 30 days (the Follow Up Evaluation) and 6 months ± 30 days (the Final Evaluation) after the initial or pre-intervention evaluation and application of integrative medicine approaches:

1. Quantitative EEG
2. Functional MRI to assess functional connectivity, tractography, brain volumes
3. Physiological measures (neopterin, 2-hydroxyguanosine, CRP)
4. Neurocognitive and psychological testing

Quantitative EEG (QEEG) is essentially a quantitative analysis of standard EEG recordings. The QEEG allows for more accurate determination of origin of electrical signals in the brain. FDG PET is performed only the first time in order to minimize radioactive exposure. We will use the PET-MRI scanner at the Marcus Institute of Integrative Health, Villanova in order to obtain simultaneous PET and fMRI data. Patients will be assigned to receive either the anti-inflammatory diet or the IV/oral NAC. They will be scanned initially and then at 3 and 6 months ± 30 days post initiation of either the diet or NAC. Subjects will be asked to continue the program (i.e. diet or NAC) until the 3 month Follow Up evaluation. At that point, subjects will resume standard of care for any persistent cTBI symptoms and will undergo the Final Evaluation 3 months after the Follow Up Evaluation (or 6 months after initiation of treatment. After three months of standard of care, and after the 3 month Follow Up Evaluation, the control subjects will be offered the opportunity to receive oral NAC and will subsequently have the final 6 month ± 30 days Final Evaluation (thus controls will be scanned initially, followed up at 3 months with standard of care, and then have the final evaluation at 6 months after initiating the integrative therapy plan). Furthermore, referring physicians will be provided the results of the diagnostic studies as available in order to potentially help with determining additional treatment options.

At the conclusion of the study, a thorough evaluation will be performed on understanding which diagnostic tests revealed clinically important abnormalities and how such findings predicted outcome and response to the treatment plan.

3.1.1 Flow Chart


Prescreening
Informed Consent Form
Initial Evaluation {N = 120 subjects}: Neurological evaluations (MMSE, POMS, TBI-39) for TBI symptoms
Meets all inclusion and exclusion criteria
YES
Eligible
NO
NOT Eligible
Completed Scans: FDG PET-MRI, QEEG, physiological measures
NO
Removed from Study
YES
Assignment
Dietary (AID) Group {N = 40}: Patient will receive extensive dietary guidance for an anti-inflammatory diet until the follow up evaluation
Intravenous/Oral NAC Group {N = 40}: IV NAC 1x a week plus oral NAC 500mg 2x/day for approximately 3 months until the follow up evaluation
Waitlist Control Group {N = 40}: for approximately 3 months until the follow up evaluation
Follow Up Evaluation approximately 90 days after initiating diet: fMRI, QEEG, neurological and physiological reevaluation to test response
Follow Up Evaluation approximately 90 days after beginning NAC: fMRI, QEEG, neurological and physiological reevaluation to test response
Follow Up Evaluation approximately 90 days into waitlist period: fMRI, QEEG, neurological and physiological reevaluation to test response
Waitlist Control Group can then take oral NAC 500mg 2x per day for 3 months
Final Evaluation approximately 90 days after Follow Up Evaluation: fMRI, QEEG, neurological and physiological reevaluation to test response
Final Evaluation approximately 90 days after Follow Up Evaluation: fMRI, QEEG, neurological and physiological reevaluation to test response
Final Evaluation approximately 90 days after Follow Up Evaluation: fMRI, QEEG, neurological and physiological reevaluation to test response

3.1.2 Inclusion criteria

1. Individuals with a history of TBI and complaints of persistent symptoms including cognitive impairment, emotional disturbances, headache, or other symptoms associated with TBI.
2. Age 18-80 years old
3. Patients had no other pre-existing history (i.e. prior to the TBI) of significant medical, neurological, or psychological disorders such as schizophrenia or active substance abuse.
4. Minor, stable health problems that should have no substantial effect on cerebral blood flow will be allowed (i.e. controlled hypertension, medication controlled diabetes).
5. Able to give informed consent and willing to complete the study.
6. Patients will be allowed to be taking medications or supplements at the initial intake, but they must be on a stable dose regimen for at least 1 month.
7. Women of childbearing potential will confirm a negative pregnancy test and must practice effective contraception during the period of pilot study. In addition, male subjects who have a partner of childbearing age should practice effective contraception.

