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Diagnosis of
Chronic Fatigue Syndrome
in Adolescents

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  Operationalized SEID-criteria
1 PREFACE

1.1 Acknowledgements

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The very start of my academic journey in the winding landscape of chronic fatigue syndrome (CFS) actually started before my medical education even was initiated. I had been walking a close relative, severely affected by this condition of unknown disabling fatigue. Watching her, a question repeatedly came to mind: What IS this? Gradually, my relative returned to life, and her way out of the darkness has obviously formed my thinking of the concept of chronic fatigue, treatment etc.

When I occasionally met my main supervisor back in 2012, my intention was to thank him for his work on CFS. The talk rapidly turned into an interesting conversation about the CFS diagnosis, and a short time after I was finding myself digging into the Canada Consensus Criteria for ME/CFS. Vegard Bruun Bratholm Wyller, from the very beginning you have performed supervision at top level. Always accessible, clear in your feedback, generous with the red markings in the manuscripts. I have learned a lot from your holistic approach to every subject, and I have appreciated our long conversations about medicine, psychology, sociology, theology, and everything in between. Thank you!

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To my wife: Solgunn – thank you ♥

To my Creator, Lord and Savior: Thank you Jesus!

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September 2021
## 1.2 Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>5-HTT</td>
<td>Serotonin transporter</td>
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<tr>
<td>ACTH</td>
<td>Adrenocorticotropic Hormone</td>
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<tr>
<td>ANCOVA</td>
<td>Analysis of covariance</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
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<tr>
<td>ASP</td>
<td>Autonomic Symptom Profile</td>
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<tr>
<td>BDS</td>
<td>Bodily Distress Syndrome</td>
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<tr>
<td>CBT</td>
<td>Cognitive Behavioral Therapy</td>
</tr>
<tr>
<td>CCC</td>
<td>Canadian Consensus Criteria</td>
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<tr>
<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
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<tr>
<td>CF</td>
<td>Chronic Fatigue</td>
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<tr>
<td>CFQ</td>
<td>Chalder Fatigue Questionnaire</td>
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<tr>
<td>CFS</td>
<td>Chronic Fatigue Syndrome</td>
</tr>
<tr>
<td>CMV</td>
<td>Cytomegalovirus</td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
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<tr>
<td>COMT</td>
<td>Catechol – o – methyltransferase</td>
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<tr>
<td>COVID</td>
<td>Corona Virus Disease</td>
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<tr>
<td>CRP</td>
<td>C-reactive protein</td>
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<tr>
<td>D-KEFS</td>
<td>Delis – Kaplan Executive Function System</td>
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<tr>
<td>DSM</td>
<td>Diagnostic and Statistical Manual of mental Disorders</td>
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<tr>
<td>EBV</td>
<td>Epstein-Barr Virus</td>
</tr>
<tr>
<td>FDI</td>
<td>Functional Disability Inventory</td>
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<tr>
<td>FSS</td>
<td>Fatigue Severity Scale</td>
</tr>
<tr>
<td>FT4</td>
<td>Free Thyroxine</td>
</tr>
<tr>
<td>HF</td>
<td>High Frequency</td>
</tr>
<tr>
<td>HPA</td>
<td>Hypothalamus-pituitary-adrenal</td>
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<tr>
<td>HR</td>
<td>Heart Rate</td>
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<tr>
<td>HRV</td>
<td>Heart Rate Variability</td>
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<td>HUT</td>
<td>Head-Up Tilt Test</td>
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<tr>
<td>HVLT-R</td>
<td>Hopkins Verbal Learning Test – Revised</td>
</tr>
<tr>
<td>IBS</td>
<td>Inflammatory Bowel Syndrome</td>
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<tr>
<td>ICC-2011</td>
<td>International Consensus Criteria</td>
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<tr>
<td>ICD</td>
<td>International Classification of Diseases</td>
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<tr>
<td>IGF-1</td>
<td>Insulin-like growth factor 1</td>
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<td>KSQ</td>
<td>Karolinska Sleep Questionnaire</td>
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<tr>
<td>LF</td>
<td>Low Frequency</td>
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<tr>
<td>LIA</td>
<td>Luminescence immunoassay</td>
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<tr>
<td>ME</td>
<td>Myalgic encephalomyelitis</td>
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<tr>
<td>MFQ</td>
<td>Mood and Feelings Questionnaire</td>
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<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
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<tr>
<td>MS</td>
<td>Multiple Sclerosis</td>
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<tr>
<td>MUS</td>
<td>Medically Unexplained Symptoms</td>
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<tr>
<td>NCR</td>
<td>Norwegian Research Council</td>
</tr>
<tr>
<td>NorCAPITAL</td>
<td>The Norwegian Study of Chronic Fatigue Syndrome in Adolescents</td>
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<tr>
<td>PAMPs</td>
<td>Pathogen-associated molecular patterns</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
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<tr>
<td>PedsQL</td>
<td>Pediatric Quality of Life Inventory</td>
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PEM = Post-exertional malaise  
RCT = Randomized controlled trial  
RRi = RR interval  
SD = Standard Deviation  
SEID = Systemic Exertion Intolerance Disease  
SNP = Single Nucleotide Polymorphism  
TSH = Thyroid Stimulating Hormone  
WISC-IV = Wechsler Intelligence Scale for Children – fourth edition

### 1.3 List of papers

**Paper 1**


**Paper 2**


**Paper 3**

1.4 Summary

Diagnostic labels such as Chronic Fatigue Syndrome (CFS), Myalgic Encephalomyelitis (ME) and Systemic Exertion Intolerance Disease (SEID) represent different approaches to the enigmatic phenomenon of long-lasting unexplained fatigue. More than 20 case definitions/diagnostic criteria for CFS/ME/SEID exist. All are based on subjective symptom reports, and the details of symptom requirement vary considerably. No one has been thoroughly validated.

The present thesis shows that adolescent CFS patients fulfilling the Canadian Consensus Criteria (CCC) or SEID-criteria do not differ from adolescent CFS patients diagnosed according to broad diagnostic criteria regarding neuroendocrine, cardiovascular, inflammatory, infectious or cognitive variables. Furthermore, there appears to be no distinct subgroups within the overarching CFS label, but rather a continuum of subjective symptom experiences and pathophysiological aberrations.

These findings question the descriptive, predictive and construction validity of the CCC and SEID-criteria, and more fundamentally question the rationale of sub-classifying chronically fatigued patients based on clinical symptoms. Rather, the results seem to suggest that all patients with an unexplained chronic fatigue may be seen as one entity in a qualitative sense, albeit with individual, quantitative differences regarding symptom severity and functional impairments.
1.5 Sammendrag

Diagnostiske kategorier som kronisk utmattelsessyndrom (CFS), myalgisk encefalomyelitt (ME) og “Systemic Exertion Intolerance Disease” (SEID) representerer ulike tilnærminger til det gåtefulle fenomenet langvarig uforklarlig utmattelse. Det finnes mer enn 20 ulike sett med diagnostiske kriterier for CFS/ME/SEID (videre brukes CFS som fellesbetegnelse). Alle er basert på subjektiv rapportering av symptomer, og det er stor grad av variasjon i hvilke symptomer som kreves for å oppfylle kriteriene. Ingen av kriteriesettene har blitt grundig validert.

Denne avhandlingen sammenlikner ungdommer med CFS som oppfyller strenge diagnosekriterier (Canada-kriteriene og SEID-kriteriene) med ungdommer med CFS som har blitt diagnostisert med vide diagnosekriterier (minimum tre måneder med uforklarlig kronisk/tilbakevendende utmattelse og ingen krav om tilleggssymptomer). Sett bort fra én kognitiv test var det ingen statistisk signifikante forskjeller mellom gruppene når man undersøkte nevroendokrine, kardiovaskulære, inflammatoriske, infeksjøse og kognitive variabler. I tillegg ser det ut til at det ikke finnes noen distinkte undergrupper innenfor CFS-kategorien, men heller et kontinuum av subjektive symptomopplevelser og patofysiologiske avvik.

Disse funnene stiller spørsmål ved deskriptiv-, prediktiv- og konstruktvaliditeten til Canada-kriteriene og SEID-kriteriene, og mer fundamentalt stiller funnene spørsmål ved grunnlaget for å gruppere kronisk utmattede pasienter basert på kliniske symptomer. Resultatene peker mer i retning av at alle pasienter med uforklarlig kronisk utmattelse kvalitativt sett er én gruppe, men pasientene har individuelle kvantitative forskjeller når det gjelder alvorlighetsgrad av symptomer og nedsatt funksjonsgrad.
2 INTRODUCTION

Fatigue in a chronic long lasting variant has existed throughout history, covered by a great variety of names, criteria and proposed pathophysiological explanations. Chronic Fatigue Syndrome (CFS) is characterized by unexplained, long lasting, disabling fatigue accompanied by several other symptoms such as musculoskeletal pain, headaches, problems with memory and concentration, and orthostatic intolerance. Today the terms CFS and ME (Myalgic Encephalomyelitis) are still used interchangeably, often referred to as CFS, CFS/ME, ME/CFS or ME, despite great controversies regarding pathophysiological understanding and conceptual framework. A new name has also been proposed in 2015 (Systemic Exertion Intolerance Disease – SEID). Throughout the present dissertation, I will use CFS when referring to this condition of long lasting chronic fatigue if no other term is more suitable.

Despite certain pathophysiological features, no biomarker is proven to be CFS specific, and a diagnosis relies on subjective symptom reports and exclusion of other medical conditions. More than 20 case definitions/diagnostic criteria for CFS/ME/SEID exist, but no one has been thoroughly validated. The present dissertation evaluates the validity of two of the diagnostic criteria, and more substantially investigate the concept of sub-classifying patients with long lasting unexplained fatigue.

2.1 Epidemiology of CFS in adolescents

Studies show a prevalence of CFS among children and adolescents varying from 0.2 % to 2.0 %. Studies collecting data from general practitioners/family doctors or pediatricians report substantially lower prevalence than population-based studies. Although recognized in children as young as 2 years old, CFS before 10 years of age is rare. Until puberty, the prevalence among girls and boys is equal, while female sex becomes a risk factor later in life, with female-male ratio 2-3:1.

CFS may have a great impact on psychosocial and academic development as well as family functioning. The reduced school attendance is associated with reduced physical functioning, rather than anxiety. Changes in the relationships to family and friends are noted due to, among others, the isolating effect of CFS. Further, adolescents with CFS reports poorer quality of life than adolescents who receive renal transplantation and children in remission after acute lymphoblastic leukemia.
The definition of recovery from CFS is somewhat disputed. Some studies require complete symptom remission, most studies have some version of significant functional improvement as enough to be classified as recovered, while another approach is to label those not fulfilling diagnostic criteria for CFS at follow up as recovered. This lack of a common definition leads to a wide range of reported recovery in adolescent CFS patients (40-94 %), but the majority report recovery rate around two-thirds and the illness duration is reported by the recovered patients to be 2-5 years.

The economic impact of CFS on the society and the individual patients appears large. High prevalence and a relatively young patient population results in a substantial loss of productivity. This is an indirect cost, but viewed as the greatest economic burden for the society. Economic studies of CFS are problematic, due to factors such as different diagnostic criteria and occurrence of unwillingness among doctors to diagnose CFS.

2.2 What is fatigue?

The word fatigue is used in different ways, commonly as a term in muscle physiology or as a description of a sensation or feeling. It is the latter understanding of the term that will be used further in this dissertation. How fast a muscle gets fatigued is measurable, but measuring fatigue as a characterization of a sensation is more complex, as it exists only as it is felt. The term does not correspond to an object in the world, and can therefore not be falsified or verified by an observer. This does not imply that fatigue is not a reality, or that it does not have a neurobiological substrate; indeed, evidence suggests that fatigue (as other sensations, such as pain and thirst) are related to certain activity states in brain networks. However, the reality of fatigue exists only for the one who experiences it, and it is therefore a categorical fallacy to doubt its presence if an underlying medical explanation cannot be readily found.

The sensation of fatigue, like all other sensations, is a net result of an automatic interpretation of multiple concomitant brain processes such as perceptions, emotions and cognitions. The brain’s predictive capabilities, shaped by prior learning, are fundamental in this regard: Our expectations strongly influence our experiences.

The interpretation ascribed to fatigue often seems to involve salience, which is the capability of detecting important signals in the continuous stream of sensory information, or danger. It
might be described as a homeostatic alarm directed towards energy preservation.\textsuperscript{38} Thus, in a context where excessive energy consumption is regarded too costly compared to the expected gain, the sensation of fatigue functions as a “brake”, promoting behavior that preserves energy. This mechanism conceivably increases survival, which in turn explains its evolutionary persistence.\textsuperscript{39}

An infection represents one such context. The acute sickness response involves a chain reaction of cells of the immune system recognizing pathogen-associated molecular patterns (PAMPs), which in turn initiate and propagate an inflammatory response by releasing a variety of cytokines.\textsuperscript{40} Both humoral and neuronal routes are used to communicate this situation to the brain, which in turn produces symptoms of sickness, where fatigue - in addition to pain, altered sleep, anorexia and fever - is a common feature.\textsuperscript{39}

2.3 Pathological fatigue

Several models have been proposed to understand pathological fatigue,\textsuperscript{41} but an explicit definition does not exist.\textsuperscript{42} A common understanding includes features such as: The fatigue sensation is not proportional to the actual physical or mental burden,\textsuperscript{31} fatigue persists for an unusual long time after the initial perpetuating event (e.g. Epstein-Barr virus (EBV) infection), fatigue does not improve by bed rest, and fatigue cannot be readily explained from an ongoing disease or demanding life event.

Understanding these patients’ reports of high perceived effort when performing simple tasks of daily living is of importance. In a normal functioning brain, a great proportion of afferent signals to the brain are attenuated.\textsuperscript{31} The salience network of the brain contributes to selecting which stimuli deserve our attention. This is due to the crucial necessity to focus on the most important stimuli for maintaining homeostasis, survival etc.\textsuperscript{43} An example – to lift a cup of tea – may illustrate this phenomenon. In a brain with normal function of the salience network and attenuation of non-important signals, the effort used to reach out and lift the cup of tea is not recognized by the conscious brain. It does not stand out enough to deserve attention.

If the ability to attenuate non-important signals is impaired, the brain will recognize an increased number of conscious experiences of perceived effort.\textsuperscript{31} In other words: the sensation of fatigue will be more prominent. This means lifting the cup of tea now may produce a conscious experience of perceived effort. The salience network is shown to be
dysfunctional in CFS-patients\textsuperscript{,44} and level of alteration is significantly correlated with the severity of the fatigue sensation\textsuperscript{.45}

### 2.4 Symptoms and pathophysiological features of CFS

#### 2.4.1 Symptoms

Besides fatigue, CFS patients report a wide variety of other clinical symptoms. Post-exertional malaise (PEM), considered a hallmark feature of CFS, is highly prevalent\textsuperscript{.6} PEM may be described as “an exacerbation of some or all of an individual’s ME/CFS symptoms that occurs after physical or cognitive exertion and leads to a reduction in functional ability”.\textsuperscript{.5}

Children and adolescents with CFS experience significantly more sleep disturbances compared to healthy controls\textsuperscript{.46} Disturbances such as delayed sleep-wake rhythm, increased time in bed and insomnia symptoms have all been reported\textsuperscript{.47} Regarding emotions, patients are prone to experience depression and anxiety\textsuperscript{,48} however, cause and effect remain unclear\textsuperscript{.49}

Adolescents with CFS report increased pain ratings for headache, abdominal pain and/or pain in muscles. This is accompanied by lowered pain threshold compared to healthy peers\textsuperscript{.50,51} Pain diminishes after successful CBT-treatment for CFS primarily focusing on fatigue symptoms, and adolescents who have not recovered from CFS after 12 months report that the pain symptoms persist\textsuperscript{.51}

There is evidence that CFS patients have increased amount of gastrointestinal symptoms,\textsuperscript{52} and that there is considerable symptom overlap with irritable bowel syndrome (IBS)\textsuperscript{.53}

#### 2.4.2 Pathophysiological features

Compared to healthy controls, both adolescent and adult studies have revealed certain non-specific pathophysiological characteristics of CFS.

Subtle alterations of neuroendocrine control mechanisms have been demonstrated. These include attenuation of the hypothalamus-pituitary-adrenal (HPA) axis characterized by lowered cortisol levels in saliva and urine\textsuperscript{,54,55} attenuated diurnal variation of cortisol, enhanced negative feedback to the HPA axis and decreased HPA responsiveness to stressors\textsuperscript{.56} Plasma catecholamine levels have been shown slightly elevated\textsuperscript{,57,58} which corresponds with the demonstrated sympathetic predominance of autonomic cardiovascular
The sympathetic predominance may be the underlying cause of orthostatic intolerance, which is reported in up to 90% of children with CFS. Improvement of symptoms and functional disabilities is associated with normalization of HPA-responses. Immunological alterations have been a major research focus, but the results are inconsistent. A significant attenuation of NK cells has been a relatively consistent finding. Also, alteration in subsets of CD8+ T-cells and B-cell differentiation and survival is reported. An RCT showed that B-cell depletion does not improve the symptoms of CFS patients. Many studies report low-grade inflammation. While cytokine expression have been inconsistent, recent findings indicate a linear relationship between pro-inflammatory cytokines and CFS severity.

Several infectious diseases, such as EBV, Lyme disease and Q-fever, have been shown to trigger acute fatigue and eventually CFS. Interestingly, common minor infections do not show the same link to CFS.

As for genetic predisposition, several markers have been reported. Some of the most prevalent findings are that a single nucleotide polymorphism (SNP) in the catecholaminergic breakdown enzyme catechol-O-methyltransferase (COMT) is linked to CFS, in addition to mutations in the serotoninergic system. Twin studies have consistently showed that genetic factors are an important risk factor for CFS, with the highest correlation among young CFS patients.

Executive functions in adolescent CFS patients seem slightly altered; impaired interference control, cognitive flexibility and working memory have been reported. Functional magnetic resonance imaging (fMRI) studies have shown differences between adult CSF patients and healthy controls when performing cognitive tasks, suggesting a greater effort and less efficient use of neural resources among patients.

Children whose mothers experience anxiety and/or depression have increased risk of developing adolescent CFS. Studies have shown that CFS patients often experience negative life events prior to disease onset.
Several models have been proposed to explain the pathophysiological features of CFS, ranging from cognitive-behavioral models to models with a strict biomedical pathophysiological understanding.

Sharpe et al. conceptualized a model where the patient’s belief that the disease is purely organic, as well as the interpretation of the activity-induced exacerbations of symptoms, leads to a behavior of rest and avoidance of activity. CFS is maintained because the behavior interferes with biological mechanisms (sleep disturbances, neuroendocrine dysfunction and deconditioning), and emotions of depressive, anxious and frustrating character appear.\textsuperscript{93} Lennart et al. present a somewhat different cognitive behavioral framework, the ALT+F model,\textsuperscript{94} were fatigue is conceptualized from an associative learning perspective: Interoceptive and exteroceptive stimuli can become associated with the fatigue experience. These stimuli may acquire the capacity to elicit fatigue as well as anticipatory fear-related avoidance behavior, and may contribute to development of chronic fatigue.

In the Sustained arousal model, proposed by Wyller et al. in 2009 \textsuperscript{95} and later revised,\textsuperscript{57} predisposing factors, such as genetics and personal traits, interact with precipitating factors such as long-lasting infections and demanding life events. These lead to a prolonged bodily stress response labeled sustained arousal. Sustained arousal in turn leads to immunological, endocrinological, autonomic and cognitive disturbances, in addition to the persistence of symptoms such as fatigue and pain. Harvey et al. have proposed a partially similar model,\textsuperscript{96} where predisposed individuals experience a trigger event which lead to fatigue. Then maintaining factors (prolonged bed rest, boom & burst activity, and biological changes) lead to CFS.

Maes et al. have presented a bio(psychosocial) model for ME/CFS.\textsuperscript{97,98} Here, inflammatory, oxidative and nitrosative pathophysiology is suggested to play an important part. In this model, viral- or bacterial infection or immune disease act as the primary trigger, and then further causes immunological and gastrointestinal aberrations. A number of biological sequels follow, and in total, this causes the characteristic ME/CFS symptoms. In this model, physiological and psychological stress function as important precipitating and perpetuating cofactors.
An even more strict biomedical model, the *neuro-immune-cellular dysfunction model* claims that energy metabolism dysfunction, neurological inflammation and systemic immune activation explain the reported symptoms of CFS.\(^9^9\)

### 2.5 The history of chronic fatigue/CFS/ME and the development of diagnostic criteria

Chronic fatigue has probably existed throughout history in various forms, labeled with various names, and seen with shifting theories of causality and pathophysiological understanding.\(^1\) Controversies regarding pathophysiological models and understanding of CFS is apparently not of new origin, and it is helpful to have insight in the historical lines when analyzing the ongoing dispute regarding diagnostic criteria for CFS. The present review of the history of CFS and diagnostic criteria intend to point out the highlights. Several names and terms for chronic fatigue exist, more than 20 diagnostic criteria for CFS/ME have been published,\(^4\) and it would be beyond the scope of this dissertation to give a thorough outlay of all of these.

#### 2.5.1 Nervous exhaustion/nervous asthenia/neurasthenia

In 1866, Austin Flint proposed nervous exhaustion or nervous asthenia, “as the name signifies, debility, prostration, or exhaustion, affecting especially the nervous system, constitutes the affection.” It occurred without anemia or any other disorder of vital functions.\(^1^0^0\) Three years later George Beard reintroduced the term *neurasthenia*,\(^1^0^1\) meaning lack of neural force. This term would have a great impact on the medical community as well as the society far into the 20\(^{th}\) century.\(^1\) Beard proposed that neurasthenia may be caused by acute or chronic diseases like “wasting fevers, exhausting wounds (…) and so forth”, but neurasthenia could also be the cause of acute and chronic diseases like dyspepsia, headaches, paralysis or insomnia.

Neurasthenia was a diagnosis of exclusion, and would be considered if symptoms like “general malaise, debility of functions, poor appetite, abiding weakness in the back and spine, fugitive neuralgic pains, hysteria, insomnia, hypochondriases, disinclination for consecutive mental labor, severe and weakening attacks of sick headache (…) *no evidence of anaemia or of any organic disease.*” In “A practical treatise on nervous exhaustion (neurasthenia): its
symptoms, nature sequences, treatment”, Beard elaborates on a long list of symptoms of neurasthenia and writes:

Attacks of a sensation of absolute exhaustion, as though the body had not strength to hold together, comes on very often in the nervously exhausted. This feeling of exhaustion, though not exactly pain in the usual sense of the word, is yet, in many cases, far worse than pain. These attacks may come on suddenly without warning, and may suddenly disappear. In the morning, one may be able, or feel able, to run on a wager; in the afternoon of the same day, sitting quietly in a chair seems to be an exhausting effort to which every nerve and bone and muscle is unequal. The going-to-die feeling is quite common in these cases, and at first causes alarm.¹⁰²

Neurasthenia as a diagnosis rapidly disappeared around 1930. A gradual shift in perspective - from a neurologic disease to a disturbance of the mind - happened during the first decades of the 20th century. Neurologists were moving in an organic direction, and a huge effort was made in uncovering the localization of the workings of the nervous system – neurasthenia remained a “functional” disorder due to the lack of structural pathology.¹⁰³ Coincidently with the fall in prevalence in 1930 is the reappearance of neurosis; clusters of patients in the 1930s diagnosed with neurosis were similar to earlier cases diagnosed as neurasthenia. The shift from a non-psychiatric neurological illness to psychiatric diagnosis was closely connected to the decline in neurasthenia.¹⁰⁴

### 2.5.2 DaCosta’s syndrome & Shell shock

In 1871 DaCosta published his study of exhaustion in soldiers from the US Civil War.¹⁰⁵ He described a syndrome of fatigue, breathlessness, palpitations, dizziness and chest pain. Headache, digestive disturbances, and difficulty sleeping were common. Often the syndrome was precipitated by febrile illnesses or gastroenteritis, but severe exertion could be the only cause. DaCosta found no structural abnormalities, and believed that the syndrome revealed “the connection between functional derangement and organic change”. During The First World War an epidemic of neurological conversion symptoms occurred, named “Shell Shock” with much of the same features as DaCosta’s syndrome.¹⁰⁶
2.5.3 *Chronic brucellosis*

Brucellosis is an infectious zoonosis, caused by a bacterium from the *Brucellae* family. During the 1930-40s there was a rising interest in the USA for a chronic form of this disease termed *chronic brucellosis*. This was regarded as a mild form of the disease, without objective signs. The main finding was complaints of chronic fatigue. Often the Brucella bacteria could not be detected, and the diagnosis relied on the subjective symptoms alone.\(^{107}\)

In his paper, *History of Chronic Fatigue Syndrome*,\(^1\) Stephen E. Straus demonstrates how several validation studies were performed in the wake of chronic brucellosis. An important finding was that “patients bordering on a personality disorder or emotional disturbance may be tipped over into a functional state of chronic ill health by an attack of acute brucellosis”.\(^{108}\) Another study by Imboden et al. concluded: “the emotional disturbance is not merely secondary to the stress of illness, but is more critically related to the pre-illness personality structure”.\(^{109}\) In 1961 Imboden et al. followed up this discovery with study of military personnel infected during the Asian influenza epidemic of 1957, and showed how prevalence of psychoneurotic traits prior to infection was greater in those who took more than three weeks to recover compared to those who took less than three weeks.\(^{110}\)

2.5.4 *Epidemic neuromyasthenia/benign myalgic encephalomyelitis*

From 1934 and onwards, multiple outbreaks of an unknown illness were recognized around the world. Initially the illness was confused with poliomyelitis, but it became clear that it differed at central pathophysiological features, such as low mortality rate and low paralytic rate.\(^{111}\) Several names were used, but initially “epidemic neuromyasthenia” was the most frequent term. *Benign myalgic encephalomyelitis (BME)* as a term for this condition was first used to describe an outbreak at Royal Free Hospital in London in 1955. The term was chosen because of the low mortality, severe muscle pain, involvement of the central nervous system, and that it appeared to be infectious.\(^6\) A variety of symptoms were reported from the different outbreaks, but in general patients reported muscular weakness and fatigability, severe pain, lassitude or fatigue on the least exertion, sensory changes, cognitive difficulties, lymphadenopathy and sleep difficulties.\(^{111-113}\) Dr. Acheson pointed out in an article in 1959 that the question of hysteria must enter into the differential diagnosis “in an illness in which there has been a selectivity for young women, no mortality and few positive laboratory findings”.\(^{114}\) He concluded, however, that mass hysteria was not a major factor due to among
others: the clinical picture was similar between those with clear objective physical signs and those without any; the mental symptoms were not typical of mass hysteria; and muscle pain and tenderness were a striking feature of the outbreaks.

