# Erythropoietin lowers C4a, corrects refractory symptoms and normalizes selected abnormal brain chemistry in Chronic Fatigue Syndrome

## INVESTIGATOR INFORMATION

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## Background

Indication/Disease State: With its origins in a landmark paper from the CDC in 1994, the common chronic illness, called Chronic Fatigue Syndrome, has been defined as a particular grouping of multiple symptoms from multiple systems with no known cause. Fatigue dominates the case definition, with chronic non-restorative, non-restful sleep, cognitive impairment and post-exertional malaise (delayed recovery from normal activity) important clinical elements. No therapies have been shown to be curative, in part because no common mechanism of disease causation has been demonstrated.

Our group, working as the Chronic Fatigue Center, under the research auspices of our non-profit organization, the Center for Research on Biotoxin Associated Illnesses (CRBAI), has been collecting data on patients with chronic, fatiguing illnesses since 1997. To date, we have evaluated over 6000 patients with chronic fatigue, collating lab data on nearly all. We do not agree that the current case definition of CFS is adequate, as we now can define the physiologic abnormalities invariably seen in CFS. Correction of the physiologic abnormalities improves the clinical status of CFS patients. We feel that the data clearly show the importance of abnormal activation of innate immune responses in the pathogenesis of CFS. We have demonstrated haplotypes of HLA DR analyzed by PCR that are found in cases at a significant increased frequency compared to controls; we know that deficiency of melanocyte stimulating hormone (MSH) is found in over 96% of cases and less than 5% of controls; elevated C4a (split product of complement activation, also called an anaphylatoxin) is found in nearly 100% of cases and less than 5% of controls; vascular endothelial growth factor (VEGF) is undetectably low in the preponderance of CFS patients and is nearly always normal in controls. A long available, but little used test from the field of neurotoxicology, visual contrast sensitivity (VCS) will show distinctive deficits in greater than 92% of CFS patients and less than 1% of controls. Correction of these abnormalities improves the clinical symptoms of patients with CFS.

Some of our patients, those labeled herein as having refractory CFS (RCFS), have not improved with any intervention to date. For them, we would have agreed with much of the published literature that asserts that RCFS has no beneficial therapies available. Anecdotal use of low doses of erythropoietin (epo), however, has demonstrated an impressive salutary response in CFS patients who have elevated levels of C4a persistent despite all biotoxin illness therapies. Improvement in symptoms, C4a levels and abnormalities in myoinositol, lactate and ratios of glutamate/glutamine demonstrated by magnetic resonance spectroscopy (MRS) in frontal cortex and hippocampus all followed concomitantly with use of low dose epo. Because (1) we found no adverse health effects and because (2) we saw impressive clinical response following use of low dose epo in RCFS patients, we now wish to investigate the possible benefits and potential adverse effects of use of low dose epo in a formal study. We will use known clinical information about potential adverse effects of epo since it has been used for over 10 years in treatment of anemia caused by renal failure, HIV, chemotherapy and the so-called, "anemia of chronic disease," as well as an adjunct to management of peri-operative blood loss.

Research into the mechanisms of illness development and persistence in our cohort of patients with chronic fatiguing illnesses has demonstrated a shared final common pathway of pathogenesis, beginning with inflammatory activation of innate immune responses, likely mediated by Toll-like receptors, particularly pro-inflammatory cytokines and release of anaphylatoxins C3a and C4a, that leads to a predictable set of laboratory abnormalities, including deficiency of the hypothalamic hormones MSH and vasoactive intestinal polypeptide (VIP); dysregulation of ACTH/cortisol; dysregulation of ADH/osmolality; colonization of deep aerobic nasal spaces with biofilm-forming, multiply antibiotic resistant coagulase negative staphylococci; deficiency of VEGF; marked increased incidence of autoantibodies to gliadin, cardiolipin and myelin basic protein compared to control populations; elevated MMP9; elevated IL-1B, IL-10 and increased alpha interferon. With the exception of elevated C4a, each of these abnormalities can be targeted and improved by a series of sequential interventions. The target populations for this study are the patients with persistently elevated levels of C4a, found in over 99% of patients with RCFS.

