NorCAPITAL

The Norwegian Study of Chronic Fatigue Syndrome in Adolescents

Pathophysiology and Intervention Trial

# Protocol

# 1. Introduction

**Epidemiology and clinical presentation**
*Chronic fatigue syndrome* (CFS) or *myalgic encephalomyelitis* (ME) is a long-lasting, disabling condition, existing in all cultures (43, 79, 90, 134). The prevalence is uncertain; however, international studies suggest a point prevalence of 9 000 to 18 000 in Norway (30), including appr. 600 children and adolescents (< 18 years of age). The male: female relation is 1:3. CFS/ME is rare in children younger than 10 years.

The clinical presentation is characterized by mental and physical fatigue, which is aggravated by minimal exercise and not relieved by rest (79, 90). Additional symptoms include headache, pain in the muscles and skeleton, attenuated concentration and memory, insomnia and orthostatic intolerance. The level of disability varies; however, a majority has long-lasting absence from school and work, and is unable to participate in normal social activities. The most disabled is permanently bedridden.

**Etiology and pathophysiology**
The underlying causes of CFS/ME remain unknown, but it is generally assumed that the condition is multifactorial, and many advocate a bio-psycho-social perspective (90). Regarding predisposing factors, increased risk seem to be related to genetic factors (18) as well as certain personality traits (121). Regarding precipitating factors, there is evidence for the importance of long-lasting infections (122) and critical life events (113). Important perpetuating factors possibly include altered immune response such as Th2 vs Th1 predominance (18) and ongoing inflammation (93), as well as endocrine alterations (22) and alterations of autonomic nervous activity (104).

**Diagnose, treatment and prognosis**
CFS/ME is diagnosed according to two main principles: 1) Identification of a typical history and symptom's description, and 2) Exclusion of differential diagnosis, preferably according to a standardized set of investigations (30). There is no established diagnostic test. Diagnostic criteria are developed for scientific purposes; the most commonly used originate from the US Center for Disease Control and Prevention (CDC-criteria) (42).

Several treatment regimens have been subjected to research. However, cognitive behavioral therapy (CBT) and graded exercise therapy (GET) remain the only ones with a documented beneficial effect in randomized controlled trials (30, 90). The patients included in these studies are generally restricted to the least disabled ones. One trial only (studying CBT) has focused specifically on adolescent CFS/ME patients (107). Intervention trials of glucocorticoids, mineralcorticoids, antidepressants, anticholinergic agents, growth hormone, immunoglobulins and nutritional regimens have not shown any beneficial effect. However, supportive patient care is recognized to play an important, independent role (36).

CFS/ME is long-lasting, and the long-term prognosis is uncertain; in a recent review of adult patients, total recovery/improvement was estimated to 5 % and 40 %, respectively (12). Adolescents seem to have a better prognosis (7), which may improve further if the patient receives professional care from an early stage (36).

**Scientific and clinical challenges, and challenges for the community**
CFS/ME constitutes a major challenge: Scientifically, there are several unanswered questions, in particular related to disease mechanisms and treatment. Clinically, the health care system encounters severely disabled patients, but has little to offer in terms of therapeutic options; this might become a source of conflict (81). Regarding the community, CFS/ME has a negative impact upon employment issues, social security systems, social networks etc.

# 2. Background and aims

**'Sustained arousal' – a model for disease mechanisms in CFS/ME**
Since 2003, our research in this area has primarily focused upon autonomic circulatory regulation and thermoregulation in adolescents with CFS/ME (125-135). The patients reported symptoms indicative of alteration of these regulatory mechanisms, and experiments revealed higher resting heart rate, blood pressure, total peripheral resistance, core body temperature and plasma concentration of catecholamines as compared to healthy controls. Orthostatic challenge resulted in a more pronounced increase in heart rate, blood pressure and total peripheral resistance among patients, whereas cooling precipitated a stronger decrease in body temperature and a smaller reduction in acral skin blood flow.

Taken together, these results indicate that CFS/ME patients have enhanced sympathetic nervous activity at rest and altered sympathetic nervous responses to moderate somatic stressors (135), in accordance with recent reports from other research groups (78, 84). The character of the responses suggests an alteration in central nervous modeling of homeostatic control circuits. More specifically, the patients seem to possess a permanent stress response – commonly labeled *'sustained arousal'* within stress theory (135).

We suggest that a state of sustained arousal might constitute a fundamental part of the underlying pathophysiology in CFS/ME, and we have developed a sustained arousal-model in an attempt of integrating research findings from different fields and traditions (127). It should be noted that this model is disputed and not "proven" in any scientific meaning. It enables, however, the deduction of hypothesis within different fields which might in turn be subjected to systematic testing. In this way, empirical evidence will accumulate that either supports or falsifies the underlying theory.

The sustained arousal-model will constitute the framework for further research projects, including those described in the following: Part A – Basic disease mechanisms in adolescents with CFS/ME, and Part B – Treatment of CFS/ME in adolescents with clonidine.

## *Part A – Basic disease mechanisms in adolescents with CFS/ME*

**Genetic predisposition in CFS/ME**
Catecholamines, corticotropin releasing hormone (CRH) and serotonin (5HT) act as neurotransmitters in various brain stem areas, influencing the stress response (46). These neurotransmitters have also been associated with the perception of fatigue (10, 15, 111). Based upon the sustained arousal-model of CFS/ME, an association to genetic variants (polymorphisms) influencing these transmitters is to be expected (127).

Twin studies have documented a moderate impact of hereditary factors in CFS/ME, but the underlying molecular mechanisms remain largely unknown. Recently, however, evidence has emerged suggesting a relation between CFS/ME and polymorphisms in COMT, MAO-A, and MAO-B (genes for enzymatic catecholamine break-down), NR3C1 (glucocorticoid receptor gene), CRHR1 and CRHR2 (CRH receptor genes), corticosteroid binding globulin gene, HTR2A (5HT receptor gene) and finally alfa-2a (norepinephrine receptor gene) (45, 73, 94, 102, 115). Furthermore, increased expression of genes involved in catecholamine effector systems has been documented (38), as well as a genetically induced activity of the 5HT transporter protein 5HTT (77).

In some areas, quite focused hypotheses regarding relations between specific polymorphisms and autonomic responses might be deduced. For instance, a well-known polymorphism of COMT reduces enzymatic activity by a factor of 4 resulting in higher plasma levels of catecholamines (67), in agreement with our observations (130). Preliminary data suggest that this polymorphism is prevalent among our CFS/ME patients (unpublished results). In this project, therefore, we will focus on possible relations between this and other well-defined polymorphisms and alterations of the stress response. Important research questions are:

a) Whether the frequency of genetic polymorphisms influencing the stress response are different among CFS/ME-patients than among healthy control subjects.
b) Whether there is an association between specific genetic polymorphisms and symptom score, in particular fatigue and pain, among CFS/ME patients.

c) Whether there is an association between specific genetic polymorphisms and objective measures of altered autonomic nervous activity among CFS/ME patients.

**Infections and immunological alterations in CFS/ME**

Strong evidence supports a patophysiological relation between infections and CFS/ME (51). Previously, Ebstein Barr virus was considered the most important pathogen (122), but recent findings indicate that several microorganisms might act as precipitating factors, such as enterovirus (17), cytomegalovirus (8), *Coxiella burnetii* (51) and *Chlamydiophila pneumoniae* (16). Studies of a Norwegian epidemic of giardiasis confirmed a relation to the parasite *Giardia lamblia* (Nina Langeland, Dept. of Infectious Medicine, Haukeland University Hospital, Bergen, Norway. Personal communication), whereas scattered case reports suggest that *Borrelia burgdorferi* and herpes hominis virus 6 may play an important role (Ola Didrik Saugstad, Dept. of Pediatrics, Rikshospitalet University Hospital, Oslo. Personal communication).