### 2.5.5 Myalgic nervosa

McEvedy and Beard, two psychiatrists in the UK, in 1970 studied in detail three of the major outbreaks of BME; Los Angeles Outbreak of 1934, Middlesex Outbreak of 1952 and the Royal Free Outbreak of 1955.\(^{115,116}\) They found encephalitis unlikely, due to lack of high temperature or prolonged disturbance of consciousness, no mortality, no involvement of meninges, no alterations in the cerebrospinal fluid and no clinical signs as classically expected in organic dysfunction of the central nervous system. They postulated hysterical epidemic as a more reasonable explanation. Due to lack of objective evidence that the brain and spinal cord are the sites of an infective or inflammatory disease process, they suggested BME discarded as a name, and proposed *myalgia nervosa* as a new name.

### 2.5.6 Myalgic encephalomyelitis (ME)

Dr. Melvin Ramsay, a leading researcher on BME, and his colleagues strongly refuted the findings of McEvedy and Beard.\(^{117}\) They did so mainly based on the clinical picture: fever (89% of patients), clearly objective neurological signs like ocular palsy (43%) and facial palsy (19%), and lymphadenopathy (79%). During the 1970-80s, Dr. Ramsay and colleagues published several works. They argued the syndrome not to be clearly benign, and emphasized the most characteristic presentation to be profound fatigue and muscular weakness coming on during the day, and increasing in severity with exercise.\(^{113,118}\) Because of this work, the prefix “Benign” was dropped. In 1986 Ramsay published his book, *Myalgic Encephalomyelitis and postviral Fatigue States*. This book included a disease definition for ME: a condition characterized by a unique form of muscle fatigability whereby, even after a minor degree of physical effort, three or more days elapse before full muscle power is restored; extraordinary variability or fluctuation of symptoms even in the course of one day; and an alarming chronicity.\(^6\)
2.5.7 **Chronic EBV-Syndrome, chronic fatigue syndrome (CFS) and the Fukuda-criteria/CDC-criteria**

Two major infectious outbreaks in Nevada and New York in 1984-85, clinically resembling infectious mononucleosis, attracted a lot of interest. A US Centers for Disease Control and Prevention (CDC) working group led by Dr. Holmes reported a combination of “severe fatigue, weakness, malaise, subjective fever, sore throat, painful lymph nodes, decreased memory, confusion, depression, decreased ability to concentrate on tasks, and various other complaints – with a remarkable absence of objective physical or laboratory abnormalities”. The link to mononucleosis had been drawn because many patients had EBV-antibody profiles suggesting reactivation of latent infection, not an acute infectious mononucleosis per se, and the term Chronic EBV-infection gained popularity. Follow-up studies cast doubt on this connection.\(^{120,121}\)

*Chronic Fatigue Syndrome (CFS)* was coined as a term in 1988, when Chronic EBV-infection failed to give a reasonable scientific explanation to this devastating condition.\(^1,119\) Holmes et al. noted that the “*chronic fatigue syndrome is currently an operational concept designed for research purposes that physicians must recognize not necessarily as a single disease but as a syndrome (…) that may have several causes.*” The new name was followed by a set of criteria, revised in 1994 by the CDC, known as the CDC-definition or the Fukuda-criteria for CFS (Figure 1).\(^2\) The Fukuda-criteria has had a major impact in the field and is still among the most frequently used in research and clinical work alike.
As pointed out by Holmes et al., reference laboratories in USA started to advertise EBV serologic tests for use in the diagnosis of the Chronic EBV-syndrome. Many physicians appear to have based their diagnoses only on detectable serum EBV antibody titer.\textsuperscript{119} It seems like this widespread laboratory-confirmed diagnosis contributed to a shift from being regarded an outbreak-related condition, to be viewed as an endemic illness. Although described as endemic by Ramsey in 1978,\textsuperscript{112} the impression is that the general opinion about ME also shifted from epidemic to endemic during the 1980s. This shift has received minimal attention in the research literature, but is conceivably of great importance regarding how CFS as a diagnostic category came in to general use.\textsuperscript{122} This subject will be more thoroughly elaborated in the discussion section of the present thesis.
Fig 2 History of chronic fatigue
Timeline of important events

DaCosta’s syndrome
Fatigue, breathlessness, palpitations, dizziness and chest pain. Headache digestive disturbances and sleeping difficulties were common.

Chronic brucellosis
Low grade infection, chronic fatigue.

Myalgic nervosa
Mass hysteria, encephalitis unlikely due to few objective signs

Chronic EBV-infection

Chronic fatigue syndrome (CFS)

CDC-criteria/Fukuda-criteria
Severe unexplained fatigue six months or more, impaired memory, sore throat, painful lymph nodes, pain in muscles and joints, headaches, unrefreshing sleep, post-exertional malaise.

Bodily distress syndrome (BDS)
Cardiopulmonary/autonomic/gastrointestinal arousal, musculoskeletal tension, general symptoms (incl. fatigue), disabling.

Systemic exertion intolerance disease (SEID)
Major reduction in daily functioning & fatigue, post-exertional malaise, unrefreshed sleep, cognitive impairment, orthostatic intolerance

Neurasthenia/
nervous exhaustion
Absolute exhaustion, fatigue, dyspepsia, headaches, paralysis, insomnia, neuralgia.

Epidemic neuromyasthenia/
benign myalgic encephalomyelitis
Muscular weakness and fatigability, severe pain, fatigue after minor exertion, sensory changes, lymphadenopathy, cognitive difficulties, sleep difficulties.

Myalgic encephalomyelitis (ME)
Profound fatigue, muscular fatigability with minor effort, long recovery, fluctuation of symptoms, alarming chronicity.

Canadian consensus criteria
Significant fatigue, post-exertional malaise and/or fatigue, sleep dysfunction, pain, neurological/ cognitive manifestations, autonomic/ neuroendocrine/immune manifestations, six months duration

International consensus criteria
Post-exertional neuroimmune exhaustion, neurological impairments, immune, gastro-intestinal and genitourinary impairments, energy production/transportation impairments

Systemic exertion intolerance disease (SEID)
Major reduction in daily functioning & fatigue, post-exertional malaise, unrefreshed sleep, cognitive impairment, orthostatic intolerance

2015

2011

2003

2007

1988

1985

1934 - 1978

1934

1871

1867
2.5.8 Canadian Consensus Criteria for ME/CFS (2003) and International Consensus Criteria for ME (2011)

In 2001, a consensus workshop was held in Canada, with the goal to establish a clinical working case definition, diagnostic protocols and treatment protocols for ME/CFS. The need for this was justified because the existing widespread Fukuda-criteria were created for research purposes, and were seen as inappropriate for distinguishing clinical diagnoses. Also, as Carruther et al. point out, fatigue is an integrated part of many illnesses, and the panel concurred that a higher number of the prominent symptoms should be compulsory. The result was a clinical working case definition of ME/CFS, later recognized as the Canadian Consensus Criteria (CCC). Seven main groups of criteria were included: fatigue, post-exertional malaise and/or fatigue, sleep dysfunction, pain, neurological/cognitive manifestations, autonomic or neuroendocrine or immune manifestations, and lastly that the illness had to persist for at least six months (three months in children) (Figure 3).

The authors write: “We hope that the clinical working case definition will encourage a consideration of the ongoing interrelationships of each patient’s symptoms and their coherence into a syndrome of related symptoms sharing a complex pathogenesis rather than presenting a “laundry list” of seemingly unrelated symptoms.”

As a general consideration, the authors point out the importance of assessing the patient’s total illness. “The diagnosis of ME/CFS is not arrived at by simply fitting a patient to a template but rather by observing and obtaining a complete description of their symptoms and interactions, as well as the total illness burden of the patient”.

An international consensus panel was formed in 2011. The leader of the panel and several members of the group were also involved in developing the CCC in 2003. The CCC were used as a starting point – but substantial changes were made, and resulted in publishing of the International Consensus Criteria (ICC 2011). The paper pointed out that ME involves profound dysregulation of the central nervous system and immune system, dysfunction of cellular energy metabolism and ion transport, and cardiovascular abnormalities. These pathophysiological changes induce abnormalities in physical and cognitive function and provide a basis for understanding the symptomatology.

The most important changes were exclusion of a previously required period of six months from onset of symptoms until diagnosis, and exclusion of fatigue as a cardinal symptom. A
new term was suggested, postexertional neuroimmune exhaustion, which encompasses several earlier features:

- Marked, rapid physical and/or cognitive fatigability in response to exertion
- Postexertional symptom exacerbation
- Postexertional exhaustion
- Prolonged recovery period
- Low threshold of physical and mental fatigability (lack of stamina) that results in a substantial reduction in pre-illness activity level.

Despite being a successor of the CCC, the ICC 2011-criteria have not gained the same popularity.

Jason et al. published a revision of the CCC in 2010, recommending using DePaul Symptom Questionnaire (DPQ) to get standardized information about symptoms. Explicit rules for determining whether a symptom meets ME/CFS criteria were made. Such operationalization, it was assumed, would increase the reliability of the revised criteria.\textsuperscript{124}
Fig 3 Diagnostic criteria
Canadian consensus criteria (2003)

1. Fatigue
The patient must have a significant degree of new onset, unexplained, persistent, or recurrent physical and mental fatigue that substantially reduces activity level.

2. Post-exertional malaise and/or fatigue
There is an inappropriate loss of physical and mental stamina, rapid muscular and cognitive fatigability, post-exertional malaise and/or fatigue and/or pain and a tendency for other symptoms within the patient’s cluster of symptoms to worsen. There is a pathologically slow recovery period, usually 24 hours or longer.

3. Sleep dysfunction
There is unrefreshed sleep or sleep quantity or rhythm disturbances such as reversed or chaotic diurnal sleep rhythms.

4. Pain
There is a significant degree of myalgia. Pain can be experienced in the muscles and/or joints, and is often widespread and migratory in nature. Often there are significant headaches of new type, pattern or severity.

5. Neurological/cognitive manifestations:
Two or more of the following difficulties should be present: confusion, impairment of concentration and short-term memory consolidation, disorientation, difficulty with information processing, categorizing and word retrieval, and perceptual and sensory disturbances – e.g., spatial instability and disorientation and inability to focus vision. Ataxia, muscle weakness and fasciculations are common. There may be overload phenomena: cognitive, sensory – e.g. photophobia and hypersensitivity to noise and/or emotional overload, which may lead to “crash” periods and/or anxiety.

6. At least one symptom from two of the following categories:
   - **Autonomic manifestations**: orthostatic intolerance-NMH, POTS, delayed postural hypotension, vertigo; light-headedness, extreme pallor; nausea and IBS; urinary frequency and bladder dysfunction; palpitations with or without cardiac arrhythmia; palpitations, and exertional dyspnea.
   - **Neuroendocrine manifestations**: loss of thermostatic stability - subnormal body temperature and/or marked diurnal fluctuation, sweating episodes, recurrent felling of feverishness and cold extremities; intolerance to heat and cold; marked weight change - anorexia or abnormal appetite; loss of adaptability and tolerance for stress, worsening of symptoms with stress and a slow recovery.
   - **Immune manifestations**: tender lymph nodes, recurrent sore throat and flu-like symptoms, general malaise, new sensitivities to food, medications and/or chemicals

7. The illness persist for at least six months.
It usually has a distinct onset, although it may be gradual. Preliminary diagnosis may be possible earlier. Three months is appropriate in children.
2.5.9 Bodily distress syndrome (BDS)

In 1999, Wessely et al. concluded that there is a substantial overlap between functional syndromes such as IBS and CFS, and argued that a dimensional classification may be more productive than the existing models.¹²⁵ This hypothesis was supported by Fink et al. in 2007,¹²⁶ who in an exploratory study identified three symptom groups - cardiopulmonary, musculoskeletal and gastrointestinal – and that all symptom groups were likely to manifest in the same patient. This suggested that all symptom groups are different facets of one phenomenon, and the term bodily distress syndrome (BDS) was introduced in an attempt to cover most relevant medically unexplained symptoms (MUS).¹²⁷ Budtz-Lilly et al. proposed a formal model of BDS in 2015,¹²⁸ which shows that predisposing bio-psycho-social factors heightens the risk of disease when an individual is exposed for precipitating factors such as infection, physical trauma, emotional trauma or longstanding stress and strains. Perpetuating factors such as dysfunctional cognition, illness worry, dysfunctional illness behavior, CNS sensitization, iatrogenicity and social factors (benefits, litigation etc.) contribute to the further development of a fully functional disorder. Some genetic and environmental factors correlate with the underlying bodily distress phenomenon, but specific environmental and genetic factors lead to different symptom clusters like CFS, IBD etc.¹²⁹

Diagnostic criteria for BDS requires 3 or more symptoms from at least one of the categories cardiopulmonary/autonomic arousal, gastrointestinal arousal, musculoskeletal tension, and general symptoms (including fatigue); furthermore, the symptoms must affect daily living, and relevant diagnoses should be ruled out. Single-organ BDS (mild-moderate) involves one or two of the symptom groups, multi-organ BDS (severe) involves three or four of the symptom groups.

2.5.10 Systemic Exertion Intolerance Disease (SEID)

The Institute Of Medicine (IOM) in the USA convened the Committee on the Diagnostic Criteria for Myalgic Encephalomyelitis/Chronic Fatigue Syndrome to conduct a study to 1) identify the evidence for various clinical diagnostic criteria for ME/CFS, 2) develop evidence-based clinical diagnostic criteria for ME/CFS for use by clinicians, 3) recommend whether new terminology for ME/CFS should be adopted and 4) disseminating the new criteria nationwide. The committee published a comprehensive report in 2015.⁶ A new set of diagnostic criteria was proposed, requiring a substantial reduction in daily functioning
persisting for more than 6 months, accompanied by profound fatigue, post-exertional malaise, unrefreshing sleep and either cognitive impairment or orthostatic intolerance. The focus on exclusionary diagnoses was more downplayed as compared to earlier diagnostic criteria. The report concludes with four recommendations:

(1) A diagnosis of ME/CFS should be made if diagnostic criteria are met, and a new ICD-10 code should be assigned – not linked to “chronic fatigue” or “neurasthenia”.
(2) A toolkit appropriate for screening and diagnosing patients with ME/CFS suitable for a variety of clinical settings encountering these patients should be developed.
(3) The diagnostic criteria set should be reexamined within no more than 5 years, and it should be considered whether a modification is necessary.
(4) ME/CFS should be renamed “Systemic Exertion Intolerance Disease” (SEID).

**Fig 4 Diagnostic criteria**

*Systemic exertion intolerance disease (2015)*

**The following three symptoms are required:**
1. A substantial reduction or impairment in the ability to engage in preillness levels of occupational, educational, social or personal activities that persists for more than 6 months and is accompanied by fatigue, which is often profound, is of new or definite onset (not lifelong), is not the result of ongoing excessive exertion, and is not substantially alleviated by rest.
2. Post-exertional malaise.*
3. Unrefreshing sleep.*

**In addition, at least one of the two following manifestations is required:**
5. Orthostatic intolerance.*

Diagnostic criteria for ME/CFS published by the Institute of Medicine.

*Frequency and severity of symptoms should be assessed. The diagnosis of ME/CFS should be questioned if patients do not have these symptoms at least half of the time with moderate, substantial or severe intensity.

CFS= Chronic Fatigue Syndrome ME= Myalgic Encephalomyelitis
2.6 Validity, reliability and utility of a diagnosis

2.6.1 Validity

The term validity is frequently used in research. Despite this, validity as a term in the context of diagnosis has never been adequately framed.\textsuperscript{122} Valid is from Latin validus and means strong. The concept was formulated by Kelly in 1927, who stated that “the problem of validity is that of whether a test really measures what it purports to measure”.\textsuperscript{130} The understanding of validity has developed through the years, and several sub-categories of the concept of validity have been proposed. Kendell formulates two main criteria for a diagnosis to be valid: \textsuperscript{122}

1. If the defining characteristic of the category is a syndrome, it must be separated from neighboring syndromes and normality by a zone of rarity.
2. If a category is defined by physiological, anatomical, histological, chromosomal or molecular abnormality, there must exist clear and qualitative difference from other categories.

First et al. find this too narrow, and postulate four facets of validity, where it may be possible to possess some but not all sub-categories of validity.\textsuperscript{131} Other terms are also in use, some of them may be synonymous (in parenthesis).

- Face validity: Whether the description of a category and its diagnostic criteria seem to accurately describe the disorder
- Descriptive validity (discriminant validity \textsuperscript{132}): Whether the features of a category are unique to that category relative to other disorders
- Predictive validity (prognostic validity): The extent to which having a diagnosis predicts future clinical course, complications and treatment response
- External or construct validity: The extent to which the diagnosis correlates with expected external validators.
2.6.2 Utility

Utility is defined by Oxford Languages as “the state of being useful, profitable or beneficial”. Kendell and Jablensky make the following statement: “A diagnostic rubric may be said to possess utility if it provides nontrivial information about prognosis and likely treatment outcomes, and/or testable propositions about biological and social correlates.” Budtz-Lilly et al. present a list of various clinical functions helpful when evaluating clinical utility of diagnostic criteria:  

- Is it used?
- Is it acceptable to users?
- Is it easy to use?
- Is it used correctly?
- Does it improve clinical outcome?
- Does it enhance communication:
  - With patients?
  - Across medical specialties?
- Does it assist in conceptualizing?

2.6.3 Reliability

Carmines and Zeller in 1979 described reliability as “the extent to which a measurement of a phenomenon provides stable and consist result”. In other words: does the test or instrument produce the same results under constant conditions? When applied to a diagnostic process, the reliability may refer to whether different investigators get the same results under the same conditions, and whether the same investigator get the same results each time. A way to increase reliability of for example a set of diagnostic criteria is to develop tools with explicit rules to decide whether a criterion is fulfilled or not. As an example, introduction of explicit definitions and decision rules in DSM-III and ICD-10 dramatically improved the reliability of psychiatric diagnoses based on structured interviews.
2.7 Existing validation studies on CCC and SEID

2.7.1 CCC

In 2014, Brurberg et al. published a systematic review of case definitions for CFS/ME. They identified 20 case definitions and identified 38 studies of sufficient quality and consistency for evaluation of validity. These studies were assigned to one of three groups, based on the applied methodology: A) Independent application of several case definitions on the same population, B) Different case definitions with assumed increasing specificity applied sequentially on the same population, and C) Indirect comparisons of prevalence estimates from several case definitions applied on different populations. Regarding CCC, one model A-study (Nacul et al.) and four model B-studies were found, none of the studies included adolescents. Nacul et al. used GP-databases to search for CFS cases; 0.10% satisfied CCC, while 0.19% satisfied the Fukuda criteria. Patients fulfilling the CCC reported more symptoms than patients fulfilling the Fukuda-criteria and not the CCC. Two studies by Jason et al. at level B showed 40-70% of Fukuda-positive were also CCC-positive, also confirming the finding of CCC-positive patients with more functional impairment and more severely fatigued. Lastly, Fluge et al. reported 93% of Fukuda-positive as CCC-positive. The diversity in prevalence estimates in level B-studies may be due to different screening processes before inclusion in the studies. Only the two studies by Jason et al. had comparison of diagnostic criteria as the primary research question.

To investigate publications published after the work of Brurberg et al. (November 2013), with potential valuable information about validity of the CCC-criteria, the following search was performed at PUBMED and EMBASE: ("Canadian Consensus Criteria" OR "Canada criteria" OR "Canada 2003-criteria") AND ("Chronic fatigue" OR "Chronic Fatigue Syndrome" OR "Myalgic Encephalomyelitis" OR "Fatigue" OR "Malaise" OR "Tired" OR "CFS" OR "ME" OR "CFS/ME" OR "ME/CFS" (The search was performed 26.05.2021). 11 relevant papers were found. The majority of these studies investigated differences in possible disease-markers between CCC-CFS patients and healthy controls, with no clear findings. Three randomized controlled trials (RCT), investigating administration of D-vitamin, Rituximab and Cyclophosphamide respectively to CFS-patients, all required the patients to adhere to the CCC to be eligible for participation. None of the treatments proved better than placebo, and thereby giving no support to the predictive validity of the CCC. Jason et al.
performed the only study comparing CCC and other diagnostic criteria, and reported the CCC to differentiate better between patients and healthy controls than the Fukuda-criteria.\textsuperscript{141}

2.7.2 SEID

Validation-studies on the SEID-criteria are scarce: Except for the second paper of this dissertation, only one study with information about validity was found through the search “SEID AND (Validity OR valid OR validation)” at PUBMED and EMBASE. All patients in the paper satisfied both CCC, ICC-2011 and SEID-criteria. The study confirmed earlier findings of increased autoantibody levels in CFS patients compared to healthy controls,\textsuperscript{142} thereby contributing to a zone of rarity to healthy controls, but not between SEID and ME. When searching broader with only “SEID” in title or abstract, three papers with some relevant information about validity were detected. Jason et al. point out that symptom overlap between existing medical illnesses as major depression and SEID combined with few exclusionary illnesses in the diagnostic criteria causes a great number of patients with major depressive disorder to be diagnosed with SEID. The prevalence rate raised 2.8 times compared to CFS diagnosed with the Fukuda-criteria.\textsuperscript{143} There are two studies that compare the prevalence of SEID-positive and Fukuda-positive individuals, and results are conflicting: In one study where patients were classified as CFS according to the Fukuda-criteria, 67 % of the adolescent patients also fulfilled the SEID-criteria,\textsuperscript{144} while equal prevalence was found in a large cross sectional study with patients from the US, Great Britain and Norway.\textsuperscript{145}

2.7.3 Reflections on existing validation studies

Most validation studies on CCC or SEID have focused on descriptive validity: do boundaries to related syndromes exist or is a zone of rarity rather absent? An important aspect to take into account is the diverging opinions whether CFS/ME/SEID are different names for the same condition, i.e. different gradients with ME at the most severe end,\textsuperscript{146} or if ME is viewed as a separate disease with different pathophysiological features than CFS/SEID/other similar syndromes.\textsuperscript{147,148}

Several aberrations from normal physiology have been demonstrated in CFS, particularly autonomic,\textsuperscript{55,56,58,61} immune\textsuperscript{57,67,71} and cognitive dysfunction.\textsuperscript{83,85,86} These and additional findings establish a zone of rarity between CFS and healthy controls. However, more challenging regarding validity, is the attempt to distinguish ME from CFS, SEID from

28
Despite the fact that these entities are proposed to be distinct from one another, there is a lack of studies supporting this view. Regarding descriptive validity, existing validation studies primarily compare symptom- and function scores. These studies seem to provide little support for ME as a separate disease, they rather indicate that ME may be viewed as the most severe version of the chronic fatigue spectrum. In general, patients diagnosed with CCC show a greater symptom burden than patients diagnosed with the Fukuda-criteria.

