Study protocol

Cervicocranial dysfunction, neuroinflammation and infection in ME / CFS compared to healthy subjects.

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Summary

ME / CFS is a disease based on criteria, with neurological ICD designation G93.3. [1] It's characterized by severe fatigue/exhaustion which is aggravated by even small amounts of exertion, widespread pain and neurological symptoms are also common. The cause of the disease is unknown, the incidence uncertain and the treatment options are few and symptom oriented.
The present project will include clinical findings, MRI findings, medical history and clinical examinations from patients with ME/CFS, and compare these with findings from healthy subjects. (MEPRO-project).

The main hypothesis to be tested, is that compared to healthy controls and other patient groups, patients with ME/CFS have space restrictions in the craniocervical area and signs of neuroinflammation in CSF. We will also investigate if the findings from the MRI examinations differs between regular and upright MRI, in some of the patients and healthy controls. (MELON project).

The projects are part of a doctoral dissertation at Karolinska Institutet.
The project is being implemented at ME-Center, a clinic commissioned by Stockholm County Council to assess, investigate and treat patients referred to us with a suspected ME/CFS diagnosis by their clinician, preferably their GP. The clinic is a unit within Bragée Clinics that, also
assigned by the Stockholm County Council, conducts rehabilitation for severe and complex pain conditions and severe fatigue syndromes.

The interest from patients, patient representatives and authorities, in us systematically reporting backgrounds and observations, have been big, and is also something that's required as this a new function, the patient group is large and

the disease has major personal and societal consequences in the form of personal suffering, sickness absence and impaired function in patients.

Background

Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) is a disease condition that is defined by criteria, which have varied over time, but is now usually defined with the Canadian criteria. These state that the condition can be determined in the absence of the existence of another explanatory disease, if certain symptoms are present. [2] Pronounced fatigue/exhaustion which is significantly impaired by even small exertion, pain and sleep disturbances are symptoms that are required for a diagnosis.

In addition, the condition must have lasted more than six months and be associated with symptoms from the nervous system and
cognitive symptoms, as well as manifestations from at least two autonomous, neuroendocrine or immunological categories. Internationally, the prevalence has been stated to be 0.2 - 0.4 percent, with more women than men being affected . [3] [4] [5] Information on prevalence in Sweden is lacking.

The pathophysiology of ME syndrome is unclear. Diagnostic biomarkers are missing and because there is no consensus on the etiology of the disease, differential diagnosis become important, the criteria for the syndrome are in most cases subjective and, in addition, common in several medical conditions such as fibromyalgia, neck injuries, heart failure, stress-triggered illness or psychiatric diagnosis such as 'burnout' or depression. Neuropathic components are frequently encountered when examining ME patients. [1]

In nerve pain, such as the allodynia that most patients with ME/CFS have, and when examining the potential impact from the cervical spine, a quantitative sensory measurement for sensitivity to cold and heat is a clinical method that can reveal abnormalities early on.

[12] Patients with ME/CFS may have a reduced ability to regulate the difference between an actual stimulus and the prediction that precedes that stimulus, e. g., make misinterpretations of the incoming signals, which in that case would lead to an unnatural reaction. For example, the brain interprets an incoming signal of heat as likely leading to sensations of pain later on, which is why the autonomic nervous system overreacts.

There is a great need to describe this, as well as clinical and laboratory findings, in an attempt to gain a greater understanding of the specific features of this condition, and also to get explanatory models that can lead to causal treatment.
The disease has received increased attention worldwide in recent years, but has been described since the 1960s. Media has reported about a number of suicides, where ME-patients described the powerlessness of having a diagnosis without a known cause, accepted treatment and with an uncertain or gloomy prognosis, which has contributed to an

increased understanding of the severity of the disease. No other major patient group has lower quality of life. [6] Sick leave and early retirement are very common . [7]

Research on ME/CFS has been relatively neglected. About 7,000 scientific articles are published, however, most have studied infections and immunological findings and only one article highlights a possible association between ME/CFS and neck injuries which affect the nerves. [8]

Hypothesis

The hypothesis is that the disease in a significant proportion of patients can be explained by traumatic and other changes in the craniocervical area, and sub hypotheses are that there are signs of neuroinflammation and/or infection in the blood and cerebrospinal fluid as well as a disturbance of so-called interoceptive signaling.

population

Patients consecutively enrolled at the ME-Center with ME/CFS according to the Canadian criteria
are, after getting a diagnosis, offered to participate in this study, as well as healthy controls. To begin with we are seeking so
called "friends controls", from participating patients'. Non-genetically related relatives and friends. Recruitment is ongoing until we have at least 80 patients, and at least 35 healthy controls have already been enrolled.

