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Plasma cytokine expression in adolescent chronic fatigue syndrome

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ABSTRACT

Chronic fatigue syndrome (CFS) is a prevalent and disabling condition among adolescents. The pathophysiology is poorly understood, but low-grade systemic inflammation has been suggested as an important component. This study compared circulating levels of individual cytokines and parameters of cytokine networks in a large set of adolescent CFS patients and healthy controls, and explored associations between cytokines and symptoms in the CFS group.

CFS patients (12–18 years old) were recruited nation-wide to a single referral center as part of the Nor-CAPITAL project (ClinicalTrials ID: NCT01040429). A broad case definition of CFS was applied, requiring three months of unexplained, disabling chronic/relapsing fatigue of new onset, whereas no accompanying symptoms were necessary. Thus, the case definition was broader than the Fukuda-criteria of CFS. Healthy controls having comparable distribution of gender and age were recruited from local schools. Twenty-seven plasma cytokines, including interleukins, chemokines and growth factors were assayed using multiplex technology. The results were subjected to network analyses using the ARACNE algorithm. Symptoms were charted by a questionnaire, and patients were subgrouped according to the Fukuda-criteria.

A total of 120 CFS patients and 68 healthy controls were included. CFS patients had higher scores for fatigue ($p < 0.001$) and inflammatory symptoms ($p < 0.001$) than healthy controls. All cytokine levels and cytokine network parameters were similar, and none of the differences were statistically different across the two groups, also when adjusting for adherence to the Fukuda criteria of CFS. Within the CFS group, there were no associations between aggregate cytokine network parameters and symptom scores.

Adolescent CFS patients are burdened by symptoms that might suggest low-grade systemic inflammation, but plasma levels of individual cytokines as well as cytokine network measures were not different from healthy controls, and there were no associations between symptoms and cytokine expression in the CFS group. Low-grade systemic inflammation does not appear to be a central part of adolescent CFS pathophysiology.

1. Background

Chronic fatigue syndrome (CFS) is characterized by unexplained, long-lasting, disabling fatigue accompanied by pain, cognitive impairments, and other symptoms (Fukuda et al., 1994; Royal College of Paediatrics and Child Health, 2004). CFS is an important cause of disability among adolescents, and may have detrimental effects on psychosocial and academic development

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(Kennedy et al., 2010), as well as family functioning (Missen et al., 2012). Adolescent CFS prevalence is estimated at 0.1–1.0% (Nijhof et al., 2011; Crawley et al., 2011; Jason et al., 2006).

The pathophysiology of CFS remains poorly understood. However, studies have reported alterations of homeostatic functions, such as enhanced sympathetic and attenuated parasympathetic cardiovascular nervous activity (Wyller et al., 2007, 2008a,b), attenuation of the hypothalamus–pituitary–adrenal axis (HPA-axis) (Segal et al., 2005) and low-grade systemic inflammation (Klimas et al., 2012; Raison et al., 2009). Inflammation might have a key role in the underlying pathophysiology, as assumed in the proposal for revised case definition of CFS (Carruthers et al., 2011). Accordingly, several studies have reported alterations in systemic cytokine expression in CFS, although the evidence is somewhat conflicting. For instance, Maes and co-workers (2012) reported increased levels of interleukin (IL)-1 and tumor necrosis factor (TNF), Fletcher and co-workers (2009) reported altered levels of several cytokines including IL-1α and IL-1β but normal levels of TNF, whereas Vollmer-Conna and co-workers (2007) did not find any alterations of cytokine expression. One reason for this discrepancy might be that CFS is associated with subtle alterations of cytokine networks rather than individual cytokines. Also, the mentioned pro-inflammatory cytokines are heavily influenced by pre-analytical factors and circulate at very low levels, requiring high sensitivity methods for reliable detection and making comparison between studies utilizing different assays difficult (Banks, 2000). Applying advanced mathematical methods on a large set of cytokine expression data in CFS patients and healthy controls, Broderick and co-workers (2010) found evidence of attenuated and Th1 and Th17 immune responses in the context of a well-established Th2 inflammatory environment in CFS.

