Physiological Effects of N-Acetyl Cysteine in Patients with Multiple Sclerosis

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1.0 INTRODUCTION

1.1 Introduction

The overall goal of this study will be to determine whether NAC will help to support cerebral function in patients with Multiple Sclerosis (MS). This PET-MRI study will utilize FDG PET to measure cerebral metabolism, along with MRI analysis, to measure metabolism and structural effects of NAC in patients with MS. We will compare these physiological changes with clinical measures to assess symptoms and quality of life. NAC will be given as per our currently open protocol in patients with Parkinson's disease (for reference refer to protocol #14D.141 entitled, Physiological Effects of Nutritional Support in Patients with Parkinson's Disease). Thus, NAC will be given as a combination of oral capsules and IV infusions of NAC which is thought to support the brain function by reducing oxidative damage associated with the pathophysiology of MS.

Multiple Sclerosis (MS) is a neurological disorder in which the white matter tracts are damaged by an autoimmune mediated inflammation process. Patients can experience one or multiple progressive episodes of MS and can have symptoms across a variety of neurological and cognitive domains. The pathophysiology of MS with regard to specific areas that are damaged is believed to be strongly associated with oxidative stress. Oxidative stress 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. When there is significant oxidative damage, as occurs in MS, there is a disruption of the blood brain barrier and destruction of neuronal cells. The brain has difficulty withstanding substantial amounts of oxidative stress because of the presence of high amounts of polyunsaturated fatty acids, low levels of antioxidants such as glutathione, and the accumulation of iron in the cells. 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 neurodegenerative process of MS.

There have been a number of approaches to treating patients with MS that primarily pertain to reducing the activity of the immune system from attacking the white matter of the brain. A number of drugs have been developed and new ones continue to be sought in the treatment of MS. However, while reducing and/or preventing immunological effects has been helpful, patients have continued to have recurrent episodes and once an episode occurs, it is difficult to determine how much functional recovery a patient might have. With regard to treatment, none of the currently available interventions attempts to modify the oxidative damage caused by the disease process itself. This could be highly important in preventing the progression of a given lesion and reducing the initial impact of future lesions.

Glutathione is the principle naturally occurring antioxidant in neurons which protects against oxidative damage. Importantly, glutathione is found to be depleted in the brain of MS patients. NAC is the N-acetyl derivative of the naturally occurring amino acid, L-cysteine, and helps to restore glutathione levels in the body and brain. In fact, an MR spectroscopy study demonstrated increased levels of glutathione result from administration of NAC.

NAC 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 (1,2), which has interesting implications for exploring fatigue and other quality of life measures related to MS patients. In animal studies, the administration of

NAC has been shown to increase glutathione levels in the mouse brain (3,4). NAC also has been shown to reduce markers of oxidative damage (5).

In humans, we have significant clinical data using NAC in patients with Parkinson's disease, a related neurodegenerative disorder that specifically affects the dopamine neurons. In our preliminary paper just published in the journal, PlosOne, we report that in our first cohort of 25 MS patients, the NAC treated group showed significantly increased dopamine function in the primary dopamine areas of the basal ganglia ranging from 4.4% to 7.8% ($p<0.05$). In addition, patients treated with NAC had significant improvements in standard clinical scores of approximately 13% ($p=0.01$). There are only a few case studies in human beings that have evaluated antioxidants in MS patients but none have explored the use of NAC. In addition, the study we are proposing will not only evaluate clinical measures of function and cognition, but will also utilize our PET-MR scanner to perform both PET imaging to evaluate overall brain metabolism and functional MRI to assess functional connectivity and the integrity of neuronal connections in the brain as the result of NAC treatment over 3 months.

This is an important aspect of the proposed study since several studies have explored the use of brain imaging in MS. MRI is typically the most useful for identifying lesions and suggesting an inflammation of the blood brain barrier. However, researchers have also noted that the MRI findings do not always show the complete picture of the pathophysiological process in MS patients. We plan to include additional MRI sequences to assess the integrity of neuronal connections and fiber tracts. The use of FDG PET has been explored in several studies that have demonstrated reduced metabolism in key brain areas including the frontal and parietal lobes, as well as the cerebellum. These effects are likely related to the disease process resulting in neuronal dysfunction. Since metabolic activity is continually changing, we anticipate being able to observe with PET imaging whether there is functional recovery in the brain of MS patients treated with NAC.

