Brain, Behavior, and Immunity xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Predictors of chronic fatigue in adolescents six months after acute Epstein-Barr virus infection: A prospective cohort study

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ARTICLE INFO

Keywords:
Chronic fatigue
Epstein-Barr virus infection
Infectious mononucleosis
Adolescents
Chronic Fatigue Syndrome

ABSTRACT

Introduction: Acute Epstein-Barr virus (EBV) infection is a trigger of chronic fatigue and Chronic Fatigue Syndrome (CFS). This study investigated baseline predictors of chronic fatigue six months after an acute EBV infection.

Materials and methods: A total of 200 adolescents (12–20 years old) with acute EBV infection were assessed for 149 possible baseline predictors and followed prospectively. We performed linear regression to assess possible associations between baseline predictors and fatigue (Chalder Fatigue Questionnaire total score) six months after the acute EBV infection. A total of 70 healthy controls were included for cross-sectional reference. This study is part of the CEBA-project (Chronic fatigue following acute Epstein-Barr virus infection in adolescents).

Results: In the final multiple linear regression model, fatigue six months after acute EBV infection was significantly and independently predicted by the following baseline variables (regression coefficient B[95% CI]): Sensory sensitivity (0.8[0.09–1.6]), pain severity (0.2[0.02–0.3]), functional impairment (1000 steps/day) (−0.3[−0.5 to −0.08]), negative emotions (anxiety) (0.4[0.2–0.6]), verbal memory (correct word recognition) (1.7[0.1–3.3]), plasma C-reactive protein (2.8[1.1–4.4] for CRP values > 0.86) and plasma Vitamin B_{12} (−0.005[−0.01 to −0.001]).

Conclusions: Development of fatigue after acute EBV infection is to a larger extent predicted by baseline variables related to symptoms and functions than to baseline variables reflecting infectious and immune processes.

Trial registration: ClinicalTrials, ID: NCT02335437, https://clinicaltrials.gov/ct2/show/NCT02335437.

1. Introduction

Chronic fatigue, defined as substantial fatigue lasting for more than six months, affects up to 18% of the adult population in Western countries (Pawlikowska et al., 1994). In adolescents, about 20% of girls and 6.5% of boys report to have been severely fatigued during the last month (Crawley, 2014). If fatigue is unexplained, long lasting, disabling and accompanied by musculoskeletal pain, orthostatic intolerance,

Abbreviations: BIPQ, Brief Illness Perception Questionnaire; BPI, Brief Pain Inventory; CAPS, Children and Adolescents Perfectionism Scale; CFQ, Chalder Fatigue Questionnaire; EBV, Epstein-Barr virus; FDI, Functional Disability Inventory; HADS, Hospital Anxiety and Depression Scale; IM, Infectious mononucleosis; KSQ, Karolinska Sleep Questionnaire; LEC, Life Event Checklist; PedsQL, Pediatric Quality of Life; PSWQ, Penn State Worry Questionnaire; TAS-20, Toronto Alexithymia Scale 20

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https://doi.org/10.1016/j.bbi.2018.09.023
Received 5 June 2018; Received in revised form 8 September 2018; Accepted 24 September 2018
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cognitive problems, post exertional malaise and other symptoms, the patient may fulfill one of the case-definitions of Chronic Fatigue Syndrome (CFS) (IOM, 2015). These case-definitions are based solely on different constellations of patient-reported symptoms, as no biomarker has been identified. CFS has two age peaks in incidence, the first during adolescence and the second from 30 to 39 years (Bakken et al., 2014), and female-to-male ratio varies from 2:1 to 5:1 across studies. The prevalence of CFS among adolescents is estimated at 0.1–1.9%, depending on the applied case definition (Jordan et al., 2006); anyhow, it constitutes one of the most significant health problems among young people.

The pathophysiology behind chronic fatigue and CFS remains largely unknown, preventing development of prophylactic measures as well as therapeutic strategies. Immunological mechanisms have been intensively researched, but results are inconclusive. Findings across some studies include attenuated natural killer (NK) cell function (Mensah et al., 2017), and low-grade systemic inflammation (Sulheim et al., 2014; Klimas et al., 2012); however, these findings are not consistent (Katz et al., 2013). Plasma cytokine levels tend to be similar between CFS patients and healthy controls, at least in the adolescent group (Wyller et al., 2015). Recently, functional alterations of cytotoxic T-cells ($CD8^+$) as well as a gene expression pattern suggesting altered B cell differentiation and survival have been reported (Brenu et al., 2016; Nguyen et al., 2017).

