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Fatigue in Epstein-Barr virus infected adolescents and healthy controls: A prospective multifactorial association study

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ABSTRACT

Objective: Acute Epstein-Barr virus (EBV) infection is a known trigger of both acute and chronic fatigue. The aim of this study was to investigate associations to fatigue in adolescents with EBV infection during the initial stage and six months after, as well as in healthy controls.

Methods: 200 adolescents (12–20 years old) with EBV infection were assessed as soon as possible after the onset of symptoms ($EBV_{baseline}$) and six months later ($EBV_{six\ months}$, 5 drop-outs). Also, 70 healthy controls (HC) were included. Associations between current fatigue and 148 different variables (including symptoms, functional abilities and biomarkers) were investigated separately for $EBV_{baseline}$, $EBV_{six\ months}$ and HC using linear regression modelling.

Results: Fatigue was associated with symptoms of sleeping difficulties, negative emotions, and quality of life under all circumstances. Fatigue was independently associated with markers of immune response at $EBV_{six\ months}$ and in HC, not at $EBV_{baseline}$. An association between fatigue and markers of autonomic cardiovascular control was only present at $EBV_{six\ months}$. Cognitive functioning shifted from a positive association to fatigue at $EBV_{baseline}$ to a negative trend at $EBV_{six\ months}$. Markers of infection were not associated with fatigue at $EBV_{baseline}$, $EBV_{six\ months}$ nor in HC.

Conclusion: Irrespective of the cause, fatigue is important for quality of life and is highly associated with negative emotions. Markers of infection and immune response had respectively none and barely any association to fatigue. Autonomic alterations and cognitive dysfunction were exclusively associated with fatigue long after infection, corroborating findings from studies of the Chronic Fatigue Syndrome.

1. Introduction

Fatigue is a common complaint in the general population, and approximately 3% of adolescents suffer from chronic fatigue [1]. A well-known trigger of fatigue is infectious mononucleosis caused by acute Epstein-Barr virus (EBV) infection, which can lead to acute fatigue, chronic fatigue (CF) and Chronic Fatigue Syndrome (CFS) [2,3]. Fatigue is an important reason for reduced quality of life [4], and contributes significantly to absence from school [5].

A number of subtle biological alterations are associated with patients with CF/CFS compared to healthy controls: Attenuated natural killer cell function [6]; low grade systemic inflammation [7,8]; reduced cognitive functioning [9]; altered autonomic cardiovascular control [10]; abnormal thermoregulatory response [11]; increased plasma catecholamine levels [12]; attenuation of the hypothalamus-pituitary-adrenal axis [13]; and sleep abnormalities [14]. Some pathophysiological theories regarding CFS incorporate these findings in a biopsychosocial model [15–17], whereas others claim a strict biological understanding [18]. Anyhow, the relationships between these findings and the subjective experience of fatigue have scarcely been explored.

The limited pathophysiological understanding of fatigue complicates development of prophylactic and therapeutic strategies. For the time being, the best documented treatment for adults as well as adolescents with CFS is cognitive behavioral therapy [19,20].

An earlier study on the present cohort investigated risk-factors during acute EBV infection for developing chronic fatigue six months later [21]. The aim of the present study was to investigate associations between current fatigue and a number of other variables (including symptoms, functional abilities and biomarkers) in adolescents during the initial stage and six months after an EBV infection, as well as in healthy adolescents. We hypothesized that fatigue in the acutely EBV

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infected adolescents would primarily be associated with markers of infection severity, that fatigue after six months would primarily be associated with traits found in CF/CFS patients (such as immunological changes, reduced cognitive functioning, altered autonomic cardiovascular control, increased plasma catecholamine levels, decreased cortisol level, and sleep abnormalities, and that fatigue in healthy controls would primarily be associated with lifestyle factors.

2. Method

This study is part of the CEBA-project (Chronic Fatigue following acute Epstein-Barr Virus Infection in Adolescents; ClinicalTrial ID: NCT02335437). Further details are reported elsewhere [21]. In the present study, results are reported from the two first examinations of the EBV patients, as well as the examination of the healthy controls. CEBA was approved by the Regional Committee for Ethics in Medical Research. Participation was based on written informed consent.

2.1. Participants

Inclusion of participants lasted from March 2015 until November 2016. During this period, Fürst Medical Laboratory and The Department of Microbiology at Akershus University Hospital reported to the CEBA-study center all individuals fulfilling the following criteria: [21] a) A serological pattern indicating acute EBV infection; b) Age between 12 and 20 years; and c) Living in one of the Norwegian counties Oslo, Akershus or Buskerud. Exclusion criteria were a) > 6 weeks since the onset of symptoms suggesting acute EBV infection; b) Any chronic disease that needed regular use of medication; c) Pregnancy.

In addition, 70 healthy controls with the same distribution of sex, age and geographical localization of residence as the EBV infected participants were included. Recruitment of these healthy controls was undertaken by asking the EBV infected participants to bring a healthy friend of similar age and gender when returning for follow-up visit (cf. below).

2.2. Investigational program

Participants were summoned to a one-day investigational program at the CEBA study center, Akershus University Hospital, Norway. For the participants with EBV infection, their first visit was scheduled as soon as possible after the onset of symptoms (baseline), with a follow-up visit six months later. The EBV infected participants underwent the same investigational program twice, and the questionnaires as well as the cognitive test were valid for retesting. The healthy controls underwent an identical investigational program, however only once. All participants met at 8 a.m. after fasting overnight. They were instructed to apply a local anesthetic ointment (EMLA®, AstraZeneca) on both antecubital areas one hour before arriving, and to bring morning spot urine in a sterile container.

The investigational program was carried out in a fixed sequence by two researchers only (MP and TTA), and 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, followed by measurement of physical activity [21]. Blood samples were obtained from antecubital venous puncture in a fixed sequence and assayed for neuroendocrinological, immunological, microbiological, and routine clinical markers. Autonomic testing encompassed continuous, non-invasive recordings by the Task Force Monitor (Model 3040i, CNSystems Medizintechnik, Graz, Austria) of blood pressure, heart rate and stroke volume during 1) supine rest, 2) supine rest with controlled breathing, and 3) upright standing. Pressure pain threshold was assessed by gradually applying increasing pressure to six predefined areas, using the Commander™ Algometer (JTECH Medical, Midvale, USA). Cognitive tests included assessment of verbal working memory (digit span forward and backward), processing speed, cognitive inhibition and flexibility (color-word interference test), verbal learning (total immediate recall) and memory (delayed recall and delayed recognition). All tests were validated for use in adolescents and designed for retesting. The questionnaire included validated inventories of fatigue and CFS (Chalder Fatigue Questionnaire, The Chronic Fatigue Syndrome (CFS) symptom inventory), pain (Brief Pain Inventory), sleep problems (Karolinska Sleep Questionnaire), anxiety and depression (Hospital Anxiety and Depression Scale), worrying (Penn State Worry Questionnaire), emotional awareness (Toronto Alexithymia Scale 20), illness perceptions (Brief Illness Perception Questionnaire), perfectionism (Children and Adolescents Perfectionism Scale), life events (Life Event Checklist), quality of life (Pediatric Quality of Life), and functional disabilities (Functional Disability Inventory), as well as clinical symptoms of EBV infection, symptoms pertaining to different case definition of CFS [22,23], and demographic and lifestyle background variables. Immediately after the one-day investigational program, physical activity was measured by an activPAL™ accelerometer device (PAL Technologies, Glasgow, Scotland) for seven consecutive days. The predefined primary endpoint in CEBA is the level of fatigue as assessed by the Chalder Fatigue Questionnaire (CFQ), consisting of 11 items rated on a four-point Likert scale; total score range is from 0 to 33 [24]. For further details on the assessments of the participants, please see Pedersen et al. 2018 [21].

2.3. Statistical analysis

All statistical analysis was performed with SPSS statistical software (IBM SPSS Statistic 24 Inc., Chicago, IL, USA). The power calculations prior to this study estimated that a total of 200 EBV infected individuals would give a power of at least 80% to detect an associated variable that explains 5% of the variance of fatigue. Correspondingly, 200 EBV infected individuals would provide a power of 80% to detect a mean difference of 0.4 standard deviations between the two categories in a binary variable with a 5% significance level. Thus, the study had sufficient power to detect small to medium effect sizes.

The primary analyses featured simple linear regression between fatigue score and a total of 148 possibly associated variables in, respectively, EBV patients at baseline ($EBV_{baseline}$), EBV patients at six months follow-up ($EBV_{six\ months}$), and healthy controls (HC). The first screening was performed without imputation and assumptions were checked by visual inspection of residual plots. In general, the vast majority of independent variables had normally distributed residual plots; those that had not were converted into categorized variables. A total of 62 variables had a *p*-value below 0.1 in at least one of the three assessment categories ($EBV_{baseline}$, $EBV_{six\ months}$ and HC), and were subjected to multiple imputation. Missing variable analysis conducted in SPSS revealed a missing at random (MAR) pattern for all three assessment categories, and imputation was done by fully conditional specification (using predefined algorithms within SPSS). A total of five complete datasets were created. For normally distributed data with a set minimum and maximum, constraints were defined.

After imputation, multiple linear regression modelling was performed separately for each of the three assessment categories ($EBV_{baseline}$, $EBV_{six\ months}$ and HC). Also, the modelling was stratified based on categories of variables, resulting in separate models for a) variables addressing symptoms, b) variables addressing functional abilities, and c) all other variables. In each multiple linear regression model, variables were selected with regard to their *p*-value and their effect on the dependent variable's variance (adjusted R [2]). A p-value < .05 was set as statistically significant in the final models.

3. Results

A total of 895 adolescents with serological pattern suggesting acute

Table 1
Cohort characteristics.