1.2 Oxidative Stress in MS

A number of studies have suggested the importance of oxidative stress in the pathophysiology of MS. 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 (6). The brain has difficulty withstanding substantial amounts of oxidative stress because of the presence of high amounts of polyunsaturated fatty acids, low levels of antioxidants such as glutathione, and increased iron content in specific areas. 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 MS may damage key cellular components such as lipids, proteins, and DNA. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are elaborated in the brain of MS patients. This process appears mediated by activated macrophages and microglia that ultimately cause demyelinisation and axon disruption. These activated macrophages and microglia produce different inflammatory and oxidative stress mediators including cytokines such as TNFα and Interleukin 1b and IL 6 along with chemokines such as macrophage inflammatory protein (MIP) 1a, monocyte chemoattractant protein (MCP) 1 and interferon (IFN) inducible protein (IP) 10). (7).

Glutathione is an important reducing agent in the neurons, which is found to be depleted in the brain of MS patients (8). 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 MS, but the magnitude of glutathione depletion appears to parallel the severity of the disease. Glial cells may also contribute to the problem of oxidative stress in MS even though such cells are protected from toxic levels of H2O2 by possessing high levels of glutathione and glutathione peroxidase that act to detoxify H2O2 to water.

Overall, there appears to be substantial evidence that oxidative stress, and associated nitrosative stress, likely play a prominent role in the pathophysiology of MS ([9]). 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 Potential Use of N-acetyl cysteine in MS Patients

NAC 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 (10,11), which has interesting implications for exploring fatigue related to MS.

Laboratory studies have suggested how NAC might have a beneficial effect in neurodegenerative disorders such as MS. For example, one study showed that NAC may reduce misfolded protein levels and ameliorate proteotoxicity through heat shock proteins (12). 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 (13). 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 fact, NAC reduced the near-complete loss of cells after exposure to dual hydrogen peroxide hits.

In humans, an MRS study of 3 patients with PD showed that blood glutathione increased after the start of an NAC infusion and reached a maximum at approximately 60 to 75 minutes (14). 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. The mean maximal percent change from baseline in brain glutathione was 55% in the MS patients and this was higher than the mean increase in patients with Gaucher's disease or healthy controls. 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 preventing oxidative damage in other neurological disorders such as MS patients.

A recent large randomized controlled trial of 123 patients in interstitial lung disease patients showed no increase in adverse effects over placebo (15). A study of 70 cystic fibrosis

patients also showed benefit to the lung function in patients receiving NAC with no significant increase in adverse events (16). All of these studies were performed with doses in ranges similar to that proposed in our study. However, in order to avoid any potential issues, we will exclude patients with a history of pulmonary hypertension.2.0

OBJECTIVES

2.1 Evaluate the physiological effects of intravenous/oral NAC in MS patients as determined by FDG PET and MRI.

2.2 To evaluate whether the physiological changes correspond with clinical changes in neurological function and quality of life.

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.

The study would recruit 25 patients (screening up to 30 in case of drop outs) with MS over a 2 year period with either relapsing remitting MS or progressive MS who do not plan to start a medication during the study, or on stable disease modifying medication (interferon, glatiramer, dimethyl fumarate, teriflunomide). We would also exclude patients who have received treatment with high dose steroids within the past 90 days for conditions other than MS. Patients will be recruited if they have a new (i.e. < 1 month) or existing lesion. Patients would undergo informed consent and then would receive a PET-MRI at our Villanova site. Scans may be performed at the Marcus Institute of Integrative Health PET-MRI scanner using standard head coils or a 32 channel research head coil (Ceresensa: London, ON) which is medically equivalent to currently available head coils, but designed specifically for the PET-MRI scanner. This head coil poses no additional risk to the patients. Patients will undergo FDG PET to assess macrophage activity in the region of the lesion(s), additional metabolic changes associated with the effects of the disease process (i.e. decreased metabolism in specific cortical areas), and MRI to assess the lesion itself.