3.1.3 Exclusion criteria

1. Previous brain surgery.
2. Score on Mini-Mental Status examination of 25 or lower.
3. Intracranial abnormalities that may complicate interpretation of the brain scans (e.g., stroke, tumor, vascular abnormality affecting the target area).
4. Pregnant or lactating women.
5. Enrollment in active clinical trial/ experimental therapy within the prior 30 days.
6. Any pre-existing medical conditions that may interfere with cerebral function.
7. Subject is unable or unwilling to lie still in the scanner (i.e. due to claustrophobia or weight > 350 pounds)
8. Subject has metal in their body or other reason that they cannot undergo magnetic resonance imaging.
9. Patients taking medications that might interact with the NAC involved in this study will be evaluated on a case by case basis by the PI or study physician.
10. Patients that have a history of uncontrolled diabetes, asthma, gastroesophageal reflex disease, or thyroid conditions.

3.2 Registration Guidelines and Recruitment

Study subjects initially will be recruited by referral from the Jefferson Department of Neurology, local neurology groups, and self-referrals. If any recruitment materials are developed, they will not be distributed without IRB approval.

The subject population is derived from the greater Philadelphia area, which represents a racially and economically diverse population. We will make efforts for this protocol to be widely accessible, including offering the procedures protocol without charge to the subject.

3.3 Treatment Plan:

3.3.1 Informed consent will be obtained from all subjects before protocol specific activities are carried out. The subject will be informed about the limited data on the anti-inflammatory diet and intravenous/oral NAC to support brain health in TBI patients, possible risks and benefits, and possible adverse events. Informed consent will be documented by use of written consent form approved by the Institutional Review Board at Thomas Jefferson University and signed by the subject or the subject's legal guardian. History, physical and neurological examination, and initial diagnostic procedures (see below) will begin within 14 days of the informed consent process. Subjects will be assigned to either the anti-inflammatory diet, NAC, or the waitlist control. Dietary counseling will take place during a 1 hour session reviewing all food intake and providing a handout with dietary recommendations. Subjects will be asked to keep a food diary for one week each month using the free website, myfitnesspal.com. Subjects will be given a specific subject username and password so that they can record their food intake. Subjects will be contacted every 2 weeks for additional guidance and support on their dietary guidelines. Subjects receiving the NAC will receive the combination of oral plus intravenous NAC for 3 months until the follow up evaluation and scan. Subjects receiving dietary counseling will follow the anti-inflammatory diet for 3 months until the follow up evaluation and scan. After 3 months, subjects can pursue additional standard of care treatment for any residual symptoms and will undergo a Final Evaluation and scan at 6 months after the initial evaluation. Patients in the control group will have a Follow up evaluation at 3 months and then will be offered the opportunity to receive oral NAC for 3 months until the Final evaluation and scan. It is hoped that for all patients, their standard of care treatment will remain constant throughout the course of the study, but their referring neurologist may adjust their medications as medically necessary.
3.3.2 N-Acetylcysteine will be obtained from the Jefferson Pharmacy (NAC is also called Acetadote; Cumberland Pharmaceuticals). NAC is an intravenous (IV) medication for the treatment of acetaminophen overdose. Acetylcysteine is the nonproprietary name for the N-acetyl derivative of the naturally occurring amino acid, L-cysteine (N-acetyl-L-cysteine, NAC). Acetadote is supplied as a sterile solution in vials containing 200 mg/mL acetylcysteine. The pH of the solution ranges from 6.0 to 7.5. Acetadote contains the following inactive ingredients: 0.5 mg/mL disodium edetate, sodium hydroxide (used for pH adjustment), and Sterile Water for Injection, USP.

For the oral supplement, NAC is obtained through the Marcus Institute produced by Ortho Molecular Products (produced under GMP; Woodstock, IL) or an equivalent supplier (produced under GMP) as a 500mg capsule of N-acetyl cysteine. The following inactive ingredients are also included: Ascorbyl Palmitate, Gelatin, Rice, and Silica. The capsules contain no coatings, binders, fillers, or dairy, wheat, eggs, soy, yeast, commercial sugars, starch, preservatives, or hydrogenated oil.