	EBV patients, baseline (n = 200)	EBV patients, six months (n = 195)	Healthy controls (n = 70)
Background			
Sex - no. males (%)	71 (35.5%)	n.a.	26 (37.1%)
Age, years - mean (SD)	16.9 (1.6)	n.a.	17.0 (1.8)
BMI, kg/m^2 - mean (SD)	21.3 (2.6)	22.2 (2.6)	21.5 (3.1)
Symptoms and functional impairment			
Days since the onset of symptoms, self reported - mean (SD)	30.2 (6.6)	n.a.	n.a.
Chalder Fatigue Questionnaire (CFQ), total score - mean (SD)[a]	19.5 (4.7)	15.2 (5.1)	10.8 (3.8)
Fatigued (CFQ dichotomously scored ≥4) - no (%)[b]	163 (87.2%)	92 (47.2%)	12 (18.2%)
Infectious Symptoms, mean score - mean (SD)	2.7 (0.9)	1.8 (0.7)	1.4 (0.4)
Functional Disability Inventory, total score - mean (SD)	16.6 (11.8)	6.6 (8.8)	3.2 (3.9)
Steps/day, number - mean (SD)	7515 (3080)	9046 (3438)	10,133 (4133)
Clinical findings			
Epstein-Barr Virus (EBV) load, copies in blood - no. (%)			
Negative (< 160)	49 (24.9%)	82 (43.6%)	60 (85.7%)
Low (1600 to 2000)	115 (58.4%)	61 (32.4%)	8 (11.4%)
Moderate/high (> 2000)	33 (16.8%)	45 (23.9%)	2 (2.9%)
EBV Viral Capsid Antigen (VCA) IgM, titer - median (IQR)	160 (73)	20 (162)	0.0 (0.0)
EBV-VCA-IgG, titer - median (IQR)	69 (67)	169 (162)	51 (195)
EBV Nuclear Antigen (EBNA) IgG, titer - median (IQR)	0 (0)	98 (205)	57 (349)
Serum total IgG, g/L - mean (SD)	12.0 (2.7)	9.9 (1.8)	9.4 (1.7)
Blood Lymphocyte count, 10^9 cells/L - median (IQR)	2.3 (0.8)	1.9 (0.7)	1.9 (0.6)
Serum Alanine Transaminase (ALT), IU/L - median (IQR)	33 (23)	24 (9)	24 (7)

n.a. = not applicable.
[a] The CFQ total score is defined as the primary endpoint for each of the three groups.
[b] All of the patients that were classified as fatigued six months after the acute EBV infection, were also classified as fatigued during the acute infection, except for four patients.

EBV infection were assessed for eligibility. In this group, 221 individuals were not contacted due to practical reasons (eg. public holidays), 134 were unavailable, 125 met exclusion criteria and 215 declined participation, leaving a total of 200 adolescents for inclusion in the CEBA-project. The participants 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. A total of 195 (97.5%) returned for the second visit six months after the acute infection (Table 1). In addition, 70 healthy controls were examined. The EBV patients and the healthy controls did not differ regarding sex and age.

The total fatigue score from Chalder Fatigue Questionnaire served as dependent variable in all analyses. Mean values improved significantly among the EBV patients from baseline (19.2) to six months follow-up (15.2), and was even lower among healthy controls (Table 1).

3.1. Symptoms

In simple linear regression analyses, all variables representing symptoms were strongly associated with fatigue for $EBV_{baseline}$, $EBV_{six\ months}$ and HC (Table 2, eTable1).

In the final models for symptoms, sleep difficulties were positively associated with fatigue for $EBV_{baseline}$ ($B = -0.38$, $p < 0.001$), $EBV_{six\ months}$ ($B = -0{,}28$, $p = 0.007$) and HC ($B = -0.20$, $p < 0.001$), and was the sole factor in the final model for HC (of note, lower scores at the Karolinska Sleep Questionnaire imply more sleep difficulties). In addition, fatigue was positively and independently associated with post-exertional malaise ($B = 0.86$, $p = 0.001$), hypersensitivity symptoms ($B = 0.75$, $p = 0.021$) and infectious symptoms ($B = 0.83$, $p = 0.013$) at $EBV_{baseline}$ (Table 3). At $EBV_{six\ months}$, the positive associations to post-exertional malaise remained ($B = 1.39$, $p = 0.001$), and a positive association to pain experience appeared ($B = 0.24$, $p = 0.003$),.

3.2. Functional abilities

In simple linear regression analyses, functional disability score and quality of life score were strongly associated with fatigue for $EBV_{baseline}$, $EBV_{six\ months}$ and HC (Table 2, eTable1). Physical activity (steps per day) had a highly significant negative association with fatigue at $EBV_{baseline}$, and a barely significant negative association at $EBV_{six\ months}$; among HC, there was no association between fatigue and physical activity.

In the final models for function, quality of life remained negatively and independently associated with fatigue for $EBV_{baseline}$ ($B = -0.14$, $p < 0.001$), $EBV_{six\ months}$ ($B = -0.19$, $p < 0.001$), and HC($B = -0.23$, $p < 0.001$), (Table 3). At $EBV_{baseline}$, functional disability also contributed positively and independently to the fatigue variance ($B = 0.09$, $p = 0.023$), whereas physical activity was not associated with fatigue in any of the three final models.

3.3. Other variables

In simple linear regression analyses, variables addressing negative life events and negative emotions (anxious mood, depressed mood and worrying) were positively associated with fatigue for $EBV_{baseline}$, $EBV_{six\ months}$ and HC) Emotional awareness and illness perception were negatively associated with fatigue (Table 2, eTable1). Also, for $EBV_{baseline}$, $EBV_{six\ months}$ and HC, fatigue showed a trend towards a positively association with female sex, lymphocyte count, subgroups of T cells counts, and insulin like growth factor. At $EBV_{baseline}$ and $EBV_{six\ months}$, the personality trait of perfectionism and total IgM were significantly positively associated with fatigue, whereas markers of EBV replication were not. Some associations were only seen at $EBV_{six\ months}$, such as the positive associations between fatigue and C-reactive protein (CRP), plasma norepinephrine and false word recognition, and the negative associations between fatigue and blood B cell fraction and HF-RRI-response to controlled breathing (a marker of parasympathetic nervous activity).

In the final models, fatigue at $EBV_{baseline}$ was characterized by independent positive associations with negative emotions (anxiety and depression, $B = 0.28$, $p = 0.012$), negative life events ($B = 0.08$, $p = 0.047$) and digit span backward (a cognitive test of working memory, $B = 0.32$, $p = 0.034$), and independent negative associations with serum creatinine kinase ($B = -0.01$, $p = 0.006$) and body mass

Table 2
Simple linear regression with current total score on Chalder Fatigue Questionnaire as dependent variable.

	EBV patients, baseline (n = 200)			EBV patients, 2.visit (n = 195)			Healthy controls (n = 70)		
		CFQ total score as dependent			CFQ total score as dependent			CFQ total score as dependent	
	Variable caracteristics	Linear regression coefficient B (CI)	p-value	Variable caracteristics	Linear regression coefficient B (CI)	p-value	Variable caracteristics	Linear regression coefficient B (CI)	p-value
Background									
Sex - no. males (%)	71 (35.5%)	1.26 (−0.14 to 2.66)	0.078	71 (35.5%)	2.1 (0.6 to 3.6)	0.006	26 (37.1%)	1.60 (−0.29 to 3.50)	0.095
Age, years - mean (SD)	16.9 (1.6)	0.24 (−0.18 to 0.67)	0.264	17.4 (1.6)	0.0 (−0.4 to 0.5)	0.917	17.0 (1.8)	0.55 (0.05 to 1.06)	0.033
BMI, kg/m^2 - mean (SD)	21.3 (2.6)	−0.26 (−0.52 to −0.00)	0.049	22.1 (2.6)	−0.0 (−0.3 to 0.3)	0.913	21.5 (3.1)	0.07 (−0.24 to 0.38)	0.646
Days since the onset of symptoms, self reported - mean (SD)	30.2 (6.6)	−0.05 (−0.16 to 0.05)	0.315		−0.0 (−0.2 to 0.1)	0.441			
Ethnicity - no. (%)			0.199			0.830			0.702
Scandinavian	184 (92.5%)	reference		184 (92.5%)[a]	reference		60 (85.7%)	reference	
Half Scandinavian	10 (5%)	0.43 (−2.59 to 3.44)	0.777	10 (5%)[a]	−0.5 (−3.7 to 2.8)		4 (5.7%)	−1.68 (−5.66 to 2.30)	0.402
Not Scandinavian	5 (2.5%)	−3.77 (−7.97 to 0.43)	0.078	5 (2.5%)[a]	1.2 (−3.3 to 5.8)		6 (8.6%)	−0.13 (−3.72 to 3.46)	0.943
Lives with… - no. (%)			0.574			0.241			0.656
…both parents	142 (71.4%)	reference		142 (71.4%)[a]	reference		43 (61.4%)	reference	
…divorced parents, alternating	17 (8.5%)	1.56 (−0.84 to 3.96)	0.202	17 (8.5%)[a]	0.4 (−2.2 to 3.0)		6 (8.6%)	−1.21 (−4.86 to 2.44)	0.510
…one parent	35 17.5%)	0.31 (−1.50 to 2.13)	0.736	35 17.5%)[a]	1.2 (−0.7 to 3.2)		19 (27.1%)	1.00 (−1.18 to 3.18)	0.361
…alone	3 (1.5%)	1.13 (−4.31 to 6.57)	0.683	3 (1.5%)[a]	5.9 (0.1 to 11.7)		0 (0%)	n.a.	n.a.
…other	2 (1%)	3.80 (−2.85 to 10.44)	0.261	2 (1%)[a]	2.2 (−4.9 to 9.4)		2 (2.9%)	0.89 (−4.69 to 6.47)	0.751
Parents' highest education - no. (%)			0.507			0.160			0.414
Primary school	1 (0.5%)	reference		1 (0.5%)[a]	reference		2 (2.9%)	reference	
Secondary school	47 (23.9%)	3.32 (−6.05 to 12.69)	0.486	47 (23.9%)[a]	1.3 (−8.8 to 11.4)		10 (14.5%)	−3.00 (−8.95 to 2.95)	0.318
Lower university	97 (49.2%)	3.91 (−5.41 to 13.23)	0.409	97 (49.2%)[a]	1.8 (−8.3 to 11.8)		36 (52.2%)	−1.96 (−7.55 to 3.64)	0.488
Higher university	52 (26.4%)	2.81 (−6.55 to 12.18)	0.554	52 (26.4%)[a]	−0.2 (−10.3 to 9.9)		21 (30.4%)	−0.75 (−6.45 to 4.95)	0.793
Siblings - no. (%)			0.198			0.517			0.642
0	29 (14.5%)	reference		29 (14.5%)[a]	reference		10 (14.3%)	reference	
1	109 (54.5%)	0.96 (−0.99 to 2.90)	0.335	109 (54.5%)[a]	0.8 (−1.4 to 2.9)		30 (42.9%)	1.76 (−1.16 to 4.69)	0.233
2	47 (47%)	0.50 (−1.72 to 2.72)	0.658	47 (47%)[a]	1.6 (−0.8 to 4.1)		25 (35.7%)	1.74 (−1.29 to 4.77)	0.218
≥3	15 (7.5%)	−1.82 (−4.83 to 1.19)	0.234	15 (7.5%)[a]	−0.1 (−3.3 to 3.1)		5 (7.1%)	0.96 (−3.32 to 5.23)	0.068
Usage of alcoholic beverages - no. (%)		1.07 (−0.33 to 2.48)	0.133		0.6 (−0.9 to 2.2)	0.442		2.20 (0.32 to 4.09)	0.023
Never	69 (36.7%)			69 (36.7%)[a]			24 (36.4%)		
Occationally	119 (63.3%)			119 (63.3%)[a]			42 (63.6%)		
Usage of tobacco products - no. (%)		1.04 (−0.33 to 2.41)	0.135		1.1 (−0.4 to 2.6)	0.164		1.32 (−0.59 to 3.22)	0.172
Never	107 (56.9%)			107 (56.9%)[a]			41 (61.2%)		
Occationally	81 (43.1%)			81 (43.1%)[a]			26 (38.8%)		
Usage of narcotics/illegal drugs - no. (%)		0.17 (−2.14 to 2.48)	0.887		0.1 (−2.4 to 2.6)	0.925		5.17 (1.85 to 8.50)	0.003
Never	170 (90.4%)			170 (90.4%)[a]			61 (92.4%)		
Occationally	18 (9.6%)			18 (9.6%)[a]			5 (7.6%)		
Personality/life events									
Child and Adolescent Perfectionism Scale (CAPS), total score - mean (SD)	36.6 (10.2)	0.09 (0.02 to 0.15)	0.009	36.6 (10.2)[a]	0.11 (0.04 to 0.18)	0.003	36.6 (9.1)	0.06 (−0.04 to 0.17)	0.223
CAPS Subscore: Self oriented striving - mean (SD)	10. 3 (2.8)	0.18 (−0.06 to 0.42)	0.143	10. 3 (2.8)[a]	0.09 (−0.18 to 0.35)	0.530	10.8 (2.8)	0.00 (−0.34 to 0.34)	0.999
CAPS Subscore: Self oriented critical - mean (SD)	9.73 (3.65)	0.20 (0.02 to 0.39)	0.033	9.73 (3.65)[a]	0.27 (0.07 to 0.47)	0.008	10.2 (3.8)	0.11 (−0.14 to 0.36)	0.378