<u>Inclusion criteria for patients are</u>

- Age 18-75 years
- Completed an investigation at the ME-Center and received the diagnosis ME / CFS

<u>Inclusion criteria for controls are</u>

- Age 18-75 years
- Healthy without current regular medication or treatment for illness

<u>Exclusion criteria for both groups</u>

Bleeding disease or treatment with blood thinners
Contraindication for MRI examination (metal implant, claustrophobia, etc.)
Pregnancy

Estimation of patient and control group size is based on power calculation, where prevalence of neurologically or radiologically studied abnormalities to date has been about 50%, against an estimated prevalence in the general population of 25% in women and 15% in men, in healthy people, probably even lower. This means a minimum group size of about 40 participants in each group.
We assume that it will be more difficult to recruit people to the control group.
Recruitment is done through written information to patients, and if necessary in a newspaper or
Internet with texts approved by EPN.

Material and method

One main purpose is to note any constrictions in the craniocervical area, that can affect the flow of CSF and give rise to symptoms common in ME/CFS, for example headache. [9] There is also a described relationship between the state of the cerebellar tonsils and the symptoms that can occur after trauma to the head and neck. [10] Another purpose is to determine whether measuring the width of the optic nerve may have a neuroradiological correlate to pressure conditions in the brain/spinal cord space, measured with lumbar punction and symptoms.

Patients and controls that have not undergone an MRI of the brain and cervical spine during the last 6 months, for the past two years, are referred for MRI examination.
The examination is conducted at the MR unit in the Stockholm region, and is assessed by two
independent radiologists, with regards to the occurrence of constriction or other abnormal changes in the area. In particular, the position of the cerebellum tonsils is measured in relation to the line between the foramen Monrois front and rear restriction (Mc Rae line), and space of the most restricted area of the neck. The width of the optic nerve 3 mm behind the exit from the eyeball is measured and compared to the width of the eyeball. The background to these measurements is presented above.

Lumbar punction

Patients and controls undergo a lumbar punction where 10 ml of cerebrospinal fluid (CSF) is collected, with blood samples taken simultaneously . This should be preceded by MRI scans of the brain as mentioned above.
The lumbar punction is not performed if there is a well-founded suspicion of a very high intracranial pressure such as MRI brain showing midline or unblotted basal cisterns, obliterated 4th ventricle or if the four-height plate is not seen. Lumbar punction is also not performed if there is a laboratory finding of bleeding disorder (INR> 1.7; TPK <50) or treatment with antithrombotic drugs such as Waran, new direct-acting oral anticoagulants (NOAK), some antiplatelet agents and or high-dose heparin, or a local infection in the lumbar region . The risks of lumbar punction are stated as low. [11] The purpose is to collect cerebrospinal fluid for analysis of signs of neuroinflammation or infection and to measure pressure in CSF.

Lumbar punction is done according to the usual aseptic technique with the patient in lateral position and the precedence of anesthesia with 5 ml of local anesthetic in the outer duct. Pressure
is measured with a vintage hose as a siphon. Then 10 ml of spinal fluid is dropped on polypropylene tube (on ice) and mixed. 2 ml is spared with cells. 8 ml are spun to obtain pure CSF (2000g for 10 min, + 4 grC), and aliquoted into 0.5 mL Nunz tube for freezing at -80 ° C and stored in the clinic's biobank for analysis. Two tubes are transferred to Uppsala Academic Hospital for analysis of neuroinfammation.

One or two tubes are also transferred to Karolinska Hospital immunology clinic for Elisa test, and one tube to the Karolinska Hospital's unit for plasmapheresis for the analysis of

antibodies. A sufficient amount is transferred to the Karolinska Hospital's laboratory for the usual analysis of cell numbers and antibodies for Borreliosis. Any remaining sampl8 are stored in the biobank as a reserve for the above or future analyzes, in the clinic's biobank, at - 80 gr C.

