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SINDROMES DE SENSIBILIDAD CENTRAL

RESEARCH PROJECT PROPOSAL

Characterization of immunological biomarkers in Encephalomyelitis Myalgic / Chronic fatigue syndrome

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Introduction

The myalgic encephalomyelitis/syndrome of chronic fatigue (CFS/EM) is a serious disease that affects in a physical and cognitive way, it is disabling and by so far is exclusively diagnosed based on symptoms (1-3).

In spite the World Health Organization (OMS) classifies it as a neurological disease (ICD G93.3), the term CFS is still mostly using, considering only one part of the whole illness, and giving greater importance to one aspect: the fatigue, which appears in many other pathologies, such as cancer or multiple sclerosis; in fact, latest research have shown a general inflammation and a systemic neuropathology (1).

Up to now, the diagnosis is carried out on the basis of a continued (of at least 6 months of duration) period of fatigue, not being the result of recent exercise, which does not subside with a rest, and that affects in a significant way the life of the affected ones (2).

The most recent criteria, those of the international consensus (1), take out the duration of 6 months, although they are not developed at the moment.

There is no consensus about the disease etiology, covering among others, the viral type, mainly the herpes virus one as EBV, CMV and HHV-6 (4-8), enter virus (9-11) once the XMRV rejected (12).

Relative improvements with antiviral treatment (13-15) has been obtained, and recently the use of Rituximab, used for the lymphoma treatment, a monoclonal antibody, has been shown effective in a significant percentage of patients (16,17) and in which it is evident the role of the autoimmunity (18).

Despite it has been failed to establish the cause of the EM/SFC, an immune involvement is notorious (19-21), as well as the profile of altered cytokine (22-24). Particularly NK cells have a lower lythic function, and an altered expression of receptors (25-29), whereas cells T has been observed a lower presence of CD8 effectors cells, and an over expression of regulatory CD4 cells (30). Finally, B cells seem to have an active role in pathology, although their functional and phenotypic alterations are not definitely established (31).

Background

By the project called "Comparison of biomarkers in EM/SFC", promoted by ASSSEM, it was the aim to create a framework in which to continue and expand the preliminary study carried out by IrsiCaixa, in relation to different immune abnormalities in a small group of sicks by EM/SFC (30).

Through this comparative study, were could determine different immune subpopulations (see methodology) in 193 participants and 20 healthy controls, as well as different herpes virus serological determinations (see methodology) in 80 of 193 participants.

For the statistical survey and analysis of the results, we had the advice and support of IrsiCaixa, and we developed a bibliographic work which is not possible to publish in absence of certain basic elements for this purpose (see objective).

The preliminary results obtained, which confirm immunological abnormalities in at least 2 of the subpopulations, which IrsiCaixa was referring in his document of 2013 from Curriu and Col. (30), it would indicate specific immunological abnormalities in EM/CFS patients, even though they wouldn't be uniform for all participants, and it could determine subgroups of involvement at this level.

Hypothesis

The analysis of these data has identified two markers of cells: Natural Killer, the CD57 and the NKp46, which present differences between patients and controls, and that could determine two subgroups of affected:

1. A major subset where NKCD57 values are low and NKP46 values are high.

2. A minority subgroup in which the values of NKCD57 are elevated and NKp46 values are low.

These observations could be explained by the reactivation of different herpes virus (CMV and EBV) that could affect in a different way the Natural Killer cells, so that about 1/3 of the affected ones, would not show altered values. On the other hand, we would like to point out that there is a correlation between high values of IgG for CMVH, and elevated values for subpopulations of T cells with the marker - CD57+

Objective

To improve certain aspects of the initial comparative study, with respect to the methodology, in order to publish the results already referred.

For this reason, at first place, it would be necessary to review the comparative transversal retrospective observational study, through two actions:

1-Increasing the number of healthy controls, in a proportion of at least 1 healthy control for each patient.

2-Improving the categorization of patients. The diagnosis of participants, both sick and healthy controls, will be made by two optional independent and sufficiently qualified physicians to do so (*), unknowing if the participant is a sick or a control one.

Criteria to be used: Canadian Consensus criteria for EM/SFC, which are internationally accepted and developed so far.

Once carried out the dual diagnosis, data will be analyzed again, elaborating a corresponding document with which to defend the hypothesis detailed and if possible, to study in depth other aspects that may be altered, to be presented to the scientific community through any specialized magazine.