In addition, adolescent CFS is associated with altered autonomic cardiovascular control characterized by enhanced sympathetic and attenuated parasympathetic cardiovascular nervous activity (IOM, 2015; Wyller et al., 2007), increased plasma catecholamine levels (IOM, 2015), and – in some studies – attenuation of the hypothalamus-pituitaryadrenal axis (HPA-axis) (Papadopoulos and Cleare, 2012). Neuropsychological studies suggest slight impairments of executive control functions (Sulheim et al., 2015; Ocon et al., 2012); furthermore, pressure pain threshold is reduced (Winger et al., 2014), and CFS patients experience more sleep difficulties than healthy individuals (Pedersen et al., 2017).

Infectious mononucleosis (IM) is a well-known trigger of chronic fatigue and CFS (Hickie et al., 2006); among adolescents, 11–13% are reported to have CFS six months after IM (Hickie et al., 2006; Katz et al., 2009). Acute Epstein-Barr virus (EBV) infection is the most common causative agent of IM (Balfour et al., 2015). Clinical symptoms include fever, pharyngitis, lymphadenopathy, malaise and spleen enlargement, whereas laboratory findings are characterized by elevated liver function test, increased number of cytotoxic T cells ($CD8^+$), and altered B cell functions (Balfour et al., 2015). Thus, acute EBV infection parallels some of the characteristics of CFS. In addition, EBV-infection is associated with other serious long-term complications, such as certain malignancies (e.g. nasopharyngeal carcinoma) and the neurological disease multiple sclerosis (Balfour et al., 2015).

Previous prospective studies report female sex to be a main predictive factor of chronic fatigue after IM (Buchwald et al., 2000; Candy et al., 2003; Petersen et al., 2006). Other reported predictors include symptom load of the initial infection (Hickie et al., 2006), days spent in bed following acute IM (Jason et al., 2014), autonomic symptoms (Jason et al., 2014), illness perception (Candy et al., 2003), premorbid mood disorders (Petersen et al., 2006), days from symptoms to diagnosis (Petersen et al., 2006), negative life events, and the level of family support (Buchwald et al., 2000). Taken together, previous findings appear somewhat inconsistent, which might be due to heterogeneous patient materials. Furthermore, well-known biological characteristics of CFS and EBV infection have hardly been explored as predictors, suggesting that a broader approach is warranted.

The problem of heterogeneity might be overcome by studying adolescents exclusively, possibly reducing confounding effects of normal ageing processes and comorbidities as compared to adults. Furthermore, an extensive biopsychosocial approach would ensure that a broad range of possible predictors are covered. These considerations underlie the project entitled Chronic Fatigue Following Acute EBV Infection in Adolescents (CEBA) (cf. Supplementary Material for details). The primary aim of the present study within the CEBA project was to investigate predictors of chronic fatigue six months after an acute EBV infection. We hypothesized the most important predictors to be a combination of viral load, immune response characteristics, and some of the previously described neuroendocrine features of CFS patients.

2. Materials and methods

2.1. Study design

The CEBA project encompasses a prospective, a cross-sectional and a randomized controlled design with a total follow-up time of 21 months (supp Fig. 1). In this paper, prospective results from the first six months are reported; data from a healthy control group are included for reference. The project has been approved by the Norwegian National Committee for Ethics in Medical research. Participation was based upon informed consent.

2.2. Participants

From March 2015 until November 2016, EBV infected individuals fulfilling the following criteria were assessed for eligibility (cf. Supplementary Material for details): a) A serological pattern indicating acute EBV infection (supp Table 1); b) Age between 12 and 20 years; and c) Living in one of the Norwegian counties Oslo, Akershus or Buskerud. Exclusion criteria were a) more than 6 weeks since debut of symptoms suggesting acute EBV infection; b) Any chronic disease that needed regular use of medication; c) Pregnancy. Because of some practical hindrances (such as the lag from patients' first symptoms to the primary serological EBV analyses were performed), the first patient encounter was on average 30 days after the first presenting symptom of EBV infection.