Only one of all the studies found in the review by Brurberg et al. and in the database searches were performed on adolescent CFS patients.

### 2.8 ME in the media

When reading about CFS/ME in media, a rather intricate picture arises. Despite not being a standard scientific contribution to the ongoing debate, certain patient groups and representatives participate in forming the general opinion on what CFS/ME is and how it should be treated. An important message advocated by some groups is that ME is a separate disease entity, different from CFS. Similar views are uttered by the Norwegian ME-association, which proclaims the importance of distinguishing between ME and Chronic Fatigue (CF) and suggests using ICC-2011 or the CCC when diagnosing ME. The Norwegian Directorate of Health has acknowledged that there is to date no convincing evidence suggesting that one set of diagnostic criteria is superior to others. Despite this officially recognized lack of evidence, the Norwegian Research Council (NCR) required the use of the CCC in order for a project to be eligible for funding when they in 2016 announced an available funding of 30 mill NOK for CFS/ME research projects. This announcement was a pilot project were patients, relatives and patient organizations had a great influence on criteria for the funding call as well as selecting research projects for funding.

While scientific studies on CFS among adolescents display a reported recovery rate of approximately two-thirds, the ME-association in UK reports a general 5 % recovery rate. Regarding adolescents, they state that the rate is better, but they do not provide a specific percentage. In 2016, the Norwegian ME-association claimed that there is no cure for ME and that the goal of interventions is to reduce the patient’s suffering as much as possible.
A great controversy in the field regards recovery and disease. Certain stakeholders (often patients suffering from ME) claim that if individuals recover from ME through psychological treatment (such as CBT), they did not have ME in the first place.157 Also, patients and professionals who defend psychological treatment strategies are sometimes subjects of intense campaigns from some patient groups.158

### 2.9 Rationale for the present studies

As described above, the CCC has gained some traction within the research field as well as among patients and advocacy groups. The lack of validity has not hindered the CCC in being used to differentiate ME-patients from CFS-patients. However, the future perspectives in CFS and ME are strikingly different, and it could be of great importance which of the labels an individual patient embraces. Identifying with ME, the patient is frequently told that there is no existing treatment, the prognosis is poor, and management of symptoms is the main focus.159 On the other hand, identifying with CFS, the patient is presented to well-documented treatment approaches (CBT, Graded Exercise Therapy), and the prognosis is rather good.

An important issue in research is to avoid biases. When the NRC required a specific non-validated diagnostic tool to be used for projects to be eligible for funding, a possible selection bias was introduced at an early stage. Validating the CCC is crucial for the interpretation of the results from the studies funded by the NCR application.

Validation studies have been performed on adult CFS patients. Although they share many pathophysiological features, adolescent CFS patients tend to differ from adult patients in some areas: e.g. genetic predisposition tends to be more important in adolescents,160 and adolescents have better prognosis than adults.20, 24 These differences increase the importance of validating the diagnostic criteria on an adolescent population.
2.10 Aim of the dissertation

The overarching aim of this dissertation is to investigate diagnostic criteria for CFS by:

1. Assessing the descriptive, prognostic and construct validity of the Canadian Consensus Criteria (CCC) and the SEID-criteria (both based on subjective symptoms only) in adolescent CFS patients utilizing objectively defined disease markers.
2. Performing a cluster analysis on disease markers within a widely defined group of fatigued adolescents in an attempt to reveal subgroups not visible at the phenotypical level.

Paper 1: The aim of this study was to explore the validity of the CCC in a sample of adolescent patients with CFS, selected according to a wide case definition. We hypothesized a difference in disease markers and prognosis between patients that satisfied the CCC (CCC-positive) and those who did not (CCC-negative).

Paper 2: The aims of this study were to a) Investigate the prevalence of SEID-positive patients in adolescent CFS-patients, b) Evaluate the SEID criteria by investigating differences in background and disease markers between SEID-positive and SEID-negative patients, and c) Evaluate prognostic impact of the SEID criteria by investigating differences in activity measure and fatigue between the groups at follow up.

Paper 3: The aims of this study were to a) Explore possible subgroups based on biological aberrations within a widely defined cohort of adolescent CFS patients, b) Investigate to what extent these subgroups are associated with constitutional factors (including genetic markers), diagnostic criteria, subjective symptoms and prognosis.
3 MATERIALS AND METHODS

3.1 NorCAPITAL at a glance

3.1.1 Study design

The NorCAPITAL project (The Norwegian Study of Chronic Fatigue Syndrome in Adolescents: Pathophysiology and Intervention Trial; ClinicalTrials ID: NCT01040429) is a combined cross-sectional and randomized controlled trial. The primary aim of NorCAPITAL was to investigate the pathophysiology of CFS in adolescents, and whether low-dose clonidine pharmacotherapy would be beneficial to this group of patients.\(^5\)

The 20 hospital pediatric departments in Norway, primary care pediatricians and general practitioners were invited to refer adolescents aged 12-18 years with CFS to the Department of Paediatrics at Oslo University Hospital, which is the national referral center in Norway for young patients with CFS. A total of 3 months of unexplained chronic/relapsing fatigue of new onset was required, and in line with clinical guidelines the patients were not required to meet any additional symptom criteria.\(^3\),\(^161\) A standard form asked the referring unit to confirm the results of clinical investigations considered compulsory to diagnose pediatric CFS according to national Norwegian recommendations (pediatric specialist assessment, comprehensive hematology and biochemistry analyses, chest x-ray, abdominal ultrasound, and brain magnetic resonance imaging). The patient should also be a) unable to follow normal school attendance, b) not permanently bedridden, c) cleared for not having a medical or psychiatric disorder that might explain the fatigue, d) not experiencing a concurring demanding life event, e) not using prescribed medication, including hormone contraceptives, regularly. Completed forms received at the study center were evaluated carefully, and patients considered eligible were summoned to the study center for a final clinical examination where findings from the previous screening was confirmed.

All participants underwent a baseline investigational program which included a 1-day assessment in hospital consisting of clinical examination, blood sampling, autonomic testing and cognitive testing. Immediately afterwards, daily physical activity was monitored for seven consecutive days using the activPAL accelerometer device (PAL Technologies, Glasgow, Scotland),\(^162\) and a self-administered questionnaire was completed. Thereafter, the patients were randomized to clonidine or placebo treatment in a 1:1-ratio. An identical
An investigational program was performed at follow-up appointments 8 and 30 weeks after inclusion. Both patients and researchers were blinded to treatment allocation at all stages.

176 adolescent CFS patients were assessed for eligibility

- 25 not eligible for randomization due to:
  - usage of other pharmaceuticals (6)
  - uncertain diagnosis (6)
  - older than age limit (5)
  - declined participation (5)
  - spontaneous recovery (3)

151 fulfilled criteria for randomization

120 fulfilled inclusion criteria and started treatment

Clonidine n=76

- 16 not included due to:
  - declined participation (7)
  - met exclusion criteria (6)
  - spontaneous recovery (3)

Placebo n=76

- 15 not included due to:
  - declined participation (4)
  - met exclusion criteria (9)
  - alternative treatment (2)

151 fulfilled criteria for randomization

120 fulfilled inclusion criteria and started treatment

Clonidine n=60

- 5 drop-outs due to:
  - headache (2)
  - syncope (1)
  - declined to take capsules (1)
  - declined participation (1)

Placebo n=60

- 9 drop-outs due to:
  - declined participation (5)
  - declined to take capsules (1)
  - suspected suicidality (1)
  - abdominal discomfort (1)
  - intercurrent illness (1)

55 completed clonidine intervention at week 8

1 drop-out due to declined participation

54 completed investigational program at week 30

51 completed placebo intervention at week 8

2 drop-outs due to declined participation

49 completed investigational program at week 30

Figure 5. Overview of the NorCAPITAL project
3.1.2 Questionnaires

A CFS symptom inventory for adults \(^{163}\) has been used to develop an inventory for adolescents \(^{57}\). A total of 24 common symptoms are evaluated in terms of frequency during the last month (5-point Likert scale ranging from never/rarer than once a month to present every day/almost every day, scored from 1 to 5). In addition, validated inventories were used to assess the following:

- **Fatigue (Chalder Fatigue Questionnaire, CFQ \(^{164}\))**: CFQ contains 11 questions reflecting different aspects of fatigue. It may be scored in two ways. We used dichotomous scoring, where the respective answers are scored 0-0-1-1, giving a maximum sum score of 11.

- **Fatigue severity (Fatigue severity scale – FSS \(^{165}\))**: Nine statements related to fatigue last month are scored on a Likert scale from 1 to 7, ranging from “strongly disagree” to “strongly agree”, giving the maximum sum score of 63.

- **Sleep disturbances (Karolinska Sleep Questionnaire – KSQ \(^{166}\))**: Each symptom is scored 1-6 on a Likert scale, lower scores indicate poorer sleep. A subscale measuring insomnia \(^{166,167}\) is constructed by taking the mean across four items addressing insomnia problems during the preceding month.

- **Symptoms of autonomic dysfunction (Autonomic Symptom Profile – ASP \(^{168}\))**: A version for children and adolescents \(^{58}\) provides sub scores on six functional areas. The score reflecting orthostatic intolerance is utilized in the present paper: Patients were asked whether they get dizzy when rising up from supine position (maximum score 2), and whether they have felt dizzy or not in seven specific situations (score of 1 each), giving a maximum total score of 9.

- **Depressive symptoms (Mood and Feelings Questionnaire – MFQ \(^{169}\))**: Patients were asked 34 questions on what they had been feeling and doing the preceding two weeks, each question was indicated as “Not true”, “Sometimes true” or “True”, scored 0, 1 and 2, giving a maximum total sum score of 68. Seven items were removed in a sensitivity analysis because they were likely to be confirmatively answered by a fatigued patient.

- **Quality of life (Pediatric Quality of Life Inventory – PedsQL \(^{170}\))**: PedsQL covers four dimensions of quality of life: physical (8 items), emotional (5 items), social (5 items) and school functioning (5 items). 23 items are scored from 0-4 on a Likert scale,
ranging from “never” to “almost always”. Raw scores are transformed, providing a mean score that ranges from 0 to 100.

- **Functional disability** (Functional Disability Inventory – FDI \(^{171}\)). FDI addresses difficulties related to participation in different activities, each item scored 0-4 on a Likert scale, extending from “No trouble” to “Impossible”. Maximum total sum score is 60.

### 3.1.3 Biological markers

The biological markers included in the NorCAPITAL-project were selected partly due to existing knowledge of potential pathophysiological mechanisms in CFS, and partly due to theoretical considerations based upon the “sustained arousal” model for CFS. The biological markers relevant for the present studies are mentioned below.

**Immunological markers** were investigated by examining plasma CRP level through a high-sensitive assay (Roche Diagnostics, Indianapolis, IN, USA), and by measuring 27 plasma cytokines, including interleukins, chemokines and growth factors, using a multiplex technique (Bio-Plex Human Cytokine 27-Plex; Bio-Rad Laboratories Inc., Hercules, CA, USA). \(^{172}\)

**Autonomic markers** were investigated using the Task Force Monitor ® (Model 3040i, CNSSystems Medizintechnic, Graz, Austria), a combined hardware and software device for noninvasive continuous recording of autonomic cardiovascular control. \(^{173}\) Instantaneous heart rate (HR) was obtained from the R-R interval (RRi) of the electrocardiogram, while photoplethysmography on the right middle finger was used to obtain a non-invasive, continuous reading of arterial blood pressure. \(^{174}\) Supine values as well as responses to a 20 deg. head-up tilt test (HUT) are reported. \(^{61}\) Power spectral analysis of heart rate variability (HRV) was calculated in the Low Frequency (LF) range (0.05 to 0.17 Hz), and High Frequency (HF) range (0.17 to 0.4 Hz). \(^{175}\) Vagal (parasympathetic) activity is the main contributor to HF variability, whereas both vagal and sympathetic activity contributes to LF variability.

**Neuroendocrine markers** included plasma and urine norepinephrine and epinephrine. These markers were assayed by high-performance liquid chromatography (HPLC) with a reversed-phase column and glassy carbon electrochemical detector (Antec, Leyden Deacade II SCC, Zoeterwoude, The Netherlands), using a commercial kit (Chromsystems, München, Germany).
Urine free cortisol (non-conjugated cortisol) was assayed by solid phase competitive luminescence immunoassay (LIA) (type Immulite® 2000, Siemens Healthcare Diagnostics, NY, USA) after extraction from the urine sample with ether. The cortisol/creatinine ratio was calculated in a morning spot urine sample, according to recommendations. Plasma cortisol, adrenocorticotrophic hormone (ACTH), thyroid-stimulating hormone (TSH), and free thyroxine (FT4), as well as serum Insuline-like Growth Factor 1 (IGF1), were determined by routine assays at the accredited Hormone laboratory at Oslo University Hospital, Norway.

Genomic DNA was extracted from whole blood samples. Single Nucleotide Polymorphism (SNP) genotyping was carried out using custom TaqMan SNP genotyping assays (Applied Biosystems, Foster City, CA, USA). Concordance rate was 100% when 10 % of the samples were re-genotyped. To determine the length of the polymorphic promoter region of the serotonin transporter (S-HTT) gene (SLC6A4), the DNA sequence was first amplified by polymerase chain reaction (PCR) and then separated by gel electrophoresis. The PCR yielded a long (529 bp) and a shorter (486 bp) fragment. After four hours separation at 100V on a 2.5% agarose gel (MetaPhor Agarose, Lonza Cologne GmbH, Cologne, Germany), GelRed dye was added and the fragments were visualized by UV light (Biotium Inc, California, USA). The PCR 100 bp low ladder (Sigma-Aldrich CO, St. Louis, Mo, USA) was used to determine the length of the fragments.

Cognitive function was assessed using three different tools for cognitive testing.

- The digit span test from the Wechsler Intelligence Scale for Children, 4th edition (WISC-IV). The test is performed in a forward and backward manner. In both occasions, strings of random digits are read out loud, starting with two strings of two digits, then two strings of three digits etc. In the forward version, the test person repeats digits in the same order as they are read. In the backward version, the digits are repeated in reverse order.

- The Color-Word Interference test from the Delis-Kaplan Executive Function System (D-KEFS). The test consists of four conditions: 1) Name the colors of different bars. 2) Read written color names. 3) Say aloud the color of the names of colors printed in dissonant colors, such as yellow (should read red) – red (should read green) – green (should read blue). 4) Switch between reading color words and naming dissonant ink colors.
•  The total recall part of Hopkins Verbal Learning Test-Revised (HVLT-R). The examiner reads aloud 12 words, the test person repeats as many words as possible in three trials.

**Pressure pain threshold** was assessed by gradually applying increasing pressure to six predefined areas (the third finger’s cuticles, the trapezius muscle and the supraspinatus muscle bilaterally), by using the force transducer Commander™ Algometer, which has a rubber tip of 0.5 cm² (JTECH Medical, Midvale, USA). Participants were asked to indicate the first sensation of pain during increasing pressure. All sites were assessed in the same order for each patient, and the pressure stimuli were applied twice to each spot and then averaged. Values were reported in Newton (N).

**Physical activity** was measured by using the activPAL accelerometer device (PAL Technologies Ltd, Glasgow, Scotland). This device provide data on step number and cadence as well as time spent on walking, standing and sitting/lying during everyday activities. The recording unit was attached midline on the anterior thigh, and was attached all the time except during showering/bathing. A recording period of seven consecutive days was selected. For each participant, all recording epochs were carefully and independently reviewed, and the mean number of steps per day was calculated for all recording epochs.

### 3.2 Operationalization of diagnostic criteria

The ME/CFS Clinical Diagnostic Worksheet for the CCC contains seven categories of symptoms which in turn are divided into subcategories (Figure 3), while the SEID-criteria has five categories (Figure 4). In the original publications, none of these diagnostic criteria were operationalized regarding explicit specifications of symptom severity, such as for instance a threshold value for pain intensity and frequency. In the present study, all elements of these two diagnostic criteria were operationalized using questionnaires completed at inclusion (cf. appendix 1 and 2 for details). If suitable, a validated inventory was used to verify if a criterion was fulfilled. Alternatively, single questions were used. Generally, in paper I, all symptoms had to be present more than once a week, corresponding to a score of three or higher on the CFS symptom inventory. In paper II, symptoms had to be scored four or higher, corresponding to 3-5 times a week. For example, the pain criterion of the CCC is phrased as follows: “There is a significant degree of myalgia. Pain can be experienced in the muscles and joints and is often migratory in nature”. The two corresponding questionnaire
items read: “How often have you experienced pain in your muscles?” and “How often have you experienced pain in several joints?” Medical records were consulted if questionnaire data were incomplete. Finally, this process enabled dichotomization of the CFS group included in the study: In paper I, one subgroup that satisfied the CCC (CCC-positive group) and one subgroup that did not (CCC-negative group), in paper II, a SEID-positive and a SEID-negative group.

3.3 Statistics

SPSS (IBM SPSS Statistics 22-26, Chicago/New York, USA), R (R Foundation, Austria) and iNZight (Dept. of Statistics, University of Auckland, New Zealand) statistical software were used for statistical analyses. In paper I and II, no imputation of missing data was performed. In paper III, missing baseline data was imputed by the last observation carried backwards-principle, using values from follow-up appointments at week 8, or (if not available) week 30. Generally, a p-value ≤ 0.05 was considered statistically significant.

3.3.1 Power calculations

The total number of adolescents with CFS in the NorCAPITAL project was 120. Assuming equal group sizes (Criteria-positive and Criteria-negative) in paper 1 and paper 2 and a significance level of 5 %, the power to detect an effect size (difference/SD) of 0.6 was 90%, while the power to detect an effect size of 0.5 was at least 75%. An unbalance in sample size of <1:2 only marginally affects the power estimates.

3.3.2 Statistics paper I

The CCC-positive and CCC-negative groups were compared applying t-test, Mann-Whitney U test or Chi-square test as appropriate on cognitive, neuroendocrine, cardiovascular, infectious and inflammatory variables. Analysis of covariance (ANCOVA) of steps per day and CFQ at week 30 was used to assess prognostic validity of the CCC.
3.3.3 Statistics paper II

Comparison of SEID-positive and SEID-negative groups regarding cardiovascular, inflammatory, infectious, neuroendocrine and cognitive variables was performed by applying t-test, Mann-Whitney U test, Chi-square test or Fisher’s exact test as appropriate. Analysis of covariance (ANCOVA) was used to evaluate group differences at week 30. Multiple regression analyses were performed to explore possible confounding effects of baseline characteristics on between-group differences. Holmes-Bonferroni correction was performed in order to account for multiple comparisons.

3.3.4 Statistics paper III

A total of 69 biological markers were selected from the NorCAPITAL database for further analyses. The selection was guided by expert knowledge of the CFS/ME scientific literature. The biological markers were grouped into five domains: endocrine (n=10), inflammatory (n=30), cardiovascular (n=18), pressure pain threshold (n=3), and cognitions (n=8).

Correlation analyses among variables under each domain were performed. When two or more variables were strongly correlated (correlation coefficient ≥ 0.7), one variable was selected for further analyses based upon an assessment of interpretability, suitability regarding statistical analyses and the size of the correlation coefficient. A final correlation analysis of all remaining variables from each domain was performed, resulting in a total of 37 variables which became the basis for subsequent cluster analyses.

Firstly, hierarchical clustering analyses were performed within each of the five domains separately, using Ward’s method, squared Euclidian distance and Z-score. Thereafter, 1-3 variables from each domain were used for a final cluster analysis across all domains. Variables were selected due to their importance in the cluster formation under each domain. The final number of clusters was decided primarily by visual inspection of dendrograms, but a preliminary validation of the possible cluster-solutions was also performed to ensure that there were meaningful differences between the clusters.

We investigated associations between clusters and simple demographic variables, constitutional factors (including genetic markers) and adherence to CFS diagnostic criteria. Baseline values of CFQ, PEM, MFQ, Steps per day, PedsQL and FDI were used to explore associations between clusters and markers of symptoms and function. Prognostic value of
clusters was assessed by evaluating changes in markers of symptoms and function from baseline to week 30.

Differences across clusters were analyzed by Fisher’s exact test, one-way ANOVA or Kruskal-Wallis test as appropriate. A p-value of <0.05 was considered statistically significant. No correction for multiple tests was performed due to the highly exploratory nature of the analyses.
4 RESULTS

*Paper I:*

A total of 46 patients were classified as CCC-positive, 69 were classified as CCC-negative, and five could not be classified. All disease markers were equal across the two groups, with the exception of a poorer performance on the digit span backward test in the CCC positive group. Also, the prognosis over a 30-week period was equal between the groups.

*Paper II:*

A total of 45 CFS patients were SEID-positive, 69 were SEID negative and six could not be classified. The SEID-positive group had significantly higher depressive symptoms score (MFQ) total score 23.2 vs 13.4, difference 9.19 (95% CI 5.78 to 12.6)). No other baseline characteristics were significantly different across the two groups when adjusting for multiple comparisons. Additionally, the prognosis over a 30-week period regarding fatigue score and steps per day was equal between the groups.

*Paper III:*

A total of 116 patients (26.7 % males, mean age 15.4 years) from the NorCAPITAL database were included. The final cluster analyses revealed six clusters labelled: ‘Pain tolerant & good cognitions’, ‘Restored HPA dynamics’, ‘Orthostatic intolerance’, ‘Low-grade inflammation’, ‘Pain intolerant & poor cognitions’, and ‘High vagal (parasympathetic) activity’. There was substantial overlap between clusters. The ‘Pain intolerant & poor cognitions’-cluster was associated with low functional abilities and quality of life, and adherence to the CCC. This cluster had the lowest score on inflammation. No other statistically significant cluster associations were discovered.
NorCAPITAL n= 120

Paper I – validation CCC
n = 5
Could not be classified due to missing data

 CCC-positive n=46
CCC-negative n=69

Baseline
Steps per day and Digit Span Forward (test of cognitive function) significantly lower in CCC-positive. Otherwise no significant differences.

Week 30
No difference in steps per day or CFQ.

Paper II – validation SEID
n = 6
Could not be classified due to missing data

 SEID-positive n=45
SEID-negative n=69

Baseline
Symptoms suggesting mood disorder significantly higher in SEID-positive. Otherwise no significant differences.

Week 30
No difference in steps per day or CFQ.

Paper III – cluster analysis
n = 4
Could not be classified due to missing data

Pain tolerant & good cognitions n=40
Low-grade inflammation n=4

Restored HPA-dynamics n=15
Pain intolerant & poor cognitions n=33

Orthostatic intolerance n=20
High vagal activity n=3

Main conclusions:
Substantial overlap between clusters, no well defined subgroups, no significant associations with symptom scores nor prognosis.

Pain intolerant & poor cognitions:
Associated with low functional abilities and quality of life, and adherence to the CCC. Low score on inflammation.

Figure 6. Flowchart of summarized results in Paper I-II. CCC=Canadian consensus criteria, SEID=systemic exertion intolerance disease, CFQ=Chalder Fatigue Questionnaire, HPA-axis =Hypothalamic-Pituitary-Adrenal axis
5 DISCUSSION

5.1 Validity, utility and reliability of symptom based diagnostic criteria in light of the present studies

5.1.1 Validity

The most important findings from paper I are: 1) The CCC-positive group walks significantly fewer steps per day than the CCC-negative group – this is in line with earlier findings of more severe disability among CCC-positive patients.\textsuperscript{4, 135, 136, 146} 2) The only significant difference between CCC-positive and CCC-negative patients regarding disease markers was on a test of cognitive functioning. No differences were found regarding autonomic cardiovascular control, endocrine markers, previous infections with EBV or Cytomegalovirus (CMV), and immunological markers (including markers of inflammation). 3) No differences were found regarding steps per day and fatigue score at week 30.