(*continued on next page*)

Table 2 (*continued*)

	EBV patients, baseline (n = 200)			EBV patients, 2.visit (n = 195)			Healthy controls (n = 70)		
		CFQ total score as dependent			CFQ total score as dependent			CFQ total score as dependent	
	Variable caracteristics	Linear regression coefficient B (CI)	p-value	Variable caracteristics	Linear regression coefficient B (CI)	p-value	Variable caracteristics	Linear regression coefficient B (CI)	p-value
CAPS Subscore: Socially perscribed - mean (SD)	16.6 (6.0)	0.14 (0.02 to 0.25)	0.015	16.6 (6.0)[a]	0.20 (0.08 to 0.32)	0.001	15.7 (5.4)	0.13 (−0.05 to 0.30)	0.150
Life Event Checklist (LEC), total score of positive events last year - median (IQR)[b]	7.0 (7.0)	0.05 (−0.06 to 0.17)	0.367	5.5 (5.0)	0.1 (−0.1 to 0.3)	0.462	6.0 (7.0)	−0.04 (−0.22 to 0.14)	0.669
LEC, total score of all positive events - median (IQR)	7.0 (8.0)	0.01 (−0.09 to 0.12)	0.790	13.0 (11.0)	0.06 (−0.04 to 0.16)	0.218	7.0 (6.0)	−0.07 (−0.24 to 0.10)	0.391
LEC, total score of negative events last year- median (IQR)[b]	5.0 (8.0)	0.19 (0.12 to 0.27)	< 0.001	4.0 (10.0)	0.3 (0.2 to 0.4)	< 0.001	4 (7.3)	0.24 (0.09 to 0.38)	0.002
LEC, total of all negative events - median (IQR)	7.0 (10.0)	0.17 (0.10 to 0.24)	< 0.001	14.0 (15.0)	0.17 (0.11 to 0.22)	< 0.001	6.0 (9.0)	0.16 (0.02 to 0.29)	0.023
Clinical symptoms									
Post-exertional Malaise, single item - mean (SD)	2.7 (1.3)	1.95 (1.53 to 2.38)	< 0.001	2.0 (1.2)	2.94 (2.43 to 3.45)	< 0.001	1.5 (0.6)	2.18 (0.73 to 3.37)	0.004
Infectious Symptoms, mean score - mean (SD)	2.7 (0.9)	2.24 (1.55 to 2.93)	< 0.001	1.8 (0.7)	4.3 (3.3 to 5.3)	< 0.001	1.4 (0.4)	3.39 (1.16 to 5.61)	0.003
Hypersensitivity Symptoms, mean score - mean (SD)	1.7 (1.0)	2.36 (1.75 to 2.96)	< 0.001	1.6 (0.9)	3.1 (2.3 to 3.9)	< 0.001	1.3 (0.6)	2.35 (0.80 to 3.89)	0.003
Brief Pain Inventory (BPI), total pain severity score - mean (SD)	10.9 (4.9)	0.41 (0.29 to 0.54)	< 0.001	9.5 (4.8)	0.5 (0.4 to 0.7)	< 0.001	8.1 (3.2)	0.47 (0.19 to 0.75)	0.001
BPI, average pain single item score - mean (SD)	3.2 (1.7)	0.78 (0.41 to 1.16)	< 0.001	2.4 (1.2)	2.1 (1.5 to 2.7)	< 0.001	2.0 (0.8)	1.17 (0.45 to 1.88)	0.002
Karolinska Sleep Questionnaire (KSQ), total score - mean (SD)	52.5 (12.4)	−0.24 (−0.28 to −0.20)	< 0.001	58.4 (13.9)	−0.2 (−0.3 to −0.2)	< 0.001	62.2 (10.6)	−0.20 (−0.27 to −0.12)	< 0.001
KSQ Subscore: Insomnia - mean (SD)	15.6 (4.8)	−0.50 (−0.62 to −0.38)	< 0.001	17.8 (4.8)	−0.5 (−0.7 to −0.3)	< 0.001	18.9 (3.2)	−0.49 (−0.76 to −0.22)	0.001
KSQ Subscore: Awakenings - mean (SD)	10.2 (3.6)	−0.74 (−0.90 to −0.58)	< 0.001	11.4 (3.8)	−0.8 (−1.0 to −0.6)	< 0.001	12.5 (3.4)	−0.56 (−0.80 to −0.32)	< 0.001
KSQ Subscore: Sleepy at daytime - mean (SD)	13.9 (4.2)	−0.64 (−0.78 to −0.51)	< 0.001	15.8 (4.6)	−0.7 (−0.8 to −0.5)	< 0.001	16.7 (4.2)	−0.40 (−0.60 to −0.20)	< 0.001
Clinical findings									
Ear temperatur, °C - mean (SD)	36.3 (0.5)	0.81 (−0.52 to 2.14)	0.232	36.2 (0.5)	0.1 (−1.2 to 1.5)	0.860	36.3 (0.4)	0.27 (−2.04 to 2.58)	0.817
Maximum spleen length, cm - mean (SD)	12.6 (1.7)	0.24 (−0.17 to 0.64)	0.245	11.7 (1.3)	0.0 (−0.5 to 0.6)	0.897	11.2 (1.8)	−0.32 (−0.83 to 0.20)	0.221
Pain Pressure Threshold finger nail, N/cm^2 - mean (SD)	10.5 (4.5)	−0.05 (−0.20 to 0.10)	0.511	11.0 (4.3)	−0.0 (−0.2 to 0.1)	0.599	11.6 (3.5)	−0.08 (−0.36 to 0.20)	0.579
Pain Pressure Threshold trapezius muscle, N/cm^2 - mean (SD)	5.4 (3.1)	−0.21 (−0.44 to 0.02)	0.078	5.5 (3.3)	−0.2 (−0.4 to 0.0)	0.123	4.8 (2.1)	−0.11 (−0.55 to 0.32)	0.604
Blood Haemoglobin concentration, g/dL - mean (SD)	12.7 (1.2)	−0.64 (−1.22 to −0.07)	0.029	12.2 (1.2)	−0.6 (−1.2 to 0.0)	0.070	13.3 (1.1)	−1.06 (−1.88 to −0.24)	0.013
Blood Platelet count, 10^9 cells/L - mean (SD)	237.5 (58.3)	0.01 (−0.01 to 0.02)	0.247	241.1 (52.0)	0.0 (−0.0 to 0.0)	0.351	238.5 (51.4)	0.01 (−0.01 to 0.03)	0.219
Serum Alanine Transaminase (ALT), IU/L - median (IQR)	33.0 (23.0)	0.01 (−0.01 to 0.03)	0.188	24.0 (9.0)	−0.0 (−0.1 to 0.1)	0.472	24.0 (7.0)	−0.12 (−0.27 to 0.04)	0.138
Serum Gamma-Glutamyl Transpeptidase (GGT), IU/L- median (IQR)	27.0 (26.0)	0.03 (0.01 to 0.05)	0.017	15.0 (6.0)	−0.0 (−0.1 to 0.1)	0.384	14.0 (4.0)	0.15 (−0.10 to 0.40)	0.232
Serum Total Bilirubin, µmol/L- median (IQR)	10.0 (6.0)	−0.11 (−0.24 to 0.03)	0.134	10.0 (5.0)	−0.1 (−0.2 to 0.0)	0.144	10.0 (8.0)	−0.06 (−0.23 to 0.11)	0.489
Plasma International Normalized Ratio (INR) - median (IQR)	1.1 (0.6)	−2.37 (−9.93 to 5.19)	0.538	1.1 (0.1)	0.5 (−7.8 to 8.8)	0.903	1.1 (0.1)	−7.20 (−17.8 to 3.40)	0.180
Serum Creatinine, µmol/L - mean (SD)	63.5 (10.0)	−0.02 (−0.09 to 0.05	0.583	66.2 (10.9)	−0.1 -0.2 to −0.0)	0.006	65.8 (10.8)	0.07 (−0.01 to 0.16)	0.096
Serum Creatinine Kinase (CK), IU/L - median (IQR)	53.0 (36)	−0.01 (−0.02 to −0.00)	0.004	86.5 (84.0)	−0.0 (−0.0 to 0.0)	0.461	91.0 (72.0)	0.00 (−0.01 to 0.01)	0.979
Serum 25-OH-Vitamin D, nmol/L - mean (SD)	57.46 (20.87)	0.01 (−0.03 to 0.04)	0.770	61.8 (24.6)	−0.0 (−0.0 to 0.0)	0.333	58.9 (21.8)	0.04 (−0.01 to 0.08)	0.118
Serum Vitamin B_{12}, pmol/L - median (IQR)	320.0 (170)	−0.00 (−0.01 to 0.00)	0.620	220 (133)	−0.0 (−0.0 to 0.0)	0.450	280.0 (140.0)	−0.00 (−0.01 to 0.01)	0.828
Emotions									