Healthy adolescents having the same distribution of sex and age as the EBV infected individuals were recruited during the same time period, mainly by asking adolescent already included in the EBV group to bring a healthy friend of the same age and sex to the six months follow-up encounter. In addition, a total of ten healthy adolescents were recruited from local schools. This healthy control group was mainly intended for cross-sectional comparison with the subgroup of fatigued EBV individuals at six months follow-up; these comparisons are not reported in the present paper.

2.3. Investigational program

Participants were summoned to a one-day investigational program at the CEBA study center, Akershus University Hospital, Norway. Encounters with the EBV-infected individuals were scheduled as soon as possible after debut of symptoms (baseline), with a follow-up visit six months later (supp Fig. 1). Healthy controls were seen only once.

All participants met at 8 a.m. after fasting overnight. They brought morning spot urine in a sterile container, and were instructed to apply a local anesthetic ointment (EMLA®, AstraZeneca) on both antecubital areas one hour before arriving. The investigational program included a clinical examination, ultrasound of the spleen, blood and throat swab sampling, autonomic cardiovascular control assessment, pressure pain threshold assessment, cognitive testing and questionnaire charting, and was followed by activity monitoring (cf. Supplementary Material for details). The program was carried out in a fixed sequence for all participants by two researchers only (MP and TTA).

2.4. Laboratory assays

After at least ten minutes of supine rest, blood samples were

obtained in a fixed sequence from antecubital venous puncture performed between 8:30 and 9:00. Samples were assayed for neuroendocrinological, immunological, microbiological, and routine clinical markers. A throat swab sample was assayed for EBV virus load.

2.5. Autonomic cardiovascular control

Three test conditions were applied to each participant in a fixed sequence: 1) Supine rest for five minutes; 2) Supine rest with controlled breathing for five minutes; 3) Active upright standing without support for three minutes. The Task Force Monitor (Model 3040i, CNSystems Medizintechnik, Graz, Austria) was used to provide continuous, beat-to-beat, non-invasive recording of blood pressure, heart rate and stroke volume. Other cardiovascular variables of relevance, such as total peripheral resistance index and heart rate variability (HRV) indices were calculated from these primary recordings.

2.6. Pressure pain threshold

Pressure pain threshold was assessed by gradually applying increasing pressure to six predefined areas, using the Commander™ Algometer (JTECH Medical, Midvale, USA).

2.7. Cognitive testing

A neuropsychological test battery assessed working memory (digit span forward and backward), processing speed, cognitive inhibition, and cognitive flexibility (color-word interference test), verbal learning (total immediate recall), and verbal memory (delayed recall and delayed recognition). In addition, estimated full-scale intelligence quotient (IQ) was assessed.

2.8. Questionnaires

The questionnaires included the following validated instruments: Chalder Fatigue Questionnaire (CFQ), The Chronic Fatigue Syndrome (CFS) symptom inventory, Penn State Worry Questionnaire (PSWQ), Karolinska Sleep Questionnaire (KSQ), Hospital Anxiety and Depression Scale (HADS), Brief Illness Perception Questionnaire (BIPQ), Children and Adolescents Perfectionism Scale (CAPS), Pediatric Quality of Life (PedsQL), Functional Disability Inventory (FDI), Life Event Checklist (LEC), Toronto Alexithymia Scale 20 (TAS-20), Brief Pain Inventory (BPI). In addition, the questionnaires assessed clinical symptoms of EBV infection (such as fever/chills, sore throat, tender lymphatic nodes), symptoms pertaining to different case definition of CFS, and demographic background variables.

2.9. Activity monitoring

Daily physical activity was monitored during seven consecutive days using the *activPAL* accelerometer device.