In Paper II, the most interesting finding was a significant higher score on depressive symptoms among the SEID-positive as compared to the SEID-negative patients. In a sensitivity analysis where MFQ-items that most likely would be confirmatory answered by a fatigued patient were removed, there still was a significant difference between the two groups. This strengthens the finding of a significant difference in depressive symptom score. It is important to note, though, that patients with known psychiatric disorder, including depression, were excluded from the NorCAPITAL-project. Additionally, the average sum score of the applied inventory (MFQ) did not exceed 28, which has been proposed a cut-off for suspicion of clinical depression.\textsuperscript{185} When adjusted for the possible confounding effect of depressive symptoms, CFQ and Steps per day, no biomarkers differed significantly between the SEID positive and the SEID negative group. Regarding prognosis, results from Paper II showed no difference in steps per day or CFQ at week 30 between the two subgroups.

Summing up, the findings from study I and II reveal only subtle differences regarding disease markers and no difference in prognostic markers between adolescent CFS patients adhering to wide criteria (> 3 months of unexplained fatigue and no additional symptoms required) and those adhering to more strict criteria (CCC and SEID). These findings question the descriptive, predictive and construction validity of the CCC and SEID criteria:
• Descriptive validity is questioned because no zone of rarity is demonstrated between patients satisfying and not satisfying the criteria
• Predictive validity is questioned because criteria adherence is not associated with prognosis.
• Construction validity is questioned because biological markers that could serve as an external validator of the criteria do not differ substantially between the groups.

Perceived together, these findings question more fundamentally the rationale of subclassifying chronically fatigued patients based on clinical symptoms. Rather, the results seem to suggest that all chronically fatigued patients may be seen as one entity in a qualitative sense, albeit with individual, quantitative differences regarding symptom severity and functional impairments.

5.1.2 Utility

A diagnosis or diagnostic criteria may not be valid, but still possess good utility. Budtz-Lilly et al. proposed seven questions to ask when evaluating utility. Two aspects may be evaluated in light of the present dissertation.

Are the diagnostic criteria used correctly? A major part of the work with paper I and II was to operationalize the CCC and SEID criteria applying the questionnaire administered in the NorCAPITAL-project (cf. appendix 1 and 2). Both the CCC and SEID-criteria use phrases such as “significant degree of (...) physical and mental fatigue”, “substantial reduction or impairment in the ability to engage in pre-illness (...) activities”, etc. The CCC describes “significant” as “if the symptom has substantial impact (approximately a 50 % reduction) on the patient’s life experience and activities”. The SEID-criteria mention the diagnosis of CFS to be “questioned if patients do not have these symptoms at least half of the time with moderate, substantial, or severe intensity.” None of these phrases offer a suitable and reliable tool for clinicians and researchers to evaluate if a symptom is present. This lack of operationalization challenges the utility of the diagnostic criteria, and restrict comparison of results across studies.

Does the diagnostic criteria improve clinical outcome?

Using a specific diagnostic set of criteria to categories CFS patients may be beneficial if adhering to the criteria set leads to, for instance, different treatment with favorable results
compared to not adhering to the criteria set. The results of paper I and II indicate no difference in clinical outcome when applying strict as compared to loose criteria. Theoretically, this lack of outcome difference does not exclude that there might exist undiscovered therapeutic options that are more effective for patients adhering to the CCC/SEID-criteria as compared to those that do not adhere. However, the lack of substantial differences in pathophysiological features as mentioned above renders this unlikely.

5.1.3 Reliability
An important aspect of reliability is the consistency and stability of a measurement. The lack of operationalized diagnostic criteria, which conceivably leads to variation in diagnostic practice between clinicians encountering CFS patients in their daily work, not only influences the utility as mentioned above - but impacts reliability as well.

5.2 Clustering of biological markers as an alternative approach
Despite numerous attempts, no specific biological markers have been found in CFS or subgroups of CFS. One possible explanation for this is that existing diagnostic criteria for CFS are based on subjective symptom reports. Thus, CFS patients diagnosed this way by definition will present the same phenotype, but may still differ regarding underlying pathophysiologic mechanisms. If so, research that relies on symptom-based criteria for inclusion may fail to detect biological markers that pertain to a subgroup only. Investigation of biological markers in a more broadly defined group of chronic fatigued patients, independent on diagnostic criteria adherence, may be an alternative strategy. If specific biological markers are discovered for a subgroup, this could in turn point to specific treatment strategies.

In paper III, these considerations were the underlying rationale for performing a hierarchical clustering technique on 69 biological markers. Firstly, clustering was performed within five subgroups or domains: endocrine, inflammatory, cardiovascular, pressure pain threshold and cognition. The primary variables driving the cluster-construction in each domain were further included in a comprehensive cluster analysis. A total of six clusters could be delineated, but there was substantial overlap between them, and no specific subgroups could be identified. Rather, the data seemed to represent a continuum in a multi-dimensional space. Framed otherwise, no one of the clusters manifested a zone of rarity as compared to the other clusters.
Figure 7. Scatterplot of the three most important variables for the final cluster formation. Each colored dot represents one individual belonging to one of the six clusters from the final cluster solution.

The cluster labelled ‘Pain intolerant & poor cognitions’ was significantly associated with the CCC, low functional ability score, low quality of life score, and low levels of inflammatory markers. This confirms earlier findings of the CCC selecting the most severely ill patients, but questions the assumption inherent in the CCC definition that these patients share inflammation as a common pathophysiological feature.\(^5\)

The findings from this novel approach of using biological markers for CFS patient classification correspond with previously identified pathophysiological features within widely defined groups of chronically fatigued patients, such as low-grade inflammation, altered HPA-dynamics and orthostatic intolerance. However, the findings do not support the often advocated proposition that the term ME corresponds to a well-defined subgroup of individuals being erroneously “lumped together” with other conditions characterized by chronic fatigue. Thus, the findings from paper III are in line with the findings from paper I and II: There appears to be no distinct subgroups within the overarching CFS label, but rather a continuum of subjective symptom experiences and pathophysiological aberrations.
5.3 General considerations regarding CFS and diagnostic criteria

A recurring theme in the ongoing debate on CFS/ME/SEID and diagnostic criteria is the belief that certain criteria can define a homogenous group, understood as homogenous on a phenotypical level but corresponding with a similarly homogeneous pathophysiological mechanism. However, the findings of the present thesis do not support this belief, and no good evidence for choosing strict/narrow diagnostic criteria has been provided. A zone of rarity, or fundamental biological differences, between CFS, ME or SEID has not been proven. Neither disease markers nor prognosis differs among CCC-positive vs negative or SEID-positive vs negative patients. Regarding symptoms and function, patients seem to group together in one continuum with no clear boundaries.146

Kendell and Jablensky write: 122

“diagnostic categories are simply concepts, justified only by whether they provide a useful framework for organizing and explaining the complexity of clinical experience in order to derive inferences about outcome and to guide decisions about treatment. (…) once a diagnostic concept (…) has come into general use, it tends to become reified. That is, people too easily assume that it is an entity of some kind that can be invoked to explain the patient’s symptoms and whose validity not be questioned. (…) the mere fact that a diagnostic concept is listed in an official nomenclature and provided with precise, complex definitions tends to encourage this insidious reification.”

This description resonates with CFS. The last decades, a huge effort has been invested in evolving symptom based diagnostic criteria, as well as questionnaires pinpointing assumed core features of CFS. An a priori-assumption, underlying this whole concept, is that CFS is something. This reification of a construct, giving it a name, diagnostic criteria and socio-economic benefits, establishes it as an own entity. One may argue that CFS as a construct has high face validity. However, the face validity has been fueled as CFS/ME/SEID has evolved in the common consciousness as an entity “invoked to explain the symptoms”. As shown in the present dissertation, this process does not seem to be initiated as a response to increasing evidence of descriptive, prognostic or construct validity. Rather, it may have been initiated because CFS has been assumed as an entity; then, this entity has been established in general use and then adapted an assumed validity.
Fink et al. have proposed bodily distress syndrome (BDS) as a common underlying phenomenon for CFS and other medically unexplained syndromes (MUS). It is proposed that the different diagnostic entities share some predisposing and perpetuating factors, whereas other environmental and genetic factors explain the phenotypic scatter. When viewing these conditions as dimensions of the same underlying phenomenon, overlap is expected. It is still of major importance to understand the specific symptom burden of the patient, but the specific name of the condition is not as crucial. The lack of difference and major overlap between subgroups inside the CFS domain presented in the present dissertation may fit well with the BDS hypothesis.

5.4 Methodological issues

5.4.1 Inclusion and exclusion criteria in the NorCAPITAL project

As stated earlier, the NorCAPITAL project required only 3 months of unexplained fatigue. No accompanying symptoms were mandatory. Patients were not allowed to have a concurrent physical or psychiatric disease, to use medications regularly, or to have a concurring demanding life event. These criteria are liberal in the context of available diagnostic criteria for CFS. A frequent critique is that this leads to a heterogeneous patient population. The underlying assumption in such a critique is the assumed pathophysiological difference between patients satisfying different criteria, implying that ME is something different than CFS. If this assumption is invalid, the critique also lacks justification. Heterogeneity in the NorCAPITAL-project would actually be beneficial when searching for subgroups among CFS adolescents. To put it pointedly, one might say that the group under study is rather homogenous, as all patients suffer from unexplained chronic fatigue.

A more relevant critique is related to the exclusion criteria in the NorCAPITAL-project. Patients with a recent adverse life event were excluded from participation, in addition to patients using prescribed medication regularly and those who were permanently bedridden. These exclusion criteria influence the representability of the sample. Both infectious triggers and adverse life events are considered precipitating factors for CFS, and exclusion based on the presence of one of these factors may have produced a skewed sample. Accordingly, by excluding the most disabled patients, the findings from the studies in the NorCAPITAL-project may have limited transferability to this patient group.
5.4.2 Sample size

A total of 120 patients were included in the NorCAPITAL project, which was initially designed as a double-blinded RCT to evaluate the effect of clonidine vs. placebo treatment between two groups with a 50:50 distribution. In paper I and II, the groups investigated were distributed approximately 40:60, which yields sufficient power to detect moderate differences between the groups.

However, in paper III, the sample size of 120 limited the number of variables that could be appropriately included in each cluster analysis. The validation analyses of the clusters also relied on group differences, where the number of patients in each group varied from 15 to 40, limiting the statistical power to detect possible differences. Thus, the nature of paper III is highly explorative.

5.4.3 Questionnaires and operationalization

The questionnaires in the NorCAPITAL-project consist of a variety of validated inventories, along with a CFS symptom inventory for adolescents. This latter inventory was developed approximately in the same period as Brown et al. invented DePauls Questionnaire (DPQ). The rationale for developing DPQ was to enhance the reliability of the diagnostic criteria for ME/CFS by operationalizing the criteria with explicit rules to determine if a criterion is fulfilled.

When the operationalization of the CCC and SEID-criteria was performed in the NorCAPITAL project, we were unaware of this parallel and relevant work from Brown and colleagues. The problem of operationalizing rather vague formulations such as “a substantial degree of” and “significant reduction”, were a common challenge in both projects. The operationalization process using the NorCAPITAL questionnaire would probably have benefited from consulting with the DPQ researchers, possibly enhancing the comparability of results between the studies.

A main issue during the operationalization process of the CCC was to decide a rational cut-off on the Likert Scale of the CFS symptom inventory. We considered a score of $\geq 3$ (more than 1-2 times a week) as a suitable cut off regarding questions like “If you consider the last month, how often have you had headache?” The formulation in the CCC-paper from 2003 was: “A symptom has significant severity if it substantially impacts (approximately a 50 %
reduction) on the patient’s life experience and activities”. Having no grading of severity, we concluded it would be too strict to require each symptom to be frequent more than half of the time.

In contrast to the CFS symptom inventory, the DPQ also requires patients to report severity. In the present study, questions on severity would most likely have enhanced the quality and applicability of the CFS symptom inventory.

When operationalization of the SEID-criteria was performed, we were uncertain whether the cut-off established for the CCC should be applied in this novel context. The phrasing used in the SEID criteria definition is somewhat different: “Frequency and severity of symptoms should be assessed. The diagnosis of ME/CFS should be questioned if patients do not have these symptoms at least half of the time with moderate, substantial, or severe intensity”. Due to the lack of severity measurements, and pinpointing each symptom to be present at least half of the time, we raised to cut off to $\geq 4$ (3-5 times a week or more).

Taken together, this post-hoc operationalization process has some clear weaknesses. The implication is that the results from paper I and II should be interpreted with caution.

5.4.4 Post-exertional malaise (PEM)

PEM is often considered a key feature of CFS.\textsuperscript{5, 6, 123, 143} However, it is questionable whether PEM is uniquely connected to CFS. Major depressive disorder shows a prevalence of PEM from 20 % - 64 %,\textsuperscript{187, 188} while 52 % of MS patients reported PEM in a study by Komaroff et al.\textsuperscript{189} While still definitely more prevalent in CFS, (69 % - 100 % depending on criteria used\textsuperscript{6}), this may also be due to inclusion of PEM in several of the diagnostic criteria, resulting in a self-fulfilling phenomenon.

Still, it should be acknowledged that patients report PEM to be qualitatively different from normal fatigue, characterized by an extreme exhaustion response to minor physical or mental effort, and often accompanied by a flu-like feeling and painful muscle ache. The questionnaire in the NorCAPITAL project did not cover all aspects of PEM, but focused on post-exertional fatigue and the long period of recovery. This may be considered inadequate; however, it is rather unlikely that worsening of additional symptoms is not followed by a worsening in the feeling of fatigue. Thus, the questionnaire item “Increased fatigue after activity” may be seen as a proxy for other PEM-symptoms.\textsuperscript{143}
5.4.5 Adolescents vs adults

The NorCAPITAL project recruited adolescent CFS patients aged 12-18. When studying an adolescent population, the risk of confounding due to comorbidity and the normal aging processes is reduced as compared to adults. While the generalizability of the results to an adult population may be questioned, the great majority of studies do not suggest strong differences in clinical features or pathophysiology between adolescent and adult CFS patients.

5.4.6 Statistics

**Paper I:** Adjustment of p-values due to multiple testing was not performed in paper I. In total, 43 p-values were calculated. Given a significance-level of 0.05 and stochastic independence between all variables, there is a high probability of getting two false positive results, which corresponds neatly to our empirical findings where Steps per day and Digit Span Backward were found to be significantly different between CCC-positive and negative patients. That said, these findings correspond to previous findings of CCC-positive patients as more severely affected, and therefore most likely represent true positives.

**Paper II:** In this paper, adjustment for multiple testing were performed, reducing the risk of type-1 errors at the cost of possible type 2-errors: When increasing the significance level from 0.05 to 0.05/44=0.001, the power drops about 67%.

When we still chose to report findings that were not significant on a nominal level, one argument was the consistent significant differences among variables reflecting HRV/autonomic cardiovascular control.

Taken together, the statistical approach applied in paper II seems reasonable, but the results should be interpreted with caution.

**Paper III:** Paper III was a highly explorative study. Accordingly, the statistical analysis required careful considerations and several strategic decisions:

- **Hierarchical clustering vs latent profile analysis.** All biological markers considered suitable for analysis (n=69) were continuously distributed. Thus, latent profile analysis was considered suitable. Still, hierarchical cluster approach was chosen due to feasibility, i.e. lack of expertise in latent profile analysis.
• **Imputation.** A total of three cases were excluded due to greater amount of missing data, and one case was excluded due to extreme and highly unlikely immunological results. For the remaining 116 cases a total of \( \frac{59}{8280} = 0.0071\% \) of the data from biological markers were missing at baseline, and imputed according to the last observation carried backward-principle (cf. above).

• **Clustering method, distance measures, normalizing of data.** When performing cluster analysis, there are multiple options regarding clustering method, distance measures and handling of data from participating variables. We chose the Ward method, squared euclidian distance and z-score for all participating variables. In a highly explorative study as paper III, one could argue that performing reanalyzes with different methodologies might have increased confidence in the results.

• **Numbers of clusters** was decided by performing visual inspection of dendrograms as well as preliminary analyses of data to ensure the solution was providing meaningful clusters.
6 CONCLUSION

- When compared to CFS patients of same age diagnosed according to wide diagnostic criteria, adolescent CFS patients fulfilling the more strict CCC or SEID-criteria do not differ on neuroendocrine, cardiovascular, inflammatory, infectious or cognitive variables.
- There appears to be no distinct subgroups within the overarching CFS label, but rather a continuum of subjective symptom experiences and pathophysiological aberrations.
- These findings question the descriptive, predictive and construction validity of the CCC and SEID-criteria, and more fundamentally question the rationale of subclassifying chronically fatigued patients based on clinical symptoms. Rather, the results seem to suggest that all patients with unexplained chronic fatigue may be seen as one entity in a qualitative sense, albeit with individual, quantitative differences regarding symptom severity and functional impairments.
7 FUTURE PERSPECTIVES

Brurberg et al. in 2014 concluded: “Development of further case definitions of CFS/ME should be given low priority”. Findings from the present dissertation strengthen this conclusion. The lack of validity of the CCC or SEID-criteria imply that future research project approaching pathophysiological and clinical features of CFS should use broad diagnostic criteria. However, the design should allow post-hoc subgrouping of patients according to more strict diagnostic criteria, in an attempt to strengthen or weaken the conclusions from the present dissertation. This will also enable a formal validation of the Fukuda-criteria, which beside the CCC is the most frequently used diagnostic criteria for CFS.

Findings from the cluster analysis in paper III indicate that future research on CFS should utilize statistical techniques compatible with the appearing continuous nature of chronic fatigue.

These findings, especially the continuous nature of chronic fatigue, should be replicated in a larger study. Both applying hierarchical, but also explore different clustering techniques to the same data would be preferable. Latent profile analysis is an interesting alternative statistical approach that should be considered.

An important next step is to do a formal validation of the CFS diagnosis. This may be performed by comparing CFS patients to patients diagnosed with neighboring conditions such as IBD and fibromyalgia regarding pathophysiological features. Such approach will also be helpful in strengthening or weakening the BDS-hypothesis.
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Study findings challenge the content validity of the Canadian Consensus Criteria for adolescent chronic fatigue syndrome

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Adolescence, Chronic fatigue syndrome, Cytokines, Diagnostic criteria, Validity

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ABSTRACT
Aim: The 2003 Canadian Consensus Criteria for chronic fatigue syndrome (CFS) are often assumed to suggest low-grade systemic inflammation, but have never been formally validated. This study explored the content validity of the Criteria in a sample of adolescents with CFS selected according to a wide case definition.

Methods: A total of 120 patients with CFS with a mean age of 15.4 years (range 12–18 years) included in the NorCAPITAL project were post hoc subgrouped according to the Canadian Consensus Criteria. Those who satisfied the criteria (Criteria positive) and those who did not (Criteria negative) were compared across a wide range of disease markers and markers of prognosis.

Results: A total of 46 patients were classified as Criteria positive, 69 were classified as Criteria negative, and five could not be classified. All disease markers were equal across the two groups, except the digit span backward test of cognitive function, which showed poorer performance in the Criteria-positive group. Also, the prognosis over a 30-week period was equal between the groups.

Conclusion: This study questions the content validity of the Canadian Consensus Criteria, as few differences were found between adolescent patients with CFS who did and did not satisfy the Criteria.

INTRODUCTION
Chronic fatigue syndrome (CFS) is a disabling condition among adolescents, characterised by unexplained, long-lasting, disabling fatigue accompanied by several other symptoms such as musculoskeletal pain, headaches, problems with memory and concentration, and orthostatic intolerance (1,2). CFS may have a detrimental effect on

Key notes
- The Canadian Consensus Criteria for chronic fatigue syndrome (CFS) are frequently used, but have not been formally validated.
- In 120 adolescent patients with CFS diagnosed according to wide criteria, the subgroup defined by the Canadian Consensus Criteria did not differ on disease markers or prognosis from those who were not defined by the Canadian Consensus Criteria.
- These results question the content validity of the Canadian Consensus Criteria in adolescent CFS.
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Validity of adolescent CFS criteria

psychosocial and academic development (3), as well as family functioning (4). Prevalence estimates vary from 0.1 to 1.3%, and more females than males are affected (5). Cognitive behavioural therapy has significant benefits (6), but no pharmacological therapy has been proven effective.

The pathophysiology of CFS remains poorly understood, but adolescent as well as adult studies have consistently revealed certain characteristics, such as hypothalamus–pituitary–adrenal (HPA) axis attenuation (7,8), sympathetic predominance of autonomic cardiovascular control (9,10) and slight cognitive impairments (11,12). The Norwegian Study of Chronic Fatigue Syndrome in Adolescents: Pathophysiology and Intervention Trial (NorCAPITAL) confirmed these findings, reporting lower urinary free cortisol/creatinine ratio (a marker of HPA axis function), stronger heart rate response to orthostasis and higher plasma noradrenaline levels (markers of autonomic cardiovascular control), and lower digit span backward test score (a marker of cognitive function) in adolescent patients with CFS as compared to healthy controls (13).

In addition, low-grade systemic inflammation is reported by many CFS researchers (14), but the evidence seems to be somewhat more conflicting. For instance, Maes et al. (15) reported increased levels of interleukin (IL)-1 and tumour necrosis factor (TNF), Fletcher et al. (16) reported altered levels of several cytokines including IL-1β and IL-1β but normal levels of tumour necrosis factor (TNF), Broderick et al. (17), found evidence of attenuated and Th1 and Th17 immune responses Sulheim et al. (13), reported slightly elevated levels of C-reactive protein (CRP) whereas Vollmer-Conna et al. (18) did not find any alterations of cytokine expression. A possible reason for long-lasting inflammatory activity is sustained infections with herpesviruses. However, the evidence for such infections is limited (13,19).

Despite certain pathophysiological characteristics, there are no established biomarkers of CFS, and a diagnosis therefore rests upon symptom-based case definitions. The Fukuda definition is most common in research, requiring at least 6 months of unexplained chronic or relapsing fatigue of new onset severely affecting daily activities and four or more of eight specific accompanying symptoms (1). The Canadian Consensus Criteria, which were introduced in 2003, have a more detailed symptom requirement (20). It is advocated by part of the scientific community and often taken to suggest that low-grade systemic inflammation is a particularly important feature of CNS pathophysiology. Other advance case definitions leave out accompanying symptoms altogether. These include the Oxford definition (21), which is also commonly used in research, and the definition from the Royal College of Paediatrics and Child Health (2), which is widespread in clinical paediatric practice.

No case definition has been thoroughly validated (22). The aim of this study was to explore the validity of the Canadian Consensus Criteria in a sample of adolescent patients with CFS selected according to a wide case definition. We hypothesised a difference in disease markers and prognosis between patients that satisfied the Canadian Consensus Criteria (Criteria positive) and those who did not (Criteria negative).

METHODS
Design
This study is part of the NorCAPITAL project (ClinicalTrials ID: NCT01040429). Adolescent patients with CFS were assessed at baseline and randomised in a one-to-one ratio to treatment with low-dose clonidine or placebo for 9 weeks and followed for 30 weeks. Double blinding was provided. Data were collected from March 2010 until October 2012. The study was approved by the Norwegian National Committee for Ethics in Medical Research and the Norwegian Medicines Agency and adhered to the Declaration of Helsinki. Details of the design and experimental methods are reported elsewhere (13).

Patients with CFS
The Department of Paediatrics at Oslo University Hospital is a national referral centre for young patients with CFS. All 20 hospital paediatric departments in Norway, as well as primary care paediatricians and general practitioners, were invited to refer patients with CFS aged 12–18 years consecutively to our department. To be included, we required only 3 months of unexplained chronic/relapsing fatigue of new onset, causing disability to a degree that prevented normal school attendance. We did not require that patients met any other accompanying symptom criteria. The referring units were required to confirm that the patient did not have any medical or psychiatric disorder that might explain the fatigue, and that they had not experienced any concurrent demanding life event. Informed consent was obtained from all participants and from parents or next-of-kin if required. In this study, we used baseline and week 30 data.

All participants underwent an investigational programme which included a 1-day assessment in hospital, consisting of clinical examination, blood sampling, autonomic testing, and cognitive tests. Immediately afterwards, daily physical activity was monitored during seven consecutive days using the activPAL accelerometer device (PAL Technologies Ltd, Glasgow, Scotland), and a self-administered questionnaire was completed.