Furthermore, immunological alterations have been documented among CFS/ME patients, characterized by increased levels of type 2-cytokines (such as IL-6), attenuated NK cell activity (18, 58, 101), immunological activation (3, 66), and persistent inflammation (93). Taken together, these findings are indicative of a stimulated humoral response and an attenuated cellular response, i.e. a Th2/Th1 predominance (18).

The possible relations between the immunological and microbiological findings remain largely unknown. Based upon the sustained arousal-model, two alternative hypotheses might be deduced: a) Infections are somatic stressors, promoting a state of sustained arousal in concert with other mental and physical stressors, which in turn results in immunological alterations through altered endocrine and autonomic mechanisms (127). In addition, long-lasting infections may cause immunological alterations directly. b) Sustained arousal causes immunological alterations which in turn result in reactivation of latent infections (19). In a), infections are regarded as a causal factor, in b) as an epi-phenomenon. A third, competing hypothesis is direct microbiological damage of the central nervous system, explaining both autonomic, endocrine and immunological alterations (31).

In order to explore these possibilities, a more thorough characterization of microbiological and immunological variables in the patients group is a necessary first step. More specifically, we ask the following research questions:

a) Whether the frequency of certain infections and the level of selected immune markers are different among CFS/ME patients than healthy controls
b) Whether there is an association between certain infections, autonomic/endocrine/immunological alterations and symptom score among CFS/ME patients.

Such an explorative approach might constitute a platform for subsequent studies of more specific relations between microorganisms and pathophysiological phenomena.

**Endocrine alterations in CFS/ME**

Previous studies of CFS/ME in this field has mainly focused on the hypothalamus-pituitary-adrenal axis (HPA axis): The patients tend to have low levels of cortisol in plasma, urine and saliva (18, 22), altered circadian rhythms (28) and attenuated HPA responsiveness during stimuli that normally increase cortisol secretion (99). Studies specifically addressing adolescents are scarce; however, similar findings have been reported in this age group as well.

Other endocrine systems remain to be explored. Some studies have reported low levels of prolactin and growth hormone (29, 83); as for endogenous opioids, reports are conflicting (22). We have documented increased resting levels of epinephrine and norepinephrine (130); this finding is in accordance with another study (58), but otherwise hardly investigated. Recently, we have provided evidence of attenuated vasopressin (ADH) secretion from the pituitary, increased plasma osmolality, and increased plasma renin activity (129). However, these studies are subjected to methodological constraints.

Taken together, the documented endocrine alterations are in agreement with the sustained arousal-model. In particular, attenuated HPA axis responses are an expected finding during chronic stress, as opposed to acute stress (137). We plan to explore endocrine abnormalities further, with a particular emphasis on the HPA axis, catecholamines and regulation of osmolality. Important research questions include:

a) Whether previously documented endocrine alterations can be confirmed in adolescent CFS/ME patients.
b) Whether there is an association between endocrine alterations, symptom score, and autonomic alterations in CFS/ME.

**Cognitive impairments, neurotransmitters and functional neuroradiologic assessment in CFS/ME**

An experience of cognitive impairments is an essential part of CFS/ME; in particular, the patients describe altered short-time memory and attention. Neuropsychological tests have revealed attenuation of information processing abilities and executive function control (27, 72). Results are somewhat conflicting, however, which may be explained from methodological issues, for instance different diagnostic criteria, confounding effects of hereditary traits (such as intelligence), and different degree of co-morbidity in the study populations. Other cognitive functions, such as intelligence and visual memory, do not appear to be different among CFS/ME patients and healthy control subjects (20).

The cognitive impairments are in agreement with the sustained arousal-model (127). In adolescents, however, such impairments are not thoroughly explored, despite a potential strong negative impact upon daily living, school functioning in particular. In addition, cognitive behavioral therapy is the intervention with best evidence of beneficial effects (90). There is, therefore, several arguments supporting further research in this area.

The relation between specific cognitive functions, neurotransmitters and well-defined brain areas is rapidly evolving field of research. The documented impairments in CFS/ME patients may indicate alterations of noradrenergic transmission (73): Physical exercise promotes brain norepinephrine secretion, and is related to further decrease in cognitive function among CFS/ME patients (9). Furthermore, pharmacological attenuation of noradrenergic transmission using clonidine, improved executive functions – such as planning abilities – in one study (73).

The majority of noradrenergic neurons in the central nervous system emanates from locus coeruleus in pons and innervates several other brain areas, including amygdala and hippocampus in the limbic system, the prefrontal cortex and rostral ventrolateral medulla (RVLM) in medulla oblongata (46). Excretion of norepinephrine in these areas modulates other cerebral functions. In amygdala, for instance, norepinephrine enhances emotional memory, including the somatic stress response associated with memory of distressing events (118). Antiadrenergic drugs, therefore, inhibit activation of the amygdala, emotional memory, and somatic stress responses, experimentally demonstrated by means of functional magnetic resonance imaging (fMRI) (117). Generally, amygdala is considered pivotal in classis and operant conditioning and has been suggested to have a key role in the pathophysiology of CFS/ME (48), in agreement with the sustained arousal-model (127).

Another possible important neurotransmitter in CFS/ME is serotonin (5HT), mainly produced by neurons emanating from the raphe nuclei. 5HT are important in CNS modulation of nociceptive sensory signals, but also appears to have a role in the perception of fatigue (111). For instance, 5HT excretion increases in relation to exhaustive physical exercise (4, 25), and there are also evidence of increased 5HT neurotransmission in CFS/ME (21). Accordingly, 5HT antagonist seems to improve the experience of fatigue, in CFS/ME (112) as well as in hepatitis (86). Furthermore, the indications of increased 5HT neurotransmission in CFS/ME are in agreement with the sustained arousal-model: experimental animal studies have shown that a distressing event increases 5HT levels in amygdala and hippocampus (1,2), and this increment correlates negatively to the animal's ability to control the event (10).

Functional neuroradiological investigations have scarcely been undertaken for CFS/ME research purposes; however, there are some evidence indicating involvement of the brain areas mentioned above. For instance, Costa and co-workers reported decreased perfusion in brain stem areas (24), Tirelli and co-workers found altered glucose metabolism in the same areas (114), whereas Yamamoto and co-workers documented altered neurotransmission in limbic structures (136).

The positive effect of cognitive behavioral therapy in CFS/ME has been interpreted in different ways: some has claimed that the effect is mainly explained by an improved adjustment to a situation of chronic illness, whether others have advocated a more profound alteration of pathophysiological processes. The first alternative is supported by a study from Knoop and co-workers, showing a subjective improvement of cognitive functioning after cognitive behavioral

therapy without a concomitant improvement of objective neuropsychological test results (62). Thus, the subjective perceptions of cognitive skills might have a greater impact on disease mechanisms in CFS/ME than the cognitive skills *per se*. Altered subjective perceptions might in turn influence more profound cognitive processes, not available for standard neuropsychological testing.

In this project, important research questions include:

a) Whether adolescents with CFS/ME have altered cognitive abilities, in particular executive control functions, as compared to healthy control subjects.
b) Whether adolescents with CFS/ME have altered activation of prefrontal cortex, amygdala, locus coeruleus and raphe nuclei when exposed to cognitive tasks of various emotional content, and when exposed to different stimuli provoking a stress response.
c) Whether there is a relation between results in a) and b), selected genetic markers (such as polymorphism of the MAO-A gene), and selected endocrine variables (such as plasma level of cortisol).

**Sensory hypersensitivity and autonomic responses in CFS/ME**

Chronic pain is a main complaint among CFS/ME patients, contributing to their disability (90, 135). The underlying pathophysiology has hardly been investigated. However, Whiteside and co-workers showed altered pain threshold in CFS/ME (123); another study indicate attenuation of brain nociceptive inhibition (70). Taken together, these results have been interpreted as evidence of central sensitization (71), corresponding neatly with the sustained arousal-model, in which sensitization for various somatic signals are regarded a core element (127). Preliminary data from a Norwegian study of adult CFS/ME patients support this hypothesis (Dr. Bjarte Stubhaug, Skånevik behandlingssenter, Hardanger, Norway. Personal communication). Thus, one should expect altered pain threshold among adolescent CFS/ME patients as well as altered autonomic responses during stimuli close to the threshold. Central sensitization might have a hereditary component, which also may be explored further in this project.