Sampling
With the usual aseptic technique, 40 mL of blood volume is collected from the patient. After centrifugation this yields about 20 mL of plasma, which is aliquoted in 0.5 mL tubes and frozen at -80 ° C and stored in the clinic's biobank no. 771. Two tubes are transferred to Uppsala Academic Hospital for analysis, two tubes are transferred to the Karolinska Hospital's unit for plasmapheresis, spare tubes are stored in the clinic's biobank for redundancy and later analysis.

Examination of blood and tissue will be performed for cytokines and autoantibodies. Among other things, the cytokines CCL11 (Eotaxin-1), CXCL1 (GROα), CXCL10 (IP 10), IFN-γ, IL-4, IL-5, IL-7, IL-12p70, IL-13, IL-17F, leptin, G-CSF, GM-CSF, LIF, NGF, SCF, TGf-β TGF-α and resistin are to be investigated with multiplex. Autoantibodies to the central nervous system will be examined with immunofluorescence and with ELISA / Western Blot technique. Cells from peripheral blood will be examined with flow cytometry where they will examine activation with phenotyping panels. Hybrid technology LC-MS / MS will also be used in the analyzes.
Infection samples with antibodies for Borrelia are analyzed. .

Medical history and clinical examination
Medical history and clinical examination are carried out in the same way for patients and controls and documented in the clinic's medical record system. These include weight, height, gender, age, other illnesses, diagnoses and medical history that can be reported at group level without disclosing the identity of the patient.
Structured neurological clinical examination including mobility assessment complements other examinations and is carried out according to a special template by the examining clinician and Physiotherapist, PhD student at the clinic, all of whom are unaware of the result of each other's examinations and the result of other examinations.

In particular, the incidence of mobility (H-EDS) should be investigated as the clinical experience is that a significant proportion of patients with ME/CFS have collagenopathy-related hypermobility.

Other laboratory investigations.
These are made within the investigation of patients as clinically motivated tests. For both controls and patients the tests are taken at Karolinska Hospital's immediate lab and analyzes at Karolinska Hospital's laboratory. If the tests were taken in the last 6 months, they are not repeated.

1.<u>Laboratory examinations include</u>
a. Blood status Hb, B-LPK
b. Inflammation Surveys SR, CRP, Transferrin

c. Endocrine studies TSH, T3, T4, TRAK, B (S) glucose, Hba1C,
S-Folate, S-Ca2, S-Cortisol (morning value)
d. Liver and renal status S-ASAT, S-ALAT, S-GT, S-CDT, S-Creatinine, U-knit
e. Immunological status IgG, IgA, IgM,
f. Infection status as the presence of serology for EBV, CMV, Borrelia, Hepatitis B and C, HIV
g. S-INR, S-TPK (bleeding samples)
h. Other directed tests after medical history and clinical examination.

2. Clinical examinations
a. Quantitative examination of pain thresholds (KST) according to protocol from American College of Rheumatology.
b. Walking test according to Cooper, 6 minutes on a treadmill, this is done on the majority in both cohorts
c. Tilt test with electronic tilting board raised 80 degrees from horizontal position while simultaneously measuring blood pressure and heart rate, this is only done on patients in the ME cohort

3. Thermotest, determination of sensation, pain and tolerance thresholds for cold and heat with electronic equipment.
In nerve pain, such as the allodynia that most patients with ME/CFS have and for examining the impact of the cervical spine, a quantitative sensory measurement for sensitivity to cold and heat is a clinical method that can show changes early on. [12]
In this project this sensitivity is measured to determine sensation threshold, pain threshold and pain tolerance for cold and for heat with electronic equipment, thermo-test. That patients have difficulty perceiving cold and heat is normally included in the criteria description and is also reported by many patients. Patients with ME / CFS may have a reduced ability to regulate the difference between actual stimulus and the prediction that precedes the stimulus, i.e. make a misinterpretation, a prediction error of input signals.

If this is the case, one can suspect that this is true also for other interoceptive and exteroceptive signals, such as muscle fatigue, autonomous over-reactions to change of body position etc. This can then be investigated in future studies.
We intend therefore, to measure not only the subjective experience of stimulation with heat and cold but also HRV, blood pressure, heart rate, sweating and finger temperature simultaneously.
The tests are harmless, and stimulated cold and heat stay below the thresholds for tissue damage, and can be momentarily interrupted in case the patient experiences major discomfort.

