FIBROMYALGIA / CHRONIC FATIGUE SYNDROME AND LEAKY GUT. SUMMARY OF CLINICAL TRIAL DESIGN. Double-blind randomized placebo-controlled challenge with gluten and milk protein in patients responders to gluten free and milk protein free diet.

The objective of this document is to propose a clinical trial on fibromyalgia and gluten sensitivity, in order to find the necessary resources to carry it out. General methodology is described.

Fibromyalgia and chronic fatigue syndrome are very common problems of unknown etiology, causing fatigue, severe chronic pain, functional limitation and disability. There is no known treatment capable of solving the problem. Treatment is symptomatic and generally has little benefit. It causes significant expenditure of health resources without clinical benefit, in addition to heavy social and labour costs.

Gluten sensitivity without diagnostic criteria for celiac disease is a recently recognized pathology, the clinical manifestations of which include symptoms of chronic fatigue syndrome and fibromyalgia. There is no diagnostic test that confirms or rules out gluten sensitivity.

In the experience observed in an open follow-up study with gluten-free diet-based intervention in more than 200 patients with fibromyalgia without diagnostic criteria for celiac disease, very significant improvement has been observed in 36% of patients. The observed improvement has included remission of fibromyalgia, recovery of normal life, and return to work. This improvement has been observed after a long period of a very strict gluten-free diet of at least six months in duration. Previous analytical, genetic, and duodenal biopsy studies have been performed. The available data allow us to hypothesize that there is a clinical - genetic - and histological profile that predicts the response to a gluten-free diet (gluten sensitivity profile). The first clinical observations have already been published.

For this clinical observation to be applicable in general clinical practice and accepted by the scientific community, it must be demonstrated in a clinical trial.

Currently, the diagnosis of gluten sensitivity or leaky gut is arrived at on clinical basis due to the absence of a specific marker. A long period of restrictive diet is needed to know if the patient responds, and the benefit can be only partial. Biological markers that predict response to diet and optimization of treatment strategy are needed. Genetic HLA typing and light microscopy study of duodenal biopsies do not meet these objectives. New technologies for research are:

Intestinal microbiome study with massive sequencing

Serological markers of intestinal permeability

Morphological markers of mucosal barrier dysfunction: electron microscopy and immunohistochemistry for tight junctions, in duodenal biopsies. The study will consist of two phases:

Open label pragmatic uncontrolled study with gluten free and milk protein free diet in patients with fibromyalgia / chronic fatigue syndrome.

Randomized, placebo-controlled double blind challenge in patients who have responded to diet.

General Objectives

To demonstrate, with adequate methodology, the efficacy of diet based treatment on the model of gluten sensitivity and leaky gut in patients with fibromyalgia and chronic fatigue syndrome.

To search for biomarkers that can predict the response to diet, and improve the understanding of physiopathology and treatment strategies for these patients.

Design of the first phase.

Pragmatic clinical trial with open intervention.

Patients who are referred to the generalized pain clinic of the Hospital Puerta de Hierro Majadahonda, Madrid, Spain, will be proposed for inclusion. Clinical and basic research variables (markers of intestinal permeability, intestinal biopsies, intestinal microbioma) will be evaluated prior to intervention.

The intervention will be gluten free and milk protein free diet. Cereal, corn and manufactured gluten free products will be restricted. Multivitamin and mineral supplementation will be recommended, particularly iron, vitamin D and B12, copper and zinc. Diet compliance will be supervised by the Madrid Celiac and Gluten Sensitive Patients Association. Withdrawal of medications that can induce musculoskeletal pain, such as statins, or intestinal injury such as ARA 2 inhibitors or Montelukast will be recommended.

This study will make it possible to assess in what proportion of patients the strategy has clinical benefit, and also if it is useful from the point of view of social and health care costs. It will also search for biomarkers that predict response to diet and improve our understanding and treatment strategies for these patients.

Inclusion and exclusion criteria

The inclusion criteria will be fibromyalgia and/or chronic fatigue syndrome of more than one year of evolution, in patients aged between 18 and 65 years, with a score on the fibromyalgia impact questionnaire higher than 50/100, and with clinical data of gluten sensitivity or leaky gut.