The sustained arousal-model implies that central sensitization does not only influence pain perception and responses; autonomic responses to other sensory signals might be altered as well (127). This, in turn, is in accordance with patients' own description of being sensitive towards all kinds of sensory input (light, smell, sounds, touch). Altered autonomic responses during orthostatic stress – which increases baroreceptor sensory input – is extensively documented (11, 104, 125, 126, 131, 132). Experiments in our own laboratory also indicate altered autonomic response towards the combined challenge of orthostatism and isometric muscle contraction; the latter implies stimulation of peripheral metaboreceptors as well as central activation of a "motor program" (132). However, the effect of isometric exercise in isolation has never been investigated. Preliminary data from other laboratories indicate an altered response to stretching of somatic nerves (such as Lasegue's maneuver) (Prof. Peter C. Rowe, Johns Hopkins School of Medicine, Baltimore, USA. Personal communication). Large scale, systematic experiments are lacking in this field as well.

The cognitive impairments in CFS/ME might be related to decreased ability to process thoughts and memories with emotional content, as discussed above. Distressing emotions are, in turn, associated with somatic stress responses (46). Thus, the finding of altered autonomic response during orthostatic challenges might in fact be caused by emotional distress in the experimental setting rather than a central sensitization phenomenon. If so, one would expect a similar autonomic response when patients just *imagine* themselves in an upright position. Preliminary data from our own laboratory indicate normal autonomic responses during an isolated mental stress test. However, an abnormal response might appear during combined challenges, such as performing a mental stress test in the upright position.

The most important research questions in this part of the projects are:

a) Whether there are signs of increased pain sensitivity and altered autonomic responses in CFS/ME patients as compared to healthy controls
b) Whether CFS/ME patients have altered autonomic responses when exposed to other kinds of sensory input, such as isometric exercise and nerve stretch maneuvers.
c) Whether CFS/ME patients have altered autonomic responses during specific mental instructions (such as imagining being in upright position), and during combined somatic and mental challenges.

d) Whether there are associations between autonomic responses and fMRI findings for identical sensory input.

**Mitochondrial function in CFS/ME**

The mitochondria are responsible for the cells' energy metabolism. The most important clinical features of CFS/ME – overwhelming fatigue combined with multi-organ affection – might indicate reduced energy production due to mitochondrial failure. Recently, a hypothesis of mitochondriopathy was suggested, supported by empirical findings such as increased level of reactive oxygen substances (ROS) and morphological changes in striated muscle fibers (6, 59, 96, 124). Furthermore, one study has demonstrated altered expression of genes related to mitochondrial function (98). However, a large-scale experimental study of mitochondrial function in CFS/ME is lacking.

Increased level of ROS is regarded a sign of dysfunctional mitochondria (103); still, small amount of ROS seem to be necessary for normal mitochondrial function. Furthermore, ROS may influence the intracellular distribution of mitochondria, and has a toxic effect on macromolecules.

As for CFS/ME, there are different possibilities for a causal relation between a suggested mitochondrial dysfunction and the sustained arousal-model: a) Mitochondrial dysfunction may cause sustained arousal through a direct effect on the central nervous system. The neurons' dependency upon a normal energy metabolism is generally recognized; only small deviations in mitochondrial function might have negative consequences for the brain. b) Sustained arousal may cause a mitochondrial dysfunction through alterations of endocrine and/or autonomic control systems. Indeed, both catecholamines and cortisol influences mitochondrial function *in vivo* (50, 91).

In this project, we plan to screen for altered mitochondrial function by assaying the level of ROS and complex 1-activity in white blood cells from CFS/ME patients. Complex 1 is the largest biochemical complex in the mitochondrion, and the one most commonly affected in mitochondriopathy. Testing of mitochondrial inhibitory activity *in vitro* might also be performed, as well as searching for associations between mitochondrial function and endocrine/autonomic markers. As this field is scarcely investigated, the approach has to be exploratory. At a later stage, more specific hypothesis might be proposed.

**Patients' experiences in CFS/ME**

CFS/ME constitutes a main health threat towards adolescent health; it is rather common, and yet more disabling than most other long-lasting diseased in this age group (36). An additional challenge relates to the relative lack of objective signs of disease, combined with subjective experience of severe symptoms. This apparent discrepancy might provoke distrust from family and friends. For the same reason, encounters with the health care system might be problematic (81).

Taken together, these issues might have negative impact upon quality of life and self confidence, and eventually disturb the normal cognitive, emotional and social development in childhood and adolescence. Furthermore, the sustained arousal-model infers a possible reinforcing process, as negative experiences of social interactions might enhance a stress response and thereby create a vicious circle. Finally, one would fear a negative influence upon treatment compliance; mutual trust between patient and health care worker is a prerequisite for cognitive behavioral therapy as well as graded exercise therapy.

The scientific knowledge of patients' experiences in CFS/ME is limited, in particular when it comes to children and adolescents. In this part of the project, therefore, we plan to start out by applying a qualitative, explorative approach. Recently, 50 CFS/ME patients 12-18 years old, diagnosed at our institution in 2007-2008, were enrolled in a clinical follow-up project (unpublished results). One aspect of this project consisted of mapping patients' advices to other adolescents with similar disorders or in a similar situation. In NorCAPITAL, we want to study such advices further, as they represent unique experiences regarding coping. More specifically, we plan to focus on the importance of empowerment and taking responsibility for one's own life. Also, based upon experiences from this previous project, more specific hypotheses, as well as guidelines for semi-structured interviews and in-depth interviews, can be developed. In particular, we plan to focus on the following research questions:

a) The experience and personal interpretation of symptoms among adolescent CFS/ME patients, with emphasis on fatigue and pain

b) The experiences among adolescent CFS/ME patients related to self confidence, quality of life and social interactions
c) The experiences among adolescent CFS/ME regarding coping – with particular emphasis on empowerment.

## *2.2 Part B - Treatment of CFS/ME in adolescents with clonidine*

**Treatment of CFS/ME with the alfa-2 adrenergic agonist *clonidine***

The sustained arousal-model implies that treatment attenuating stress responses might have a beneficial effect in CFS/ME (127, 135). This is in accordance with the documented effect of cognitive behavioral therapy (30, 90), and is also in line with case reports of strong beneficial effect from the so-called "Lightning Process", in which different mental techniques is pin-pointed towards reducing somatic stress (Phil Parker, founder of Lightning Process, London, UK. Personal communication).

Several pharmaceuticals have been subjected to systematic trial in CFS/ME; so far, no one – including glucocorticoids, mineralcorticoids, antidepressants and immunomodulators - has proven useful (30, 90). Thus, further research in this area is strongly needed. Based upon the sustained arousal-model, one would assume a beneficial effect from drugs inhibiting stress responses by antagonizing sympathetic nervous activity (127).

Clonidine is an agonist to the adrenergic, inhibitory alfa-2 receptor protein; thus, clonidine has a general inhibitory influence on the sympathetic nervous system, in particular due to its effect in the central nervous system (82). Consequences include a lowering of heart rate and total peripheral resistance causing blood pressure reduction, and clonidine has therefore been used as an anti-hypertensive drug for decades. Other approved indications in Norway include prophylaxis against migraine and post-menopausal flushing.