Surveys:
1.1. Questionnaire included in the National Register for Pain Rehabilitation
1.1.1. NRS initial background issues,
1.1.2. RAND 36r

1.1.3. EQ-5D (EuroQol 5 dimension)
1.1.4. MPI, (Multidimensional Pain Inventory)
1.1.5. HADS, Hospital Anxiety and Depression Scale
1.2. Canada criteria,
1.3. ME symptoms questionnaire
1.4. The clinic's questionnaire Includes pain sketch.
1.5. Örebro Musculoskeletal Pain Questionnaire
1.6. Questionnaire on Autonomic Symptoms
1.7. MAIA, Multidimensional Assessment of Interoceptive Awareness
1.8. Fatigue severity scale, Swedish version
1.9. PIPS Psychological Inflexibility in Pain Scale [13]
Assessment of pain and pain drawings according to different methods, in particular, we intend to assess the patient's own drawings of their symptoms, as these often are corresponds to bodily correlates to nerve pain. . [14, 15]

Prospective study of findings from upright MRI with ME / CFS compared to healthy controls. "MELON"
Patients included in the study are also offered to, along with matching controls, undergo an MRI examination with the possibility of serial scans in upright position.
The reason is that the method of examination with upright MRI has become all the more common, as it provides much additional information about conditions in a body position that in ME patients often causes problems .
One can demonstrate the constriction of nerve roots, and craniocervical compression in a physiologically loaded position. [16]

The contribution of the head and musculature
corresponds to a weight load of 10-15 kilos, thus you can get a different picture of the occurrence
of Chiari malformation . [17] Patients often state that they assume a supine position mainly because it provides symptomatic relief, not due to fatigue, this would also speak to the benefits of using an upright MRI. No such study has been conducted in ME / CFS.
This study is done in a fully open camera with little risk of claustrophobia, and the equipment also allows for pictures in extension and flexion, with an extended possibility to see space ratio for spinal cord and surrounding tissues.

There is no such equipment in Sweden. The investigation is therefore done at Serena Medical Center, Cromwell Road, London, with prof. Francis Smith as primary assessor. Prof. Smith has published 250 articles on the subject since 1984 and led the first clinical the 1980 MRI study.
For financial and resource reasons, this study is limited to 20 willing consecutive patients and respective healthy subjects.

Follow up
Patients referred for ME/CFS and where the diagnosis is established are assigned RAND-36

and EQ5D 6 months and 12 months after established diagnosis, and are invited to send these in the enclosed response envelope or leave them when visiting the clinic. These are also provided to the controls.

Data protection in the studies

The data is stored in the Take Care journal system which is commonly used and approved in health care, and partly in a research database protected by the clinic where remote login is not possible other than by the main administrator, and dual identification is necessary to access the server, or individual research database. It goes through daily backups and access is limited to those who participate in research, or have
delegation to protect the technology. The clinic is ISO-9000 and ISO-14001 certified and follows
the county council and GDPR regulations as well as having a special quality assured IT consulting firm that provides backup and updated protection. Each test subject is offered to block their data with access only to doctors and affected staff at the ME-Center.

Ethical aspects

An ethical question is whether it is reasonable to include friends as controls, something that occurs in many case-control type studies [18]. Then there is uncertainty about the genetic background, we wish to refrain from subjects who have a genetic relationship. We mean that recruitment among friends and acquaintances is a support for patients. The method is widely used.

All procedures, questionnaires or examinations used in the study are made on clinical indications based on the guidelines available for these conditions, or included in routine care for these for optimal examinations and treatment. There are more than 200 differential diagnoses
which are more closely related, where fatigue and stress/exertion-induced deterioration can occur.

Findings from the studies are planned to be presented in several peer reviewed articles in scientific journals, and is to be shared in popular science and in lectures / seminars.

Clinical benefit of the studies

By establishing which clinical findings are common or distinctive to the diseaseME/CFS, diagnostics can be improved, which is mainly performed in primary care . We intend to present the results and to hypothesize about ME-specific findings or combinations of findings in the clinical examination, which can significantly improve the diagnostic specificity for a long-neglected group in health care. In the past, a diagnosis of ME/CFS has often been late and uncertain and the patients we have so far assessed at the clinic have on average ten years from symptom onset to definitive diagnosis. If our hypotheses about the relationship between ME/CFS and neck spinal cord injury are confirmed it means direct access to more targeted treatment for root causes of the disease.