NAC doses will be prepared for each patient by the study nurse. The dose will be 50mg/kg in approximately 200ml of D5W infused over approximately one hour 1x per week. The IV bag containing NAC is labeled as a research medication. Subjects will also receive 500mg NAC tablets supplied by the Jefferson Pharmacy and will take 1 tablet 2x per day on the days that they do not receive the IV NAC.

3.4 Criteria for Removal from / Cessation of Protocol

3.4.1 Measuring Endpoints: Endpoints will be measured after receiving either nutritional supplementation, intravenous NAC, or being on the waitlist for 90 days. These will include the results of the MRI, QEEG, physiological measures, and neurological evaluation. Any serious adverse events also will result in immediate discontinuation of the subject from the study.

3.4.2 Subject Withdrawal: The subject may withdraw from the study at any time for any reason.

3.4.3 Missing Appointments: Subjects may be removed from the study for missing a total of 5 or more days of the oral or the intravenous NAC as per the PI. Patients may also be removed if they refuse to follow the integrative diet program.

All reasons for discontinuation of procedure will be documented in study flow sheets.

3.5 Adverse Events

The OHRP defines an adverse event as "any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign, symptom, or disease, temporally associated with the subject's participation in the research." Adverse events can additionally be classified as an unanticipated problem, meaning it was not expected to occur during the course of the research. If an unexpected adverse event were to occur, it then needs to be determined whether or not it is due to the research being conducted. If the event is a result of the research procedures, most likely the event is directly connected to the subject's participation in the research. It is also vital to determine whether an adverse event is serious. The OHRP defines a serious adverse event as one that a) results in death, b) is life-threatening, c) results in patient hospitalization or prolongation of existing hospitalization, d) results in persistent or significant disability/incapacity, e) results in congenital anomaly, or f) jeopardizes the subject's health to the point where they may need medical or surgical intervention. If the adverse event is unexpected, related to the research, and serious (where it is causing harm to subjects), then it is also classified as an unanticipated problem and must be reported to the Thomas Jefferson University Hospital IRB. All adverse events will be reported in accordance with Jefferson IRB Adverse Events Report. The IRB shall be notified in a written safety report if any serious and unexpected adverse experience associated with the use of the oral or intravenous nutritional supplements occurs.

3.6 Data Collection and Submission Schedule

3.6.1 Data Submission: Data must be submitted according to the protocol requirements for all subjects registered, whether or not assigned treatment is administered.

3.6.2 Master files, such as case report forms and progress reports, are prepared, and updated, by the study coordinator. Case report forms will include eligibility checklist, demographic data, baseline history and physical laboratory results, adverse events, and off-study document. These will be completed by the study coordinator under the supervision of the principal investigator.

3.7 Measurement of effect of the integrative medicine plan

3.7.1 Clinical Response: Neuropsychological and cognitive testing

Subjects will be evaluated initially and then at the 3 and 6 month time points. All evaluations will be performed by the PI or a research staff member qualified to administer the tests. All subjects will be administered the Speilberger State Trait Anxiety Inventory (STAI) at the time of study. The STAI contains a total of 40 questions, half of which relate to the way subjects are feeling at the moment and half of which ask them to describe how they usually feel. The Profile of Moods Scale (POMS) will be administered and the Beck Depression Inventory. The TBI-39 is a standardized questionnaire that evaluates the subject's TBI symptoms. Cognitive testing will include the Delis Kaplan Executive Function System (DKEFS) color-word interference (the Stroop effect task), Trails A & B, forward and reverse digit span. Quality of life measures will include the Epworth Sleepiness Scale, Mayo-Portland Adaptability Inventory-4 (a very nice 3-page quality-of-life oriented review), and Rivermead Post-Concussion Symptoms Questionnaire (just one page symptom checklist, also very helpful to track patients longitudinally).