There are no randomized controlled trials of clonidine in CFS/ME. However, case reports suggest a beneficial effect of sympatho-inhibition achieved by beta-antagonists (11, 57, 133). The antiadrenergic properties of clonidine are commonly used in pediatric anesthesia for analgesic purposes (34, 55) to alleviate abstinence responses (54), and against spasticity (68). Recently, clonidine has been proven effective in AD/HD (26, 75, 85) and tic disorders (32) by large randomized controlled trials focusing on children and adolescents. Furthermore, one study suggests a beneficial effect in post traumatic stress disorder (PTSD) (76); relevant in this regard, as the somatic responses in PTSD and CFS/ME appears to have a several aspects in common (37, 87, 116). Based upon these lines of evidence, we have tried low-dose clonidine for short periods of time to selected CFS/ME patients as part of our regular outpatient service, and some report a significant improvement of symptoms and functional level.

Given as a prophylactic against migraine, recommended clonidine dosage/day is in the range 50-150 micrograms (40). The risk of serious side-effects in children and adolescents using such dosages appears to be very small (55, 66); in one study, even a dosage of 600 micrograms/day was well tolerated (26).

In this project, we plan to conduct a randomized, placebo controlled, double-blinded trial of clonidine in adolescent CFS/ME. The primary gain of such a trial would be increased knowledge of pharmaceutical treatment options in CFS/ME; however, it may also yield increased insight into underlying disease mechanism. Important research questions include:

a) Whether treatment with clonidine increase activity level (primary end point) as compared to placebo in adolescents with CFS/ME
b) Whether treatment with clonidine improves symptom scores, school attendance, quality of life, autonomic responses and endocrine responses (secondary end points) as compared to placebo in adolescents with CFS/ME
c) Whether treatment with clonidine improves cognitive functions (executive control functions and emotional memory in particular) as compared to placebo in adolescents with CFS/ME
d) Whether eventual differences among the treatment group and the placebo group persist after discontinuation of the drug

**Associations between population characteristics and treatment responses**
CFS/ME probably has a multifactorial etiology, as described above (135). The sustained arousal-model suggests a 'common pathway'; still, it is not known whether sub-grouping of patients based upon the dominating etiological component might be useful (44). For instance, different sub-groups might show a different treatment response. One important predisposing factor, possible suitable for sub-grouping, are certain genetic traits, as discussed in depth previously. It is well known that genetic variations (such as polymorphism of certain signal proteins) may influence pharmacodynamics, as has been demonstrated experimentally for drugs acting on adrenergic systems (100). Other important etiological factors are long-lasting infections and critical life events.

A similar question relates to the highly disputed CFS/ME diagnostic criteria. The CDC-criteria has a dominating position within the scientific community (42); however, recent evidence indicates that they do not correspond to a well-defined, homogenous patient population (110). Furthermore, their validity for children and adolescents has been seriously questioned (69); recently, a separate pediatric case definition has been proposed (53). This trial has wide inclusion criteria; thus, a relevant question is whether the treatment response differs in sub-groups based upon different diagnostic criteria.

An important empirical basis for the sustained arousal-model is the documented changes in autonomic nervous activity upon orthostatic challenge (127). Therefore, an association between treatment response and degree of autonomic alterations is to be expected.

Taken together, important research questions in this project are:

a) Whether there is an association between certain etiological factors and the treatment response to clonidine among adolescent CFS/ME patients
b) Whether there is an association between various diagnostic criteria and the treatment response to clonidine among adolescent CFS/ME patients
c) Whether there is an association between the degree of autonomic alterations and the treatment response to clonidine among adolescent CFS/ME patients

# 3. Design and methods

**Design overview**
A design overview is given in Figure 1. After a pilot study of 5 CFS/ME patients (not shown in the figure), 120 CFS/ME patients are consecutively included in part B, which is a randomized, placebo controlled, double-blinded intervention trial of clonidine. A randomly selected sample of 30 patients from part B is also enrolled in part A, which is an experimental study of pathophysiology. In addition, 30 volunteer healthy control subjects are included in part A.

Thus, a total of 150 individuals (+ 5 pilots) are subjected to baseline program at the point of inclusion (cf. below). 30 patients and 30 controls will also go through an extended program confined to part A, whereas all 120 patients are accomplishing a part B specific program.

**Recruitment, inclusion and exclusion**
Based upon our nation-wide, outpatient referral service (established in 2003), we have a close collaboration with all local pediatric departments in Norway, and a network of interested colleagues. A guideline for diagnostic investigations has previously been developed at our institutions; these guidelines are now implemented locally (128).

Colleagues at local hospitals will provide written and oral information to possibly eligible patients and their parents/next-of-kin. Those fulfilling inclusion criteria (cf. below) will be referred by a standardized letter to the Unit for Chronic Fatigue Syndrom (UCSF) at Dept. of Pediatrics, Rikshospitalet University Hospital. A final decision on inclusion will be taken following an independent examination by the responsible pediatrician at UCSF.

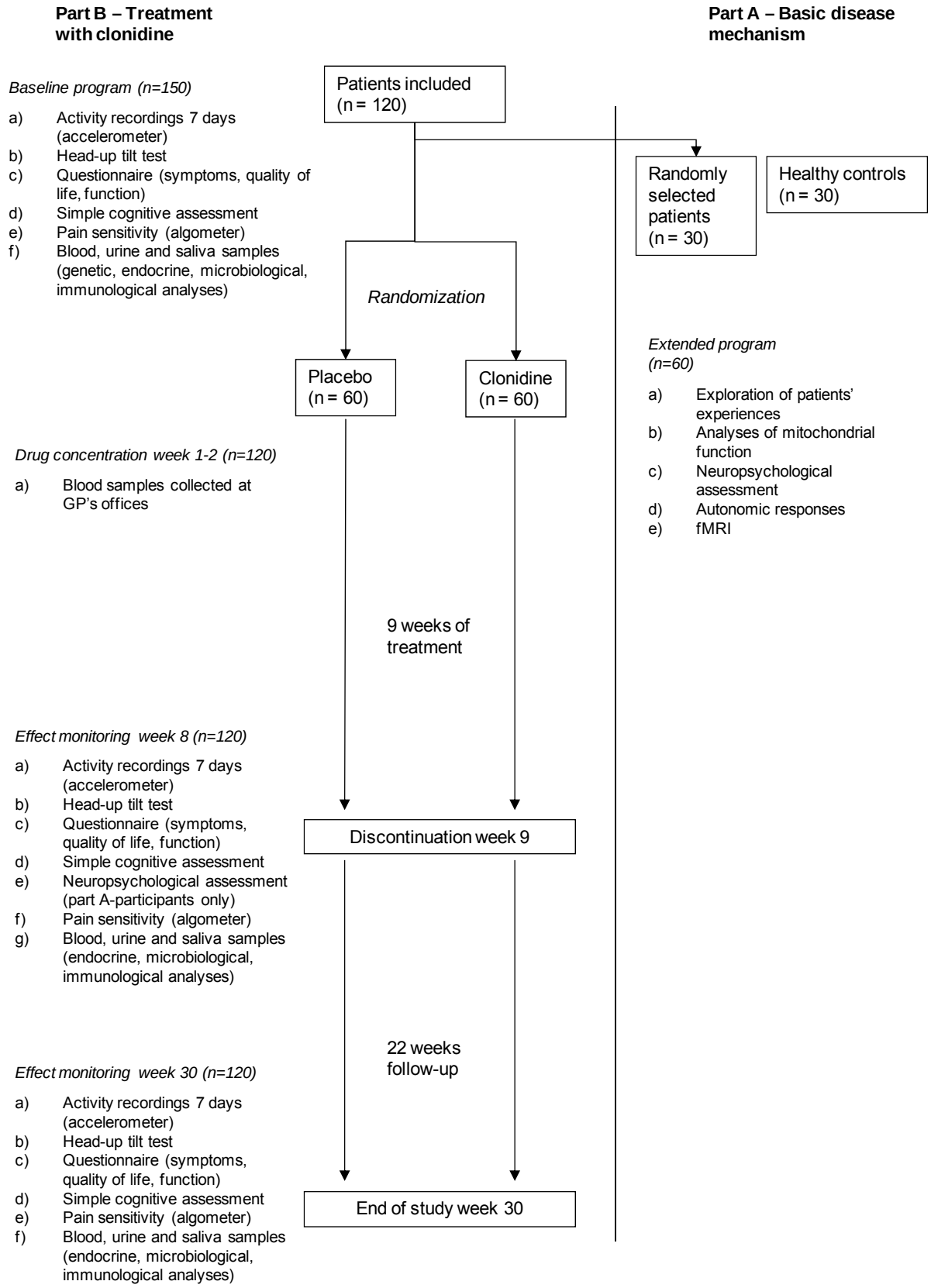


*Figure 1. Design overview. The figure does not show the pilot experiments*

Healthy volunteers for part A will be recruited from local schools according to a procedure assuring an equal distribution of gender, age and bodily proportions in the two groups (135).