(*continued on next page*)

Table 2 (*continued*)

	EBV patients, baseline (n = 200)			EBV patients, 2.visit (n = 195)			Healthy controls (n = 70)		
		CFQ total score as dependent			CFQ total score as dependent			CFQ total score as dependent	
	Variable caracteristics	Linear regression coefficient B (CI)	p-value	Variable caracteristics	Linear regression coefficient B (CI)	p-value	Variable caracteristics	Linear regression coefficient B (CI)	p-value
Hospital Anxiety and Depression Scale (HADS), total score - mean (SD)	11.2 (5.8)	0.38 (0.27 to 0.48)	< 0.001	10.5 (6.4)	0.5 (0.4 to 0.6)	< 0.001	10.6 (4.6)	0.27 (0.07 to 0.46)	0.009
HADS Subscore: Anxiety - mean (SD)	6.4 (3.2)	0.55 (0.35 to 0.75)	< 0.001	6.1 (3.6)	0.7 (0.5 to 0.9)	< 0.001	6.8 (3.0)	0.40 (0.10 to 0.70)	0.009
HADS Subscore: Depression - mean (SD)	4.8 (3.6)	0.57 (0.39 to 0.74)	< 0.001	4.4 (3.7)	0.8 (0.6 to 0.9)	< 0.001	3.8 (2.3)	0.37 (−0.03 to 0.78)	0.070
Toronto Alexithymia Scale-20 (TAS-20), total score - mean (SD)	51.9 (10.8)	0.11 (0.05 to 0.18)	< 0.001	49.4 (12.5)	0.2 (0.1 to 0.2)	< 0.001	53.6 (8.9)	0.15 (0.05 to 0.25)	0.005
TAS-20 Subscore: Difficulty identifying feelings - mean (SD)	19.5 (6.9)	0.22 (0.12 to 0.31)	< 0.001	17.6 (8.0)	0.3 (0.3 to 0.4)	< 0.001	20.2 (6.3)	0.21 (0.07 to 0.35)	0.005
TAS-20 Subscore: Difficulty describing feelings - mean (SD)	21.4 (6.9)	0.10 (−0.04 to 0.25)	0.164	20.7 (5.2)	0.1 (−0.0 to 0.3)	0.140	22.3 (4.1)	0.20 (−0.02 to 0.42)	0.080
TAS-20 Subscore: Externally oriented thinking - mean (SD)	11.0 (2.7)	0.10 (−0.15 to 0.35)	0.441	11.1 (2.9)	0.2 (−0.1 to 0.5)	0.126	11.2 (2.2)	0.01 (−0.43 to 0.45)	0.965
Penn State Worry Questionnaire, total score - mean (SD)	43.1 (12.5)	0.11 (0.05 to 0.16)	< 0.001	43.1 (12.5)[a]	0.14 (0.08 to 0.20)	< 0.001	45.7 (12.0)	0.10 (0.02 to 0.18)	0.010
Brief Illness Perception Questionnaire, total score - mean (SD)	40.9 (10.8)	0.17 (0.11 to 0.23)	< 0.001	40.9 (10.8)[a]	0.17 (0.11 to 0.24)	< 0.001	26.4 (11.1)	0.13 (0.05 to 0.21)	0.002
Infection									
Epstein-Barr Virus (EBV) load, copies in blood - no. (%)			0.798			0.297			0.239
Negative (< 160)	49 (24.9%)	reference		82 (43.6)	referance		60 (85.7%)	reference	
Low (1600 to 2000)	115 (58.4%)	0.56 (−1.10 to 2.22)	0.506	61 (32.4%)	−1.3 (−3.0 to 0.4)	0.138	8 (11.4%)	2.14 (−0.72 to 5.00)	0.139
Moderate/high (> 2000)	33 (16.8%)	0.32 (−1.87 to 2.50)	0.774	45 (23.9%)	−1.0 (−2.8 to 0.9)	0.312	2 (2.9%)	2.52 (−2.92 to 7.96)	0.359
EBV virus load, copies in throat - no. (%)			0.125			0.269			0.260
Negative	10 (5.2%)	reference		28 (15.1%)	referance		50 (74.6%)	reference	
Low (threshold cycle in PCR (CT), values > 32)	26 (13.5%)	−1.00 (−4.46 to 2.46)	0.569	35 (18.9%)	1.2 (−1.4 to 3.7)	0.377	5 (7.5%)	−0.94 (−4.52 to 2.63)	0.599
Moderate (CT values 28 to 32)	128 (66.7%)	1.23 (−1.81 to 4.28)	0.425	120 (64.9%)	0.6 (−1.5 to 2.7)	0.573	11 (16.4%)	2.28 (−0.27 to 4.82)	0.079
High (CT values < 28)	28 (14.6%)	−0.12 (−3.58 to 3.34)	0.946	2 (1.1%)	7.1 (−0.3 to 14.5)	0.060	1 (1.5%)	2.46 (−5.21 to 10.1)	0.524
EBV Viral Capsid Antigen (VCA) IgM, titer - median (IQR)	160 (73)	0.01 (−0.00 to 0.02)	0.176	20 (45)	0.0 (−0.0 to 0.0)	0.247	0 (0)	0.04 (−0.06 to 0.14)	0.378
EBV-VCA-IgG, titer - median (IQR)	69 (67)	−0.00 (−0.01 to 0.00)	0.285	169 (162)	0.0 (−0.0 to 0.0)	0.440	51 (195)	0.00 (−0.00 to 0.01)	0.399
EBV Nuclear Antigen (EBNA) IgG, titer - median (IQR)	0 (0)	0.08 (−0.11 to 0.27)	0.392	98 (205)	0.0 (0.0 to 0.0)	0.033	57 (349)	0.01 (0.00 to 0.01)	0.010
Cytomegalovirus (CMV) IgM, titer - median (IQR)	0 (0)	−0.16 (−0.94 to 0.61)	0.675	0 (0)	0.2 (−0.5 to 0.8)	0.613	0 (0)	1.75 (−1.55 to 5.04)	0.294
CMV IgG, titer - median (IQR)	0 (323)	0.00 (0.00 to 0.01)	0.028	0 (293)	−0.0 (−0.0 to 0.0)	0.635	37 (267)	0.00 (−0.00 to 0.01)	0.219
Borrelia burgdorferi IgM, titer - no. (%)			0.134			0.322			0.946
Negative	99 (50%)	reference		150 (78.9%)	referance		61 (87.1%)	reference	
Reactive	61 (30.8%)	1.63 (0.073 to 3.18)	0.040	22 (11.6%)	0.3 (−2.0 to 2.6)	0.788	3 (4.3%)	−0.46 (−5.06 to 4.14)	0.842
Greyzone	17 (8.6%)	1.49 (−0.96 to 3.94)	0.233	7 (3.7%)	−0.6 (−4.5 to 3.3)	0.751	1 (1.4%)	2.21 (−5.64 to 10.1)	0.576
Positive	21 (10.6%)	−0.34 (−2.68 to 2.00)	0.774	11 (5.8%)	2.9 (−0.2 to 6.0)	0.070	5 (7.1%)	0.21 (−3.81 to 4.23)	0.918
B. burgdorferi IgG, titer - no. (%)			0.793			0.160		n.a.	
Negative	191 (96.5%)	reference		184 (96.8%)	referance		70 (100%)		
Greyzone	5 (2.5%)	−0.68 (−4.93 to 3.57)	0.751	4 (2.1%)	3.7 (−1.4 to 8.8)	0.153			
Positive	2 (1%)	2.02 (−4.65 to 8.68)	0.551	2 (1.1%)	−4.6 (−11.7 to 2.6)	0.208			
Immunity									
Serum high sensitive CRP, mg/L - median (IQR)	0.40 (0.86)	0.07 (−0.18 to 0.33)	0.559	0.44 (0.73)	0.18 (0.02 to 0.35)	0.030	0.56 (0.41)	0.27 (−0.67 to 1.21)	0.570
Serum total IgG, g/L - mean (SD)	12.0 (2.7)	0.05 (−0.21 to 0.30)	0.728	9.9 (1.8)	−0.1 (−0.5 to 0.3)	0.655	9.4 (1.7)	−0.08 (−0.67 to 0.50)	0.774
Serum total IgM, g/L - mean (SD)	1.5 (0.7)	1.49 (0.58 to 2.40)	0.001	1.0 (0.5)	1.8 (0.2 to 3.3)	0.027	1.0 (0.5)	0.75 (−1.37 to 2.87)	0.484
Serum total IgA, g/L - mean (SD)	2.2 (0.9)	−0.04 (−0.78 to 0.69)	0.906	1.4 (0.6)	−0.1 (−1.3 to 1.2)	0.929	1.4 (0.6)	0.71 (−0.84 to 2.26)	0.364
Blood Leukocyte total count, 10^9 cells/L - median (IQR)	5.2 (1.7)	0.11 (−0.40 to 0.62)	0.671	5.5 (2.1)	0.4 (0.0 to 0.9)	0.037	5.4 (2.4)	0.13 (−0.38 to 0.64)	0.615