2.10. Statistical analysis

The CFQ total score at six months was predefined as the primary dependent variable in the prospective analyses. Adherence to case definitions of unspecific chronic fatigue (White et al., 2011), CFS according to the Fukuda-criteria (Fukuda et al., 1994), and CFS according to the Canada-criteria at six months follow-up (Carruthers et al., 2003), respectively, were set as secondary dependent variables. We estimated that a total of 200 EBV infected individuals would give a power of at least 80% for detecting a predictor variable that explains 5% of the variance in CFQ total score at six months. Correspondingly, when assessing associations with a binary predictor at a 5% significance level, a total of 200 patients would give a power of 80% to detect a mean difference of 0.4 SD between the two categories. Assuming a standard deviation of 5 on the CFQ score, this corresponds to a mean difference of approximately 2. Thus, the study was considered to have sufficient power to detect small to medium effect sizes. Furthermore, we estimated that about 70 EBV infected individuals would be classified as fatigue cases at six months follow-up (Hickie et al., 2006); recruitment of an equal number of healthy controls was regarded sufficient for detecting cross-sectional differences of clinical relevance, in line with previous studies from our group (Sulheim et al., 2014; Wyller et al., 2015; Sulheim et al., 2015; Winger et al., 2014).

All statistical analyses were carried out using SPSS statistical software (IBM SPSS Statistic 22 Inc., Chicago, IL, USA). Missing values were replaced by multiple imputation. The primary analyses featured simple linear regression between CFQ total score and a total of 149 possible baseline predictors, the selection of which was based on previous research findings in CFS and IM as well as theoretical considerations. The bivariate analyses were followed by multiple linear regression modelling assessing each variable's p-value and the effect on the dependent variable variance (adjusted R^2, cf. Supplementary Material for details). In the final models, a p-value < 0.05 was considered statistically significant. Binary logistic regression was used for the case-definition analysis.

3. Results

A total of 895 adolescents with a serological pattern suggesting acute EBV infection were assessed for eligibility (Fig. 1). A total of 200 were included; they were younger (mean age 16.9 vs 17.5, $p < 0.001$) and the percentage of females was higher (64.5% vs 54.7%, $p = 0.013$) as compared to the non-included group. The EBV serology patterns did not differ.

A total of 70 healthy controls were also included. Background characteristics did not differ significantly between patients and controls (Table 1, supp Table 2). The patients reported more clinical symptoms and functional impairments at baseline; serology analyses indicated acute EBV infection in all patients and in no healthy controls, corroborating slight lymphocytosis and elevated antibody levels and liver function tests in the patient group. C-reactive protein (CRP) level (high-sensitive assay) was significantly lower among the EBV infected individuals.

At six months follow-up in the EBV infected group, a total of five did not attend the scheduled visit (Fig. 1), leaving 195 individuals for the final analyses. A total of 91 was classified as chronic fatigue cases (a total score of 4 or more at the Chalder Fatigue Questionnaire, each item scored dichotomously), whereas 27 and 20 individuals, respectively, fulfilled the Fukuda-definition and Canada-definition of CFS. In simple linear regression analyses, clinical symptoms, functional impairments, negative emotions, previous negative life events, and elevated plasma CRP at baseline were strongly associated with fatigue score at six months (supp Table 2). Other associations were weak and statistically non-significant when adjusting for test multiplicity. Of 98 baseline biomarkers related to infection, immunity, neuroendocrinology and autonomic cardiovascular control, only nine showed any association to fatigue at six months.

In the final multiple linear regression model, a total of seven baseline variables were identified as predictors of fatigue score at six months (Table 2): Sensory sensitivity, pain severity, steps/day, anxiety and elevated plasma CRP at baseline, which were all strongly associated in simple regression analyses, remained predictors in the multivariate model; in addition, correct word recognition performance (an index of verbal memory) were positively associated and plasma vitamin B_{12} negatively associated with fatigue.