Criteria for inclusion and exclusion are given in Table 1. A demand of at least three months fatigue duration is in accordance with international pediatric recommendations (36). The exclusion of bed-ridden patients is due to their own welfare as well as practical considerations; in addition, inactivity might constitute a confounding factor (135). Hormone drugs, including contraceptive pills, are also potential confounders; information on alternative methods for anticonception will be provided to those who chose to discontinue drug use temporarily in order to participate in the study. The exclusion criteria concerning pregnancy test, other disease states, hypersensitivity to capsula, ECG, supine heart rate and supine/standing blood pressure are safety precautions related to clonidine treatment, as thoroughly described below.

**Table 1. Criteria for inclusion and exclusion**

| | *Inclusion criteria* | *Exclusion criteria* |
|---|---|---|
| CFS/ME patients | Persisting or constantly relapsing fatigue lasting 3 months or more.<br>Functional disability resulting from fatigue to a degree that prevent normal school attendance<br>Age ≥ 12 years and < 18 years | Another disease process or current demanding life event that might explain the fatigue<br>Another chronic disease<br>Permanent use of pharmaceuticals (including hormone drugs)<br>Permanently bed-ridden<br>Positive pregnancy test<br>Pheochromocytoma<br>Evidence of reduced cerebral and/or peripheral circulation due to vessel disease<br>Polyneuropathy<br>Renal insufficiency<br>Know hypersensitivity towards clonidine or inert substances (lactose, sakkarose) in capsula<br>Abnormal ECG (in particular bradyarrythmias due to sick sinus syndrome or AV-block. Isolated ectopic beats do not lead to exclusion)<br>Supine heart rate < 50 beats/min<br>Supine systolic blood pressure < 85 mmHg<br>Systolic blood pressure fall upon standing > 30 mmHg |
| Healthy control subjects | Age ≥ 12 years and < 18 years | Another chronic disease<br>Permanent use of pharmaceuticals (including hormone drugs) |

**Power calculation**

The primary end-point in part B is patients' functional capacity, operationalized as mean steps/day count during a seven day period after 8 weeks of clonidine treatment. Mean (standard deviation) steps/day count for healthy adolescents is reported to be approximately 10 000 (4 000) (106). An increment of 2 500 steps/day count for the patients receiving clonidine is considered a clinically significant improvement. 106 patients are to be included based upon this calculation only (level of significance = 0.05, test power = 0.9). However, we expect appr. 10 % drop-outs; thus, the total number of patients needed is estimated to 120.

Part A is analogous to our previously conducted experimental CFS/ME studies (135). Based upon experiences from those studies, there are strong reasons to assume that a sample of 30 patients and 30 healthy controls would be sufficient to discover significant and clinically interesting differences among the two groups. The genetic analyses represent an exception; here, the number of healthy controls needed is estimated to 100, due to the relative low prevalence of certain relevant polymorphisms in the general population.

## *3.1. Baseline program (all 150 participants + 5 pilot patients)*

**Baseline recordings**
All referred CFS/ME patients will be received by an experienced physician at UCSF for additional examination and final evaluation according to the inclusion and exclusion criteria. The patients as well as and parents/next of kin will be thoroughly informed; inclusion is based upon written informed consent.

Clinical examination includes measurement of supine heart rate, supine/standing blood pressure and pregnancy test (cf. Table 1). For included subjects, the following tests are added:

- Standard ECG (12 channels)
- Percentile scores for weight/height and height/age
- Assessment of pain sensitivity by means of an algometer. Anatomically well-defined "trigger-points" are subjected to increasing pressure; the patients alert at the point where the pressure is perceived to be painful (49).
- Subjective grading of pain intensity, quality and distribution by means of the validated questionnaire Brief Pain Inventory (61).
- Validated cognitive tests of working memory (WISC-IV), executive function (STROOP, BRIEF), and learning abilities (Hopkins Verbal Learning Test)

**Blood sample routines**
Blood samples are collected between 07.30 and 08.30 a.m. for genetic, microbiological and endocrine assessments, and for analyses of mitochondrial function in part A. The participants will be instructed to fast overnight, and to abstain from tobacco products and caffeine at least 48 hours.

An ointment containing the local anesthetic lidocaine (Emla®) is to be applied on the skin in the elbows one hour prior to blood sample collection. The patients rest supine at least 15 minutes; thereafter, blood samples are drawn from an anticubital vein. This routine has been considered feasible in previous experiments (135). A total of 70 ml. blood is drawn

**Genetic analyses**
Genomic DNA will be extracted from blood or form oral epithelium (cf. below) by standard laboratory procedures. As some of the relevant polymorphism has a relative low prevalence among the general population, a control population of 100 persons is necessary; this will be achieved by providing DNA from classmates of the healthy controls included. These extra controls will not be subjected to any other experiments, and the data will be registered and stored with no personal identification. In addition, we will consider including previously collected DNA from healthy controls during the Oslo Health Survey (HUBRO 2000) or the North-Trøndelag County Health Survey (HUNT); such material is available at the National Institute of Occupational Health (STAMI). The frequency of interesting genetic variants will be assayed by real-time PCR TaqMan methods, and the genotype determined by automatic scoring using SDS software (Applied Biosystems).

These methods have for years been part of the routine laboratory repertoire at STAMI. Also, for this particular patient group, a pilot project has been launched analyzing specific polymorphism in the genes encoding COMT, beta-1 receptor protein, beta-2 receptor protein, and alfa-2 receptor protein.

**Microbiological analyses in blood**
Microbiological analyses are conducted in EDTA blood and serum. The following microorganisms are assayed by PCR methodology:

- Epstein Barr virus (EBV)
- Cytomegalovirus (CMV)
- Human herpes virus 6 (HHV-6)
- Enterovirus
- Parvovirus B19
- XMRV retrovirus
- Borrrelia burgdorferi sp.

- Bartonella sp.
- Bebesia sp.

In addition, antibodies towards the following microorganisms are assayed:

- EBV
- CMV
- Parvovirus B19
- Mycoplasma pneumonia

All analyses are based upon standard laboratory methods. A pilot project has been established, performing the same analyses on CFS/ME patients routinely evaluated at our outpatient clinic.

**Immunological analyses in blood**

The following immunological signal substances will be assayed, based upon EIA methodology:

- IL-1-beta
- IL-6
- IL-8
- TNF alfa
- IL-4
- IL-10
- INF-gamma

In addtion, leukocytes will be extracted on PAXgene tubes for assessment of gene expression of selected immunological markers.

**Endocrine analyses in blood**

Catecholamines analyses are performed in plasma from blood collected on ice-cold gluthation-EGTA tubes, centrifuged and thereafter stored at -80 $^{o}$C. Norepinephrine and epinephrine are assayed using HPLC technology; a well-established method at Dept. of Endocrinology, and successfully applied in previous studies of CFS/ME patients at our institution (135). Additional blood samples will be stored for subsequent analyses of DHPG (dihydroxyphenylglycol), a metabolite in the MAO breakdown pathway; such analyses might provide additional information on sympathetic nervous activity (46).