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Table 2 (*continued*)

	EBV patients, baseline (n = 200)			EBV patients, 2.visit (n = 195)			Healthy controls (n = 70)		
		CFQ total score as dependent			CFQ total score as dependent			CFQ total score as dependent	
	Variable caracteristics	Linear regression coefficient B (CI)	p-value	Variable caracteristics	Linear regression coefficient B (CI)	p-value	Variable caracteristics	Linear regression coefficient B (CI)	p-value
Blood Lymphocyte count, 10^9 cells/L - median (IQR)	2.3 (0.8)	1.15 (0.25 to 2.04)	0.012	1.9 (0.7)	1.2 (−0.2 to 2.5)	0.092	1.9 (0.6)	2.50 (0.56 to 4.44)	0.012
Blood Monocyte count, 10^9 cells/L - median (IQR)	0.5 (0.3)	1.05 (−2.48 to 4.58)	0.558	0.5 (0.2)	−0.9 (−5.0 to 3.1)	0.658	0.5 (0.2)	1.79 (−4.54 to 8.11)	0.574
Blood Neutrophil count, 10^9 cells/L - median (IQR)	2.2 (1.2)	−0.42 (−1.10 to 0.25)	0.216	2.8 (1.3)	0.4 (−0.0 to 0.9)	0.078	2.9 (1.9)	−0.07 (−0.67 to 0.52)	0.810
Blood Eosinophil count, 10^9 cells/L - median (IQR)	0.1 (0.1)	−3.53 (−8.29 to 1.23)	0.145	0.1 (0.1)	2.7 (−4.2 to 9.6)	0.441	0.1 (0.1)	4.20 (−4.83 to 13.2)	0.356
Blood Basophil count, 10^9 cells/L - median (IQR)	0.0 (0.1)	8.54 (−5.26 to 22.3)	0.224	0.0 (0.0)	2.8 (−18.4 to 24.0)	0.793	0.0 (0.1)	16.4 (−12.1 to 44.9)	0.253
Blood T cell ($CD3^+$) total count, 10^6 cells/L - median (IQR)	1792.5 (710)	0.00 (0.00 to 0.00)	0.008	1495 (572)	0.0 (0.0 to 0.0)	0.013	1333 (424)	0.00 (0.00 to 0.01)	0.005
Blood T cell ($CD3^+$) fraction (of lymfocyte count), % - mean (SD)	81.3 (8.3)	0.07 (−0.01 to 0.15)	0.088	78.2 (5.5)	0.2 (0.1 to 0.3)	0.006	74.7 (6.3)	0.13 (−0.03 to 0.28)	0.100
Blood double negative T cell ($CD4^-CD8^-$) subset (of $CD3^+$ count), % - median (IQR)	0.7 (0.5)	−0.69 (−2.65 to 1.28)	0.490	0.8 (0.5)	−0.6 (−2.2 to 1.1)	0.491	0.9 (0.5)	−0.46 (−2.46 to 1.55)	0.649
Blood cytotoxic T cell ($CD8^+$) count, 10^6 cells/L- median (IQR)	856 (514)	0.00 (0.00 to 0.00)	0.015	584 (282)	0.0 (−0.0 to 0.0)	0.066	506 (783)	0.01 (0.00 to 0.01)	0.005
Blood cytotoxic T cell ($CD8^+$) fraction (of lymfocyte count), % - mean (SD)	40.5 (9.7)	0.04 (−0.03 to 0.11)	0.270	31.0 (6.2)	0.0 (−0.1 to 0.1)	0.953	27.0 (4.8)	0.19 (−0.01 to 0.38)	0.065
Blood early effector memory T cell subset (of $CD8^+$ count), % - mean (SD)	28.5 (10.5)	0.02 (−0.05 to 0.08)	0.590	20.0 (7.7)	0.0 (−0.1 to 0.1)	0.349	10.6 (3.9)	0.01 (−0.24 to 0.25)	0.943
Blood late effector memory T cell subset (of $CD8^+$ count), % - median (IQR)	5.2 (5.8)	−0.03 (−0.16 to 0.11)	0.703	4.6 (11.1)	0.0 (−0.1 to 0.1)	0.667	7.0 (18.1)	0.02 (−0.05 to 0.10)	0.561
Blood helper T cell ($CD4^+$) count, 10^6 cells/L - median (IQR)	750 (334)	0.00 (0.00 to 0.01)	0.008	768 (321)	0.0 (0.0 to 0.0)	0.012	781 (332)	0.01 (0.00 to 0.01)	0.004
Blood helper T cell ($CD4^+$) fraction (of lymfocyte count), % - mean (SD)	34.3 (7.9)	0.02 (−0.07 to 0.10)	0.731	41.3 (6.7)	0.1 (−0.0 to 0.2)	0.118	43.7 (6.0)	0.08 (−0.08 to 0.24)	0.327
Blood recent thymic emigrant T cell subset (of $CD4^+$ CD45RA+ T cell count), % - mean (SD)	69.0 (10.3)	−0.05 (−0.12 to 0.02)	0.130	68.2 (9.9)	−0.0 (−0.1 to 0.1)	0.995	69.5 (9.2)	0.04 (−0.07 to 0.14)	0.489
Blood naïve T cell subset (of $CD4^+$ count), % - mean (SD)	61.4 (12.2)	0.00 (−0.05 to 0.06)	0.875	62.5 (9.7)	−0.0 (−0.1 to 0.1)	0.756	64.9 (10.3)	−0.04 (−0.14 to 0.05)	0.344
Blood follicular T cell subset (of $CD4^+$ count), % - median (IQR)	6.4 (2.9)	−0.04 (−0.23 to 0.15)	0.691	7.8 (3.8)	0.0 (−0.2 to 0.3)	0.753	7.8 (3.6)	0.02 (−0.37 to 0.42)	0.910
Blood regulatory T cell subset (of $CD4^+$ count), %- median (IQR)	5.3 (2.0)	0.03 (−0.42 to 0.46)	0.912	6.0 (1.8)	−0.5 (−1.0 to 0.0)	0.057	6.6 (1.9)	−0.19 (−0.81 to 0.44)	0.554
Blood memory T cell subset (of $CD4^+$ count), % - mean (SD)	51.0 (11.4)	0.04 (−0.02 to 0.10)	0.191	50.0 (10.2)	0.0 (−0.1 to 0.1)	0.747	49.2 (9.8)	0.06 (−0.04 to 0.16)	0.223
Blood B cell ($CD19^+$) total count, 10^6 cells/L - median (IQR)	159 (107)	−0.01 (−0.01 to 0.00)	0.135	237 (149)	−0.0 (−0.0 to 0.0)	0.445	251 (114)	0.01 (−0.00 to 0.02)	0.224
Blood B cell ($CD19^+$) fraction (of lymfocyte count), % - median (IQR)	7.4 (4.9)	−0.09 (−0.18 to 0.01)	0.086	12.8 (5.9)	−0.2 (−0.4 to −0.0)	0.017	14.3 (3.9)	−0.12 (−0.36 to 0.13)	0.342
Blood naïve B cell subset (of $CD19^+$ count), % - mean (SD)	81.4 (8.1)	−0.03 (−0.11 to 0.06)	0.504	69.5 (12.1)	−0.1 (−0.2 to 0.0)	0.176	77.7 (10.0)	−0.04 (−0.14 to 0.05)	0.351
Blood transitoric B cell subset (of $CD19^+$ count), % - median (IQR)	4.3 (4.7)	−0.16 (−0.36 to 0.04)	0.105	2.5 (1.8)	0.0 (−0.4 to 0.4)	0.875	2.6 (1.7)	−0.14 (−0.62 to 0.35)	0.573
Blood class switch B cell subset (of $CD19^+$ count), % - median (IQR)	4.1 (4.0)	0.08 (−0.11 to 0.27)	0.388	5.5 (3.8)	0.1 (−0.1 to 0.4)	0.258	6.3 (4.8)	0.05 (−0.16 to 0.26)	0.651
Blood IgM memory B cell subset (of $CD19^+$ count), % - median (IQR)	8.1 (5.4)	0.16 (−0.00 to 0.33)	0.055	8.8 (5.6)	0.1 (−0.0 to 0.3)	0.110	9.6 (6.5)	0.14 (−0.06 to 0.33)	0.163
Blood plasmablast subset (of $CD19^+$ count), % - median (IQR)	0.3 (0.5)	0.12 (−0.46 to 0.70)	0.681	0.3 (0.7)	0.4 (−0.2 to 1.0)	0.156	0.4 (0.8)	−0.05 (−0.80 to 0.71)	0.904
Blood $CD21^{low}$ B cell subset (of CD19+ count), % - median (IQR)	1.8 (1.8)	0.22 (−0.20 to 0.64)	0.309	1.8 (1.5)	0.3 (−0.2 to 0.7)	0.239	1.6 (1.3)	0.49 (−0.29 to 1.26)	0.213
Blood NK cells ($CD16^+CD56^+CD3^-$) count, 10^6 cells/L - median (IQR)	199 (166)	0.00 (−0.00 to 0.01)	0.782	132.5 (107.0)	−0.0 (−0.0 to 0.0)	0.791	159.5 (96.0)	0.00 (−0.01 to 0.01)	0.895
NK cell function fraction (of NK cell count), % - mean (SD)	26.6 (7.3)	0.01 (−0.09 to 0.10)	0.907	27.1 (7.8)	−0.1 (−0.2 to 0.0)	0.167	21.9 (7.0)	−0.07 (−0.21 to 0.07)	0.299

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Table 2 (*continued*)