Applying a less strict procedure for explanatory variable selection (cf. supp Fig. 2, point g), two immune biomarkers at baseline (T cell ($CD3^+$) fraction and IgM memory B cell subset) could be considered to have some independent predictive value of fatigue score at six months in addition to the seven baseline variables already identified (supp

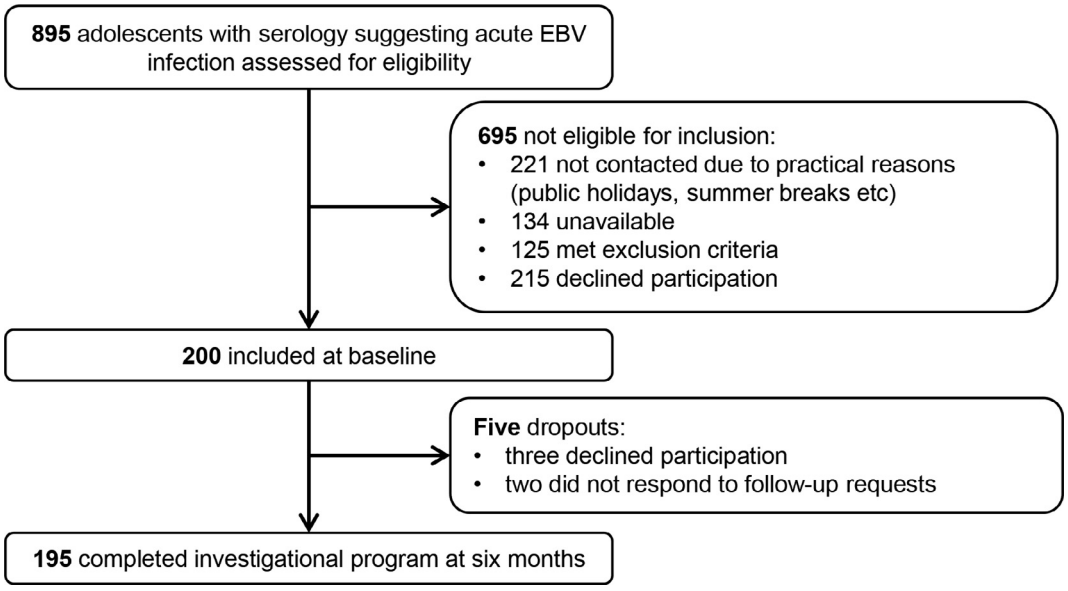

Fig. 1. Study flowchart.

Table 3). Analyses stratified by sex seemed to indicate differences between males and females, although no interaction term remained statistically significant in the adjusted model. Among males, only baseline anxiety score remained a significant predictor of fatigue at six months, and the explanatory power of the model was much lower than in females (supp Table 3).

In unadjusted logistic regression analyses, a total of 38 baseline variables were associated with either chronic fatigue caseness, CFS according to the Fukuda criteria, or CFS according to the Canada criteria at six months (supp Table 4). In general, the associated variables

Table 1
Cohort characteristics.

	At inclusion (Baseline)			At six months	
	Patients (n = 200)	Healthy controls (n = 70)	p-value (patients vs controls)[1]	Patients (n = 195)	p-value (patients at inclusion vs patients at six months)[2]
Background					
Sex – no. males (%)	71 (35.5%)	26 (37.1%)	0.805	n.a.	n.a.
Age, years – mean (SD)	16.9 (1.6)	17.0 (1.8)	0.236	n.a.	n.a.
BMI, kg/m^2 – mean (SD)	21.3 (2.6)	21.5 (3.1)	0.058	22.2 (2.6)	< 0.001
Symptoms and functional impairment					
Days since debut of symptoms, self reported – mean (SD)	30.2 (6.6)	n.a.	n.a.	n.a.	n.a.
Chalder Fatigue Questionnaire (CFQ), total score – mean (SD)[3]	19.5 (4.7)	10.8 (3.8)	< 0.001	15.2 (5.1)	< 0.001
Infectious Symptoms, total score – mean (SD)	2.7 (0.9)	1.4 (0.4)	< 0.001	1.8 (0.7)	< 0.001
Functional Disability Inventory, total score – mean (SD)	16.6 (11.8)	3.2 (3.9)	< 0.001	6.6 (8.8)	< 0.001
Steps/day, number – mean (SD)	7515 (3080)	10,133 (4133)	0.138	9046 (3438)	< 0.001
Clinical findings					
Epstein-Barr Virus (EBV) load, copies in blood – no. (%)			< 0.001		0.111
Negative (< 160)	49 (24.9%)	60 (85.7%)		82 (43.6%)	
Low (1600–2000)	115 (58.4%)	8 (11.4%)		61 (32.4%)	
Moderate/high (> 2000)	33 (16.8%)	2 (2.9%)		45 (23.9%)	
EBV Viral Capsid Antigen (VCA) IgM, titer – median (IQR)	160 (73)	0.0 (0.0)	< 0.001	20 (162)	< 0.001
EBV-VCA-IgG, titer – median (IQR)	69 (67)	51 (195)	0.430	169 (162)	< 0.001
EBV Nuclear Antigen (EBNA) IgG, titer – median (IQR)	0 (0)	57 (349)	< 0.001	98 (205)	< 0.001
Serum total IgG, g/L – mean (SD)	12.0 (2.7)	9.4 (1.7)	< 0.001	9.9 (1.8)	< 0.001
Blood Lymphocyte count, 10^9 cells/L – median (IQR)	2.3 (0.8)	1.9 (0.6)	0.041	1.9 (0.7)	< 0.001
Serum Alanine Transaminase (ALT), IU/L – median (IQR)	33 (23)	24 (7)	< 0.001	24 (9)	< 0.001