Patients on sick leave or diagnosed with depression, dysthymia or somatoform disorder not excluded. Patients with systemic autoimmune disease will be considered for inclusion if conventional treatment of the autoimmune disease has not achieved relevant improvement of fibromyalgia / chronic fatigue.

Patients with diseases that are a confounding factor for the evaluation of clinical improvement, such as previous spinal surgery, polyarthritis or severe psychiatric illness, will be excluded. Patients with B, C or HIV viruses comorbidity with serious medical conditions such as cancer or infection will be excluded.

Outcomes

The primary outcome will be relevant clinical improvement defined as:

> The patient reports that he/she has clearly improved and has also achieved at least one of the following goals: Being asymptomatic; remission of permanent generalized chronic pain, resumption of normal life if previously impaired; return to work if unable to work; transition from limited life in bed or wheelchair to walking; recovery of independence for personal hygiene care if dependent on others; withdrawal of opioids, remission of severe asthenia that limits life, remission of mental fatigue that limits life.

The secondary outcome variable will be the change in pain and function assessment questionnaires (Fibromyalgia impact questionnaire, and visual analog scales of pain, fatigue, and mental fatigue).

Definition of gluten sensitivity / leaky gut profile: In the patient selection phase, a gluten sensitivity profile study will be performed, which is defined as meeting at least one of the following characteristics, and exclusion of celiac disease. Anti-transglutaminase antibodies and anti-gliadin-deaminated peptide antibodies must be negative and villous atrophy must have been excluded in duodenal biopsy.

<u>Characteristics:</u>

Presence of HLA DQ2.5 (DQA1*05 DQB1*02) in cis or trans, or DQ2.2 (DQA1*02 DQB1*02) or DQ8 ((DQA1*03 DQB1*0302).

Marsh type 1 enteropathy in duodenal biopsy defined as: at least 25 intraepithelial lymphocytes per 100 enterocytes in anti-CD3 staining.

Family member in the first or second degree with a diagnosis of celiac disease.

IgA deficit.

Anti-gliadin IgG or IgA positive antibodies, or PDG + TGT positive screening test, with specific serology (anti transglutaminase and anti gliadin-deaminated peptide) negative.

Personal history of psoriasis.

Recurrent oral aphthae

Recurrent vaginal candidiasis.

Digestive symptoms (irritable bowel, abdominal distention, dysmotility dyspepsia, gastroesophageal reflux) refractory to conventional treatment.

History of chronic or repeated iron deficiency and/or anemia.

Severe migraine.

Persistent unexplained liver transaminases elevation.

Lower than normal levels of at least two of the following: cholesterol, ferritin, folic acid, B12, Cu, Zn.

Pre-inclusion evaluation

Clinical evaluation

Analytical evaluation

HLA typing, locus DQA1, DQB1, DRB1.

Gastroscopy and duodenal biopsies. Nonsteroidal anti-inflammatory drugs (NSAIDs) will be withdrawn prior to gastroscopy. A helicobacter test (breath test, urease test, and gastric biopsy) will be performed, but there will be no need to treat the helicobacter and repeat duodenal biopsy.

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Exclusion criteria: infection with C virus, B virus, severe tumor or infectious disease, rheumatological disease defined as rheumatoid arthritis, Sjogren's or systemic lupus

erythematosus, or generalized osteoarthritis that may explain the patient's symptoms. The inclusion of patients with fibromyalgia and autoimmune analytical data without defined rheumatological disease is permitted. Previous psychiatric diagnoses such as depression, anxiety, adaptive disorder, somatization, somatoform disorder, personality disorder are not criteria for exclusion.

Design of the second phase.

It will be a double-blind randomized placebo-controlled clinical trial challenge with gluten and milk proteins.

During the trial, the treatment and diet with which clinical improvement had been achieved will remain unchanged.

The inclusion criteria will be relevant clinical improvement in the first phase of the study. The duration of the provocation will be three months if the patient tolerates it, or until clinical deterioration. The main outcome measure will be worsening of fibromyalgia / chronic fatigue syndrome.