	EBV patients, baseline (n = 200)			EBV patients, 2.visit (n = 195)			Healthy controls (n = 70)		
		CFQ total score as dependent			CFQ total score as dependent			CFQ total score as dependent	
	Variable caracteristics	Linear regression coefficient B (CI)	p-value	Variable caracteristics	Linear regression coefficient B (CI)	p-value	Variable caracteristics	Linear regression coefficient B (CI)	p-value
Plasma Norepinephrine, pmol/L - mean (SD)	1564 (534)	−0.00 (−0.00 to 0.00)	0.151	1252(691)	0.0 (0.0 to 0.0)	0.004	1237 (575)	0.00 (−0.00 to 0.00)	0.747
Urine Norepinephrine:Creatinine ratio, nmol/mmol - median (IQR)	10.39 (5.99)	0.11 (−0.02 to 0.24)	0.095	0.0110 (0.00)	104.4 (−41.7 to 250.6)	0.160	0.0113 (0.01)	0.30 (−0.17 to 0.23)	0.763
Plasma Epinephrine, pmol/L - median (IQR)	360.0 (237.8)	−0.00 (−0.01 to 0.00)	0.109	286.0 (231.0)	0.0 (−0.0 to 0.0)	0.063	282.0 (153.5)	0.00 (−0.01 to 0.01)	0.916
Urine Epinephrine:Creatinine ratio, nmol/mmol - median (IQR)	1.69 (1.51)	0.17 (−0.29 to 0.63)	0.466	0.0015 (0.00)	185.7 (−260.2 to 631.7)	0.412	1.49 (1.05)	−0.18 (−0.85 to 0.49)	0.591
Plasma Adrenocorticotropic Hormone (ACTH), pmol/L - median (IQR)	4.2 (3.2)	0.01 (−0.22 to 0.25)	0.910	4.5 (3.1)	−0.2 (−0.5 to 0.1)	0.125	5.3 (3.6)	−0.25 (−0.61 to 0.10)	0.162
Serum Cortisol, nmol/L - median (IQR)	335 (190)	0.00 (−0.00 to 0.01)	0.109	360.0 (230.0)	0.0 (−0.0 to 0.0)	0.081	330 (160)	0.01 (−0.00 to 0.01)	0.163
Urine Cortisol:Creatinine ratio, nmol/mmol - median (IQR)	2.79 (2.86)	−0.01 (−0.15 to 0.13)	0.908	3.04 (3.02)	−0.01 (−0.10 to 0.09)	0.911	3.29 (4.31)	−0.10 (−0.25 to 0.05)	0.197
Serum Thyroid Stimulating Hormone (TSH), mIE/L - median (IQR)	2.1 (1.3)	0.31 (−0.35 to 0.96)	0.353	2.0 (1.3)	−0.2 (−0.9 to 0.6)	0.654	2.1 (1.0)	0.80 (−0.04 to 1.64)	0.063
Serum free Thyroxine, pmol/L - median (IQR)	11.0 (2)	0.09 (−0.30 to 0.49)	0.637	12.0 (2.0)	0.2 (−0.1 to 0.6)	0.179	13.8 (4.0)	0.18 (−0.19 to 0.55)	0.340
Serum Growth Hormone, µg/L - median (IQR)	1.0 (4.8)	−0.06 (−0.24 to 0.12)	0.512	0.7 (3.5)	0.0 (−0.2 to 0.2)	0.885	1.4 (5.1)	0.01 (−0.19 to 0.21)	0.924
Serum Insulin-like Growth Factor (IGF-1), nmol/L - median (IQR)	49.3 (21.8)	−0.05 (−0.09 to −0.01)	0.023	43.2 (24.1)	−0.0 (−0.1 to 0.0)	0.070	39.5 (20.5)	−0.05 (−0.11 to 0.01)	0.087
Serum Prolactine, mIU/L - median (IQR)	200 (100)	−0.01 (−0.01 to 0.00)	0.296	210.0 (100.0)	0.0 (−0.0 to 0.0)	0.775	230 (133)	0.00 (−0.01 to 0.01)	0.997
Cognition									
Digit Span Forward, total sum score - mean (SD)	9.0 (1.9)	−0.15 (−0.50 to 0.21)	0.415	9.4 (1.8)	−0.1 (−0.5 to 0.3)	0.657	9.2 (1.8)	0.06 (−0.45 to 0.57)	0.817
Digit Span Backward, total sum score - mean (SD)	6.2 (1.9)	0.29 (−0.06 to 0.64)	0.099	6.4 (2.0)	−0.3 (−0.7 to 0.0)	0.068	6.1 (2.0)	0.12 (−0.36 to 0.61)	0.610
Hopskin s Verbal Learning Test-Revised (HVLT-R) Learning/ Immediate Recall, total sum score - mean (SD)	28.1 (4.0)	0.09 (−0.09 to 0.26)	0.334	27.6 (4.0)	−0.2 (−0.3 to 0.0)	0.089	27.5 (3.8)	0.08 (−0.17 to 0.33)	0.517
HVLT-R Delayed Recall, total sum score - mean (SD)	9.9 (1.8)	0.21 (−0.18 to 0.60)	0.281	9.7 (1.8)	−0.2 (−0.6 to 0.2)	0.245	9.6 (2.0)	0.50 (0.03 to 0.97)	0.036
HVLT-R Correct Recognition		−0.49 (−2.23 to 1.26)	0.581		−1.4 (−3.4 to 0.4)	0.133		0.51 (−4.02 to 5.04)	0.824
All correct - no. (%)	162 (81%)			162 (83.1%)			67 (97.7%)		
Less than all correct - no. (%)	38 (19%)			33 (16.9%)			3 (4.3%)		
Word False Recognition		−1.07 (−2.74 to 0.60)	0.209		2.8 (0.2 to 5.4)	0.037		−1.86 (−4.35 to 0.64)	0.142
No false recognition - no. (%)	158 (79)			179 (91.8%)			58 (82.9%)		
One or more false recognition - no. (%)	42 (21)			16 (8.2%)			12 (17.1%)		
Color-Word Interference (CWI) condition 1, T-score - median (IQR)	8.0 (3.0)	−0.07 (−0.34 to 0.19)	0.589	10.0 (3.0)	−0.19 (−0.51 to 0.14)	0.261	9.0 (4.0)	−0.04 (−0.38 to 0.30)	0.798
CWI condition 2, T-score - median (IQR)	9.0 (4.0)	−0.25 (−0.51 to 0.02)	0.065	10.0 (3.0)	−0.11 (−0.41 to 0.20)	0.484	10.0 (2.3)	−0.18 (−0.57 to 0.20)	0.349
CWI condition 3, T-score - median (IQR)	10.0 (4.0)	−0.12 (−0.35 to 0.11)	0.303	11.0 (2.0)	−0.05 (−0.40 to 0.31)	0.789	10.5 (4.3)	−0.07 (−0.40 to 0.26)	0.670
CWI condition 3, no. of errors - median (IQR)	2 (2)	−0.01 (−0.34 to 0.33)	0.972	1.0 (2.0)	0.4 (−0.1 to 0.9)	0.146	2.0 (2.0)	−0.16 (−0.61 to 0.29)	0.478
CWI condition 4, T-score - median (IQR)	10.0 (3.0)	0.04 (−0.21 to 0.29)	0.738	12.0 (3.0)	0.09 (−0.25 to 0.42)	0.613	10.0 (3.0)	−0.06 (−0.41 to 0.28)	0.707
CWI condition 4, no. of errors - median (IQR)	2 (3)	−0.30 (−0.65 to 0.05)	0.092	1.0 (2.0)	0.0 (−0.4 to 0.4)	0.922	3.0 (3.0)	−0.13 (−0.56 to 0.29)	0.532
Wechsler Abbreviated Scale of Intelligence, 4th edition (WASI-IV) Matrix Reasoning, T-scores - mean (SD)	52.5 (8.4)	0.00 (−0.08 to 0.08)	0.974	52.5 (8.4)[a]	−0.03 (−0.12 to 0.06)	0.489	54.5 (5.8)	0.16 (−0.00 to 0.32)	0.050

(*continued on next page*)

Table 2 (*continued*)