ADH and ACTH, as well as osmolality, are analyzed subsequently (no storing) in plasma from blood collected on ice-cold tubes and centrifuged immediately. ADH is assayed by RIA techniques in accordance with previous projects; ACTH and osmolality are assayed by standard laboratory methods.

In addition, serum are provided for the following analyses:

- Cortisol
- Beta-endorphins
- IGF-1
- TSH
- Thyroxin, free fraction (FT4)
- Triiodthyronin, free fraction (FT3)

**Endocrine analyses in urine and saliva**

At the time of blood sample collection, the patients also provide a sample of urine on a sterile tube, and a sample of saliva on special collection tubes (Salivette). In urine, the following analyses are carried out:

- Norepinephrine
- Epinephrine
- Cortisol
- Creatinine
- Osmolality

An assay of cortisol is applied to the sample of saliva; this method has previously been successful in CFS/ME patients (80).

**Head-up tilt-test**
The head-up tilt-test (HUT) is performed between 8 and 10 a.m. The participants will be instructed to fast overnight, and to abstain from tobacco products and caffeine at least 48 hours. The testing room is calm with dimmed light and holds a stable temperature of 25 $^{o}$C.

The subjects rest in supine position for at least 15 minutes. Thereafter, a head-up tilt of 20 degrees is performed for 15 minutes, after which the subjects are tilted back to horizontal position for another 5 minutes. Throughout the test, subjects are attached to the Task Force Monitor, providing continuous, beat-to-beat, non-invasive recording of blood pressure (plethysmography of index finger), heart rate (ECG) and stroke volume (thoracic impedance). Other cardiovascular variables of relevance, such as cardiac output, total peripheral resistance and LF/HF-ratio (based upon spectral analyses of the RR-interval), are calculated from these primary recordings.

We have many years of experience with this method; it is thoroughly validated, and has been successfully applied in previous CFS/ME research projects (125, 126, 131).

**Activity recordings**
Participants' level of activity is recorded by *activPAL*, an accelerometer of established validity (Associate Professor Jorun Helbostad, University of Trondheim, Norway. Personal communication). The recording unit, having the size of a credit card, is applied to the front of the thigh by adhesive strips at the time of inclusion. After seven days it is to be returned to UCSF by mail in a pre-stamped envelope.

Data from the recording unit is automatically transferred to a computer running producer developed software; available output includes mean steps/day count, which is the primary end-point in the intervention trial of clonidine. In addition, the software provides for instance the number of hours in supine vs. upright position.

**Questionnaire**
A questionnaire is distributed to all participants, being composed of the following validated instruments:

- Autonomic Symptom Profile (108), translated and slightly modified (135)
- Chalder Fatigue Questionnaire (14), translated and validated for a Norwegian population (65)
- Fatigue Severity Scale (63), translated and validated for a Norwegian population (64), and thereafter slightly modified in order to fit a pediatric sample (135)
- PedsQL (119), translated and validated for a Norwegian population (95)
- Functional Disability Inventory (120), translated and slightly modified.

Furthermore, single items from the following instruments are included as well: Childhood Health Assessment Questionnaire (41), and Bath Adolescent Pain Inventory (33). Finally, there are questions specifically related to the different diagnostic criteria, including the CDC-criteria (42), the Canadian criteria (13) and the currently proposed pediatric criteria (53), and simple questions regarding life style and demographics. The main part of this questionnaire has been routinely applied in our outpatient CFS/ME program, and has been subjected to several minor adjustments according to our practical experiences.

The questionnaire is to be returned to the UCSF in a pre-stamped envelope.

## *3.2 Extended program – Part A (30 patients and 30 healthy controls)*

**Analyses of mitochondrial function**
Leukocytes are extracted from blood collected on EDTA tubes and are thereafter subjected to routine assays of mitochondrial function at Dept. of Medical Biochemistry, Rikshospitalet University Hospital. The assays include assessment of complex 1-activity and ROS production.

**Exploration of patients' experiences**
The participants' experiences regarding somatic complaints, self confidence, quality of life and social interaction are explored through a semistructured, qualitative interview. As for the patients, their

experiences regarding encounters with the health care system will be explored as well. All interviews are tape recorded and thereafter analyzed by means of qualitative techniques. In addition, all participants are to answer the Norwegian version of Hospital Anxiety and Depression Scale (HADS); this is a thoroughly validated instrument, here serving to supplement the qualitative data.

**Neuropsychological and functional neuroradiological assessment**

The participants are to complete CANTAB (97); a validated, computer-based test battery of executive control functions (shift, inhibition, attention). In addition, test of episodic memory and mentalization will be applied (5). Furthermore, participants are subjected to structured interviews mapping critical life events (KIDDIE-SADS), and they are to answer the first part of UCLA's PTSD instrument (92). Finally, interviews will explore thoughts/images that are emotionally neutral vs. distressing in each individual.

Functional neuroradiological assessments by means of fMRI are performed at the Intervention Centre, Rikshospitalet University Hospital, by a whole-body 3-Tesla MRI unit (Philips Medical Systems, Best, The Netherlands). Blood Oxygen Level Dependent-data (BOLD) are used to construct T2-weghted, functional images, whereas T1-wheighted images are used for visualization of the relevant anatomical structures (prefrontal cortex, amygdala, locus coeruleus, raphe nuclei). Specially developed software (MRIConvert, MRIicroN, SPM) is applied for electronic imaging modification, allowing better definition of anatomical areas, standardization of measurements in accordance with inter-individual variability, and statistical analyses. In order to investigate flexibility and inhibition related to prefrontal cortex, the participants will be instructed to perform simple exercises ('go/no-go paradigm'). Emotional control will be assess in relation to executive areas (prefrontal cortex) as well deeper structures (amygdala) by a specially designed exercise which includes exposing the participants to emotionally arousing pictures on a PC screen. The exercise is developed by our collaborating institution Dept. of Psychology, University of Oslo; it has previously been validated for the same age group.

**Assessment of autonomic responses**

Instrumentation and general experimental conditions are equal to those described for head-up tilt-test (cf. above). Participants are attached to the Task Force Monitor; following 15 minutes of supine rest, they perform the following tests in subsequent order:

- Laying supine, participants are instructed to imagine being in supine, relaxing position (for instance, being on a beach). Thereafter, participants are instructed to imagine being in upright position for 30 seconds. For each individual, the sequence is repeated twice.
- Laying supine, participants are subjected to algometer pressure on the nail of the left 3. finger. The pressure applied is to be 50 % of the pain threshold for the same trigger point, calculated from the baseline recordings (cf. above). Pressure is kept stable for 30 seconds. For each individual, the sequence is repeated twice.
- Laying supine, participants are exposed to emotionally arousing pictures on a PC screen. The test is analogous to the one applied during fMRI experiments, enabling correlation between central and peripheral autonomic responses.
- Laying supine, participants are instructed to perform isometric exercise with the left hand (handgrip), using 30 % of maximum voluntary contraction for 60 seconds (132). The force of the handgrip is continuously displayed by an electronic device. For each individual, handgrip is repeated twice.
- Laying supine, the left arm is passively abducted to 110 degrees; the shoulder girdle is held in neutral position. Thereafter, the wrist is maximally dorsiflexed, the elbow supinated and the shoulder rotated laterally. The participants then start to extend the elbow; they are instructed to notify the researcher when perception of neural stretch occurs, and the angel of the elbow is measured at the same moment. Finally, maximum extension of the elbow is performed; the angle is measured once more, and the position is hold for 30 seconds. It's repeated twice (23).
- After quiet standing for 3 minutes, participants are asked to perform a simple mental exercise (repeated subtraction of 7, starting from 100). Previously, this exercise has been performed by CFS/ME patients and healthy controls in supine position at our laboratory (unpublished results).

## *3.3. Intervention – Part B (120 patients + 5 pilot patients)*

**Pilot study**
A pilot study specifically related to the intervention trial will be carried out, designed in accordance with analogous previous pilot studies of CFS/ME (112). The aim of the pilot study is twofold: to ascertain the dosage plan of clonidine for the main trial, and to assess our routines for activity measurement, questionnaire distribution, tilt-test protocol etc.