3.7.2 PET Imaging Procedure

FDG PET Imaging Procedure – Subjects will receive a standard of care FDG PET scan initially. Subjects will be asked to arrive at the Marcus Institute of Integrative Health in the morning on the day of the study. A signed informed consent form will be documented after all questions have been answered. Women of childbearing potential must have had a negative pregnancy test within 48 hours before proceeding with the PET study. An intravenous catheter will be inserted and capped. FDG (4-8 mCi) will be injected intravenously. After injection of the FDG, the venous catheter will be removed, and then the subject will be asked to sit comfortably in a chair in a dimly lit room for approximately 30 minutes to allow for the uptake of the FDG. Subjects will receive an MRI to assist with anatomic delineation. In addition, the MRI session will be utilized in order to obtain data assessing functional connectivity, tractography, and brain volumes.

Image Acquisition and Processing – The FDG PET scan component will be obtained over approximately 30 minutes on the Siemens mMR PET-MRI scanner. This will allow for simultaneous acquisition of both the FDG PET data and MRI data (please note that the MRI acquisition is longer so some scanning will take place after the PET scan is completed while the patient continues to lie on the imaging table). The FDG PET scan will enable us to obtain quantitative regional metabolic values as determined by a commercially available software program called MIM neuro that quantifies uptake and compares the results to a normative database.

3.7.3 MRI Procedure

The imaging protocol will be performed simultaneously with the FDG PET scan and will include the following scans: On follow up days, only the functional MRI sequences will be run. The following scans will be obtained: (1) Localizer scan. (2) T1-weighted MPRAGE sequence. (3) DTI scan with 64-gradient diffusion-weighted (HARDI) sequence. (4) Resting state BOLD scan.

(5) Perfusion ASL imaging for perfusion assessment. The MRI components will be performed at 3 and 6 months after the initial scan. Total scan time including set-up will be approximately 1 hour. The MRI data will be analyzed at each time point (0, 3, and 6 months) according to the following:

3.7.4 Blood Work

The patient will need to have blood drawn to check for markers of inflammation. This will require approximately 4-6 T. (2 Tubes) of blood which will be sent off to the lab.

3.8 Statistical Considerations

The initial analyses of the data will be descriptive in nature. Using means, standard deviations, median, and range, the clinical scores will be described for each time point. Graphical methods, such as plots of measurements over time, histograms, and boxplots are important tools for understanding the quality of the data, and assessing assumptions underlying statistical models (such as normality). Transformations will be applied as necessary to satisfy these assumptions. Plots of all of the measured variables over time will be important to assess longitudinal patterns of change.

FDG PET data is only obtained initially so the PET data will be utilized for evaluating initial brain function and particularly to assess for focally increased or decreased metabolism. Scans will be evaluated visually by a trained nuclear medicine physician to observe for specific areas that have increased or decreased metabolic activity. PET scans will be coregistered with the MRI to aid in anatomical localization. In addition, all PET scans will be analyzed using the commercially available MIMneuro software program that determines metabolic activity in over 100 regions and compares them to a normative database. Metabolic activity is scored according to standard deviations above or below the mean of the normative group. This allows for a quantitative assessment of areas that are increased or decreased in metabolic activity. Patients will be assessed as to whether the majority of abnormal brain structures have increased metabolic activity suggesting neuroexcitotoxicity and inflammation or decreased metabolic activity suggesting neuronal damage.

3.8.1 Functional Connectivity Analysis Using the Resting-state BOLD Imaging:

In an effort to uniquely describe the communication between resting state networks without the influence of noise contaminants, a specialized analysis pipeline is required. This process starts with spatial preprocessing using SPM12 (Wellcome Group, UCL) in the Matlab environment (Mathworks, Inc.). Realignment and slice timing correction will be performed, ideally concurrently, to ensure proper voxel to voxel correspondence as well as adjusting for timing inconsistencies within single-shot EPI data. The data is next segmented to create gray matter, white matter, and cerebro-spinal fluid (CSF) maps to co-vary out confounding temporal effects. Warping to a standard template space (MNI) will be performed as well as spatial smoothing with a Gaussian kernel. Seed regions of interest (ROIs) will be defined by the brain areas which are found to be activated and/or deactivated during task performance in the fMRI scans (BOLD, or

ASL). Specifically, time series from the resting-state BOLD scan will be extracted from the activated/deactivated ROIs (such as PCC, vACC, MPFC, and MTLs) defined in the fMRI scans. Each such time series will then be used as a covariate of interest in a whole-brain, linear regression, statistical parametric analysis. In particular, we will be most interested in DMN which were found to involve brain regions that were deactivated during task performance.