	EBV patients, baseline (n = 200)			EBV patients, 2.visit (n = 195)			Healthy controls (n = 70)		
		CFQ total score as dependent			CFQ total score as dependent			CFQ total score as dependent	
	Variable caracteristics	Linear regression coefficient B (CI)	p-value	Variable caracteristics	Linear regression coefficient B (CI)	p-value	Variable caracteristics	Linear regression coefficient B (CI)	p-value
WASI-IV Vocabulary, T-scores - mean (SD)	59.7 (8.2)	0.06 (−0.02 to 0.15)	0.139	59.7 (8.2)[a]	−0.09 (−0.18 to 0.00)	0.052	60.8 (6.5)	0.06 (−0.09 to 0.20)	0.442
Estimated Full-Scale Intelligence Quotient (IQ) - mean (SD)	110.8 (11.9)	0.02 (−0.04 to 0.08)	0.443	110.8 (11.9)[a]	−0.05 (−0.11 to 0.01)	0.117	113.4 (8.8)	0.09 (−0.01 to 0.19)	0.084
Autonomic cardiovascular control									
Heart Rate (HR) supine, beats/min - mean (SD)	65.1 (9.3)	0.07 (−0.01 to 0.139)	0.071	61.4 (9.6)	0.1 (−0.0 to 0.1)	0.061	60.3 (8.4)	−0.04 (0.0.15 to 0.07)	0.440
Systolic Blood Pressure (SBP) supine, mmHg - mean (SD)	98.9 (8.6)	−0.03 (−0.11 to 0.05)	0.421	98.3 (7.8)	−0.1 (−0.2 to 0.0)	0.187	100.4 (8.1)	−0.06 (−0.18 to 0.06)	0.311
Diastolic Blood Pressure (DBP) supine, mmHg - mean (SD)	59.5 (6.7)	−0.05 (−0.15 to 0.05)	0.341	59.4 (6.3)	0.0 (−0.1 to 0.1)	0.762	59.5 (6.3)	−0.03 (−0.19 to 0.12)	0.655
Total Periferal Resistance Index (TPRI) supine, mmHg/L/min/m^2 - mean (SD)	12.7 (2.2)	−0.16 (−0.60 to 0.28)	0.473	7.6 (1.6)	−0.1 (−0.5 to 0.4)	0.715	7.8 (2.6)	0.08 (−0.30 to 0.45)	0.686
High Frequency Variability of the RR-interval (HF-RRI) supine, ms^2 - median (IQR)	1052 (1715)	0.00 (−0.00 to 0.00)	0.391	1338 (1854)	0.0 (−0.0 to 0.0)	0.209	1275 (1489)	−0.00 (−0.00 to 0.00)	0.509
Low Frequency Variability of the RR-interval (LF-RRI) supine, ms^2 - median (IQR)	660 (913)	0.00 (−0.00 to 0.00)	0.439	925 (1218)	0.0 (−0.0 to 0.0)	0.359	783 (874)	0.00 (−0.00 to 0.00)	0.586
LF-RRI:HF-RRI ratio supine - median (IQR)	0.63 (0.56)	−0.19 (−1.30 to 0.93)	0.739	0.7 (0.6)	−0.1 (−1.3 to 1.0)	0.811	0.54 (0.56)	−1.03 (−2.28 to 0.22)	0.104
Low Frequency Variability of Diastolic Blood Pressure (LF-DBP) supine, $mmHg^2$ - median (IQR)	3.0 (3.0)	−0.18 (−0.35 to 0.00)	0.055	7.3 (25.1)	0.0 (−0.0 to 0.0)	0.882	3.2 (6.0)	0.04 (−0.04 to 0.11)	0.333
HR response to Controlled Breathing (CB), beats/min - mean (SD)	0.96 (2.8)	−0.13 (−0.37 to 0.11)	0.295	0.3 (2.7)	−0.0 (−0.3 to 0.2)	0.836	1.7 (2.8)	0.03 (−0.31 to 0.38)	0.846
SBP response to CB, mmHg - mean (SD)	0.14 (5.6)	0.01 (−0.11 to 0.13)	0.860	0.7 (4.1)	0.0 (−0.1 to 0.2)	0.689	0.8 (5.6)	0.02 (−0.15 to 0.19)	0.784
DBP response to CB, mmHg - mean (SD)	−0.38 (4.74)	0.04 (−0.10 to 0.19)	0.539	−0.6 (3.8)	0.0 (−0.2 to 0.2)	0.980	0.24 (4.6)	0.04 (−0.17 to 0.25)	0.687
TPRI response to CB, mmHg/L/min/m^2 - mean (SD)	−0.18 (0.61)	0.45 (−0.67 to 1.57)	0.430	−0.1 (0.5)	0.6 (−0.8 to 1.9)	0.412	−0.2 (0.7)	−0.38 (−1.75 to 0.99)	0.580
HF-RRI response to CB, ms^2 - median (IQR)	20.5 (630.7)	0.00 (−0.00 to 0.00)	0.313	60.4 (754.4)	−0.0 (−0.0 to −0.0)	0.011	39.0 (847)	0.00 (−0.00 to 0.00)	0.191
LF-RRI response to CB, ms^2 - mean (SD)	−333 (842)	0.00 (−0.00 to 0.00)	0.707	−240 (824)	−0.0 (−0.0 to 0.0)	0.836	−473.8 (1162)	0.00 (−0.00 to 0.00)	0.998
LF-RRI:HF-RRI ratio response to CB - mean (SD)	−0.21 (0.56)	0.24 (0.95 to 1.43)	0.691	−0.23 (0.34)	−0.1 (−1.6 to 1.3)	0.850	−0.29 (0.55)	1.85 (0.10 to 3.60)	0.039
LF-DBP response to CB, $mmHg^2$ - mean (SD)	−0.75 (1.91)	0.17 (−0.20 to 0.54)	0.358	−1.8 (6.1)	−0.0 (−0.1 to 0.1)	0.988	−1.6 (4.2)	−0.09 (−0.31 to 0.14)	0.448
HR response to Orthostatic Challenge (OC), beats/min - mean (SD)	30.2 (11.5)	−0.01 (−0.07 to 0.05)	0.736	27.5 (9.5)	0.0 (−0.1 to 0.1)	0.605	26.9 (14.1)	−0.01 (−0.08 to 0.06)	0.811
SBP response to OC, mmHg - mean (SD)	−2.0 (12.6)	−0.01 (−0.06 to 0.05)	0.802	2.2 (9.3)	0.0 (−0.1 to 0.1)	0.491	3.2 (25.7)	−0.01 (−0.04 to 0.03)	0.769
DBP response to OC, mmHg - mean (SD)	7.1 (9.0)	−0.01 (−0.08 to 0.07)	0.856	10.0 (7.4)	0.1 (−0.0 to 0.2)	0.275	10.4 (15.9)	−0.01 (−0.07 to 0.05)	0.760
TPRI response to OC, mmHg/L/min/m^2 - mean (SD)	0.8 (1.6)	−0.24 (−0.68 to 0.19)	0.266	1.3 (1.4)	0.4 (−0.2 to 0.9)	0.210	1.5 (3.2)	−0.19 (−0.49 to 0.11)	0.220
HF-RRI response to OC, ms^2 - median (IQR)	−749 (1465)	−0.00 (−0.00 to 0.00)	0.424	−841 (1470)	0.0 (−0.0 to 0.0)	0.385	−1231 (1514)	0.00 (−0.00 to 0.00)	0.448
LF-RRI response to OC, ms^2 - mean (SD)	−105 (1632)	−0.00 (−0.00 to 0.00)	0.132	−34.0 (1357)	−0.0 (−0.0 to 0.0)	0.717	−17.1 (1861)	0.00 (−0.00 to 0.00)	0.929
LF-RRI:HF-RRI ratio response to OC - mean (SD)	1.9 (1.7)	−0.35 (−0.75 to 0.06)	0.091	1.9 (1.6)	−0.3 (−0.7 to 0.1)	0.203	2.4 (1.9)	0.18 (−0.36 to 0.72)	0.504
LF-DBP response to OC, $mmHg^2$ - mean (SD)	−0.5 (2.5)	0.10 (−0.18 to 0.38)	0.486	−1.8 (10.9)	−0.0 (−0.1 to 0.1)	0.951	−1.2 (8.4)	−0.04 (−0.16 to 0.08)	0.493
Function									

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Table 2 (*continued*)

	EBV patients, baseline (n = 200)			EBV patients, 2.visit (n = 195)			Healthy controls (n = 70)		
		CFQ total score as dependent			CFQ total score as dependent			CFQ total score as dependent	
	Variable caracteristics	Linear regression coefficient B (CI)	p-value	Variable caracteristics	Linear regression coefficient B (CI)	p-value	Variable caracteristics	Linear regression coefficient B (CI)	p-value
Functional Disability Inventory, total score - mean (SD)	16.6 (11.8)	0.26 (0.22 to 0.31)	< 0.001	6.3 (8.6)	0.4 (0.3 to 0.5)	< 0.001	3.2 (3.9)	0.33 (0.10 to 0.56)	0.005
Pediatric Quality of Life (PedsQL), total score - mean (SD)	66.5 (17.4)	−0.19 (−0.22 to −0.17)	< 0.001	78.9 (17.9)	−0.20 (−0.24 to −0.17)	< 0.001	85.3 (10.1)	−0.23 (−0.30 to −0.16)	< 0.001
Steps/day, number linear regression per 10,000 steps - mean (SD)	7515 (3080)	−4.10 (−6.37 to −1.83)	< 0.001	9046 (3438)	−2.29 (−4.52 to −0.06)	0.044	10,133 (4133)	0.67 (−2.84 to 1.51)	0.541

[a] Only measured under baseline examination.

[b] During the examination at six months follow-up, the EBV patients were asked about life events during the preceding six months.

index (B = −0.24, p = 0.014),. At $EBV_{six\ months}$, fatigue remained independently and positively associated with negative emotions (depression, B = 0.39, p = .004), and also with reduced emotional awareness (B = 0.16, p = 0.001), and illness perception (B = 0.09, p = 0.006); furthermore, fatigue was positively associated with plasma norepinephrine (B = 1.19, p = 0.021) and total T cell count (B = 2.26, p = 0.008), and negatively associated with HF-RRI-response to controlled breathing (B = −0.00, p = 0.037). Among HC, fatigue was also positively associated with negative emotions (anxious mood, B = 0.30, p = 0.017) and illness perception (B = 0.11, p = .002); in addition, an association to cytotoxic T cell count (B = 6.19, p = 0.007) and usage of illicit drugs were found (B = 3.66, p = 0.011, Table 3).

4. Discussion

The most important findings of the present study are: a) Across all three assessment categories ($EBV_{baseline}$, $EBV_{six\ months}$ and HC), fatigue was associated with symptoms of sleeping difficulties, negative emotions and low quality of life, but not with reduced physical activity or markers of infection; b) Fatigue at $EBV_{baseline}$ was not associated with immune response markers; at $EBV_{six\ months}$ and in HC, fatigue was independently associated with markers of cellular adaptive immunity; c) Fatigue at $EBV_{six\ months}$ was associated with markers of enhanced sympathetic and attenuated parasympathetic activity; d) Fatigue at $EBV_{baseline}$ was positively associated with cognitive functions, whereas there was a negative association with cognitive functions at $EBV_{six\ months}$.

4.1. Symptoms

The associations between fatigue and other symptoms for $EBV_{baseline}$, $EBV_{six\ months}$ and HC immediately suggest that the experience of fatigue normally occurs in concert with other bodily complaints. Accordingly, a scatter of baseline symptoms predict future level of fatigue, as was shown in a previous study from the same cohort [21].

The symptom of post-exertional malaise (PEM) seems to have some specificity to fatigue in relation to EBV-infection, especially at $EBV_{six\ months}$, corroborating a characteristic of CFS [25]. Concurrently, this finding indicates that the experience of PEM is not a specific marker that is useful in delineating distinct subgroups of CFS or chronically fatigued individuals, in contrast to what is sometimes claimed [23,25]. A possible explanation for the difference between $EBV_{six\ months}$ and HC with regards to PEM might be that the subjective notion of fatigue is contextualized differently according to differences in previous experiences. For instance, fatigue at $EBV_{six\ months}$ may be mentally coupled to other symptoms of infection and the sickness response, whereas fatigue in the HC group might be more related to “tiredness” and “sleepiness”.