Patients referred to our routine outpatient service are eligible; criteria for inclusion and exclusion are as described above. There is no blinding or randomization. Total study period is three weeks, during which clonidine will be administered orally as follows: 25 microgram x 2/day for patients < 35 kg and 50 microgram x 2/day for patients > 35 kg for a total of two weeks, and thereafter one half of this dosage for one week (cf. dosage plan for the main trial below). A total of 5 patients will be included; they are to undergo the baseline program as described previously. Patients will be hospitalized for 5 hours after administration of the first clonidine dosage in order to observe eventual harmful effects. In addition, blood samples for drug concentration measurements and a repeated head-up tilt-test for hemodynamic assessment will be performed just after administration of the first clonidine dosage. After 2 weeks of treatment, patients will undergo clinical reassessment including a repeated head-up tilt test, clonidine concentration assays and a detailed assessment of possible harmful effects. Safety precautions are as described for the main intervention trial (cf. paragraph 4, below)

We aim at publishing the results from the pilot study in an international medical journal. In addition, the results will be used to refine our routines and eventually alter the planned dosage plan for clonidine in the intervention trial. If so, or if other substantial protocol changes are indicated, permission will be sought from the relevant authorities.

**Randomizing and blinding**
The intervention trial is to be carried out according to the CONSORT guidelines (cf. www.consort-statement.org/?o=1001). Patients are randomized to treatment with either clonidine capsula or identically looking placebo capsula, produced by Apoteket Produktion & Laboratorier, Sweden. The randomization will be stratified according to disease duration, which is regarded an important prognostic factor among adolescent CFS/ME patients (69). Each patient receives treatment for 9 weeks (63 days); dosage is reduced during the last week, cf. below.

A computer-based routine for stratified block randomization is provided by the Dept. of Research Support at the Norwegian University of Science and Technology, Trondheim. Each participant is allocated to clonidine or placebo in a 1:1 probability, and also to participation in part A or non part A in a 1:3 probability. The program is operated by Pelle Rohdin, RN, Dept. of Pediatrics, Oslo University Hospital Rikshospitalet, who is not affiliated with any other part of the study. The result of the clonidine/placebo allocation is forwarded to the Pharmacy of Rikshospitalet (Sykehusapoteket), which is responsible for the import of clonidine and placebo from Apoteket Produktion & Laboratorier, and which in turn will provide every study patient with the correct pharmaceutical. Sealed envelopes providing information on allocation of every single patient (clonidine or placebo) will be available at the Section of Congenital Heart Diseases (Barnehjerteseksjonen), Oslo University Hospital Rikshospitalet, in case of emergencies (cf. below).The result of the part A/non part A allocation is forwarded to the NorCAPITAL study secretariat, being responsible for coordinating the investigational program for each participant.

Thus, as for the clonidine/placebo randomization procedure, patients are blinded for group allocation as well as end-point evaluation. Likewise, the responsible researchers are blinded for allocation during the stages of inclusion, intervention and end-point evaluation. In order to reduce variability of procedures, as few persons as possible are to be involved in the practical patient work.

**Dosage plan**
Given as a prophylactic against migraine, recommended clonidine dosage/day is in the range 50-150 micrograms (40). In trials of other pediatric condition, such as AD/HD and tic disorders, much higher

dosages have been applied. Abrupt discontinuation may cause rebound-effects. Generally, CFS/ME patients are considered sensitive to pharmaceutical agents (128). The following dosage plan will be applied:

- Day 1-56 (week 1-8): 25 microgram x 2/day for patients < 35 kg; 50 microgram x 2/day for patients > 35 kg.
- Day 57-63 (week 9): 25 microgram x 1/day for patients < 35 kg; 25 microgram x 2/day for patients > 35 kg.

Every second week, each patient is telephoned by are research nurse and systematically inquired about possible side effects. Treatment with clonidine/placebo commences one week after inclusion, immediate following the baseline activity recording (cf. above).

**Drug concentration**

The pharmacokinetics of clonidine varies interindividually (88), possible influencing individual treatment responses. Therefore, measurement of drug concentration is warranted. During week 1-2 of the intervention period, blood sample will be collected (at the local hospital or local general practitioner's office) and submitted to Dept. of Clinical Pharmacology, Rikshospitalet, for assaying (74). In addition, supine heart rate and blood pressure are to be measured and reported to the study secretariat. A second concentration measurement will be applied in conjunction with effect monitoring during week 8.

Analyses to explore a possible relation between drug concentration and effect of treatment will be carried out together with the general end-point evaluation. During the course of the intervention stage, the responsible researchers will be blinded for results of drug concentration measurements. However, if an individual drug concentration is found to be more than 3 standard deviations higher than the mean value, this patient is to be automatically excluded from further participation.

**Effect monitoring**

The patients are thoroughly assessed at UCFS during week 8 (day 56-63) and week 30 (day 203-210). At these occasions, most elements from the baseline program (cf. above) are to be repeated:

- Clinical examination: Supine heart rate, supine/standing blood pressure
- ECG
- Algometer assessment
- Brief Pain Inventory
- Validated cognitive tests of working memory (WISC-IV), executive function (STROOP, BRIEF), and learning abilities (Hopkins Verbal Learning Test)
- Blood samples for microbiological, immunological, endocrine and drug concentration analyses. A total of 50 ml. blood is collected from ordinary venepuncture.
- Endocrine analyses in urine and saliva.
- Head-up tilt-test

For patients included in part A, the CANTAB test of cognitive function is repeated as well. In addition, the following assessment is carried out in all participants:

- Activity recording by accelerometer (*activePAL*); the device is attached by adhesive strips to the thigh for 7 days.
- Assessment of symptoms, quality of life and disability by means of the composite questionnaire also administered at baseline.

Accelerometer and questionnaire are to be returned to UCFS by ordinary mail in pre-stamped envelopes.

Clonidine/placebo are to be discontinued gradually (half dosage one week), starting just after finishing of the activity recording in week 8. At week 30, the trial is completed, and the patient dismissed after having completed the final activity recording.

**Side-effects and unexpected events**

A separate questionnaire addressing possible side effect, unexpected events, complication etc. related to clonidine treatment are developed. This questionnaire will be used for telephone interviews by a research nurse every second week during the intervention period. In addition, the patients will

complete the questionnaire themselves during the UCFS assessment in week 8. The answers to the questionnaire will be analyzed and published together with the rest of the trial results.

**End-points**
The primary end-point is:

- Mean steps/day count during one week 8 weeks after inclusion

Secondary end-points are:

- Symptoms
  - Fatigue scores 8 and 30 weeks after inclusion
  - Pain scores 8 and 30 weeks after inclusion
  - Algometer testing response 8 and 30 weeks after inclusion
  - Autonomic symptom scores 8 and 30 weeks after inclusion
  - Quality of life-score 8 and 30 weeks after inclusion
- Functional level
  - Disability scores 8 and 30 weeks after inclusion
  - School attendance 8 and 30 weeks after inclusion
  - Mean steps/day count during one week 30 weeks after inclusion
  - Scores on cognitive function tests 8 and 30 weeks after inclusion
- Autonomic cardiovascular regulation
  - The change in mean arterial pressure (MAP) during head-up tilt-test 8 and 30 weeks after inclusion
  - The change in heart rate during head-up tilt-test 8 and 30 weeks after inclusion
  - The change in LF/HF-ratio (a measure of autonomic control) during head-up tilt-test 8 and 30 weeks after inclusion
- Additional biological markers
  - Hormonal levels 8 and 30 weeks after inclusion
  - Microbiological analyses 8 and 30 weeks after inclusion

**Analyses**
Data are to be analyzed according to the intention-to-treat-principle. In addition, analyses focusing on relations between individual treatment responses and specific characteristics (such as hereditary traits, microbiological results, diagnostic criteria fulfillment etc) will be carried out. Most variables are continuous, and we assume a normal distribution of the primary end-point and a majority of the secondary end-points. Thus, standard parametric statistical procedures are expected to be feasible.