n.a. = not applicable.
[1] Based on *t*-test, Mann-Whitney test or chi-square test, as appropriate.
[2] Based on paired *t*-test or Wilcoxon's test, as appropriate.
[3] The CFQ total score at 6 months is defined as the primary endpoint for the prediction analyses, cf. Table 2.

Table 2
Baseline independent predictors of fatigue score[1] six months after acute EBV infection. Final multiple linear regression model.

Baseline independent predictors	Linear regression coefficient B (CI)	p-value	Δadj.R^2
Anxiety symptoms score[2]	0.4 (0.2–0.6)	< 0.001	0.041
Plasma high-sensitive CRP, mg/L[3]			0.036
< 0.2	*Reference*		
0.2–0.4	0.8 (−0.9 to 2.4)	0.354	
0.4–0.86	2.0 (0.4–3.6)	0.012	
> 0.86	2.8 (1.1–4.4)	0.001	
Steps/day, number/1000 steps	−0.3 (−0.5 to −0.08)	0.007	0.023
Sensory sensitivity score[4]	0.8 (0.09–1.6)	0.028	0.016
Serum Vitamin B_{12}, pmol/L	−0.005 (−0.01 to −0.001)	0.029	0.016
Total pain severity score[5]	0.2 (0.02–0.3)	0.032	0.015
Correct word recognition[6]	1.7 (0.1–3.3)	0.033	0.014
Explained variance (adjusted R^2) of model	0.34		

The average effect size of each baseline predictors on the fatigue score at six months is given by the linear regression coefficient B. For instance, a one point increase in baseline anxiety score increases the six month fatigue score by 0.4 points. Missing data were replaced by multiple imputation; a detailed explanation of the procedures for model generating is given in the Supplementary. Explained variance (adjusted R^2) is calculated as the pooled average from 5 imputed dataset. The Δadj. R^2-value indicates the change in explained variance of the entire model when one variable is removed from the model. EBV = Epstein-Barr virus, CI = Confidence Interval, CRP = C-reactive protein.

[1] Fatigue score (dependent variable) is from the Chalder Fatigue Questionnaire and defined as the total linear score (i.e. the sum across all 11 items, each item scored on a zero to three Likert scale). Total range is from zero to 33; higher scores imply more severe fatigue.

[2] Anxiety symptoms score is a subscore from the Hospital Anxiety and Depression Scale (HADS). Total range is from zero to 21; higher scores implies more severe anxiety symptoms

[3] The distribution of plasma high-sensitivity CRP deviated strongly from normality; thus, a dummy-variable based on 25th-centiles was created (cf. Supplementary for details).

[4] Sensory sensitivity score encompasses sensitivity towards light and sounds. Total range is from zero to five; higher scores implies more severe symptoms.

[5] Total pain severity score is from the Brief Pain Inventory (BPI). Total range is from zero to 40; higher scores implies more severe pain.

[6] Correct word recognition is part of the Hopkins Verbal Learning Test-Revised (HVLT-R). Total range is from zero to 12; higher scores implies better performance.

and the direction of association were similar to the prediction models for fatigue score. For each variable, the odds Ratio (OR) estimates were similar across all three case definitions.