Questionnaire
In accordance with a CFS symptom inventory for adults (23), we previously developed a CFS symptom inventory for adolescents, assessing the frequency of 24 common symptoms during the preceding month. Each symptom was rated on a five-point Likert scale, ranging from never/rarely present to present all of the time. In addition, the questionnaire encompassed validated inventories assessing fatigue (Chalder Fatigue Questionnaire), sleep disturbances (Karolinska Sleep Questionnaire), symptoms of autonomic dysfunction (Autonomic Symptom Profile), depressive symptoms (Mood and Feelings Questionnaire), quality of life (Pediatric Quality of Life Inventory), and functional
disability (Functional Disability Inventory). A detailed description is available (13).

Subgrouping according to Canadian Consensus Criteria of CFS
The ME/CFS Clinical Diagnostic Worksheet for the Canadian Consensus Criteria of CFS contains seven categories of symptoms, which in turn are divided into subcategories (20). All parts of this worksheet correspond to items in the questionnaire of this study, and questionnaire results were therefore used to split the patients with CFS into one subgroup that satisfied the Canadian Consensus Criteria (Criteria-positive group) and one subgroup that did not (Criteria-negative group). As a general rule, all symptoms required in the Canadian Consensus Criteria had to be present more than once a week for patients in the Criteria-positive group, corresponding to a score of three or higher on the CFS symptom inventory. Medical records were consulted if there was incomplete questionnaire data. The subgrouping was performed by only one researcher (TTA).

Disease markers
Cognitive function was assessed by the digit span backward test (24). The examiner reads aloud strings of random digits: The first two strings consist of two digits, the next two strings of three digits, etc. The test person is required to repeat the digits in reverse order. The total score ranges from zero to 14, with higher scores implying better cognitive function.

Autonomic cardiovascular control was assessed by heart rate response to orthostasis and plasma noradrenaline concentration. Regarding the former, participants were asked to sit with their feet elevated to 45° and during 20° head-up tilt. The difference reflects the heart rate response (9). Plasma noradrenaline was assayed by high-performance liquid chromatography with a reversed-phase column and glassy carbon electrochemical detector (Antec, Leyden) using a commercial kit (Chromsystems, München, Germany). All samples were measured in singlets, with serial samples from a given individual run at the same time to minimise run-to-run variability.

HPA axis function was assessed by urinary free cortisol/creatinine ratio. Urine free cortisol was assayed by solid-phase competitive luminescence immunoassay (type Immulite® 2000; Siemens Healthcare Diagnostics, New York, USA). The urine levels of creatinine were analysed using standard automatic analyser techniques, and the cortisol/creatinine ratio was calculated in accordance with recent recommendations (25).

Inflammatory responses were assessed by plasma CRP and cytokines. The plasma CRP level was determined by a high-sensitive assay (Roche Diagnostics, Indianapolis, IN, USA). Plasma cytokines were measured by multiplex technique (Bio-Plex Human Cytokine 27-Plex; Bio-Rad Laboratories Inc., Hercules, CA, USA), performed according to the manufacturer’s instructions. Cytokine networks were estimated with a learning algorithm (17).

Specific antibody responses to the herpesviruses Epstein–Barr virus (EBV) and cytomegalovirus (CMV) were assessed using anti-EBV EBNA IgG (Bio-Rad, Dreieich, Germany) and anti-CMV IgG and IgM (Architect, Abbott, IL, USA).

Statistical analysis
The total number of patients with CFS included in the NorCAPITAL trial was 120. Assuming the number of Criteria-positive and Criteria-negative patients was approximately equal, and a significance level of 5%, the power to

<table>
<thead>
<tr>
<th>Background characteristics of patients with CFS at baseline</th>
<th>Criteria-positive group (n = 46)</th>
<th>Criteria-negative group (n = 69)</th>
<th>p-Value (Criteria-positive group versus Criteria-negative group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender – no. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>13 (28)</td>
<td>21 (30)</td>
<td>0.802</td>
</tr>
<tr>
<td>Female</td>
<td>33 (72)</td>
<td>48 (70)</td>
<td></td>
</tr>
<tr>
<td>Age – years, mean (SD)</td>
<td>15.2 (1.7)</td>
<td>15.4 (1.6)</td>
<td>0.387</td>
</tr>
<tr>
<td>BMI – kg/m², mean (SD)</td>
<td>20.7 (5.3)</td>
<td>22.0 (4.4)</td>
<td>0.081</td>
</tr>
<tr>
<td>Symptoms suggesting mood disorder – no. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>22 (48)</td>
<td>45 (65)</td>
<td>0.064</td>
</tr>
<tr>
<td>No</td>
<td>24 (52)</td>
<td>24 (35)</td>
<td></td>
</tr>
<tr>
<td>Disease duration – months, median (range)</td>
<td>16 (4–104)</td>
<td>18 (5–72)</td>
<td>0.367</td>
</tr>
<tr>
<td>Steps per day – number, mean (SD)</td>
<td>3958 (2142)</td>
<td>5144 (2482)</td>
<td>0.006</td>
</tr>
<tr>
<td>School absence (%) mean (SD)</td>
<td>57.8 (36.9)</td>
<td>56.3 (34.6)</td>
<td>0.795</td>
</tr>
</tbody>
</table>

p-Values are based on chi-square test, Fisher’s exact test, Student t-test or Mann-Whitney’s U test, as appropriate. Criteria-positive group = adheres to the Canadian Consensus Criteria; Criteria-negative group = does not adhere to the Canadian Consensus Criteria; SD = standard deviation; BMI = body mass index.
detect an effect size of 0.6 (difference/SD) was 90%. Correspondingly, the power to detect an effect size of 0.5 would have been at least 75%. An unbalance in sample size of <1:2 only marginally affects the power estimates. SPSS (SPSS Inc., Chicago, IL, USA) and R (R Foundation, Austria) softwares were used for statistical analyses. The two groups (Criteria positive and Criteria negative) were compared applying t-test, Mann–Whitney U test or chi-square test as appropriate, and ANCOVA was used to assess prognostic validity of the Canadian Consensus Criteria. A p-Value ≤0.05 was considered statistically significant. All tests were two sided.

Table 2 Disease markers

<table>
<thead>
<tr>
<th>Regular variables</th>
<th>Criteria-positive group</th>
<th>Criteria negative group</th>
<th>95% confidence interval for difference*</th>
<th>p-Value† (Criteria-positive group versus Criteria negative group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digit span backwards – total sum score, mean (SD)</td>
<td>5.24 (1.39)</td>
<td>6.16 (2.17)</td>
<td>(−1.58, −0.26)</td>
<td>0.007</td>
</tr>
<tr>
<td>Plasma noradrenaline – pmol/L, median (range)</td>
<td>1752 (940–5051)</td>
<td>1802 (862–3769)</td>
<td>(−317, 193)</td>
<td>0.658</td>
</tr>
<tr>
<td>Heart rate responsivenes – beats/min, mean (SD)</td>
<td>4.84 (3.65)</td>
<td>5.18 (4.78)</td>
<td>(−1.90, 1.22)</td>
<td>0.683</td>
</tr>
<tr>
<td>Urine cortisol/creatinine ratio – nmol/mmol, median (IQR)</td>
<td>3.4 (2.6)</td>
<td>3.5 (3.5)</td>
<td>(−0.92, 0.72)</td>
<td>0.930</td>
</tr>
<tr>
<td>Anti-EBV EBNA IgG – no. (%)</td>
<td>No</td>
<td>24 (55.8)</td>
<td>26 (43.3)</td>
<td>(−0.18, 0.11)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>19 (44.2)</td>
<td>34 (56.7)</td>
<td>0.221</td>
</tr>
<tr>
<td>Anti-CMV IgG – no. (%)</td>
<td>No</td>
<td>22 (48.9)</td>
<td>39 (57.4)</td>
<td>0.377</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>23 (51.1)</td>
<td>29 (42.6)</td>
<td>0.766</td>
</tr>
<tr>
<td>Serum CRP – mg/L, median (IQR)</td>
<td>0.41 (0.52)</td>
<td>0.46 (0.97)</td>
<td>(−0.30, 0.79)</td>
<td>0.374</td>
</tr>
<tr>
<td>Plasma cytokines</td>
<td>IL-1β – pg/mL, median (IQR)</td>
<td>2.4 (2.3)</td>
<td>2.0 (1.9)</td>
<td>(−0.30, 0.79)</td>
</tr>
<tr>
<td></td>
<td>IL-1α – pg/mL, median (IQR)</td>
<td>87.2 (92.7)</td>
<td>70.9 (69.9)</td>
<td>(−9.43, 33.1)</td>
</tr>
<tr>
<td></td>
<td>IL-2 – pg/mL, median (IQR)</td>
<td>4.18 (5.86)</td>
<td>3.85 (5.61)</td>
<td>(−0.80, 2.18)</td>
</tr>
<tr>
<td></td>
<td>IL-4 – pg/mL, median (IQR)</td>
<td>1.96 (1.70)</td>
<td>1.97 (1.49)</td>
<td>(−0.38, 0.50)</td>
</tr>
<tr>
<td></td>
<td>IL-5 – pg/mL, median (IQR)</td>
<td>3.09 (3.05)</td>
<td>2.94 (2.75)</td>
<td>(−0.42, 1.11)</td>
</tr>
<tr>
<td></td>
<td>IL-6 – pg/mL, median (IQR)</td>
<td>7.89 (6.20)</td>
<td>6.56 (5.63)</td>
<td>(−0.81, 2.46)</td>
</tr>
<tr>
<td></td>
<td>IL-7 – pg/mL, median (IQR)</td>
<td>9.64 (6.54)</td>
<td>8.43 (7.07)</td>
<td>(−0.97, 3.35)</td>
</tr>
<tr>
<td></td>
<td>IL-8 – pg/mL, mean (SD)</td>
<td>7.09 (5.28)</td>
<td>7.47 (8.46)</td>
<td>(−2.95, 2.18)</td>
</tr>
<tr>
<td></td>
<td>IL-9 – pg/mL, median (IQR)</td>
<td>11.7 (8.97)</td>
<td>11.1 (10.2)</td>
<td>(−2.18, 3.34)</td>
</tr>
<tr>
<td></td>
<td>IL-10 – pg/mL, median (IQR)</td>
<td>3.26 (6.67)</td>
<td>3.08 (5.99)</td>
<td>(−1.01, 1.64)</td>
</tr>
<tr>
<td></td>
<td>IL-12 – pg/mL, median (IQR)</td>
<td>9.44 (12.8)</td>
<td>8.31 (9.77)</td>
<td>(−1.89, 4.13)</td>
</tr>
<tr>
<td></td>
<td>IL-13 – pg/mL, median (IQR)</td>
<td>3.09 (3.39)</td>
<td>3.07 (2.94)</td>
<td>(−0.77, 0.97)</td>
</tr>
<tr>
<td></td>
<td>IL-17 – pg/mL, median (IQR)</td>
<td>30.3 (47.1)</td>
<td>27.1 (35.8)</td>
<td>(−10.6, 10.4)</td>
</tr>
<tr>
<td></td>
<td>FGF – pg/mL, median (IQR)</td>
<td>36.2 (32.2)</td>
<td>34.3 (33.0)</td>
<td>(−7.41, 10.55)</td>
</tr>
<tr>
<td></td>
<td>INFγ – pg/mL, median (IQR)</td>
<td>92.8 (107)</td>
<td>86.9 (73.1)</td>
<td>(−18.6, 28.5)</td>
</tr>
<tr>
<td></td>
<td>IP-10 – pg/mL, median (IQR)</td>
<td>352 (257)</td>
<td>334 (259)</td>
<td>(−44.0, 98.9)</td>
</tr>
<tr>
<td></td>
<td>MCP1 – pg/mL, median (IQR)</td>
<td>10.1 (9.28)</td>
<td>10.5 (7.15)</td>
<td>(−2.46, 2.05)</td>
</tr>
<tr>
<td></td>
<td>MIP-1α – pg/mL, mean (SD)</td>
<td>5.77 (2.62)</td>
<td>5.92 (3.77)</td>
<td>(−1.34, 1.04)</td>
</tr>
<tr>
<td></td>
<td>MIP-1β – pg/mL, median (IQR)</td>
<td>40.3 (16.9)</td>
<td>40.0 (16.9)</td>
<td>(−5.38, 6.06)</td>
</tr>
<tr>
<td></td>
<td>PDGF-BB – pg/mL, median (IQR)</td>
<td>215 (254)</td>
<td>256 (345)</td>
<td>(−91.2, 64.6)</td>
</tr>
<tr>
<td></td>
<td>RANTES – pg/mL, mean (SD)</td>
<td>12878 (9324)</td>
<td>10109 (8138)</td>
<td>(−586, 6114)</td>
</tr>
<tr>
<td></td>
<td>TNF – pg/mL, median (IQR)</td>
<td>48.3 (40.1)</td>
<td>43.1 (42.6)</td>
<td>(−7.26, 15.5)</td>
</tr>
<tr>
<td></td>
<td>VEGF – pg/mL, mean (SD)</td>
<td>143.7 (12.4)</td>
<td>165.2 (29.5)</td>
<td>(−10.2, 5.86)</td>
</tr>
</tbody>
</table>

Cytokine network centralisation measures

| | Degree centrality | 0.12 | 0.11 | (−0.09, 0.13) | 0.822 |
| | Closeness centrality | 0.27 | 0.18 | (−0.08, 0.22) | 0.310 |
| | Eigenvector centrality | 0.72 | 0.72 | (−0.17, 0.19) | 0.998 |
| | Mean links per node | 2.07 | 2.00 | (−0.17, 0.35) | 0.798 |

SD = standard deviation; IQR = interquartile range; CRP = C-reactive protein; IL-1β = Interleukin-1 beta; IL-1α = Interleukin-1 receptor antagonist; IL-2 = Interleukin-2; IL-4 = Interleukin-4; IL-5 = Interleukin-5; IL-6 = Interleukin-6; IL-7 = Interleukin-7; IL-8 = Interleukin-8; IL-9 = Interleukin-9; IL-10 = Interleukin-10; IL-12 = Interleukin-12; IL-13 = Interleukin-13; IL-17 = Interleukin-17; FGF = fibroblast growth factor; INFγ = interferon gamma; IP-10 = interferon gamma-induced protein 10; MCP-1 = monocyte chemotactic protein-1; MIP-1β = macrophage inflammatory protein 1beta; PDGF-BB = platelet-derived growth factor-BB; RANTES = regulated on activation, normal T-cell expressed and secreted; TNF = tumour necrosis factor; VEGF = vascular endothelial growth factor.

*Confidence intervals for the cytokine network parameters are based on the bootstrap.

†p-Values are based on chi-square test, Fisher’s exact test, Student t-test or Mann–Whitney’s U test, as appropriate.
RESULTS
A total of 120 adolescent patients with CFS were included in NorCAPITAL: 46 were classified as Criteria positive, 69 as Criteria negative and five could not be classified due to insufficient data (Table 1). Steps per day were lower in the Criteria-positive group.

At baseline, the Criteria-positive group scored poorer on the digit span backward test of cognitive function (Table 2). All other disease markers were equal across the two groups. At week 30, neither steps per day nor fatigue score was significantly different among the two groups when adjusted for baseline values and allocation to clonidine/placebo (p = 0.649 and p = 0.831, respectively).

DISCUSSION
Several subcategories of validity exist. In this study, two aspects of content validity were explored: discriminate and prognostic validity. Discriminate validity concerns whether two phenomena that are claimed to be unrelated, in fact, are unrelated. In this case, this is whether the two groups of CFS adolescent separated by the Canadian Consensus Criteria are distinct entities. Prognostic validity concerns whether there is a significant difference in outcome for the two groups.

This study shows that there were hardly any differences in disease markers between adolescent patients with CFS that adhered to the Canadian Consensus Criteria definition versus those who did not. Of note, CPR level and cytokine expression were identical, suggesting no differences in systemic inflammation, as opposed to the strong emphasis on a possible inflammatory pathophysiology by the proponents of the Canadian Consensus Criteria (20). The difference on the digit span backward test of cognitive function could be explained by higher symptom burden and lower functional abilities in the Criteria-positive group, which follows directly from the case definition and is reflected in lower number of steps per day. Furthermore, the prognosis over a 30-week observational period was equal. Thus, the Canadian Consensus Criteria seem to have poor content validity, questioning its usefulness in research as well as in clinical practice.

To the best of our knowledge, no previous studies have explored the content validity of the Canadian Consensus Criteria of CFS in adolescents or adults. However, our results are in line with recent findings concerning the validity of the Fukuda diagnostic definition (1). For instance, a formal factor analysis of symptoms in a broadly defined group of patients with chronic fatigue syndrome did not show a strong correspondence with the Fukuda accompanying symptoms (26). A study based upon the Swedish twin registry concluded that there was no empirical support for the requirement of four out eight Fukuda accompanying symptoms (27). A report on a broadly defined population of adolescent patients with CFS concluded that the subgroup adhering to the Fukuda criteria was not characterised by a certain level of disability nor was this subgroup specifically related to characteristics of underlying pathophysiology (alteration of cardiovascular autonomic control) (28). Accordingly, subgrouping based upon the Fukuda criteria did not influence the cross-sectional comparisons or the intervention effects in previously reported results from the NorCAPITAL project (13). Recently, Fink and co-authors provided evidence that bodily distress disorders, which in their study included CFS, might be regard as one entity instead of separate ones (29).

The results of the present study seem to support this view. This study had some limitations. The questionnaire items used to subgroup patients with CFS have not been formally validated for this purpose. However, they correspond closely to the ME/CFS clinical diagnostic worksheet (21). Furthermore, the International Consensus Criteria (30), which is considered a successor of the Canadian Consensus Criteria, were not evaluated here because they were published after the initiation of NorCAPITAL. As the two case definitions are similar, we find it likely that our results are extensible to the ICC as well.

Taken together, the results of this study question the content validity of the Canadian Consensus Criteria in adolescent CFS. Further validation studies are warranted.

ACKNOWLEDGEMENTS
We thank Anette Winger for blood sampling, Kari Gjersum for secretary assistance, Hamsana Chandrakumar, Esther Gangsø, Adelheid Holm, Anna Marie Thorendal Ryenbakken and Marianne Svendsen for practical assistance and Anette Winger, Thor Ueland, Pål Aukrust, Fredrik Müller, Kristin Godang, J Philip Saul and Peter C. Rowe for discussions on study design and results. This study was funded by: Health South-East Hospital Trust and The University of Oslo.

CONFLICT OF INTERESTS
None of the authors have any conflict of interests relevant to this study.

FUNDING
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References


Systemic exertion intolerance disease diagnostic criteria applied on an adolescent chronic fatigue syndrome cohort: evaluation of subgroup differences and prognostic utility

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ABSTRACT
Objective Existing case definitions for chronic fatigue syndrome (CFS) all have disputed validity. The present study investigates differences between adolescent patients with CFS who satisfy the systemic exertion intolerance disease (SEID) diagnostic criteria (SEID-positive) and those who do not satisfy the criteria (SEID-negative).

Methods 120 adolescent patients with CFS with a mean age of 15.4 years (range 12–18 years) included in the NorCAPITAL project (ClinicalTrials ID: NCT01040429) were post-hoc subgrouped according to the SEID criteria based on a comprehensive questionnaire. The two subgroups were compared across baseline characteristics, as well as a wide range of cardiovascular, inflammatory, infectious, neuroendocrine and cognitive variables. Data from 30-week follow-up were used to investigate prognostic differences between SEID-positive and SEID-negative patients.

Results A total of 45 patients with CFS were SEID-positive, 69 were SEID-negative and 6 could not be classified. Despite the fact that clinically depressed patients were excluded in the NorCAPITAL project, the SEID-positive group had significantly higher score on symptoms suggesting a mood disorder (Mood and Feelings Questionnaire): 23.2 vs 13.4, difference 9.19 (95% CI 5.78 to 12.6). No other baseline characteristics showed any group differences. When accounting for multiple comparisons, there were no statistically significant differences between the groups regarding cardiovascular, inflammatory, infectious, neuroendocrine and cognitive variables. Steps per day and Chalder Fatigue Questionnaire at week 30 showed no differences between the groups.

Conclusion The findings question the discriminant and prognostic validity of the SEID diagnostic criteria in adolescent CFS, and suggest that the criteria tend to select patients with depressive symptoms.

BACKGROUND
Chronic fatigue syndrome (CFS) is a disabling and long-lasting disorder characterised by symptoms such as fatigue, postexertional malaise (PEM), sleeping difficulties, widespread pain, cognitive problems and orthostatic intolerance.1–3 The prevalence estimates among adolescents vary from 0.1% to 1.0%,4 5 and the disorder may have a substantial negative impact on school attendance,5 quality of life6 and family functioning.7 The pathophysiology of CFS remains poorly understood. However, some studies report certain characteristics such as attenuation of the hypothalamus–pituitary–adrenal axis,8 9 which may be associated with PEM,10 altered autonomic cardiovascular control8 11 12 and impaired cognitive function.13 14

No biomarker association has been established in CFS, and a diagnosis therefore depends on symptom-based diagnostic criteria only. More than 20 case definitions exist. Most of them require between 3 and 6 months of unexplained fatigue, but vary considerably regarding requirement of additional symptoms.1 3 15 16 In a systematic review

Box 1 Systemic exertion intolerance disease criteria

The following three symptoms are required:

1. A substantial reduction or impairment in the ability to engage in preillness levels of occupational, educational, social or personal activities that persists for more than 6 months and is accompanied by fatigue, which is often profound, is of new or definite onset (not lifelong), is not the result of ongoing excessive exertion, and is not substantially alleviated by rest.

2. Postexertional malaise.*

3. Unrefreshing sleep.*

In addition, at least one of the two following manifestations is required:


5. Orthostatic intolerance.

Diagnostic criteria for ME/CFS published by the Institute of Medicine.*

*Frequency and severity of symptoms should be assessed. The diagnosis of ME/CFS should be questioned if patients do not have these symptoms at least half of the time with moderate, substantial or severe intensity.

From 2014, Brurberg et al 16 could not draw firm conclusions concerning the validity of any of these criteria due to weak methodology and inconsistent results of the 38 included validation studies.

In 2015, the Institute of Medicine (IOM) in the USA proposed new diagnostic criteria for CFS (box 1) and coined a new term: systemic exertion intolerance disease (SEID).2 In line with previous CFS criteria, the SEID criteria are also based on the requirement of specific symptoms assumed to correspond to certain pathophysiological characteristics.

The IOM report found strong evidence of slowed cognitive processing speed and orthostatic intolerance in CFS. Evidence also suggests immune dysfunction in CFS and that certain infections (such as Epstein-Barr virus (EBV) infection) often precipitate the disorder. The IOM report underlined the importance of empirically testing the SEID criteria, and that a multidisciplinary committee review should be undertaken within 5 years.

A diagnostic category should be regarded valid when at least one of two conditions is met: (1) if the diagnostic entity is clearly separated from neighbouring conditions and (2) if the diagnostic entity can be associated with a specific underlying disease process.17 Discriminant validity in this study concerns whether the two groups defined by the SEID criteria (SEID-positive and SEID-negative) differ in terms of variables reflecting underlying disease mechanisms, whereas prognostic validity concerns to what degree there are differences in outcomes between the two groups.

Some studies have compared the SEID criteria with existing case definitions, showing differences in prevalence, symptom severity and grade of impairment,18 19 but to the best of our knowledge the SEID definition has not been firmly validated, either in adolescent or adult patients with CFS. The aims of this study were to (1) investigate the prevalence of SEID-positive patients in a group of 120 adolescent patients with CFS, (2) evaluate the SEID criteria by investigating differences in background and disease markers between SEID-positive and SEID-negative patients, and (3) evaluate the prognostic impact of the SEID criteria by investigating differences in activity measure and fatigue between the groups at 30-week follow-up.

METHODS

Design

This study is part of the NorCAPITAL project (The Norwegian Study of Chronic Fatigue Syndrome in Adolescents: Pathophysiology and Intervention Trial; ClinicalTrials ID: NCT01040429, post-results). NorCAPITAL is a combined cross-sectional and randomised controlled trial that primarily aimed to investigate the pathophysiology of adolescent CFS and to assess low-dose clonidine pharmacotherapy to this group of patients; the design has been described in detail elsewhere.5 In the present study, we used baseline data and follow-up data from week 30. Data were collected between March 2010 and October 2012. Informed, written consent was obtained from all participants and from parents or next of kin if required.