# 4. Ethical considerations, monitoring and publishing policy

**Informed consent and data storage**
Participation in the project is based upon the principle of informed consent, and thorough information will be provided orally as well as in writing. Persons younger than 16 years old will receive separate information material, adjusted to their level of maturation. In addition, parents/next-of-kin will be informed. Consent from both parties is a prerequisite for inclusion. It is reason to emphasize, however, that Norwegian law grants children ≥ 12 years (the lower age limit in this project) considerable influence over their own health issues.

Specific study data are treated and stored without personal identifying information, and in accordance with national directives. All participants are informed of the procedures for data storage. For each participant, written data (signature of informed consent, baseline medical data, completed questionnaires etc.) will be stored in an archive at the study secretariat. This archive will also contain detailed descriptions of any adverse and unexpected events, cf. below. Digitalized data (such as tilt-test results) will be stored on a server dedicated to research purposes at Oslo University Hospital Rikshospitalet in accordance with guidelines from the Norwegian Data Inspectorate. In addition, information relevant to medical treatment will be stored in the ordinary patient records at the hospital.

Approval to conduct the project is granted from the Norwegian National Committee for Ethics in Medical Research, the Norwegian Data Inspectorate, the Norwegian Health Directorate, and the Norwegian Medicines Agency. The project will be carried out in accordance with Norwegian laws and precepts (FOR 2009-30-10), as well as the consolidated guidelines of Good Clinical Practice

**Safety considerations**

The baseline and part A-specific program includes painful venous puncture; therefore, an ointment containing the local anesthetic lidocaine (Emla®) is routinely given as a prophylactic. Algometer testing implies pressure up to the pain threshold; however, when patients signalize pain, the pressure terminates immediately. fMRI does not imply radiation, and is considered totally free of risk. The interviews addressing patient experiences may uncover distressing thoughts and/or emotions; if so, the patients will be offered a separate encounter with psychologically skilled health care professional.

The intervention trial raises specific concerns. Recently, clondine has become a commonly used drug in several pediatric conditions, and the risk of serious side-effects is generally considered to be minimal (26, 55, 60). However, in persons suffering from certain diseases, such as bradyarrythmias and orthostatic hypotension, clonidine may have harmful effects; here is the reason for the exclusion criteria regarding ECG, heart rate and supine/standing blood pressure (cf. above). Also, the pilot study – which includes detailed pharmacokinetic and hemodynamic assessments in relation to administration of the first dosage – provides detailed insight into eventual side effects specifically concerning CFS/ME patients and gives a rationale for an eventual adjustment the dosage plan. In addition, patients having a well-known chronic condition other than CFS/ME are excluded from participation. Furthermore, the planned dosages are smaller than what is commonly used in other conditions. Finally, the drug concentration measurements in week 1-2 and the telephone interview with research nurse every second week constitute additional precautions, allowing patients to be excluded from further participation on an early stage if high drug concentration and/or serious side-effects are discovered.

Despite these precautions, harmful effects of clonidine might occur. In addition, study participants might be exposed to serious events (such as a trauma) coincidentally, making it necessary to know whether clonidine or placebo was given. Therefore, patients/next of kin are instructed (orally and in writing) to contact the NorCAPITAL secretariat immediately if a possible harmful side effect or a serious coincidental event occurs. In case of a medical emergency, normal routine (dial 113) should be used. The local emergency department might in turn contact the doctor on call at the Section of Congenital Cardiology, Oslo University Hospital Rikshospitalet, who is available at a 24 hours basis, and who will be able to give information on group allocation (clonidine vs. placebo) based upon an archive of sealed envelopes (cf. above).

All potential harmful effects of clonidine will be described in detail in the patient's specific study files and in the ordinary hospital record, and will be reported to the Norwegian Medicines Agency according to present precepts. In addition, harmful effects will be reported in scientific publications. Generally, the knowledge on effects and risks of pediatric drug use is sparse, severely affecting clinically decision making in many fields (56). Thus, there are scientific as well as ethical arguments for carrying out pharmaceutical randomized controlled trials in children.

**Clinical follow-up**

Several CFS/ME sufferers do not have an optimal rehabilitation and follow-up program in their local communities (30). Participation in this project implies close contact with UCSF, serving as a national referral center, and may thus be of benefit for all patients.

If the intervention trial proves a beneficial effect of clonidine, such treatment will be offered to the placebo group as well.

**Study monitoring and surveillance**

Study monitoring and surveillance will be provided by a committee of four senior consultants and researchers, who are not otherwise affiliated with the study. The committee will have free access to all data and analyses, including the list of randomized numbers, and will be consecutively informed of each included participants. Thus, the monitoring and surveillance committee will be in a position to ascertain that each included participant is correctly allocated to intervention/placebo. Also, the committee will be consecutively informed of any adverse event, and will receive the results of the drug

concentration measurements in week 1-2. Exclusion of single participants during the course of the study will be discussed with the committee if practically feasible (cf. above). Furthermore, the committee will participate in discussions on statistical approach and publication policy.

The study group will meet regularly with the monitoring and surveillance committee during the entire study period to discuss the above-mentioned issues and eventual improvements of the protocol.

**Publication policy**

All results from this project will be published in international, peer-reviewed medical journal. We will also report negative results. Co-authorship will be granted according to the Vancouver guidelines. Furthermore, the project will provide data for at least three PhD-dissertations.

A various kind of information material has been developed in relation to our routine outpatient service. Results from this project will be implemented in such material. CFS/ME is a condition of public interest, and we will therefore attempt at presenting the main project results in the mass media.

# 5. Project administration and collaborators

## *Primary study group*

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## *Other collaborators*

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## *Information on the institutions*

Since 2003, children and adolescent with CFS/ME has been a field of high priority at the Division of Paediatrics, Rikshospitalet University Hospital, Oslo, Norway. The Unit for Chronic Fatigue Syndrome (UCFS) was formally established in 2007, and has been assigned a national responsibility for scientific and clinical development among children and adolescents with CFS. The unit serves as a national referral center, receiving two CFS patients < 18 years for outpatient evaluation each week. There is a close scientific collaboration with highly reputed international institutions: Medical University of South Carolina, Charleston, USA (Prof. J. Philip Saul); Johns Hopkins University School of Medicine, Baltimore, USA (Prof. Peter C. Rowe); Massachusetts General Hospital/Harvard Medical School, Boston, USA (Dr. Riccardo Barbieri). Nationally, the unit has a central role in the research network "Chronic fatigue syndrome in theory and practice" (headed by Dr. Kirsti Malterud, Bergen). UCSF hosted a national conference on CFS/ME in 2008, and was a major contributor to the HTA-report from the Norwegian Health Knowledge Centre in 2006.

Dept. of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, USA, is a leading international institution for pediatric CFS/ME research, and particular concerned with the field of autonomic assessment. Dept. of Pediatrics, Medical University of South Carolina, Charleston, USA, is highly reputed for their competence in experimental studies of autonomic cardiovascular control and related data analyses. The Cognitive Developmental Research Unit, Dept. of Psychology, University of Oslo is especially established to study cognitive function such as memory, emotion, executive function, and social-cognitive functions. The methods and the laboratory facilities are particularly suited to the study of the possible problems that might be predicted in adolescents with CFS/ME. Dept. of Medical Biochemistry, Rikshospitalet University Hospital, Oslo, has as special focus on mitochondrial function and a close collaboration with Center of Molecular Biology and Neuroscience (a national centre of excellence). The Pediatric Research Institute, Dept. of Microbiology, and Dept. of Endocrinology, Rikshospitalet University Hospital, are leading scientific institutions, regularly publishing in high-impact medical journal. The National Institute of Occupational Health has years of experience with similar research projects among adult patients.

# 6. References

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