3.8.2 DTI Analyses:

The proposed acquisition scheme for diffusion imaging will enable for analysis of three distinct datasets. Rigid body motion correction will be performed using SPM to facilitate a more accurate tensor estimate. Quantitative DTI maps will be calculated for each slice, including three eigen value maps (λ 1, λ 2, λ 3), radial diffusivity ((λ 1 + λ 2)/2), mean diffusivity or apparent diffusion coefficient (ADC) ((λ1+ λ 2+ λ 3)/3) and fractional anisotropy. These indices will be calculated using the 30 directions at a b-value of 700s/mm2 through a non-linear least squares fit using dipy. Outlier rejection will also be used to eliminate spurious signal fluctuations to again ensure a more accurate tensor calculation. High angular resolution diffusion imaging (HARDI) data will be used for fiber tracking of the white matter structures associated with the DMN as well as resolving fiber crossings. Analysis will be performed in dipy using the first 4 spherical harmonic terms of the diffusion signal decomposition for calculation of the orientation distribution functions. Tractography will be used to delineate tracts of interest such as those connecting the frontal lobe and amygdala by normalizing subject data to MNI space and delineating seed regions. The same seeds will be used on every participant so as to eliminate any bias from repeated drawing of ROIs. Lastly, the two shell diffusion data will be used in calculating neurite orientation and dispersion (NODDI). Using a three compartment model will enable characterization of intracellular (space bounded by membranes of neurites), extracellular (space around neurites), and CSF space. NODDI analysis will be conducted through the Camino toolbox.

3.8.3 Statistical Parametric Mapping (SPM) Method for the ASL fMRI data:

We will perform a number of analyses on the ASL fMRI data. Images will all be analyzed in SPM which will be used for both a voxel-wise analysis in order to assess changes in CBF throughout the brain, and also a region of interest (ROI) approach that will focus on the structures delineated in the specific aims. Thus, for each subject, functional ASL images will be realigned to correct for head motion. Perfusion-weighted image series are generated by pairwise subtraction of the label and control images, followed by conversion to absolute CBF image series. A mean CBF map will be generated for each experimental condition for each individual subject by averaging the CBF image series. Each subject will have multiple CBF maps corresponding to the resting, neutral, and traumatic memory conditions pre and post the intervention programs. These CBF images will be co-registered with corresponding high-resolution structural MRI, and then normalized into a canonical space (Montreal Neurological Institute standard brain) using SPM. Voxel-based analyses of the normalized CBF data will be carried out using the ANCOVA model provided in SPM. The voxel-based analysis will be performed to address the same specific aims as elaborated below regarding the ROI analysis. The CBF in each voxel at baseline will be correlated to the change in the various cognitive and

emotional scales as covariates. Third, a comparison between the change in CBF will be compared to the change in the various emotional scale scores. In all the above analyses, global mean CBF will be included as a covariate along with age and gender.

3.8.4 Physiological and Clinical Measures:

To accommodate the longitudinal nature of the data, a Heterogeneous Random Coefficients Model (33,34) will be fit for the physiological values (neopterin, 2-hydroxyguanosine, CRP), and each neurocognitive score. This model can also account for both between and within subject heterogeneity and will accommodate modeling potentially nonlinear therapeutic effects over time. The random coefficients model is more flexible in terms of modeling the changes in physiological and neuropsychological tests over time than repeated measures analyses of variance. The random coefficients model also has fewer restrictions on the correlation structure between multiple measures within subjects. ANCOVA will be used in order to determine if there are correlations between the pre-intervention neuropsychological and clinical measures and the longitudinal outcome measures after receiving the therapeutic plan. To determine if there is a correlation between changes in physiological measures and symptom severity, Pearson product-moment correlations will be computed unless the normality assumption is violated, in which case the Spearman rank correlation will be computed.