Patients would be randomized to either receive NAC or be placed in a waitlist control group. Those patients receiving NAC would receive a combination of IV and oral NAC for 2

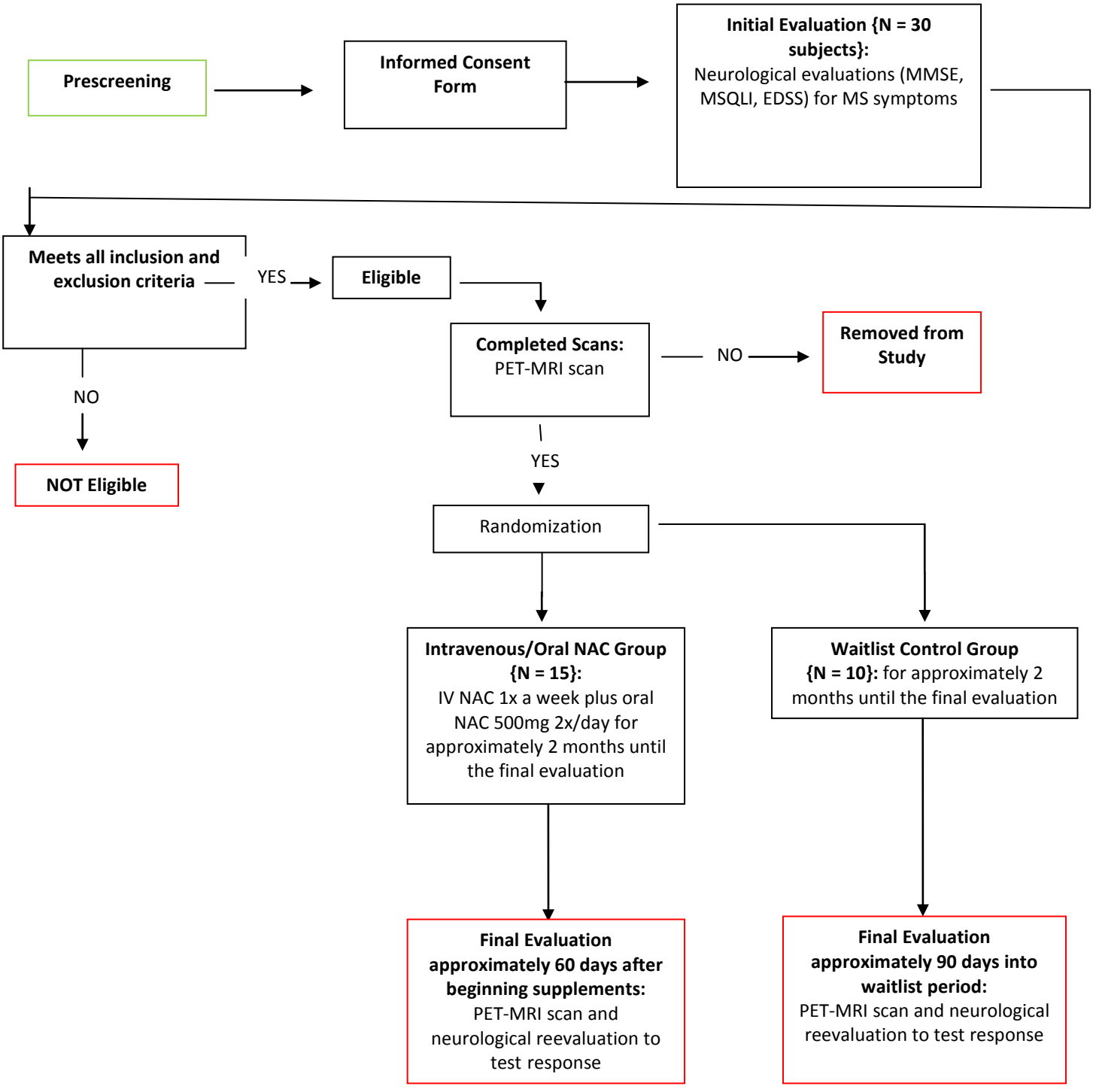
months using a model similar to what we have used in the Parkinson's study (50mg/kg IV 1x per week and 500mg oral 2x per day). Patients would undergo the same PET-MRI scanning at the conclusion of the treatment. Control subjects would undergo a follow-up PET-MRI scan two months after the initial scan. Patients would also receive a clinical evaluation which would include several questionnaires to determine the quality of life effects and clinical symptoms. The questionnaires will include the Multiple Sclerosis Quality of Life Inventory (MSQLI) and the Kurtzke Expanded Disability Status Scale (EDSS).