Methodology

Immunofenotipated, made in *Centro de Diagnóstico Biomédico* (CDB) of Hospital Clinic in Barcelona (**) of the following immunological subpopulations in %: Lymphocytes T(CD3+CD19-); Lymphocytes B(CD3-CD19+); Lymphocytes CD4(CD3+CD4+); Lymphocytes CD8(CD3+CD8+); Ratio CD4/CD8; Lymphocytes TCD16 or CD56(CD3+CD16_56+); Lymphocytes NKT(CD3+CD57+); Lymphocytes CD8(CD3+CD8+CD57-); Lymphocytes CD8 (CD3+CD8+CD57+); Ratio CD8(CD3+CD8+CD57-)/CD8(CD3+CD8+CD57+); NK true (CD3-CD16_56+); NKactivated(CD16_56+CD3-CD69+); NKbright(CD16_56 bright); BCD38 high in IgG+; Treg classic CD25hi FOXP3+; CD5 bright on CD8+; CD69 on CD56+CD16+; NKp46 on CD56+CD16+; CD57 on CD56+CD16+.

(*) Dra. Milagros García, immunologist and family doctor, and Dr. Juan Carlos Mejía, reumatologist.

Incorporating as well the determination of % perforines in NK and in CD8 (19, 32):

Perforines in NK

Perforines in CD8

At the same time, 4 determinations more were included, related with a publication of Bradley and col. (35), in which alterations of sub-populations of lymphocytes B are detailed, as of methodologies used in the immunodeficiency common variable (CVID) (33,34)

% Lymphocytes B Naïve (over % lymphocytes) or B naïve

% Lymphocytes L Naive (over % B cells) or L naïve

% Transitional (over % B cells B) or B transitional

% Plasmablast (over % B cells B) o B plasmablast

Euroclass groups

Virical serologies made by *Laboratorios Labco/General Labs* in Barcelona (**)

IgG-CMVH; IgG VCA EBV; IgG EA EBV; IgG EBNA; IgG herpes 1; IgG herpes 2; IgG herpes 6; IgG VZT; IgG Parvo B19

Statistical treatment used

Four types of analysis will be made:

Comparative between groups, for each parameter studied. Using the Mann-Whitney test. This test indicates us whether there are differences between the median values of each group.

Of correlation in the SFC GROUP. This analysis allows us to identify parameters with a related behaviour. We use the non-parametrical Spearman test that indicates the association degree between two parameters.

Longitudinal study of the parameters, that correlationates the stability of them along the time, and for this reason the possibility as a possible biomarker.

Clusterization of the results based on the research of possible common groupings.

The statistical analysis will be made in collaboration with *Dr. Julià Blanco*, IrsiCaixa, Hospital Universitari Germans Tries i Pujol of Badalona (Barcelona).

(**) Analytical metodology used that will be included in the final document.

Budget

1. personnel expenses	Euros
Two physicians for patients diagnosis, taking part both in the research and all phases of the study (*) (*) ASSSEM will assume 3000 € of the expenses of this entry.	
SUBTOTAL	4200 €- 3000 €= 1200 €
2.- analytical expenses	
Immunological analysis to cover the ideal proportion of: 1 healthy control by 2 illnesses ones.	
SUBTOTAL 1	10.800 €
TOTAL 1	**(*) 12.000 €**

Important disclaimer: this budget is developed based on the diagnosis of 150 participants (100 illnesses patients and 50 healthy controls), and the cost of developing 30 more analytical in healthy controls. In case of overcome these ratios, the economic cost would increase proportionally to the number of participants.

(*) With the campaign promoted by ASSSEM *#objetivo12.000*, we are trying to reach the minimum amount necessary to cover the costs for the study publication.

You can contribute to this study by making donations through the website ASSSEM: www.asssem.org

Benefits of the project

If after the funding accomplishment, and after increasing the healthy controls, the results would remain on the line that we have determined with current samples, those subgroups of patients affected by EM/CFS, based on well-defined immunophenotypic criteria, could be categorized.

At the same time, inmunofenotípical characterization based on the markers described, could place the basis for more in depth studies, e.g. with the determination of subpopulations NKG2C, NKG2A and K.I.R., and/or other techniques of direct detection as the EBER-DNA in EBV, that would clarify more, reactivations by EBV and/or CMVH, which directly or indirectly – and by auto-antibodies - they could influence the etiopathogenesis of EM/CFS and other SSC, and could justify trials with rituximab in indicated cases.

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