4. Discussion

The main finding of this study is that fatigue six months after acute EBV infection is significantly and independently predicted by baseline clinical symptoms, functional impairments, negative emotions, verbal memory, plasma CRP and plasma vitamin B_{12}. Our finding of symptoms (sensory sensitivity, pain severity), functional impairments (steps/day) and negative emotions (anxiety) as predictors of fatigue are in line with previous research (Hickie et al., 2006; Buchwald et al., 2000; Jason et al., 2014). In particular, high comorbidity with anxiety and depressive disorders are well described in the adolescent CFS literature (Lievesley et al., 2014).

The weak predictive value of baseline biological markers such as viral load and immune response characteristics counteracts our initial hypothesis; plasma CRP being a notable exception. Low-grade systemic inflammation has been reported as a CFS characteristic in adults (Klimas et al., 2012) as well as adolescents (Sulheim et al., 2014). Also, plasma CRP levels are prospectively associated with fatigue in young adults (Cho et al., 2009); however, this is the first study to suggest such an association in adolescents. Intriguingly, on average, baseline CRP levels were significantly lower in the acute EBV infection group as compared to healthy controls (supp Table 2); this finding corroborates a report of attenuated inflammation response in the weeks succeeding the initial phase of infectious mononucleosis (Macsween et al., 2010). Thus, the slightly raised CRP levels that increase risk of fatigue development in certain individuals seem most likely to be related to other factors than the infection *per se*; one possibility is the well-described subtle inflammatory enhancement caused by negative life events (Steptoe et al., 2007). Also, the predictive value of plasma vitamin B_{12} is a novel finding, although a relationship to fatigue has been suggested from some previous studies (Regland et al., 2015; Ellis and Nasser, 1973).

Finally, to the best of our knowledge, a positive association between verbal memory (as assessed by correct word recognition) and fatigue development has not been reported previously. This finding seems to support a theory of unconscious learning processes (such as Pavlovnian conditioning) underlying chronic fatigue development (Lenaert et al., 2017) (cf. below); of note, recent evidence suggest that declarative memory (of which verbal recognition is an example) impacts on conditioning (Connor and Gould, 2016). Of note, the correct word recognition test was the only cognitive test scored poorer by the acute EBV infection group than the healthy control group, (supp Table 2), corroborating previous findings of attenuated cognitive functioning in infectious mononucleosis (Cvejlic et al., 2014) and suggesting complex interrelations between infection, cognition, fatigue development and possibly other factors. These interrelations deserve attention in further studies.

There was a striking similarity between baseline predictors for six month chronic fatigue caseness, CFS according to the Fukuda-criteria and CFS according to the Canada-criteria. In particular, no pattern of a stepwise progression in the strength of predictor association across the different case definitions could be identified. This is in line with previous findings questioning the discriminating validity of the different CFS case definitions and suggesting similar underlying disease mechanisms (Asprusten et al., 2015; Brurberg et al., 2014). It might be more appropriate to regard fatigue as a continuously distributed variable in the population (Pawlikowska et al., 1994), with chronic fatigue and CFS denoting the upper tail.

A consistent finding in previous studies is that female sex predicts fatigue development after acute IM (Buchwald et al., 2000; Candy et al., 2003; Petersen et al., 2006). In the present study, female sex was associated with six months fatigue in unadjusted analyses, but not in the final multiple regression model (supp Table 2, Table 2). A more detailed exploration of the dataset revealed that sex interacted significantly with several variables, such as baseline perfectionism, illness perception, emotional awareness, insulin like growth factor-1, quality of life, and physical activity, which were significantly associated with six months fatigue among females only (results not shown). Likewise, the multiple regression analysis had much better explanatory power in females than in males; among the latter, only anxiety score was identified as a statistically significant predictor (supp Table 3). This discrepancy might partly be explained by the lower number of males than females, reducing the statistical power of the multiple regression model; still, sex differences in post-EBV chronic fatigue development are suggested.