The analysis would include evaluation of the PET and MRI findings before and after receiving the NAC as compared to controls. The goal would be to find a shorter duration of active lesions, reduced impact of the lesions on metabolic activity in the brain, and improved parameters with regard to the inflammation associated with the active lesions based on both MRI and PET findings. Changes on the PET and MRI scans would be correlated with changes in clinical findings and quality of life measures.

Overall, this study would: 1) test the effect of NAC on active lesions in MS patients using PET-MR and 2) would provide a framework for studying the effect of additional compounds using a similar study paradigm.

The hope is that the use of NAC will physiologically support brain function and neuronal integrity in MS patients and, therefore, improve clinical symptoms. This groundbreaking study will be the first of its kind to assess the use of NAC in MS patients and evaluate the potential physiological and clinical effects.

3.1.1 Flow Chart



3.1.2 Inclusion criteria

1. Clinical diagnosis of relapsing remitting MS or progressive MS who do not plan to start a medication during the study, or on stable disease modifying medication (interferon, glatiramer, dimethyl fumarate, teriflunomide).
2. Age 30-80 years old
3. Physically independent, ambulatory
4. 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. Patients are excluded who have received treatment with intravenous steroids within the past 90 days for reasons other than MS.
2. Previous brain surgery that would interfere with determination of cerebral metabolism or structure on the FDG PET-MRI.
3. Score on Mini-Mental Status examination of 20 or lower.
4. Wheelchair-bound or bed-ridden, non-ambulatory.
5. Intracranial abnormalities that may complicate interpretation of the brain scans (e.g., stroke, tumor, vascular abnormality affecting the target area).
6. History of head trauma with loss of consciousness > 48 hours.
7. History of asthma requiring daily medications for adequate management.
8. Any medical disorder or physical condition that could reasonably be expected to interfere with the assessment of MS symptoms, or with any of the study assessments including the PET-MRI imaging.
9. Patients with evidence of a significant psychiatric disorder by history/examination that would prevent completion of the study will not be allowed to participate.
10. Patients with current alcohol or drug abuse
11. Pregnant or lactating women.
12. Enrollment in active clinical trial/ experimental therapy within the prior 30 days.
13. Pending surgery during the course of the study.
14. Patients taking medications that might interact with NAC involved in this study will be evaluated on a case by case basis by the PI or study physician. These medications include: Medications for high blood pressure; Medications that slow blood clotting; Medications for diabetes; Nitroglycerin.
15. Patients with history of pulmonary hypertension.

3.2 Registration Guidelines and Recruitment

Study subjects initially will be recruited by referral from the Jefferson Department of Neurology

and local neurology groups. 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 intravenous/oral NAC to support brain health in MS 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 PET-MRI (see below) will begin within approximately 14 business days of the informed consent process. Subjects will then receive either intravenous/oral NAC, or be placed on the waitlist control.

After approximately 60 days of receiving the NAC, subjects will undergo a follow up evaluation, which includes repeat neurological assessments, and repeat PET-MRI. Note that subjects will continue taking the oral NAC until the scans are completed. Subjects will also continue to take their current MS medication regimen so that they will continue to receive standard of care treatment throughout the study. It is hoped that 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 supplied by the Jefferson Myrna Brind Center of Integrative Medicine (produced for Jefferson by Ortho Molecular Products under GMP; Woodstock, IL) as a 500mg capsule of N-acetyl cysteine.

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 and will take 1 tablet 2 times 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 oral/intravenous NAC, or being on the waitlist for 60 days. 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: Missing a total of 5 or more days of the supplements or 5 doses of the intravenous NAC as per the PI.

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 monitored by the study manager, Nancy Wintering or other qualified staff who are designated by the Principal Investigator. Case report forms will include eligibility checklist, demographic data, baseline history, adverse events, and off-study documents. These will be completed by study staff under the supervision of the investigators or the project manager.

3.7. Clinical Response

Subjects will be evaluated utilizing the Multiple Sclerosis Quality of Life Inventory (MSQLI) and the Kurtzke Expanded Disability Status Scale (EDSS) scores to determine any improvements in MS symptoms. These will be obtained initially and after approximately 60 days of taking the supplements.