In this proposal, we will introduce the concept of low-dose epo therapy to demonstrate efficacy in reducing symptoms and C4a in three weeks in a group of patients that, on average, have seen 10 health care providers previously without benefit. This study will be quickly followed by another study on titration of epo over time in the 80% of these patients who relapse when epo is discontinued after the first round of five injections. This second study will follow patients over 6 months to show stability of benefit and safety. A third study will be a double blinded, placebo controlled, crossover clinical trial to confirm safety and efficacy. A fourth study, use of epo to block hyperacute effects of rising C4a following environmental exposures, would be possible simply by formalizing what the author does now on a daily basis. The data set on these patients would be relatively easy to collect, given the patient base of the author's practice.

C4a assays will be performed in the lab of Dr. Patricia Giclas at National Jewish Research and Medical Center, Denver, Colorado. Dr. Giclas has studied C4a for over 5

years; she has agreed to be a co-author of any paper that would be generated by this work.

**Expected or anticipated clinical benefit:**

Marked clinical improvement correlates precisely with reduction of C4a, an effect that we can correlate solely with the intervention with epo. We will include recording of neurologic effects, as resting tremor is present in about 50% of patients, and rheumatologic effects, as inflammatory joint disease that looks like RA, but isn't, responds markedly to C4a reduction with epo therapy. Anecdotally, the purported "neuroprotective effects" of epo correlate precisely with C4a reduction, including (1) patients to date with Parkinsonian-like tremors; (2) atypical complex partial seizures; (3) symmetric peripheral neuropathy of unknown cause and in Type II diabetics; and (4) cognitive disorders labeled as "toxic encephalopathy."

Benefits of the study include, but are not limited to:
(1) identification of differences in clinical course before and after treatment
(2) identification of reduction of C4a after treatment
(3) identification of change in CNS metabolites after treatment
(4) there may be no benefits to an individual patient for participation in the study.

**Rationale for dose or dosing schema**

Using 8000 units SC twice weekly for 5 doses, we have observed symptom reduction and reduction of C4a in > 90% of the RCFS patients, with sustained benefit after 5 doses found in 15%. Of the 10% who do not respond with symptom reduction, all to date have had C4a levels that were significantly higher than the group mean of 10,000 (normal < 2830 ng/ml). A second course of 5 injections of 8000 units given twice weekly has shown that the failure of the first round of injections was due to the magnitude of the abnormality in C4a. For those who relapse after the initial protocol, re-treatment with 4000 units given every 4-5 days has shown ongoing maintenance of benefit.

**Methods**

An open label study design will be employed. Patients presenting at a single clinic for ongoing treatment of refractory chronic fatiguing illness for whom an extensive database is already present will be offered treatment with low dose epo in a non-randomized trial.

Data will be extracted by the investigator to an Excel file that does not include any protected health information. No increased risk to previously treated patients will accrue from data extraction and data analysis.

**Study Population**

Subjects may be enrolled into the clinical study regardless of gender, race or occupation.

## Objectives

Primary objective: Reduction of C4a by use of low dose epo, 8000 units given subcutaneously twice weekly for a total of 5 doses

Secondary objectives: (1) reduction of symptoms (2) delineation of mechanisms of CFS involving innate immune responses (3) documentation of safety of low-dose epo in patients without anemia (4) expansion of data base on neuroprotective effects of epo (5) establishment of data base on anti-inflammatory effects of epo in patients with innate immune response basis of rheumatologic disease (6) expansion of knowledge of correlation of abnormalities in central nervous system metabolites with peripheral physiologic disturbances

## Overview of study

**Sample Size:** 50 patients, not randomized, known cases of CFS, previously treated without benefit, not enrolled sequentially. The study will evaluate C4a response, levels of CNS metabolites and symptoms in patients without improvement from any prior therapy.