3.8.5 Power Analysis:

Repeated measures of the FDG PET and fMRI imaging in 20 regions before and after treatment will be analyzed in a linear mixed effects model accounting for the random effect of patient and correlation between before and after treatment measures. Modeling all 20 regions jointly will allow estimating the correlations among the region as part of the model estimation. The fixed effects will include the group (active treatment or controls), region, time (pre or post treatment) and their interactions, as appropriate. The fitted model will be used to compare pre-to-post differences between active treatment and controls groups separately in each region using the model-based two-sample t-test and controlling for family-wise type I error at the level 0.05. The sample size of 40 controls, and 40 in each of the treatment groups, provides 90% power to detect an effect size of 0.4 using alpha 0.05. This is consistent with our previous PET and fMRI studies that have shown a mean effect size of approximately 0.4. These power calculations assume two-sided two-sample equal-variance t-test applied to the pre-to-post Tx changes. The effect size is the ratio of the mean difference between pre-to-post treatment changes in active treatment and controls divided by the standard deviation of the pre-to-post treatment changes.

3.8.6 Assignment:

Assignments will occur via a 1:1:1 ratio of receiving the anti-inflammatory diet, NAC, or being placed into the waitlist control group.

4.0 RISKS

4.1 N-acetyl cysteine: Oral NAC has few side-effects and is commonly used as an over-the-counter supplement worldwide. Oral NAC has good, but variable absorption from the gut

(35,36), making it problematic as the sole mode of supplementation in a clinical study. Injectable NAC is used at lower doses for exercise fatigue and higher doses primarily as a liver protector. The dosages in this study are consistent with those used in the exercise physiology literature (37). Side effects increase with higher dosage, and the most common associated adverse reactions in the literature attributed to IV NAC administration are rash, urticaria, and pruritus. The frequency of adverse events has been reported to be between 0.2% and 20.8%, and they most commonly occur during the initial loading dose of acetylcysteine at dosages higher than what will be used in this study. Other side effects with greater than 1% occurrence include nausea and bronchospasm, again at higher dosages. A hypersensitivity reaction to NAC has been reported as a rare occurrence. NAC should be used with caution in patients with asthma. Please note that the oral and intravenous NAC dose used in this study is the same as what we have used in a study of NAC in women with breast cancer, patients with Parkinson's disease, and patients with multiple sclerosis. We have found the NAC to be generally well tolerated at the doses we will be using in the current study.

4.2 Anti-Inflammatory Diet

There are no known risks for following an anti-inflammatory diet. This is the standard clinical diet we recommend to all clinical patients at or Center and consists of eating more healthy foods such as vegetables, fruits, nuts, and lean meats. Perhaps the only risks of any dietary intervention are changes in the gastrointestinal system such as bloating, gas, nausea, constipation, or diarrhea.

4.3 Potential Risks of FDG PET Scan: The FDG is a commercially available radioactive tracer that will be used according to its dose, route, and indication, but results in some exposure to ionizing radiation. The amount is acceptable for the research subjects who will directly benefit by receiving full clinical reads of these scans that their referring physician can utilize for determination of prognosis and treatment planning. Subjects will be required to lie still on the imaging table for 30 minutes, which can be uncomfortable.

4.4 Risks of venous cannulation: Venous cannulation is a routine clinical procedure that carries minimal risks when performed by trained personnel. It is possible that bruising could occur in some subjects. There is a theoretical risk of phlebitis or infection, which is very remote.

4.5 Magnetic Resonance Imaging: An MRI scanner requires a strong magnetic field. MRI can be dangerous if a person has metal or metallic objects in their body. Subjects will be thoroughly screened to ensure that they have no metal in their body. Because of the magnetic field, metallic objects can move into the scanner and potentially injure the patient. All precautions are taken to ensure that no such metallic objects are in the scanning room that could result in an injury. The MRI requires the patient to lie still for approximately 1 hour, which can be uncomfortable, or be claustrophobic.

4.6 EEG: For the EEG, a cap with electrodes will be placed on the subject's head. This requires a gel to be applied to parts of the scalp and the cap can sometimes be uncomfortable or pull the patient's hair. Then, the patient will have to rest quietly for approximately 20 minutes while the EEG is recorded.

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