Taken together, the findings of the present study seem to support a biopsychosocial rather than a biomedical perspective on development of chronic fatigue and CFS. All though speculative, the independent prospective predictions of clinical symptoms, functional impairments, negative emotions, verbal memory, and biomarkers may fit with theories suggesting that chronic fatigue might develop due to unconscious learning processes in which a wide range of interoceptive and exteroceptive stimuli become associated with the fatigue experience (Lenaert et al., 2017). Attempts to validate such theories might be appropriate aims for further research. Accordingly, the present findings

suggest that some factors related to chronic fatigue development, such as functional impairments and negative emotions, might be malleable and a target for prophylactic interventions. This, too, should be addressed in further research, taking into account the possibly different trajectories of fatigue development among males and females.

4.1. Strengths and limitations

Strengths of the present study include a large group of EBV-infected adolescents recruited soon after debut of IM, few dropouts, and a comprehensive assessment of possible baseline prediction variables. A limitation is limited knowledge of background variables; ideally, the cohort should have been recruited prior to the infectious event, but this strategy would have demanded a much larger number of participants and was not practically feasible. The baseline examination was executed on average one month after the first symptoms of infection; while this time lag is shorter than in comparable studies (Katz et al., 2009), it would have been beneficial to have had the baseline examination even closer to the initial infectious episode. The large number of baseline variables compared to the number of participants demanded grouping of the variables prior to the multiple regression modelling; even though this grouping was used as a starting point only, it may have affected the results. Of note, the final multiple regression models only explained about one third of the variance in six months fatigue, suggesting that non-charted variables – ranging from genetic markers to family dynamics – might have important impact, and calling for cautious interpretation of the results. As relatively few individuals in the EBV infected group adhered to case definitions of CFS at 6 months follow-up, a multiple logistic regression analysis featuring CFS caseness as dependent variable was not statistically feasible. Finally, included EBV-infected individuals differed in age and sex distribution from the eligible, non-included group, raising a concern for selection bias.

5. Conclusion

In adolescents with acute EBV infection, fatigue after six months is predicted by baseline clinical symptoms, functional impairments, negative emotions, verbal memory, plasma CRP and plasma vitamin B_{12}. Thus, development of fatigue is to a larger extent predicted by baseline variables related to symptoms and functions than to baseline variables reflecting infectious and immune processes.

Declarations

Competing interests

None of the authors have conflict of interest or financial relationships relevant to this article to disclose.

Funding

This study was founded by the Health South–East Hospital Trust, Norway.

Authors' contribution

Maria Pedersen and Tarjei Tørre Asprusten collected clinical data, contributed to study design and participated in data analyses. Kristin Godang, Truls Leegaard, Liv Toril Osnes, and Trygve Tjade carried out laboratory analyses and contributed to study design. Merete Glenne Øie supervised cognitive tests and contributed to study design. Eva Skovlund supervised data analyses. Vegard Bruun Bratholm Wyller conceived of the study, contributed to study design and participated in data analyses. All authors contributed to data interpretation and drafting of the manuscript.

The principal investigator (VBBW) had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis, and had final responsibility for the decision to submit it for publication. No one of the authors has any conflicts of interests relevant to this study.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Acknowledgements

We thank Stine Andersen Ness for invaluable secretary assistance; Zarina Aslam, Janne Sølvernes, Savka Repac and Marit Holmefjord Pedersen at the Research Support Unit, Dept. of Cardiology, Akershus University Hospital and Lene Gustavsen Berget and the remaining members of the team at Section for Cellular Immunology, Dept. of Immunology and Transfusion Medicine, Oslo University Hospital for blood sampling and laboratory assistance; Nurettin Erman for practical assistance; Even Fagermoen, Børre Fevang, Jacob Seth Fiskå, Karina Heldal, Henrik Børsting Jacobsen, Kristine Jacobsen, Sadaf Jamil Malik, Julie Mangersnes, Chinh BKrong Nguyen, Hilde Loge Nilsen, Kari Otterdal, Silje Endresen Reme, Betty van Roy, Peter C Rowe, Linn Nilsen Rødevand, J Philip Saul, Line Sletner, Dag Sulheim and Laura Wortinger for discussion on study design and results; Jan Petter Odden and Erik Borge Skei for institutional support; all the general practitioners providing information on individual participants; and finally all participants and their parents/next-of-kin.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2018.09.023.

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