**Criteria to be used for response evaluation:**

- symptom recording by physician (not a checklist) at each visit
- physical exam at each dose to include recording evidence of clotting
- hemoglobin at each office visit to assess absence of erythrocytosis, defined as capillary hemoglobin measurement > 16.5.
- blood pressure at each visit to show stability
- measurement of C4a at each visit to show rate of decline of C4a with treatment.
- repeat magnetic resonance spectroscopy following therapy
- A final assessment in-office at 7 days post completion to review durability of improvement.

## Study design

Open-label, non-randomized clinical treatment trial in known refractory CFS patients

## Statistics

The statistical analysis of the data for each patient reported in the study will consist of multivariate analysis of variance methods appropriate for repeated measures designs. This will include both across subject comparisons to assess both the effect of the treatment with low dose epo on (1) symptoms, (2) VCS, (3) C4a and (4) CNS metabolites and within subject comparisons to assess importance of lab test results on individual

illness. The Bonferroni correction will be applied to the results of the analysis of each study endpoint to correct for the fact that multiple tests for each patient are being evaluated. The level of significance used will be $p < 0.05$.

**Inclusion criteria:**

- Diagnosis of CFS refractory to all prior therapies
- C4a > 2830 ng/ml in RIA assay performed by Dr. Giclas
- MSH level < 35 ng/ml in assay performed by LabCorp
- Age > 17
- No contraindication to use of epo
- No evidence of hemoglobin > 16.5 before or during study
- Normal renal functions
- No recent surgery
- No recent blood loss requiring transfusion
- No history of hypertension
- No clinical diagnosis of HIV

**Exclusion criteria:**

- Pediatric age group
- Pregnant or nursing
- Not using contraception if female and of child-bearing age
- Uncontrolled hypertension
- Polycythemia; entry hemoglobin > 15.5 or hemoglobin exceeding 16.5 during study
- Osmolality >305
- History of myocardial infarction, stroke or deep venous thrombosis
- History of renal failure
- History of HIV
- Presence of hepatitis B or C
- Diagnosis of cirrhosis, any type
- History of orthopedic, central nervous system, head and neck, abdominal, thoracic or pelvic surgery within 60 days
- History of placement of intravascular stents
- Ongoing immobility or paralysis
- Diagnosis of cancer requiring chemotherapy, radiation or surgery other than basal cell and squamous cell carcinoma
- History of major blunt force trauma within 90 days
- Presence of indwelling vascular lines, including PICC and CVP lines
- History of ischemic or congestive cardiomyopathy
- History of pulmonary embolus
- History of retinal arterial or venous thrombosis
- History of thombocytosis and platelet-induced illness ITP, and TTP.
- History of vasculitis
- History of collagen vascular diseases rheumatoid arthritis and SLE

- Current untreated iron deficiency
- Diagnosis of drug abuse
- Diagnosis of alcoholism
- Participation in experimental drug trial within 60 days

**Dosing**

**Dose: 8000 units Frequency: Twice weekly Duration: 5 doses**

**Safety and efficacy parameters**

- Safety Data:

(1) Absence of significant rise in hemoglobin/hematocrit
(2) Absence of thrombotic events
(3) Absence of elevated blood pressure
(4) Absence of development of iron deficiency

Assessed during each office visit

- Efficacy endpoints: Each office visit would record
  (1) symptoms
  (2) hemoglobin,
  (3) blood pressure
  (4) lack of evidence of clot formation (deep vein assessment, adverse events of any kind)
  (5) Endpoints are reduction of symptoms, reduction of C4a and correction of abnormalities in CNS metabolites myoinositol, lactate and glutamate/glutamine
  (6) Absence of change in VCS
  (7) Absence of change of CNS metabolites N-acetyl aspartate; creatinine; choline

**Laboratory studies:**

Nationally recognized, CLIA-approved high complexity including LabCorp and Quest Diagnostics

**MR spectroscopy:**

Performed with 1.5 Tesla coil at Progressive MRI, Salisbury, Maryland

**Follow-Up Evaluations:**

No follow-up evaluation is reported.