3.7.1 PET Imaging Procedure

(a) Subject Preparation - An indwelling catheter needle will be inserted into an antecubital vein. FDG will be administered through the indwelling line.

(b) FDG PET-MRI Imaging Procedure – Subjects will receive a standard of care FDG PET scan initially and after completing the NAC regimen. Subjects will be asked to arrive at the Brind Marcus Center of Integrative Medicine 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-MRI study. The intravenous catheter will be inserted and capped. FDG (4-8 mCi) will be injected intravenously. After injection of the FDG, the intravenous catheter will be removed, and then the subject will rest quietly in a dimly lit room with limited environmental stimuli for 30 minutes. Subjects will then be brought into the PET-MRI scanner. The PET acquisition will be approximately 20 minutes. The MRI component that will occur simultaneously will include standard sequences with/without contrast for MS patients.

(c) Image Processing – FDG PET analysis will be performed using MIMneuro software to quantify activity in a set of standardized regions of interest that are compared to a normative database. Specific ROIs will be placed on active lesions as detected by the MRI. The quantitative values of the glucose metabolism as determined by the FDG PET scan can then be compared between the pre and post NAC scans and between the NAC and waitlist control groups. MRI analysis will be performed to assess functional connectivity and diffusion tensor imaging results. Specifically, functional connectivity analysis using the resting-state BOLD Imaging will be spatially preprocessed using SPM12 (Wellcome Group, UCL) in the Matlab environment (Mathworks, Inc.). Seed ROIs will be defined by the brain areas which are found to be activated and/or deactivated on the FDG PET scan. 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. Psychophysiologic interaction (PPI), is also of interest and will be performed using the Conn toolbox, and is a measure of effective connectivity and how it is affected by clinical variables. 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. High angular resolution diffusion imaging (HARDI) data will be used for fiber tracking of the white matter structures associated with MS 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.

3.8 Additional Statistical Considerations

3.8.1 Descriptive and Exploratory Analyses: The initial analyses of the data will be descriptive in nature. Using means, standard deviations, median, and range, the distribution volume ratios (DVRs) will be described for each time point before and after subjects have used the nutritional supplementation for approximately 90 days. Similarly, 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.

3.8.2 Analyses for Primary Objectives: To evaluate whether intravenous/oral NAC helps to support brain function in the brain of patients with MS by measuring changes in the metabolic activity in specified regions as well as in the target lesions. To accommodate the longitudinal nature of the data, a Heterogeneous Random Coefficients Model (17,18) will be fit for the metabolic activity in each region and each neurobehavioral score (i.e. MSQLI and EDSS scores. 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 metabolic activity 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 oral or intravenous supplementation. This analysis will also evaluate whether the FDG PET or fMRI measures can be utilized as a predictor for outcome. To determine if there is a correlation between metabolic activity or fMRI 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.

3.8.3 Power Analysis: We view this study primarily as a pilot study to assess changes in physiological and clinical measures and determine the effect size for powering future, larger trials. However, we estimated the sample size needed based upon prior research we have performed evaluating FDG PET imaging in the study of neurological patients. We have previously found that the neurological patients can have variability in their cerebral glucose uptake measures of approximately 10%. In reviewing the current data, we have found approximately a 7-10% difference between the NAC and control group. If we expect a 7% improvement in the NAC group, for an 80% power to detect a significant change of $p=0.05$, we would need approximately 15 subjects in the treatment arms and approximately 10 subjects in the control arm. Therefore we will request to recruit up to 30 subjects in case of dropouts.

3.8.5 Randomization: Randomization will occur via a 3:2 ratio of the intravenous/oral NAC or waitlist control groups using the method of random permuted blocks with random block sizes without stratification.

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 (19,20), 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 (21). 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 one we have used in a study of NAC in Parkinson's patients and also women with breast cancer. We have found the NAC to be generally well tolerated at the doses we will be using in the current study.

4.2 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. Few, if any, adverse effects have been reported with the use of FDG. Subjects will be required to lie still on the imaging table for ~30 minutes, which can be uncomfortable.

4.3 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.

4.4 Magnetic Resonance Imaging: MRI will be performed in our PET-MRI scanner that requires a 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 45 minutes, which can be uncomfortable, or be claustrophobic. Due to the strength of the magnetic field of the MRI, there is a risk of being injured by receiving a burn on your skin.

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