Recruitment of patients with CFS

All hospital paediatric departments in Norway (n=20), primary care paediatricians and general practitioners were invited to refer adolescents with CFS aged 12–18 years consecutively to our department, which is a national referral centre for young patients with CFS. To be eligible for the NorCAPITAL project, we required 3 months of unexplained chronic/relapsing fatigue of new onset, and in line with clinical guidelines the patients were not required to meet any additional symptom criteria.13 15 A standard form required the referral unit to confirm the result of clinical investigations considered compulsory to diagnose paediatric CFS according to national Norwegian recommendations (evaluation by paediatric specialist, extensive haematology and biochemistry analyses, chest X-ray, abdominal ultrasound, and MRI of the brain).

Also, the referring units were required to confirm that the patient (1) was hindered from normal school attendance due to fatigue; (2) was not permanently bedridden; (3) was not struck by a medical or psychiatric disorder (including depression) and/or did not go through any concurrent demanding life event, both could possibly account for the present fatigue; and (4) did not use medicines (including hormone contraceptives) regularly. Patients considered eligible were summoned to our study centre; a final decision on inclusion was made after a separate clinical examination combined with quality assessment of the previously conducted screening programme. Details of the recruitment procedure and inclusion/exclusion criteria are described elsewhere.8

All participants underwent an identical investigational programme at baseline, 8 weeks and 30 weeks, which included a 1-day assessment in hospital consisting of
clinical examination, blood sampling, autonomic testing and cognitive testing. Immediately afterwards, daily physical activity was monitored for seven consecutive days using the activPAL accelerometer device (PAL Technologies, Glasgow, Scotland), and a self-administered questionnaire was completed.

**Questionnaires**

A CFS symptom inventory for adults has previously been used to develop an analogous inventory for adolescents. A total of 24 common symptoms are evaluated in terms of frequency during the last month (5-point Likert scale ranging from never/rarer than once a month to present every day/almost every day, scored from 1 to 5).

1. Fatigue (Chalder Fatigue Questionnaire, CFQ): CFQ contains 11 questions reflecting different aspects of fatigue. It is scored in two ways; we used dichotomous scoring, where the respective answers are scored 0-0-1-1, giving a maximum score of 11.

2. Fatigue Severity Scale: Nine statements related to fatigue last month are scored on a Likert scale from ‘strongly disagree’ to ‘strongly agree’, giving a maximum sum score of 63.

3. Sleep disturbances (Karolinska Sleep Questionnaire): Each symptom is scored 1–6 on a Likert scale measuring insomnia is constructed by taking the mean across four items addressing insomnia problems during the preceding month.

4. Symptoms of autonomic dysfunction (Autonomic Symptom Profile): A version for children and adolescents provides subscores on six functional areas. The score reflecting orthostatic intolerance is used in the present paper. Patients were asked whether they get dizzy when rising up from supine position (maximum score of 2), and whether they have felt dizzy or not in seven specific situations (score of 1 each), giving a maximum total score of 9.

5. Depressive symptoms (Mood and Feelings Questionnaire, MFQ): Patients were asked 34 questions on what they had been feeling and doing the preceding 2 weeks; each question was indicated as ‘Not true’, ‘Sometimes true’ or ‘True’, scored 0, 1 and 2, giving a maximum total sum score of 68. Seven items were removed in a sensitivity analysis because they were likely to be positively answered by a fatigued patient.

6. Quality of life (Pediatric Quality of Life Inventory, PedsQL): PedsQL covers four dimensions of quality of life: physical (eight items), emotional (five items), social (five items) and school functioning (five items). Twenty-three items are scored from 0 to 4 on a Likert scale, ranging from ‘never’ to ‘almost always’. Raw scores are transformed, providing a mean score that ranges from 0 to 100.

7. Functional disability (Functional Disability Inventory, FDI): FDI addresses difficulties related to participation in different activities, each item scored 0–4 on a Likert scale, extending from ‘No trouble’ to ‘Impossible’. The maximum total score is 60.

**Subgrouping according to the SEID criteria**

The IOM report presents the SEID criteria with an explanation of presumed core symptoms; these symptoms are considered mandatory to receive the diagnosis. We used variables from the above-mentioned set of questionnaires to operationalise the criteria, and then used baseline data to decide whether a patient fulfilled the SEID criteria or not (see online supplementary table 1).

**Disease markers**

All methods for disease marker investigation have been thoroughly described in previous publications from the NorCAPITAL project. In short, inflammation markers were investigated by examining plasma CRP (C-reactive protein) level through a high-sensitive assay (Roche Diagnostics, Indianapolis, Indiana, USA), and by measuring 27 plasma cytokines using a multiplex technique (Bio-Plex Human Cytokine 27-Plex; Bio-Rad Laboratories, Hercules, California, USA). Specific antibody responses against EBV and Cytomegalovirus (CMV) were assessed using anti-EBV EBNA IgG (Bio-Rad, Dreieich, Germany), anti-EBV VCA IgG and IgM (Hiss Diagnostics, Freiburg, Germany), and anti-CMV IgG and IgM (Architect, Abbott, Illinois, USA). Autonomic cardiovascular control of orthostasis was investigated using the Task Force Monitor (TFM; Model 3040i, CNSystems Medizintechnik, Graz, Austria), a combined hardware and software device for non-invasive continuous recording of cardiovascular variables. The patients were subjected to a low-intensity 20° head-up tilt test. Power spectral analysis of heart rate variability (HRV) was automatically provided by the TFM; power was calculated in the low-frequency (LF) range (0.05–0.17 Hz) and high-frequency (HF) range (0.17–0.4 Hz). Vagal (parasympathetic) activity is the main contributor to HF variability, whereas both vagal and sympathetic activities contribute to LF variability; the LF:HF ratio is considered an index of sympathovagal balance. Cognitive function was assessed using the digit span test from the Wechsler Intelligence Scale for Children, Fourth Edition, and the conditions 1–3 of Color-Span Interference Test from the Delis-Kaplan Executive Function System, and the total recall part of Hopkins Verbal Learning Test-Revised (HVLT-R).

**Statistical analysis**

One hundred and twenty adolescent patients with CFS were included in the NorCAPITAL project. Presupposing the same number of SEID criteria positive and negative patients and a significance level of 5%, the power to detect an effect size of 0.6 (difference/SD) was estimated to be 90%; the power to detect an effect size of 0.5 would be a minimum of 75%. A difference in sample size of <2:1 only had insignificant impact on the power estimates.
IBM SPSS statistics 24 (IBM, New York, USA) and iNZight (Department of Statistics, University of Auckland, New Zealand) were used for statistical analyses. Comparison of the SEID-positive and SEID-negative groups was performed by applying t-test, Mann-Whitney U test, $\chi^2$ test or Fisher’s exact test as appropriate, and analysis of covariance (ANCOVA) was used to evaluate group differences at week 30. Multiple linear regression analyses were performed to explore possible confounding effects of baseline characteristics on between-group differences. A P value $\leq 0.05$ was considered statistically significant. Due to multiple comparisons, a Holmes-Bonferroni correction was considered appropriate for all across-group tests (a total of 44), resulting in a level of significance equal to $0.05/44=0.00114$. All tests were two-sided.

**RESULTS**

Of the 120 adolescent patients with CFS included in NorCAPITAL, 45 patients were classified as SEID-positive and 69 as SEID-negative. Six patients were excluded due to insufficient data (table 1).

The SEID-positive group had statistically significantly higher score on symptoms suggesting a mood disorder from the MFQ inventory (total score 23.2 vs 13.4, $P\leq0.001$). We performed a sensitivity analysis by removing seven items from the MFQ likely to be positively answered by any fatigued person, but the difference remained statistically significant (total score 14.8 vs 8.46, $P\leq0.001$). No other baseline characteristics were different between the two groups.

Preliminary analyses showed statistically significant differences at baseline on variables reflecting HF power, LF power, LF:HF ratio, plasma cortisol level and digit span sum score (table 2). However, when multiple comparisons were taken into account, none of the differences were considered statistically significant. Also, when adjusting for the possible confounding effects of total score of MFQ, total score of CFQ and steps per day in multiple linear regression analyses, all $P$ values were $>0.05$.

An ANCOVA model featuring steps per day and CFQ at week 30 as outcome variables showed no differences between SEID groups (table 3).

**DISCUSSION**

The following are the main findings of this study: (1) No cardiovascular, infectious, inflammatory, neuroendocrine or cognitive biomarker differed significantly between the SEID-positive and the SEID-negative groups. (2) When controlled for baseline values, there were no differences in steps per day or CFQ at 30 weeks between the SEID-positive and the SEID-negative groups. (3) The SEID-positive group had significantly more depressive symptoms. Taken together, the findings question the validity of the SEID diagnostic criteria in adolescent CFS, and suggest that the criteria tend to select patients with depressive symptoms.

The SEID criteria have been criticised for not having predefined exclusion criteria, enabling patients with major depressive disorders to be diagnosed with CFS. The present sample should not contain patients with

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**Table 1** Baseline characteristics

<table>
<thead>
<tr>
<th>Patients with CFS, baseline</th>
<th>SEID-negative (n=69)</th>
<th>SEID-positive (n=45)</th>
<th>Difference/OR</th>
<th>95% CI of difference/OR</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, n (%), Male</td>
<td>22 (32)</td>
<td>10 (22)</td>
<td>1.64</td>
<td>0.69 to 3.90</td>
<td>0.262</td>
</tr>
<tr>
<td></td>
<td>47 (68)</td>
<td>35 (78)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years, mean (SD)</td>
<td>15.5 (1.6)</td>
<td>15.1 (1.6)</td>
<td>−0.35</td>
<td>−0.95 to 0.26</td>
<td>0.264</td>
</tr>
<tr>
<td></td>
<td>21.8 (3.9)</td>
<td>21.2 (4.7)</td>
<td>0.54</td>
<td>−2.16 to 1.07</td>
<td>0.507</td>
</tr>
<tr>
<td>BMI, kg/m$^2$, mean (SD)</td>
<td>18 (17)</td>
<td>15 (16)</td>
<td>3</td>
<td>−2 to 11</td>
<td>0.101</td>
</tr>
<tr>
<td>Disease duration, months, median (IQR)</td>
<td>13.4 (7.6)</td>
<td>23.2 (10.8)</td>
<td>9.19</td>
<td>5.78 to 12.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Symptoms suggesting a mood disorder, total score, mean (SD)</td>
<td>4824 (2507)</td>
<td>4342 (2276)</td>
<td>−481</td>
<td>−1409 to 446</td>
<td>0.306</td>
</tr>
<tr>
<td>Chalder Fatigue Questionnaire, total score, mean (SD)</td>
<td>18.1 (5.8)</td>
<td>20.7 (6.0)</td>
<td>2.67</td>
<td>0.40 to 4.94</td>
<td>0.022</td>
</tr>
<tr>
<td>School absence, %, median (IQR)</td>
<td>50.0 (65)</td>
<td>75.0 (65)</td>
<td>25.0</td>
<td>0.00 to 37.5</td>
<td>0.069</td>
</tr>
<tr>
<td>Allocation to clonidine vs placebo, n (%)</td>
<td>33 (48)</td>
<td>25 (56)</td>
<td>0.73</td>
<td>0.34 to 1.56</td>
<td>0.420</td>
</tr>
<tr>
<td>Placebo</td>
<td>36 (52)</td>
<td>20 (44)</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

P values are based on $\chi^2$ test, Student’s t-test or Mann-Whitney’s test, as appropriate. Due to multiple comparisons, the level of significance is considered equal to $0.05/44=0.00114$. BMI, body mass index; CFS, chronic fatigue syndrome; SEID, systemic exertion intolerance disease.
### Table 2  Biomarkers possibly associated with the SEID diagnostic criteria

<table>
<thead>
<tr>
<th></th>
<th>Patients with CFS, baseline</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SEID-negative</td>
<td>SEID-positive</td>
<td>Difference/ OR</td>
<td>95%CI of difference/OR</td>
<td>P value, not adjusted</td>
<td>P value, adjusted*</td>
</tr>
<tr>
<td><strong>Cardiovascular variables, supine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate, beats/min, mean (SD)</td>
<td>69.5 (9.0)</td>
<td>73.7 (13.1)</td>
<td>4.21</td>
<td>–0.23 to 8.64</td>
<td>0.063</td>
<td></td>
</tr>
<tr>
<td>MAP, mm Hg, mean (SD)</td>
<td>78.7 (7.9)</td>
<td>79.2 (9.2)</td>
<td>0.54</td>
<td>–2.66 to 3.73</td>
<td>0.740</td>
<td></td>
</tr>
<tr>
<td>TPRI, mm Hg/L/min/m² x 10⁻³, mean (SD)</td>
<td>8.70 (2.12)</td>
<td>9.39 (16.6)</td>
<td>0.69</td>
<td>–0.05 to 1.43</td>
<td>0.067</td>
<td></td>
</tr>
<tr>
<td>LFnuRRI, normalised units, mean (SD)</td>
<td>38.7 (16.2)</td>
<td>46.1 (13.0)</td>
<td>7.43</td>
<td>1.72 to 13.1</td>
<td>0.011</td>
<td>0.104</td>
</tr>
<tr>
<td>HFnuRRI, normalised units, mean (SD)</td>
<td>61.3 (16.3)</td>
<td>53.9 (13.0)</td>
<td>–7.39</td>
<td>–13.1 to –1.67</td>
<td>0.012</td>
<td>0.106</td>
</tr>
<tr>
<td>LFabsRRI, ms², median (IQR)</td>
<td>632 (805)</td>
<td>451 (774)</td>
<td>–182</td>
<td>–516 to 136</td>
<td>0.159</td>
<td></td>
</tr>
<tr>
<td>HFabsRRI, ms², median (IQR)</td>
<td>1016 (1974)</td>
<td>495 (1662)</td>
<td>–521</td>
<td>–1239 to 22</td>
<td>0.014</td>
<td>0.051</td>
</tr>
<tr>
<td>LF:HF ratio, median (IQR)</td>
<td>0.63 (0.56)</td>
<td>0.92 (0.88)</td>
<td>0.29</td>
<td>0.05 to 0.52</td>
<td>0.008</td>
<td>0.082</td>
</tr>
<tr>
<td><strong>Cardiovascular variables, delta values†</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate, beats/min, mean (SD)</td>
<td>5.19 (3.49)</td>
<td>4.60 (3.22)</td>
<td>–0.58</td>
<td>–2.09 to 0.93</td>
<td>0.418</td>
<td></td>
</tr>
<tr>
<td>MAP, mm Hg, mean (SD)</td>
<td>1.02 (4.02)</td>
<td>1.32 (3.43)</td>
<td>0.30</td>
<td>–1.14 to 1.74</td>
<td>0.684</td>
<td></td>
</tr>
<tr>
<td>TPRI, mm Hg/L/min/m² x 10⁻³, mean (SD)</td>
<td>6.24 (8.09)</td>
<td>6.46 (8.63)</td>
<td>0.021</td>
<td>–0.29 to 0.34</td>
<td>0.895</td>
<td></td>
</tr>
<tr>
<td>LFnRRI, normalised units, mean (SD)</td>
<td>9.22 (10.1)</td>
<td>5.32 (12.5)</td>
<td>–3.90</td>
<td>–8.12 to 0.32</td>
<td>0.083</td>
<td></td>
</tr>
<tr>
<td>HFnuRRI, normalised units, mean (SD)</td>
<td>–9.19 (10.1)</td>
<td>–5.32 (12.5)</td>
<td>3.87</td>
<td>–0.55 to –8.28</td>
<td>0.086</td>
<td></td>
</tr>
<tr>
<td>LFabsRRI, ms², median (IQR)</td>
<td>–94.3 (428)</td>
<td>–101 (316)</td>
<td>–7.2</td>
<td>–126 to 166</td>
<td>0.739</td>
<td></td>
</tr>
<tr>
<td>HFabsRRI, ms², median (IQR)</td>
<td>–355 (961)</td>
<td>–153 (815)</td>
<td>202</td>
<td>–103 to 539</td>
<td>0.075</td>
<td></td>
</tr>
<tr>
<td>LF:HF ratio, median (IQR)</td>
<td>0.24 (0.66)</td>
<td>0.21 (0.80)</td>
<td>–0.02</td>
<td>–0.43 to 0.29</td>
<td>0.092</td>
<td></td>
</tr>
<tr>
<td><strong>Infectious variables</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Anti-EBV EBNA IgG, n (%)</td>
<td>Negative 32 (49.2)</td>
<td>25 (56.8)</td>
<td>0.74</td>
<td>0.34 to 1.59</td>
<td>0.436</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Positive 33 (50.8)</td>
<td>19 (43.2)</td>
<td> </td>
<td> </td>
<td> </td>
<td> </td>
</tr>
<tr>
<td>Anti-EBV VCA IgM, n (%)</td>
<td>Negative 67 (98.5)</td>
<td>43 (95.6)</td>
<td>0.36</td>
<td>0.37 to 35.4</td>
<td>0.562</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Positive 1 (1.5)</td>
<td>2 (4.4)</td>
<td> </td>
<td> </td>
<td> </td>
<td> </td>
</tr>
<tr>
<td>Anti-EBV VCA IgG, n (%)</td>
<td>Negative 29 (65.9)</td>
<td>21 (67.7)</td>
<td>0.92</td>
<td>0.35 to 2.45</td>
<td>0.868</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Positive 15 (34.1)</td>
<td>10 (32.3)</td>
<td> </td>
<td> </td>
<td> </td>
<td> </td>
</tr>
<tr>
<td>Anti-CMV IgM, n (%)</td>
<td>Negative 67 (100)</td>
<td>45 (100)</td>
<td>0.00</td>
<td> </td>
<td> </td>
<td> </td>
</tr>
<tr>
<td></td>
<td>Positive 0 (0)</td>
<td>0 (0)</td>
<td> </td>
<td> </td>
<td> </td>
<td> </td>
</tr>
<tr>
<td>Anti-CMV IgG, n (%)</td>
<td>Negative 38 (55.9)</td>
<td>24 (53.3)</td>
<td>1.11</td>
<td>0.52 to 2.36</td>
<td>0.790</td>
<td></td>
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<tr>
<td></td>
<td>Positive 30 (44.1)</td>
<td>21 (46.7)</td>
<td> </td>
<td> </td>
<td> </td>
<td> </td>
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<tr>
<td><strong>Inflammatory variables</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Serum hsCRP, mg/L, median (IQR)</td>
<td>0.44 (0.97)</td>
<td>0.46 (0.62)</td>
<td>0.02</td>
<td>–0.25 to 0.21</td>
<td>0.526</td>
<td></td>
</tr>
<tr>
<td>Serum IL-1β, pg/mL, median (IQR)</td>
<td>2.03 (2.12)</td>
<td>2.31 (2.31)</td>
<td>0.28</td>
<td>–0.92 to 1.06</td>
<td>0.620</td>
<td></td>
</tr>
<tr>
<td>Serum IL-6, pg/mL, median (IQR)</td>
<td>6.56 (5.54)</td>
<td>7.39 (7.29)</td>
<td>0.83</td>
<td>–1.66 to 3.00</td>
<td>0.481</td>
<td></td>
</tr>
<tr>
<td>Serum IL-10, pg/mL, median (IQR)</td>
<td>3.49 (3.35)</td>
<td>4.07 (6.68)</td>
<td>0.59</td>
<td>–1.25 to 3.16</td>
<td>0.936</td>
<td></td>
</tr>
<tr>
<td>Serum TNF, pg/mL, median (IQR)</td>
<td>45.5 (39.1)</td>
<td>46.8 (46.1)</td>
<td>1.34</td>
<td>–13.3 to 15.5</td>
<td>0.674</td>
<td></td>
</tr>
<tr>
<td><strong>Neuroendocrine variables</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma norepinephrine, pmol/L, mean (SD)</td>
<td>1972 (722)</td>
<td>2017 (893)</td>
<td>45</td>
<td>–258 to 348</td>
<td>0.770</td>
<td></td>
</tr>
<tr>
<td>Plasma epinephrine, pmol/L, mean (SD)</td>
<td>316 (104)</td>
<td>323 (125)</td>
<td>6.36</td>
<td>–37.4 to 50.1</td>
<td>0.774</td>
<td></td>
</tr>
<tr>
<td>Plasma cortisol, nmol/L, mean (SD)</td>
<td>345 (135)</td>
<td>400 (156)</td>
<td>55</td>
<td>–0.06 to 110</td>
<td>0.050</td>
<td></td>
</tr>
</tbody>
</table>

Continued
clinical depression disorder, given the predefined exclusion criteria of NorCAPITAL; however, patients with varying degrees of depressive symptoms were eligible. Our finding of higher depressive symptom scores among SEID-positive patients might theoretically be explained from overlapping symptoms in depression and chronic fatigue states. However, in a sensitivity analysis removing possibly overlapping items, the differences between the groups remained, strengthening the finding that the SEID-positive group has a greater depressive symptom burden.

Opinions diverge whether chronic fatigue is a general, continuous phenomenon, or may be divided into discrete subgroups that are separate entities with regard to biological profile, treatment and prognosis.38 39 The Fukuda et al criteria1 are the most frequently used in both clinical practice and research, but questionable validity has been revealed.16 A recently published validation study on the Canadian Consensus Criteria reported few differences in biomarkers and no prognostic difference between adolescent patients with CFS who did and did not satisfy the criteria.40 The results from the present study corroborate these previous findings, and taken together these findings question more fundamentally the validity of classifying chronic fatigued patients based on symptom expressions alone.

Despite not being detected as statistically significant in the present study, variables reflecting HRV give the impression that autonomous cardiovascular control may be of importance in the further search for relevant and valid subgrouping of patients with chronic fatigue. This goes well with earlier findings showing significant changes in autonomous cardiovascular control in patients with CFS.11

### Strengths and limitations

A strength of this study is the low rate of missing data. A limitation might be that data acquisition in the NorCAPITAL project was carried out before the SEID criteria were published. In particular, the phenomenon of PEM,

---

**Table 2** Continued

<table>
<thead>
<tr>
<th>Patients with CFS, baseline</th>
<th>SEID-negative (n=69)</th>
<th>SEID-positive (n=45)</th>
<th>Difference/</th>
<th>95% CI of difference/</th>
<th>P value, not adjusted</th>
<th>P value, adjusted*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine norepinephrine:creatinine ratio, pmol/mmol, mean (SD)</td>
<td>13.0 (4.80)</td>
<td>12.4 (4.23)</td>
<td>−0.55</td>
<td>−2.31 to 1.21</td>
<td>0.539</td>
<td></td>
</tr>
<tr>
<td>Urine epinephrine:creatinine ratio, pmol/mmol, median (IQR)</td>
<td>1.22 (1.27)</td>
<td>1.27 (1.06)</td>
<td>0.06</td>
<td>−0.40 to 0.59</td>
<td>0.948</td>
<td></td>
</tr>
<tr>
<td>Urine cortisol:creatinine ratio, nmol/mmol, median (IQR)</td>
<td>3.61 (2.56)</td>
<td>3.16 (3.45)</td>
<td>−0.45</td>
<td>−1.69 to 0.57</td>
<td>0.451</td>
<td></td>
</tr>
</tbody>
</table>

Cognitive variables

| Digit span test, sum score, mean (SD) | 14.7 (3.70) | 13.2 (2.92) | −1.51 | −2.81 to −0.21 | 0.023 |
| D-KEFS conditions 1 and 2 mean, s, mean (SD) | 29.7 (4.85) | 30.9 (4.67) | 1.20 | −0.65 to 3.04 | 0.201 |
| D-KEFS condition 3, s, mean (SD) | 57.3 (12.3) | 61.0 (12.5) | 3.69 | −1.01 to 8.39 | 0.123 |
| HVLT 1–3, sum score, mean (SD) | 27.8 (3.94) | 26.4 (4.14) | −1.33 | −2.86 to 0.19 | 0.086 |

P values are based on χ² test, Fisher’s exact test, Student’s t-test or Mann-Whitney’s test, as appropriate. Due to multiple comparisons, the level of significance is considered equal to 0.05/44=0.00114.