**Withdrawal/Premature Discontinuation**

No evaluation beyond treatment with five doses of epo and one week follow-up will be reported. There is no penalty for voluntary withdrawal by the research subject. Patients may be withdrawn by the investigator if there is evidence of adverse effects following use of low dose epo.

**Privacy**

All study data will be reported without use of any protected health information. Patients will be identified by number assigned by the site and kept confidential by the site. Identification of subjects by sequential numbers will be kept in two registries. Data will be protected in a data base password secured in the study computer. Patient records are stored as other charts in the office under lock and key.

**Confidentiality**

No results will be released by any individual to any individual, organization or entity. The FDA or Copernicus Group IRB may review study charts at their discretion, **so absolute confidentiality cannot be guaranteed**.

**Informed consent**

Each individual must sign an informed consent document approved by Copernicus Group IRB before inclusion of the individual data in the group data can reported unless a waiver is obtained through the Copernicus Group IRB. The waiver applies under Code of Federal Regulations 46.116 section (d), as this study meets the following criteria

1. the research involves no more than minimal risk to the subjects
2. the waiver or alteration will not adversely affect the rights and welfare of the subjects
3. the research could not practicably be carried out without the waiver or alteration
4. the subjects will be provided with additional pertinent information after participation whenever appropriate

**Definition of an Adverse Event**

An AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (that could include a clinically significant abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

An AE does include a/an:

- exacerbation of a pre-existing illness.
- increase in frequency or intensity of a pre-existing episodic event or condition.
- condition detected or diagnosed after study drug administration even though it may have been present prior to the start of the study.
- continuous persistent disease or symptoms present at baseline that worsen following the start of the study.

An AE does not include a/an:

- medical or surgical procedure (e.g., surgery, endoscopy, tooth extraction, transfusion); the condition that leads to the procedure is an AE.
- pre-existing disease or conditions present or detected at the start of the study that do not worsen.
- situations where an untoward medical occurrence has not occurred (e.g., hospitalizations for cosmetic elective surgery, social and/or convenience admissions).
- the disease or disorder being studied or sign or symptom associated with the disease or disorder unless more severe than expected for the subject's condition.
- overdose of either study drug or concurrent medication without any signs or symptoms.

**Definition of a Serious Adverse Event**

An SAE is any adverse event occurring at any dose that results in any of the following outcomes:

- death
- a life-threatening adverse event
- inpatient hospitalization or prolongation of existing hospitalization
- a disability/incapacity
- a congenital anomaly in the offspring of a subject who received drug
- important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse

Clarifications:

- "Occurring at any dose" does not imply that the subject is receiving study drug.
- Life-threatening means that the subject was, in the view of the investigator, at immediate risk of death from the event as it occurred. This definition does not include an event that, had it occurred in a more severe form, might have caused death.

- Hospitalization for elective treatment of a pre-existing condition that did not worsen during the study is not considered an AE.
- Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization, the event is an SAE.

"Inpatient" hospitalization means the subject has been formally admitted to a hospital for medical reasons. This may or may not be overnight. It does not include presentation at a casualty or emergency room.

- With regard to the last bullet above, medical and scientific judgment should be used in deciding whether prompt reporting is appropriate in this situation.

Death occurring for any reason during a study, including death due to a disease-related event, will always be reported promptly.

**Emergency procedures**

The test center is an outpatient physician's office. RCFS patients are seen as part of the daily patient mix of a busy Family Practice office. There is no reason to expect study patients to present a greater risk for emergency care than other patients in the practice. The center is close to the local ambulance squad, with 911 response time less than 5 minutes. The office is equipped with basic life support equipment.

## References

Attached is a partial list of papers on use of erythropoietin as a neuroprotective agent and an anti-inflammatory agent reviewed in support of this application

## Questions

Any questions regarding participation should be referred to the study investigator.

## Study Investigator

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