4.2. Functional abilities

The strong association between fatigue and quality of life across all three assessment categories ($EBV_{baseline}$, $EBV_{six\ months}$ and HC) are in line with previous research [4,26,27], emphasizing the serious disabling consequences of chronic fatigue states.

The overall weak association between fatigue and physical activity (steps per day) was surprising, but in accordance with previous research from the same cohort indicating different prediction models of sedentary behavior and fatigue, respectively, after EBV infection [21,28]. In addition, Huang et al. reported similar findings in their adolescent acute infectious mononucleosis cohort [29]. Earlier studies have used steps per day as a proxy for fatigue, and for assessment of treatment effects in CFS patients [8]. The present finding suggests that reduced physical activity is not a simple reflection of high level of fatigue, and questions the validity of steps per day as a proxy variable.

Table 3
Final models - the best fitted multiple regression models.

Symptoms

	EBV patients, baseline			EBV patients, six months				Healthy controls			
	Linear regression coefficient B (CI)	p-value	Δadj.R^2 [d]		Linear regression coefficient B (CI)	p-value	Δadj.R^2 [d]		Linear regression coefficient B (CI)	p-value	Δadj.R^2 [d]
Karolinska Sleep Questionnaire Subscore: Sleepy at daytime[a]	−0.38 (−0.52 to −0.23)	< 0.001	0.086	Post-exertional Malaise, single item[b]	1.39 (0.61 to 2.18)	0.001	0.068	Karolinska Sleep Questionnaire, total score[a]	−0.20 (−0.27 to −0.13)	< 0.001	0.288
Post-exertional Malaise, single item[b]	0.86 (0.35 to 1.37)	0.001	0.044	Brief Pain Inventory (BPI), total pain severity score - mean (SD)	0.24 (0.09 to 0.40)	0.003	0.040				
Infectious Symptoms, mean score	0.83 (0.18 to 1.49)	0.013	0.016	Karolinska Sleep Questionnaire Subscore: Sleepy at daytime[a]	−0.28 (−0.47 to −0.08)	0.007	0.034				
Hypersensitivity Symptoms, mean score	0.75 (0.11 to 1.38)	0.021	0.015								
Explained variance (adjusted R^2) of model[c]		0.439		Explained variance (adjusted R^2) of model[c]		0.395		Explained variance (adjusted R^2) of model[c]		0.288	

Function

	EBV patients, baseline			EBV patients, six months				Healthy controls			
	Linear regression coefficient B (CI)	p-value	Δadj.R^2 [d]		Linear regression coefficient B (CI)	p-value	Δadj.R^2 [d]		Linear regression coefficient B (CI)	p-value	Δadj.R^2 [d]
Pediatric Quality of Life, total score	−0.14 (−0.19 to −0.09)	< 0.001	0.094	Pediatric Quality of Life, total score	−0.19 (−0.23 to −0.15)	< 0.001	0.404	Pediatric Quality of Life, total score	−0.23 (−0.30 to −0.16)	< 0.001	0.362
Functional Disability Inventory, total score	0.09 (0.01 to 0.17)	0.023	0.017								
Explained variance (adjusted R^2) of model[c]		0.467		Explained variance (adjusted R^2) of model[c]		0.404		Explained variance (adjusted R^2) of model[c]		0.362	

All other variable

	EBV patients, baseline			EBV patients, six months				Healthy controls			
	Linear regression coefficient B (CI)	p-value	Δadj.R^2 [d]		Linear regression coefficient B (CI)	p-value	Δadj.R^2 [d]		Linear regression coefficient B (CI)	p-value	Δadj.R^2 [d]
Hospital Anxiety and Depression Scale, total score	0.28 (0.08 to 0.48)	0.012	0.092	Hospital Anxiety and Depression Scale, Subscore: Depression	0.39 (0.14 to 0.64)	0.004	0.051	Brief Illness Perception Questionnaire, total score	0.11 (0.04 to 0.17)	0.002	0.086
Serum Creatinine Kinase (CK), IU/L	−0.01 (−0.01 to −0.00)	0.006	0.026	HF-RRI response to CB, ms^2	−0.00 (−0.00 to −0.00)	0.037	0.042	Blood cytotoxic T cell ($CD8^+$) count, 10^6 cells/L	6.19 (1.73 to 10.6)	0.007	0.065
Life Event Checklist, total score, all negative events	0.08 (0.00 to 0.16)	0.047	0.017	TAS-20 Subscore: Difficulty identifying feelings	0.16 (0.06 to 0.26)	0.001	0.037	Usage of narcotics/illegal drugs	3.66 (0.84 to 6.48)	0.011	0.059

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Table 3 (*continued*)

All other variable	EBV patients, baseline				EBV patients, six months				Healthy controls		
	Linear regression coefficient B (CI)	p-value	Δadj.R^2 [d]		Linear regression coefficient B (CI)	p-value	Δadj.R^2 [d]		Linear regression coefficient B (CI)	p-value	Δadj.R^2 [d]
BMI, kg/m^2	−0.24 (−0.47 to −0.02)	0.037	0.014	Plasma Norepinephrine, nmol/L	1.19 (0.20 to 2.18)	0.021	0.033	Hospital Anxiety and Depression Scale (HADS), Subscore: Anxiety	0.30 (0.05 to 0.55)	0.017	0.047
Digit Span Backward, total sum score	0.32 (0.02 to 0.62)	0.034	0.014	Blood T cell ($CD3^+$) total count, 10^9 cells/L	2.26 (0.63 to 3.88)	0.008	0.033				
				Brief Illness Perception Questionnaire, total score	0.09 (0.03 to 0.15)	0.006	0.027				
Explained variance (adjusted R^2) of model[c]		0.249		Explained variance (adjusted R^2) of model[c]		0.391		Explained variance (adjusted R^2) of model[c]		0.336	

[a] Karolina Sleep Questionnaire has a reversed scale; the more sleeping difficulties, the lower score.
[b] Post-exertional Malaise is a single item score "how often do you feel more fatigued the day after an exertion?".
[c] Explained variance of model is the mean explained variance for the five imputed datasets.
[d] Δadj.R^2 equals the difference in explained variance of the model with and without the respective variable.

4.3. Other variables

There were strong associations between fatigue and negative emotions (depression, anxiety) for $EBV_{baseline}$, $EBV_{six\ months}$ and HC. Accordingly, a previous CEBA sub-study showed that anxiety at baseline was the most important predictor for fatigue six months later [21]. Interestingly, six months after the acute infection, fatigue was primarily associated with depression rather than anxiety. This finding is in line with general assumption of a temporal relationship between anxiety and depression where anxiety often comes first [30]. These associations deserve attention in further research. Fatigue is a common feature of emotional distress, in particular depression [31–33]; furthermore, reports suggest that CFS patients have reduced ability to identify feelings [34]. Thus, it is possible that the findings in the present study simply reflect that fatigue and negative emotions are two facets of the same underlying construct.

Fatigue was positively associated with a marker of sympathetic nervous activity (plasma norepinephrine), and negatively associated with a marker of parasympathetic nervous activity (HR-RRI-response to controlled breathing) at $EBV_{six\ months}$ only. These findings corroborate several empirical studies on CFS showing enhanced sympathetic and attenuated parasympathetic activity [10,35,36], in line with the "sustained arousal"-model [16]. Of note, this model suggests that immune alterations in CFS are secondary to autonomic alterations; recent evidence suggests that adolescents with CFS are characterized by upregulated innate immunity and downregulated adaptive humoral immunity which in turn are associated with autonomic and neuroendocrine markers [37]. Accordingly, in the bivariate analyses of the present study, there was a positive association between fatigue and CRP (a marker of innate immunity) and a negative association between fatigue and B cell fraction (a marker of adaptive humoral immunity) at $EBV_{six\ months}$ only. Furthermore, these associations disappeared in the multivariate model, as expected if they were driven by alterations of sympathovagal balance.

In simple linear regression analyses, positive associations between fatigue and markers of adaptive cellular immunity, in particular T-cell counts, were found across all three assessment categories ($EBV_{baseline}$, $EBV_{six\ months}$ and HC). The underlying mechanisms for this association should be explored in further studies. However, all associations between immune response and fatigue disappeared in the adjusted model at $EBV_{baseline}$, while it remained at $EBV_{six\ months}$ and in HC. This is surprising since an acute EBV infection mounts a strong immune response including an increased level of cytotoxic T cells. Thus the present results suggest that fatigue during acute EBV infection is primarily related to factors other than immunity. The general lack of associations between infectious markers and fatigue in the EBV infected individuals (both during baseline assessment and six months later) are also somewhat surprising, but corroborates findings from previous CFS research [38].

Working memory (digit span backward) was positively associated with fatigue in the final model at $EBV_{baseline}$. Similarly, a previous study on the same cohort has documented a positive predictive value of baseline cognitive function for later fatigue development [21]. Interestingly, at $EBV_{six\ months}$, most cognitive function tests were negatively associated with fatigue indicating that more fatigue was associated with worse cognition, in line with empirical findings in CFS [9]. Taken together, the results indicate that the reduction of cognitive functioning in CFS patients is a consequence of, rather than a risk factor for, the disorder. This corroborates evidence provided by Nijhof et al. comparing current IQ test results of adolescent CFS patients, with pre-CFS school-test results [39].

The associations between female sex and fatigue in bivariate analyses under all three conditions are in line with several previous empirical studies [40–42]. However, the associations disappears in the adjusted models. Sex differences in fatigue deserve more attention in further studies.

4.4. Strengths and limitations

Strengths of this study include the well-defined cohort, the large number of participants, the low number of drop-outs and the extensive investigational program. Limitations include some missing data in most variables; therefore, multiple imputation was performed to reduce the risk of bias. In addition, the external validity of the study is uncertain as only about 22% of eligible patients were included.

5. Conclusion

Irrespective of the cause, fatigue is important for quality of life and is highly associated with negative emotions. Viral load does not seem to contribute to fatigue, and there is a general scarcity of associations between fatigue and immune response markers. Autonomic alterations (sympathetic enhancement, parasympathetic attenuation) and cognitive dysfunction are exclusively associated with fatigue long after infection, corroborating findings from studies of the chronic fatigue syndrome.

Declarations of interest

None.

Competing interests

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

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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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpsychores.2019.04.008.

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