*Multiple linear regression models, adjusting for MFQ, CFQ total score and steps per day.
†Response to 20° head-up tilt (delta values).
abs, absolute; CFQ, Chalder Fatigue Questionnaire; D-KEFS, Delis-Kaplan Executive Function System; hsCRP, high-sensitivity C-reactive protein; HF, high frequency; HVLT, Hopkins Verbal Learning Test; IL, interleukin; LF, low frequency; MAP, mean arterial pressure; MFQ, Mood and Feelings Questionnaire; NA, not applicable; nu, normalised units; RRI, RR-interval; SEID, systemic exertion intolerance disease; TNF, tumour necrosis factor; TPRI, Total Peripheral Resistance Index.

---

**Table 3** Differences in physical activity (steps per day) and fatigue (CFQ score) between SEID-positive and SEID-negative patients 30 weeks after inclusion

<table>
<thead>
<tr>
<th>Variables</th>
<th>SEID-positive, mean</th>
<th>SEID-negative, mean</th>
<th>Difference*</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steps per day, number</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>4836</td>
<td>4823</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 30</td>
<td>4667</td>
<td>4518</td>
<td>−149</td>
<td>0.326</td>
</tr>
<tr>
<td>CFQ total sum score</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>20.7</td>
<td>18.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 30</td>
<td>19.0</td>
<td>20.4</td>
<td>1.46</td>
<td>0.413</td>
</tr>
</tbody>
</table>

*Based on analysis of covariance models in which differences and P values are adjusted for baseline values of outcome variables as well as allocation to clonidine/placebo during the first 8 weeks of the trial.

CFQ, Chalder Fatigue Questionnaire; SEID, systemic exertion intolerance disease.
CONCLUSION

This study questions the discriminant and prognostic validity of the SEID diagnostic criteria in adolescent CFS, and suggests that the criteria tend to select patients with depressive symptoms. These results corroborate earlier findings and question the concept of classifying fatigued patients based on symptom phenotype. A new approach may be to perform cluster analysis on biological markers to look for subgroups on a basal level with potentially different treatments, prognosis and others.

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Contributors JIA and SVN conceptualised and designed the study, carried out the statistical analyses, drafted the initial manuscript, and reviewed and revised the manuscript. ES supervised the statistical analyses, and critically reviewed and revised the manuscript.

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Competing interests None declared.

Patient consent Obtained.

Ethics approval The study was approved by the Norwegian National Committee for Ethics in Medical Research and the Norwegian Medicines Agency and adhered to the Declaration of Helsinki.

Provenance and peer review: Not commissioned; externally peer reviewed.

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Are there subgroups of chronic fatigue syndrome? An exploratory cluster analysis of biological markers

Tarjei Tørre Asprusten¹, Line Sletner¹ and Vegard Bruun Bratholm Wyller¹,²*

Abstract

Background: Chronic fatigue syndrome (CFS) is defined according to subjective symptoms only, and several conflicting case definition exist. Previous research has discovered certain biological alterations. The aim of the present study was to explore possible subgroups based on biological markers within a widely defined cohort of adolescent CFS patients and investigate to what extent eventual subgroups are associated with other variables.

Methods: The Norwegian Study of Chronic Fatigue Syndrome in Adolescents: Pathophysiology and Intervention Trial (NorCAPITAL) has previously performed detailed investigation of immunological, autonomic, neuroendocrine, cognitive and sensory processing functions in an adolescent group of CFS patients recruited according to wide diagnostic criteria. In the present study, hierarchical cluster analyses (Ward’s method) were performed using representative variables from all these domains. Associations between clusters and constitutional factors (including candidate genetic markers), diagnostic criteria, subjective symptoms and prognosis were explored by standard statistical methods.

Results: A total of 116 patients (26.7% males, mean age 15.4 years) were included. The final cluster analyses revealed six clusters labelled pain tolerant & good cognitions, restored HPA dynamics, orthostatic intolerance, low-grade inflammation, pain intolerant & poor cognitions, and high vagal (parasympathetic) activity, respectively. There was substantial overlap between clusters. The pain intolerant & poor cognitions-cluster was associated with low functional abilities and quality of life, and adherence to the Canada 2003 diagnostic criteria for CFS. No other statistically significant cluster associations were discovered.

Conclusion: Within a widely defined cohort of adolescent CFS patients, clusters could be delineated, but no distinct subgroups could be identified. Associations between clusters and constitutional factors, subjective symptoms and prognosis were scarce. These results question the clinical usefulness of searching for CFS subgroups, as well as the validity of the most “narrow” CFS diagnostic criteria.

Trial registration: Clinical Trials NCT01040429

Keywords: Chronic fatigue syndrome, Adolescent, Cluster analysis, Diagnostic criteria

Background

Chronic Fatigue (CF) affects a substantial proportion of the population. In adolescents, about 20% of girls and 6.5% of boys report to have been severely fatigued during the last month [1, 2]. The label Chronic Fatigue Syndrome (CFS), sometimes referred to as Myalgic Encephalomyelitis (ME), may be appropriate if the fatigue is unexplained,
long lasting, disabling and accompanied by other symp-
toms such as post exertional malaise, musculoskeletal
pain, orthostatic intolerance, and cognitive problems [3].
Adolescent CFS prevalence is estimated at 0.1 to 1.0%
[4–6], and CFS may have detrimental effects on psycho-
social and academic development [7,8], as well as family
functioning [8].

The diagnostic criteria of CFS has been a scientific
controversy for decades. As no diagnostic biomarker
has been discovered, the diagnosis depends upon spe-
cific constellation of symptoms. One part of the sci-
centific community has promoted wide diagnostic criteria
[9–11], and also maintained that CFS is most properly
understood as a variant belonging to an even broader
category, such as Functional Somatic Syndrome [12] or
Bodily Distress Syndrome [13]. This "lumping together"
tendency has been strongly opposed by another part
of the scientific community, advocating CFS as a het-
ergeneous group of patients with different diseases
and pathophysiological features, e.g., ME is claimed as
a distinct unique entity different from other fatiguing
conditions, such as reflected in the Canadian diagno-
sic criteria of CFS (sometimes referred to as the Inter-
national Consensus Criteria of ME/CFS) [14, 15]. The
commonly used Fukuda-criteria [16] as well as the more
recently proposed SEID-criteria [3] may be taken to rep-
resent pragmatic compromises.

Nevertheless, the net result has been a confusing exist-
ence of at least 20 case definitions. Most of them require
between 3 and 6 months of unexplained fatigue but vary
considerably regarding requirement of additional symp-
toms. In a systematic review from 2014, Brurberg et al.
could not draw firm conclusions concerning the valid-
ity of any of these criteria due to weak methodology and
inconsistent results of the 38 included validation studies
[17]. Accordingly, studies from our own institution ques-
tion the validity of the Canadian-criteria [18] as well as
the SEID-criteria [19] for diagnosing CFS in adolescents.

In an attempt of investigating possible subgroups
within widely defined CFS cohorts, latent class analyses
have been applied; recent reports suggest the presence
of discrete endophenotypes [20–23]. However, these
approaches still rely on subjective reporting of symp-
toms, and it remains unclear to what extent a specific
endophenotype corresponds with certain pathophys-
iological mechanisms or etiological factors. The presence
or not of such correspondence may be considered essen-
tial for a proper understanding of the underlying disease
mechanisms of CFS.

Despite the absence of diagnostic biomarkers, assos-
ciations between CFS and candidate genetic mark-
ers as well as certain aberrations of immunological,
autonomic, neuroendocrine, cognitive and sensory
processing functions have been firmly established in
previous research [24]. As for genetic markers, a single
nucleotide polymorphisms (SNP) in the gene encod-
ing the catecholaminergic breakdown enzyme COMT
(catechol-O-methyltransferase) has been linked to CFS
in several reports [25, 26]. In addition, mutations in the
serotonergic system are one of the most consistently
reported findings in genetic studies of CFS [27–29].

As for immunological aberration, the most consistent
finding appears to be a tendency towards low-grade
systemic inflammation, as reflected in elevated serum
C-reactive protein (CRP) [30], elevated pro-inflamma-
tory cytokines [31, 32], and increased levels of innate
immunity gene products in whole blood gene expres-
sion analyses [33]. Also, low-grade inflammation has
been hypothesized as a common pathophysiological
phenomenon across fatigue states in general [34]. As
for autonomic aberrations, most studies suggest a sym-
pathetic predominance, reflected in increased sym-
pathetic cardiovascular activity [35–38], decreased
parasympathetic (vagal) heart rate control [39], altered
sympathetic thermoregulatory responses [39], and
increased plasma and urine catecholamines [30, 40].
This sympathetic predominance may be the underlying
cause of the Postural Orthostatic Intolerance Syndrome
(POTS) phenomenon, which is frequently observed
among CFS patients [3]. As for neuroendocrine aber-
rations, attenuated hypothalamic–pituitary–adrenal
[HPA] axis dynamics is a consistent finding across adult
and adolescent CFS studies [30, 41–44]. Interestingly,
normalization of HPA responses may be associated
with improvement of symptoms and functional dis-
abilities [43, 45]. As for cognitive functions, previous
research has provided evidence of aberrations in the
domains of attention, memory and reaction time [46–
48]. Studies specifically addressing executive functions
in adolescent CFS patients have reported impaired
interference control [49, 50], cognitive flexibility [51],
and working memory [50, 52]. Finally, as for sensory
processing functions, three studies have reported
strongly reduced pressure pain thresholds [53–55], sug-
gestive of central sensitization to afferent sensory stim-
uli [56]. Accordingly, functional brain imaging studies
have demonstrated differences across CFS patients and
healthy controls [57].

Thus, an alternative approach for delineating possible
CFS subgroups would be to use the above-mentioned
biological aberrations as a point of departure instead of
subjective symptoms when performing subgroup-gener-
ating statistical analyses. To the best of our knowledge,
such an approach is novel in the field of CFS. In the pre-
sent study, we aimed to: a) Explore possible subgroups
based on biological aberrations within a widely defined
Methods
Study design and ethics
This study is part of the Norwegian Study of Chronic Fatigue Syndrome in Adolescents: Pathophysiology and Intervention Trial (NorCAPITAL) (ClinicalTrials ID: NCT01040429), which is a combined cross-sectional and randomized controlled trial of low-dose clonidine in adolescent CFS; the design has been described in detail elsewhere [30]. In the present study, we used baseline data and follow-up data from week 30, collected between March 2010 and October 2012. The study was approved by the Regional Committee for Medical and Health Research Ethics for South-East Norway and the Norwegian Medicines Agency and adhered to the Declaration of Helsinki. Informed, written consent was obtained from all participants and from parents or next-of-kin if required.

Recruitment of CFS patients
All 20 hospital paediatric departments in Norway primary care paediatricians and general practitioners were invited to refer adolescents with CFS aged 12 to 18 years consecutively to the Department of Paediatrics at Oslo University Hospital, which served as a national referral center for young patients with CFS. To be eligible for the NorCAPITAL project, we required 3 months of unexplained chronic/relapsing fatigue of new onset. The patients were not required to meet any additional symptom criteria, in line with clinical Paediatric guidelines [9]. A standard form required the referral unit to confirm the result of clinical investigations considered compulsory to diagnose pediatric CFS (specialist evaluation, extensive hematolgy and biochemistry work-up, chest X-ray, abdominal ultrasound, and brain MRI). Also, the referring units were required to confirm that the patient (a) was hindered from normal school attendance due to fatigue; (b) was not permanently bedridden; (c) was not stroked by a medical or psychiatric disorder (including depression) and/or did not go through any concurrent demanding life event; and (d) did not use medicines (including hormone contraceptives) regularly. Patients considered eligible were summoned to our study center; a final decision on inclusion was made after a separate clinical examination combined with quality assessment of the previously conducted screening program. Details of the recruitment procedure and inclusion/exclusion criteria are described elsewhere [29].

All participants underwent an identical investigational program at baseline, 8 weeks and 30 weeks, which included a one day in-hospital assessment encompassing clinical examination, blood sampling, autonomic testing, and cognitive testing. Immediately afterwards, daily physical activity was monitored during seven consecutive days, and a self-administered questionnaire was completed.

Markers of biological aberrations
All methods for assessing markers of biological aberrations have been thoroughly described in previous publications from the NorCAPITAL project [30, 45, 50, 55, 58, 59]; a brief description is provided below.

Immunological markers were investigated by examining plasma CRP level through a high-sensitive assay (Roche Diagnostics, Indianapolis, IN, USA), and by measuring 27 plasma cytokines, including interleukins, chemokines and growth factors, using a multiplex technique (Bio-Plex Human Cytokine 27-Plex; Bio-Rad Laboratories Inc., Hercules, CA, USA) [58].

Autonomic markers were investigated using the Task Force Monitor® (Model 3040i, CNSystems Medizintechnic, Graz, Austria), a combined hardware and software device for noninvasive continuous recording of autonomic cardiovascular control [60]. Supine values as well as responses to a low intensity 20 deg. head-up tilt test (HUT) are reported [59]. Power spectral analysis of heart rate variability (HRV) was calculated in the Low Frequency (LF) range (0.05 to 0.17 Hz), and High Frequency (HF) range (0.17 to 0.4 Hz) [61]. Vagal (parasympathetic) activity is the main contributor to HF variability, whereas both vagal and sympathetic activity contributes to LF variability.

Neuroendocrine markers included plasma and urine norepinephrine and epinephrine. These markers were assayed by high-performance liquid chromatography (HPLC) with a reversed-phase column and glassy carbon electrochemical detector (Antec, Leyden Decade II SCC, Zoeterwoude, The Netherlands), using a commercial kit (Chromsystems, München, Germany) [62]. Urine free cortisol (non-conjugated cortisol) was assayed by solid phase competitive luminescence immunoassay (LIA) (type Immulite® 2000, Siemens Healthcare Diagnostics, NY, USA) after extraction from the urine sample with ether [63]. Plasma cortisol, adrenocorticotropic hormone (ACTH), thyroid-stimulating hormone (TSH), and free thyroxine (FT4), as well as serum Insulin-like Growth Factor 1 (IGF1), were determined by routine assays at the accredited Hormone laboratory at Oslo University Hospital, Norway.

Cognitive function was assessed using the digit span test from the Wechsler Intelligence Scale for Children,
symptoms and disabilities. Pressure pain threshold was assessed by gradually applying increasing pressure to six predefined areas (the third finger’s cuticles, the trapezius muscle and the supraspinatus muscle bilaterally), by using the force transducer Commander™ Algometer, which has a rubber tip of 0.5 cm² (JTECH Medical, Midvale, USA) [54]. Participants were asked to indicate the first sensation of pain during increasing pressure. All sites were assessed in the same order for each patient, and the pressure stimuli were applied twice to each spot and then averaged. Values were reported in Newton (N).

Genotyping
Procedures for genotyping in the NorCAPITAL project have been described in detail elsewhere [29]. In short, genomic DNA was extracted from whole blood samples. Single Nucleotide Polymorphism (SNP) genotyping was carried out using custom TaqMan SNP genotyping assays (Applied Biosystems, Foster City, CA, USA). Approximately 10% of the samples were re-genotyped and the concordance rate was 100%. To determine the length of the polymorphic promoter region of the serotonin transporter (5-HTT)-gene (SLC6A4), the DNA sequence was first amplified by polymerase chain reaction (PCR) and then separated by gel electrophoresis. The PCR yielded a long (529 bp) and a shorter (486 bp) fragment [67]. After four hours separation at 100 V on a 2.5% agarose gel (MetaPhor Agarose, Lonza cologne GmbH, Cologne, Germany), GelRed dye was added and the fragments were visualized by UV light (Biotium Inc, California, USA). The PCR 100 bp low ladder (Sigma-Aldrich CO, St. Louis, Mo, USA) was used to determine the length of the fragments.

Questionnaires
A CFS symptom inventory for adults [68] has previously been used to develop an analogous inventory for adolescents [30]. A total of 24 common symptoms are evaluated in terms of frequency during the last month (five-point Likert scale ranging from never/rarer than once a month to present every day/almost every day, scored from 1 to 5). The questionnaire includes case defining symptoms of CFS according to the Canada as well as the Fukuda definition. As a general rule, all symptoms required in the definitions had to be present more than once a week (corresponding to a score of three or higher) for patients to be categorized as CFS [18].

In addition, validated inventories were used to assess symptoms and disabilities.

4th edition (WISC-IV) [64], the conditions 1–3 of Color-Word Interference test from the Delis-Kaplan Executive Function System (D-KEFS) [65], and the Total recall part of Hopkins Verbal Learning Test-Revised (HVLT-R) [66].

Fatigue was assessed by the Chalder Fatigue Questionnaire (CFQ), which encompasses 11 items scored on a 4-point (0 to 3) Likert scale [69]; total sum score is applied. Depressive symptoms were charted with the Mood and Feelings Questionnaire (MFQ) consisting of 34 items scored on a 3-point (0 to 2) Likert scale [70]; total sum score is applied. Quality of life was assessed with the Pediatric Quality of Life Inventory (PedsQL), consisting of 23 items scored on a 5-point (0-25-50-75-100) Likert scale [71]; mean score across all items is applied. Functional disability was assessed using the Functional Disability Inventory (FDI) encompassing 15 items scored on a 5-point (0-4) Likert scale [72]; total sum score is applied.

Finally, the symptom of post-exertional malaise (PEM) was charted by a single item: “How often do you experience more fatigue the day after an exertion?”, scored on a 5-point [1–5] Likert scale.

Daily physical activity
The activPAL accelerometer device (PAL Technologies Ltd, Glasgow, Scotland) was used to provide data on step number and cadence as well as time spent on walking, standing and sitting/lying during everyday activities [73]. A recording period of seven consecutive days was selected. For each participant, all recording epochs were carefully and independently reviewed, and the mean number of steps per day was calculated for all recording epochs. Details on the activity recording procedure have been reported elsewhere [30].

Cluster construction
A total of 69 different biomarkers was selected from the NorCAPITAL database for analyses in the present study; the selection was guided by expert knowledge of the CFS/ME scientific literature. The biomarkers were grouped into five domains: endocrine (n = 10), inflammatory (n = 30), cardiovascular (n = 18), pressure pain threshold (n = 3), and cognitions (n = 8) (Fig. 1). Thereafter, in order to reduce the number of variables, correlation analyses among variables under each domain were performed. When two or more variables were strongly correlated (correlation coefficient ≥ 0.7), interpretability, suitability regarding statistical analyses and the size of the correlation coefficient were evaluated. The variable in total considered most suitable was kept for further analyses. A final correlation analysis of all remaining variables from each domain were performed, resulting in a total of 37 variables which become the basis for subsequent cluster analyses (Fig. 1, Additional file 1).

Firstly, hierarchical clustering analyses were performed within each of the five domains separately, using Ward's method, squared Euclidian distance and Z-score. Thereafter, 1-3 variables from each domain were used for a
final cluster analysis across all domains [74]. Variables were selected due to their importance in the cluster formation under each domain. The final number of clusters was decided primarily by visual inspection of dendrograms, but a preliminary validation of the possible cluster-solutions was also performed to ensure that there were meaningful differences between the clusters.

Cluster validation
Associations between clusters and simple demographic variables, constitutional factors (including genetic markers) and adherence to CFS diagnostic criteria were explored (Table 3). Baseline values of CFQ, PEM, MFQ, Steps per day, PedsQL and FDI were used to investigate associations between clusters and markers of symptoms and function. Changes in markers of symptoms and function from baseline to week 30 were used to assess prognostic value of clusters.

Generally, differences across clusters were analyzed by Fisher’s exact test, one-way ANOVA or Kruskal–Wallis test as appropriate. All statistical analyses were carried out by SPSS statistical software. A p-value of < 0.05 was considered statistically significant. No correction for multiple testing was performed due to the exploratory nature of the analyses.

Results
Of the 120 CFS/ME patients included in the NorCAPITAL project, four were excluded from further analyses due to lack of valid data, leaving 116 for analyses in the present study. In this group, 28% were males, and mean age was 15.4 years (Table 1).

Separate cluster analyses within each domain of variables revealed substantial cluster overlap as well as few statistically significant associations with symptoms, functional abilities and prognosis. However, from each analysis it was possible to identify the most important variables driving the cluster formation, which in turn were carried over to the final cluster analysis across all domains (Additional file 2).

The final cluster solution revealed six clusters based on a total of 10 variables (Fig. 2, Table 2). Cluster 1 is characterized by high pressure pain threshold levels and high scores on cognitive function tests, and was labelled pain tolerant & good cognitions. Cluster 2 is
characterized by high urine cortisol:creatinin ratio, which signalizes restored HPA dynamics. Cluster 3 is characterized by a strong tachycardia response and corresponding fall in stroke volume during orthostatic challenge, typical of orthostatic intolerance. Cluster 4 is characterized by high levels of interferon gamma (INFγ) and interferon gamma-induced protein 10 (IP-10), indicative of low-grade inflammation. Cluster 5 is characterized by low pressure pain threshold levels and low scores on cognitive function tests; this “mirror image” of cluster one is labelled pain intolerant & poor cognitions. Cluster 6 is characterized by strong power of heart rate variability within the high-frequency (HF) domain.

There were no significant differences between clusters regarding demographic and constitutional variables, including candidate genetic markers (Table 3). However, individuals belonging to Cluster 5—Pain intolerant & poor cognitions—were significantly more prone to adhere to the Canada 2003 diagnostic criteria for CFS. Also, this cluster had significantly poorer scores on the FDI and PedsQL functional inventories as compared to clusters 1–3 (Table 4). Symptoms scores for fatigue (CFQ), post-exertional malaise and depressive thoughts (MFQ) did not differ significantly across clusters. As for changes in symptoms and function over a 30-week follow-up period, there were no significant differences between the clusters, but a non-significant tendency for stronger functional improvement among Cluster 5—Pain intolerant & poor cognitions (Table 5).

A scatterplot of the three most important variables for the final cluster formation (urine cortisol:creatinine ratio, Δ HR orthostatic response and digit span forward) revealed a substantial overlap between the clusters (Fig. 3).

Discussion

The most important finding of the present study is that within a widely defined cohort of adolescent CFS patients, clusters corresponding to certain pathophysiological characteristics could be delineated, but overlap between clusters were substantial and no distinct subgroups could be identified. Also, there were scarce associations between clusters and constitutional factors, subjective symptoms and prognosis.
Medical diagnoses remain the foundations for treatment, rehabilitation, and prognostic assumptions; hence, the importance of valid diagnostic entities can hardly be underestimated. Sadly, the lack of objective criteria for CFS has contributed to the co-existence of multiple sets of diagnostic criteria, all of which are based on subjective reporting of symptoms. It is frequently maintained that certain case definitions correspond to specific underlying disease mechanisms; for instance, the Canada 2003 definition put strong emphasis on a possible inflammatory pathophysiology [14]. Accordingly, it is often argued that studies based on a wide diagnostic definition of CFS (i.e., a definition that requires a minimum of accompanying symptoms), such as the Oxford criteria [11], are at risk of introducing substantial heterogeneity in the patient sample which in turn may obscure results that pertain to a specific subgroup only. Specifically, it is frequently maintained that the use of a wide diagnostic definition in clinical trials tend to select a large portion of patients suffering from mental distress who may benefit from psychological/behavioural interventions, whereas such interventions are claimed to be unhelpful (or even harmful) for the potential subgroup of patients suffering from another (such as inflammatory) disease mechanism [75].