In adolescent CFS, cytokine expression has hardly been investigated. In a small study of fatigued adolescents ($n = 9$) following Epstein Barr virus infection, standard comparative analyses indicated increased levels of IL-8 and reduced levels of IL-23 as compared to recovered controls (Broderick et al., 2012). In a previous report from the NorCAPITAL project, we reported slightly elevated levels of C-reactive protein (CRP) in adolescent CFS patients (Sulheim et al., 2014). To the best of our knowledge, no other studies have specifically addressed circulating cytokine expression in adolescent CFS.

Thus, the aims of this study were to compare individual cytokines and parameters of cytokine networks in plasma from a large set of adolescent CFS patients and healthy controls, and to explore associations between cytokines and symptoms in the CFS group. We hypothesized altered cytokine expression in CFS consistent with low-grade systemic inflammation, and a positive association between levels of proinflammatory cytokines and symptoms of inflammation.

2. Materials and methods

2.1. CFS patients

The Department of Paediatrics at Oslo University Hospital is a national referral center for young CFS patients. For this study, all hospital paediatric departments in Norway ($n = 20$), as well as primary care paediatricians and general practitioners, were invited to refer CFS patients aged 12–18 years consecutively to our department. The referring units were equipped with written information for distribution to potential study participants and their parents/next-of-kin. If consent was given, a standard form required the referral unit to confirm the result 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). Also, the referring units were required to confirm that the patient (a) was unable to follow normal school routines due to fatigue; (b) was not permanently bedridden; (c) did not have any concurrent medical or psychiatric disorder that might explain the fatigue; (d) did not experience any concurrent demanding life event (such as parents' divorce) that might explain the fatigue; (e) did not use prescribed pharmaceuticals (including hormone contraceptives) regularly. A previous demanding life event was not an exclusion criteria. Completed forms were consecutively conveyed to the study center and carefully evaluated by either of two authors (DS or EF). Patients considered eligible to this study were summoned to a clinical encounter at our study center after which a final decision on inclusion was made.

In agreement with clinical guidelines (Royal College of Paediatrics and Child Health, 2004; National Institute for Health and Clinical Excellence, 2007), we applied a 'broad' case definition of CFS, requiring three months of unexplained, disabling chronic/relapsing fatigue of new onset. We did not require that patients meet any other accompanying symptom criteria, in contrast to the case definition from the International Chronic Fatigue Syndrome Study Group at the Centers for Disease Control and Prevention (commonly referred to as the Fukuda-definition), which appears to be most frequently used in the scientific community (Fukuda et al., 1994). The Fukuda-definition requires at least six months of unexplained chronic or relapsing fatigue of new onset, severely affecting daily activities, as well as four or more of eight specific accompanying symptoms (headache, muscle pain, joint pain, sore throat, tender lymph nodes, impaired memory or concentration, unrefreshing sleep, and malaise after exertion). However, the validity of this definition has not been established (Brurberg et al., 2014). In fact, several empirical findings raise concerns about the validity, in particular among adolescents: A formal factor analysis of symptoms in a broadly defined group of chronic fatigued patients did not show a strong correspondence with the Fukuda accompanying symptoms (Nisenbaum et al., 2004). A study based upon the Swedish twin registry concluded that there was no empirical support for the requirement of four out of eight Fukuda accompanying symptoms (Sullivan et al., 2005). A report on a broadly defined population of adolescent CFS patients concluded that the subgroup adhering to the Fukuda criteria was not characterized by a certain level of disability, nor was this subgroup specifically related to characteristics of underlying pathophysiology (alteration of cardiovascular autonomic control) (Wyller and Helland, 2013). Accordingly, subgrouping based upon the Fukuda criteria did not influence the cross-sectional comparisons nor the intervention effects in previously reported results from the NorCAPITAL project (Sulheim et al., 2014).

Thus, the inclusion criteria in this study are wider than the Fukuda criteria. Subgrouping of the participants according to the Fukuda case definition was performed *post hoc*, based on questionnaire results (cf. below). The main reason for not adhering to the Fukuda case definition was too few accompanying symptoms.

2.2. Healthy controls

A group of healthy controls with a comparable distribution of gender and age were recruited from local schools. Controls were not matched to cases on any variable. No chronic disease and no regular use of pharmaceuticals were allowed.

2.3. Study design and ethics

A one-day in-hospital assessment included clinical examination and blood sampling, and always commenced between 7.30 and