This argument seems to rely upon an a priori-assumption of the existence of subgroups within a widely defined CFS cohort, and the related research efforts tend to focus on how such subgroups can be found from analysis of patients’ symptoms (or biomarkers yet to be discovered). However, a more fundamental scientific question, which has scarcely been addressed in previous research, is whether such subgroups exist at all. In the present study, while the cluster analyses did suggest some delineation corresponding to previously identified characteristics of CFS pathophysiology, such as low-grade inflammation, altered HPA dynamics, and orthostatic intolerance, the most striking finding is the absence of well-defined subgroups. Rather, the data seems to represent continuous variables in a multidimensional space. Accordingly, the clusters were not significantly associated with symptom scores nor prognosis. Taken together, the findings of the present paper favor a “lumping together” rather than “splitting apart” approach to CFS caseness, and question

<table>
<thead>
<tr>
<th>Table 2 Final cluster solution-contributing variables</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>1: Pain tolerant &amp; good cognitions (n = 40)</td>
</tr>
<tr>
<td>2: Restored HPA dynamics (n = 15)</td>
</tr>
<tr>
<td>3: Orthostatic intolerance (n = 20)</td>
</tr>
<tr>
<td>4: Low-grade inflammation (n = 4)</td>
</tr>
<tr>
<td>5: Pain intolerant &amp; poor cognitions (n = 33)</td>
</tr>
<tr>
<td>6: High vagal activity (n = 3)</td>
</tr>
</tbody>
</table>

| Plasma cortisol, nmol/L-mean (SD) | 329 (118) | 314 (132) | 366 (132) | 407 (136) | 443 (154) | 462 (169) |
| Urine cortisol/creatinine ratio, nmol/mmol-median (IQR) | 3.2 (2.2, 4.4) | 14.5 (7.0, 18.4) | 3.2 (2.5, 4.9) | 3.9 (2.8, 6.2) | 3.2 (1.8, 4.4) | 3.7 (n.a) |
| Interferon gamma, pg/ml-median (IQR) | 84 (57, 119) | 102 (63, 199) | 125 (92, 250) | 174 (79, 223) | 65 (45, 116) | 167 (n.a) |
| IP-10, pg/ml-median (IQR) | 345 (261, 479) | 419 (225, 467) | 314 (323, 410) | 2735 (2359, 3803) | 313 (174, 502) | 557 (n.a) |
| HF-HRV supine, ms²-median (IQR) | 1264 (422, 3262) | 1743 (147, 2630) | 869 (526, 2209) | 101 (52, 126) | 588 (198, 868) | 11052 (n.a) |
| Δ HR orthostatic response, beats/min-mean (SD) | 3.5 (3.2) | 2.5 (4.2) | 9.9 (4.1) | 3.9 (2.6) | 4.7 (3.7) | 7.8 (6.3) |
| Δ SI orthostatic response, mL/m²-mean (SD) | −4.3 (2.9) | −3.0 (5.6) | −11.4 (4.1) | −2.8 (5.4) | −4.3 (3.3) | −9.4 (5.0) |
| Pressure pain threshold, N/cm²-mean (SD) | 20.1 (7.6) | 16.5 (6.9) | 15.2 (5.2) | 13.2 (5.7) | 9.8 (3.9) | 17.1 (7.1) |
| Digit span forward, total score-mean (SD) | 9.3 (2.1) | 8.7 (1.5) | 8.0 (2.1) | 8.5 (2.4) | 7.2 (1.2) | 8.0 (1.0) |
| Color-Word interference test condition 2, sec-mean (SD) | 25.7 (4.2) | 22.9 (4.5) | 25.9 (4.4) | 25.3 (4.3) | 30.2 (9.2) | 23.7 (4.2) |

HPA: hypothalamus–pituitary–adrenal; CI: confidence interval; IP-10: Interferon gamma-induced protein 10; HR: heart rate; SI: stroke index; HF-HRV: high-frequency power of heart rate variability; IQR: interquartile range (25 and 75 percentile). Δ denotes the response (upright–supine) to orthostatic challenge. The characterizing variable scores within each cluster is highlighted with italics.
the clinical usefulness of searching for CFS subgroups as well as the validity of the most "narrow" CFS diagnostic criteria. If confirmed by future research, this finding may have important clinical implications. It would suggest, for instance, that well-documented rehabilitation strategies might be applicable to a wide range of CFS sufferers.
That said, the cluster analysis did reveal some interesting associations, such as the positive association between restored HPA axis and functional abilities, confirming findings from previous reports [43, 45]. Also, there was an association between low pain tolerance and cognitive functions, poor functional abilities and quality of life, and adherence to the Canada 2003 diagnostic definition of CFS [14]. The causality of these associations remains to be clarified; for instance, functional disability may have a negative impact on cognitive test performance, as well as

Table 5 Final cluster solution--differences in development of symptoms and functions over time (baseline to week 30 follow-up)

<table>
<thead>
<tr>
<th>Cluster</th>
<th>1: Pain tolerant &amp; good cognitions (n = 40)</th>
<th>2: Restored HPA dynamics (n = 15)</th>
<th>3: Orthostatic intolerance (n = 20)</th>
<th>4: Low-grade inflammation (n = 4)</th>
<th>5: Pain intolerant &amp; poor cognitions (n = 33)</th>
<th>6: High vagal activity (n = 3)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \Delta ) CFQ-mean [95% CI]</td>
<td>-5.0 [-7.6, -2.4]</td>
<td>-5.0 [-10.5, 0.5]</td>
<td>-4.8 [-7.6, -2.0]</td>
<td>-6.5 [n.a]</td>
<td>-5.2 [-8.1, -2.2]</td>
<td>4.0 [n.a]</td>
<td>0.999</td>
</tr>
<tr>
<td>( \Delta ) PEM-mean [95% CI]</td>
<td>-0.48 [-0.9, 0.0]</td>
<td>-0.36 [-1.4, 0.6]</td>
<td>0.12 [-0.2, 0.4]</td>
<td>-1.00 [n.a]</td>
<td>-0.73 [-1.2, -0.3]</td>
<td>-1.0 [n.a]</td>
<td>0.130</td>
</tr>
<tr>
<td>( \Delta ) MFI-mean [95% CI]</td>
<td>-1.0 [-4.1, 2.1]</td>
<td>-1.7 [-7.4, 3.9]</td>
<td>-0.2 [-3.6, 3.1]</td>
<td>-4.7 [n.a]</td>
<td>-3.1 [-6.8, 0.5]</td>
<td>-3.0 [n.a]</td>
<td>0.685</td>
</tr>
<tr>
<td>( \Delta ) Steps per day-mean [95% CI]</td>
<td>526 [-593, 1644]</td>
<td>-1107 [-3004, 789]</td>
<td>-578 [-1506, 350]</td>
<td>1228 [n.a]</td>
<td>283 [-532, 1099]</td>
<td>-657 [n.a]</td>
<td>0.218</td>
</tr>
<tr>
<td>( \Delta ) FDI-mean [95% CI]</td>
<td>-3.1 [-6.2, 0.1]</td>
<td>-1.9 [-8.2, 4.4]</td>
<td>0.6 [-3.4, 4.6]</td>
<td>-13.0 [n.a]</td>
<td>-6.7 [-10.8, -2.5]</td>
<td>-9.5 [n.a]</td>
<td>0.079</td>
</tr>
<tr>
<td>( \Delta ) PedsQL-mean [95% CI]</td>
<td>6.6 [0.8, 12.5]</td>
<td>5.8 [-6.4, 18.1]</td>
<td>2.5 [-2.7, 7.7]</td>
<td>6.9 [n.a]</td>
<td>8.4 [4.3, 12.5]</td>
<td>14.2 [n.a]</td>
<td>0.584</td>
</tr>
</tbody>
</table>

HPA: hypothalamus–pituitary–adrenal; CI: Confidence Interval; n.a.: not applicable; CFQ: Chalder Fatigue Questionnaire; PEM: Post Exertional Malaise; MFI: Moods and Feelings Questionnaire; FDI: Function and Disability Inventory; PedsQL: Pediatric Quality of Life

* Unadjusted p-values. The p-values are based on one-way ANOVA or Kruskal–Wallis test, as appropriate. Only group 1–3 and 5 were used in the statistical analyses due to few participants in group 4 and 6

Fig. 3 Scatterplot of the three most important variables for the final cluster formation. Each colored dot represents one individual belonging to one of the six clusters from the final cluster solution. Even though the three most important variables driving the cluster formation are used as coordinates, there is substantial overlap between the clusters.
the other way round. Anyway, a better characterization of this particularly vulnerable group of CFS patients may help to tailor clinical rehabilitation programs.

Interestingly, while low-grade inflammation is advocated as an important pathophysiological feature of CFS patients adhering to the Canada 2003 diagnostic criteria [14], results from the present study oppose these assumptions. The cluster characterized by low-grade inflammation was not associated with the Canada 2003 case definition for CFS, while the cluster characterized as Pain intolerant & poor cognitions, which actually was associated with the Canada 2003 case definition, had the lowest score on inflammation variables. This result corroborates previous finding from our group [18], and further questions the validity of the Canada 2003 case definition.

**Study strengths and limitations**

A strength of the present study is the detailed characterization of CFS pathophysiology within several domains. Limitations include the relatively low number of CFS patients, leaving some of the clusters with few participants, and the study should therefore be regarded exploratory. Also, the study included adolescent patients only, and it is unknown to what extent results can be generalized to adults. Further research should seek to validate the present findings in a larger cohort of adult CFS patients.

The question on how to measure fatigue is a controversy in the field of CFS. The present study assumed a priori that fatigue is best conceptualized as a subjective sensation [76]; accordingly, a validated instrument based on self report (the Chalder Fatigue Questionnaire) was selected to operationalize fatigue. We acknowledge, however, that other researchers maintain that fatigue should be measured by objective standards (e.g. activity recordings). Also, recent findings suggest that the symptom of post-exertional malaise (PEM) is even more central to the phenomenon of CFS than previously understood, and that it should be assessed with comprehensive, validated instruments [77]. Unfortunately, these instruments were not available when the present study was planned.

**Conclusion**

Within a widely defined cohort of adolescent CFS patients, clusters could be delineated based on biological markers, but no distinct subgroups could be identified. Associations between clusters and constitutional factors, subjective symptoms and prognosis were scarce. These results question the clinical usefulness of searching for CFS subgroups, as well as the validity of the most "narrow” CFS diagnostic criteria.

**Supplementary Information**

The online version contains supplementary material available at https://doi.org/10.1186/s12967-021-02713-9.

**Additional file 1.** Initial correlation analyses of all variables considered for hierarchical cluster analysis.

**Additional file 2.** Results of hierarchical cluster analyses within each subdomain of variables (immunological, autonomic, neuroendocrine, cognitive and sensory processing functions), as well as associations between clusters and constitutional factors, diagnostic criteria, subjective symptoms and prognosis.

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**Authors’ contributions**

Conceived and designed the study: TTA, VBW. Analyzed the data: TTA. Interpreted the results and wrote the paper: TTA, LS, VBW. All authors read and approved the final manuscript.

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**Availability of data and materials**

The dataset generated and analyzed during the current study is available from the corresponding author upon reasonable request.

**Ethics approval and consent to participate**

The study was approved by the Regional Committee for Medical and Health Research Ethics for South-East Norway and the Norwegian Medicines Agency. Written informed consent was obtained from all participants and from parents/next-of-kin if required.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that that they have no competing interests.

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### APPENDICES

**Appendix 1: Operationalized Canadian Consensus Criteria**

<table>
<thead>
<tr>
<th>Source of information in the present study (Corresponding variable in the questionnaire or other source)</th>
<th>Rule for adhering to Canada Consensus Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1) Fatigue</strong></td>
<td>Patients must fulfill all specifications 1.a) - 1.d)</td>
</tr>
<tr>
<td>Patients must have a significant degree of new onset</td>
<td>Total score ≥ 5'</td>
</tr>
<tr>
<td>1.a) CFQ total sum score</td>
<td>Not tick off “as long as I can remember”</td>
</tr>
<tr>
<td>Patients must have a significant degree of unexplained, persistent or recurrent physical and mental fatigue</td>
<td>Score ≥ 2. Also covered by the inclusion criteria in the NorCAPITAL project.</td>
</tr>
<tr>
<td>1.c) Item: “How often are you fatigued?”</td>
<td>Total sum score ≥ 7</td>
</tr>
<tr>
<td>1.d) FDI total sum score</td>
<td></td>
</tr>
<tr>
<td><strong>2) Post-exertional Malaise and Fatigue:</strong></td>
<td>Patients must fulfill all specifications 2.a) - 2.d)</td>
</tr>
<tr>
<td>There is an inappropriate loss of physical and mental stamina, rapid muscular and cognitive fatigability, post-exertional fatigue and/or malaise and/or pain and a tendency for other associated symptoms within the patient’s cluster to worsen.</td>
<td></td>
</tr>
<tr>
<td>2.a) Item from FSS: “I am easily fatigued”</td>
<td>Score ≥ 5</td>
</tr>
<tr>
<td>2.b) Item from FSS: “Exercise brings on my fatigue”</td>
<td>Score ≥ 5</td>
</tr>
<tr>
<td>2.c) Items from CFS symptom inventory:</td>
<td>Total score ≥ 8</td>
</tr>
<tr>
<td>“Does the fatigue get better or worse after walking slowly?”</td>
<td></td>
</tr>
<tr>
<td>“Does the fatigue gets better or worse after doing hard school work”?</td>
<td></td>
</tr>
<tr>
<td>2.d) Item from CFS symptom inventory:</td>
<td>Score ≥ 3</td>
</tr>
<tr>
<td>“If you think about the last month, how often have you been more fatigued the day after an exertion?”</td>
<td></td>
</tr>
</tbody>
</table>

### Appendix 1: Operationalized Canadian Consensus Criteria - continued

<table>
<thead>
<tr>
<th>3) Sleep dysfunction:</th>
<th>Patients must fulfill one of specifications 3.a) – 3.c)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.a) Item from CFS symptom inventory:</td>
</tr>
<tr>
<td></td>
<td>“If you think about the last month, how often have you don’t felt refreshed after you’ve slept?” Score ≥ 3</td>
</tr>
<tr>
<td></td>
<td>3.b) Item from CFS symptom inventory:</td>
</tr>
<tr>
<td></td>
<td>“If you think about the last month, how often have you woken up early and not been able to sleep again?” Score ≥ 3</td>
</tr>
<tr>
<td></td>
<td>3.c) Karolinska Sleep Questionnaire: Insomnia index</td>
</tr>
<tr>
<td></td>
<td>Score ≤ 3,5</td>
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<table>
<thead>
<tr>
<th>4) Pain:</th>
<th>Patients must fulfill specification 4.a) and specification 4.b) or 4.c)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4.a) Item from CFS symptom inventory:</td>
</tr>
<tr>
<td></td>
<td>“If you think about the last month, how often have you had pain in your muscles?” Score ≥ 3</td>
</tr>
<tr>
<td></td>
<td>4.b) Item from CFS symptom inventory:</td>
</tr>
<tr>
<td></td>
<td>“If you think about the last month, how often have you had pain in several joints?” Score ≥ 3</td>
</tr>
<tr>
<td></td>
<td>4.c) Item from CFS symptom inventory:</td>
</tr>
<tr>
<td></td>
<td>“If you think about the last month, how often have you had headache?” Score ≥ 3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>5) Neurological/Cognitive Manifestations:</th>
<th>Patients must fulfill two of specifications 5.a) – 5.g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two or more of the following difficulties should be present:</td>
<td></td>
</tr>
<tr>
<td>Confusion</td>
<td>5.a) Item from CFS symptom inventory:</td>
</tr>
<tr>
<td></td>
<td>“If you think about the last month, how often have you felt confused or disoriented?” Score ≥ 3</td>
</tr>
</tbody>
</table>
### Appendix 1: Operationalized Canadian Consensus Criteria - continued

<table>
<thead>
<tr>
<th>Impairment of concentration and short-term memory consolidation</th>
<th>5.b) Item from CFS symptom inventory: “If you think about the last month, how often have you had problems with remembering?”</th>
<th>Score ≥ 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.c) Item from CFS symptom inventory: “If you think about the last month, how often have you had problems concentrating?”</td>
<td>Score ≥ 3</td>
<td></td>
</tr>
<tr>
<td>Disorientation</td>
<td>Covered by 5.a)</td>
<td></td>
</tr>
<tr>
<td>Difficulty with information processing, categorizing and word retrieval, and perceptual and sensory disturbances- e.g. spatial instability, and inability to focus vision.</td>
<td>5.d) Item from PedsQL: “It is hard for me to pay attention during classes.”</td>
<td>Score ≥ 3</td>
</tr>
<tr>
<td>5.e) Item from CFS symptom inventory: “If you think about the last month, how often have you had problems with focusing vision or to see sharp?”</td>
<td>Score ≥ 3</td>
<td></td>
</tr>
<tr>
<td>Ataxia, muscle weakness and fasciculations are common.</td>
<td>Not covered in questionnaire</td>
<td></td>
</tr>
<tr>
<td>There may be overload phenomena: cognitive, sensory- e.g. photophobia and hypersensitivity to noise and/or emotional overload, which may lead to “crash”-periods and/or anxiety.</td>
<td>5.f) Item from CFS symptom inventory: “If you think about the last month, how often have you felt uncomfortable with loud noises?”</td>
<td>Score ≥ 3</td>
</tr>
<tr>
<td>5.g) Item from CFS symptom inventory: “If you think about the last month, how often have you felt unwell with normal indoor lightning?”</td>
<td>Score ≥ 3</td>
<td></td>
</tr>
<tr>
<td>6) At least one symptom from two of the following categories:</td>
<td><strong>Patient must fulfill one of specifications 6.a) – 6.c)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Autonomic Manifestations</strong></td>
<td>6.a) Item asking about autonomic complaints: “How often do you get dizzy when you raise up?”</td>
<td>Score ≥ 4 (3–5 times a week)</td>
</tr>
<tr>
<td>Orthostatic intolerance – NMH, POTS, delayed postural hypotension, vertigo</td>
<td>Not covered in questionnaire</td>
<td></td>
</tr>
</tbody>
</table>
### Appendix 1: Operationalized Canadian Consensus Criteria - continued

<table>
<thead>
<tr>
<th>Condition</th>
<th>Item Description</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nausea and IBS</td>
<td>6.b) Item from CFS symptom inventory: “If you think about the last month, how often have you had nausea?”</td>
<td>≥ 3</td>
</tr>
<tr>
<td>Urinary frequency and bladder dysfunction</td>
<td>Not covered in questionnaire</td>
<td></td>
</tr>
<tr>
<td>Palpitations with or without cardiac arrhythmia</td>
<td>6.c) Item from CFS symptom inventory: “If you think about the last month, how often have you had palpitations (heart beats unusually fast or strong)?”</td>
<td>≥ 3</td>
</tr>
<tr>
<td>Palpitations, and exertional dyspnea.</td>
<td>Not covered in questionnaire</td>
<td></td>
</tr>
<tr>
<td><strong>Neuroendocrine Manifestations:</strong></td>
<td>Patient must fulfill one of specifications 6.d) – 6.j)</td>
<td></td>
</tr>
<tr>
<td>Loss of thermostatic stability subnormal body temperature and/or marked diurnal fluctuations,</td>
<td>6.d) Item from CFS symptom inventory: “If you think about the last month, how often have you felt shifting between warm and cold?”</td>
<td>≥ 3</td>
</tr>
<tr>
<td>sweating episodes,</td>
<td>6.e) Item asking about autonomic complaints: “Are you easily freezing, or do you often feel to warm – compared to others?”</td>
<td>Score 1 (I’m very easily freezing) or 5 (I very often feel to warm)</td>
</tr>
<tr>
<td>Recurrent feeling of feverishness and cold extremities</td>
<td>6.g) Item from CFS symptom inventory: “If you think about the last month, how often have you had pale or cold hands?”</td>
<td>≥ 3</td>
</tr>
<tr>
<td>Intolerance to heat and cold</td>
<td>Not covered in questionnaire</td>
<td></td>
</tr>
</tbody>
</table>
**Appendix 1: Operationalized Canadian Consensus Criteria - continued**

| Marked weight change-anorexia or abnormal appetite | 6.i) Item from CFS symptom inventory:  
“If you think about the last month, how often have you had reduced appetite?”  
Score ≥ 3 |
| --- | --- |
| 6.j) Item from CFS symptom inventory:  
“If you think about the last month, how often have you had good appetite?”  
Score ≥ 3 |
| Loss of adaptability and tolerance for stress, worsening of symptoms with stress and a slow recovery. | Not covered in questionnaire |

### Immune manifestations:

| Tender lymph nodes | 6.k) Item from CFS symptom inventory:  
“If you think about the last month, how often have you had tender lymph-nodes?”  
Score ≥ 3 |
| --- | --- |
| Recurrent sore throat and flu-like symptoms | 6.l) Item from CFS symptom inventory:  
“If you think about the last month, how often have you had sore throat/felt painful to swallow?”  
Score ≥ 3 |
| General malaise | Not covered in questionnaire |
| New sensitivities to food, medications and/or chemicals | Not covered in questionnaire |

7) **The illness persists for at least six months. It usually has a distinct onset, although it may be gradual.** Preliminary diagnosis may be possible earlier. Three months is appropriate for children.  
Fulfilled by all patients included in the NorCAPITAL-project
### Appendix 2. Operationalized SEID criteria

<table>
<thead>
<tr>
<th>Source of information in the present study</th>
<th>Rule for adhering to SEID criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1) Fatigue</strong></td>
<td>Patient must fulfill <strong>all</strong></td>
</tr>
<tr>
<td>Source of information in the present study (Corresponding variable in the questionnaire or other source)</td>
<td>specifications 1.a) – 1.f)</td>
</tr>
<tr>
<td>A substantial reduction or</td>
<td></td>
</tr>
<tr>
<td>impairment in the ability to</td>
<td></td>
</tr>
<tr>
<td>engage in pre-illness levels of</td>
<td></td>
</tr>
<tr>
<td>occupational, educational, social,</td>
<td></td>
</tr>
<tr>
<td>or personal activities,</td>
<td></td>
</tr>
<tr>
<td>that persist for more than 6 months,</td>
<td>1.a) FDI total sum score</td>
</tr>
<tr>
<td>and is accompanied by fatigue,</td>
<td>Total sum score ≥ 7</td>
</tr>
<tr>
<td>which is often profound,</td>
<td>1.b) Item: “When did you recognize this fatigue for the first time?”</td>
</tr>
<tr>
<td>and is of new or definitive onset (not</td>
<td>6 months or more</td>
</tr>
<tr>
<td>lifelong),</td>
<td></td>
</tr>
<tr>
<td>and is not the result of ongoing</td>
<td>1.d) Item from CFS symptom inventory:</td>
</tr>
<tr>
<td>excessive exertion,</td>
<td>“When did you recognize the fatigue for the first time?”</td>
</tr>
<tr>
<td>and is not substantially alleviated by</td>
<td>1.e) Covered by the inclusion criteria of the NorCapital project</td>
</tr>
<tr>
<td>rest.</td>
<td>n.a.</td>
</tr>
<tr>
<td>1.f) Item from FSS: “Although I rest a lot, I am still fatigued afterwards”.</td>
<td>Score ≥ 5</td>
</tr>
</tbody>
</table>

| **2. Post-exertional malaise (PEM)**       |                                   |
| 2.a) Item from FSS: “I am easily fatigued”. | Score ≥ 5                         |
| 2.b) Item from FSS: “Exercise brings on my fatigue”. | Score ≥ 5                         |
| 2.c) Item from CFS symptom inventory:      | Score ≥ 4                          |
| “Does the fatigue get better or worse after walking slowly” | }
**Appendix 2. Operationalized SEID criteria - continued**

### 2. Operationalized SEID criteria - continued

2.d) Item from CFS symptom inventory:  
“Does the fatigue get better or worse after doing hard school work”?  
Score ≥ 4

2.e) Item from CFS symptom inventory:  
“If you think about the last month, how often have you been more fatigued the day after an exertion?”  
Score ≥ 4

### 3. Unrefreshing sleep

Patient must fulfill **one of** the specifications 3.a) – 3.c)

3.a) Item from CFS symptom inventory:  
“If you think about the last month, how often have you don’t felt refreshed after you’ve slept?”  
Score ≥ 4

3.b) Item from CFS symptom inventory:  
“If you think about the last month, how often have you woken up early and not been able to sleep again?”  
Score ≥ 4

3.c) Karolinska Sleep Questionnaire: Insomnia index  
Score ≤ 3,5

### 4. Cognitive impairment

Patient must fulfill **two of** the specifications 4.a) – 4.d)

4.a) Item from CFS symptom inventory:  
“If you think about the last month, how often have you felt confused or disoriented?”  
Score ≥ 4

4.b) Item from CFS symptom inventory:  
“If you think about the last month, how often have you had problems with remembering?”  
Score ≥ 4

4.c) Item from CFS symptom inventory: “If you think about the last month, how often have you had problems concentrating?”  
Score ≥ 4
### Appendix 2. Operationalized SEID criteria - continued

<table>
<thead>
<tr>
<th>4.d) Item from PedsQL: “It is difficult for me to keep up with the class.”</th>
<th>Score ≥ 4</th>
</tr>
</thead>
</table>

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<thead>
<tr>
<th>5. Orthostatic intolerance</th>
<th>Patient must fulfill the specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASP, orthostatic sub score</td>
<td>Score ≥ 3</td>
</tr>
</tbody>
</table>

n.a. = not applicable