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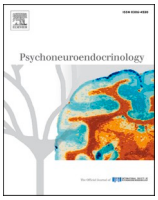

Biomarkers of brain injury in patients with stress-related exhaustion: A longitudinal study

Caroline Hansson [a,b,*], Henrik Zetterberg [b,c,d,e,f], Anniina Snellman [b,g], Kaj Blennow [b,c], Ingibjörg H. Jonsdottir [a,h]

[a] The Institute of Stress Medicine, Region Västra Götaland, Gothenburg, Sweden
[b] Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden
[c] Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Gothenburg, Sweden
[d] Department of Neurodegenerative Disease, Institute of Neurology, University College London, London, UK
[e] UK Dementia Research Institute, University College London, London, UK
[f] Hong Kong Center for Neurodegenerative Diseases, Hong Kong
[g] Turku PET Centre, University of Turku and Turku University Hospital, Turku, Finland
[h] School of Public Health and Community Medicine, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

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ABSTRACT

Introduction: Exhaustion Disorder (ED) is a stress-induced disorder, characterized by extreme fatigue, cognitive impairments, and intolerance to stress. These symptoms can be long-lasting, suggesting that the long-term stress may have initiated pathophysiological processes in the brains of patients with ED. The aims of the study were I) to investigate if plasma levels of neurofilament light (NfL), glial fibrillary acidic protein (GFAP), and phosphorylated tau (p-tau181) differ between patients with ED and healthy controls, and II) to investigate if these differences persist over time.

Method: Plasma NfL, GFAP and p-tau181 were quantified in 150 patients with ED at the time of diagnosis (baseline), 149 patients at long-term follow-up (7–12 years later, median follow-up time 9 years and 5 months), and 100 healthy controls.

Results: Plasma levels of NfL and GFAP were significantly higher in the ED group at baseline compared with controls (mean difference of NfL 0.167, 95 % CI 0.055–0.279; mean difference of GFAP 0.132, 95 % CI 0.008–0.257), while p-tau181 did not differ between the groups. Plasma levels of NfL were significantly lower in the ED group at follow-up than in the same group at baseline (mean difference −0.115, 95 % CI −0.186-(−0.045)), while plasma levels of GFAP did not differ between the groups, and plasma levels of p-tau181 were significantly higher in the ED group at follow-up than in the same group at baseline (mean difference 0.083, 95 % CI 0.016–0.151). At follow-up, there were no significant differences between the ED group and the control group for any of the proteins.

Conclusion: Plasma levels of NfL and GFAP were increased in patients with ED during the first months of the disease, indicative of axonal and glial pathophysiological processes, but had normalized at long-term follow-up.

1. Introduction

Long-term sick leave due to psychiatric diagnoses has increased in many countries, including Sweden, and psychiatric diagnoses are now the most common cause of long-term sick leave (Försäkringskassan [The Swedish Social Insurance Agency], 2020). In Sweden, stress-related mental disorders have increased the most, and among these, exhaustion disorder (ED) is the most common diagnosis (Försäkringskassan [The Swedish Social Insurance Agency], 2020). ED is a stress-induced disorder characterized by physical and mental symptoms of exhaustion, markedly reduced mental energy, memory impairment, sleep disturbance, emotional instability, and intolerance to stress (National Board of Health and Welfare, 2003). The symptoms of ED and burnout are overlapping and most patients with ED also report high burnout scores (Jonsdottir et al., 2009). In addition,

* Correspondence to: Institute of Stress Medicine, Carl Skottsbergs gata 22B, SE-413 19 Gothenburg, Sweden.
E-mail address: caroline.e.hansson@vgregion.se (C. Hansson).

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Nomenclature

BDNF	Brain-derived neurotrophic factor.
BMI	Body mass index.
CI	Confidence interval.
CNS	Central nervous system.
CSF	Cerebrospinal fluid.
ED	Exhaustion disorder.
EGF	Epidermal growth factor.
GFAP	Glial fibrillary acidic protein.
HPA	Hypothalamus-pituitary-adrenal.
ISM	Institute of stress medicine.
NfL	Neurofilament light.
p-tau181	Tau phosphorylated at threonine 181.
SD	Standard deviation.
TBI	Traumatic brain injury.
VEGF	Vascular endothelial growth factor.

comorbid anxiety and depression are common (Glise et al., 2012). The diagnostic criteria were established by the Swedish National Board of Health and Welfare in 2003 and ED was assigned the code F43.8A in the Swedish version of ICD-10.

Patients fulfilling criteria for ED often report long-lasting problems (Glise et al., 2020), with sustained symptoms of stress intolerance, fatigue, memory problems (Glise et al., 2020; Stenlund et al., 2012), and cognitive difficulties (Ellbin et al., 2021), resulting in long-term sick--leave (Grossi and Santell, 2009; Stenlund et al., 2012). These long-lasting symptoms of fatigue and cognitive impairment poses the question whether the brain has been injured in patients with ED and, if so, whether the injury is permanent or not. Therefore, we have investigated three markers of brain injury or disease: neurofilament light (NfL), glial fibrillary acidic protein (GFAP), and tau phosphorylated at threonine 181 (p-tau181). NfL is one of the components of the axonal cytoskeleton. Upon axonal injury, NfL leaks out into the extracellular fluid, and the levels of NfL in body fluids reflect the extent of axonal damage. Increased NfL levels in the cerebrospinal fluid (CSF) is a general marker of neurodegeneration (Rosengren et al., 1996). Newer methods have made it possible to measure NfL also in blood, and the correlation between plasma NfL and CSF NfL is very high (Gisslen et al., 2016). GFAP is an intermediate filament protein that is mainly expressed in astrocytes. In response to brain injury and disease, astrocytes become reactive and rapidly increase the synthesis of GFAP (Middeldorp and Hol, 2011). GFAP is thus a biomarker of astrocyte injury and activation, measurable in blood and in CSF. Blood levels of GFAP correlate with clinical severity in, e.g., traumatic brain injury (TBI) (Abdelhak et al., 2022). Tau is a protein that is essential for microtubule assembly and stabilization, located in the neuronal axons (Weingarten et al., 1975). The concentration of phosphorylated tau reflects the formation of neurofibrillary tangles, which is a core pathological feature of Alzheimer's disease (Blennow and Hampel, 2003). While NfL is a general marker of neurodegeneration, p-tau181 is a highly specific biomarker for Alzheimer's disease, which can be measured both in CSF (Skillback et al., 2015) and in blood (Karikari et al., 2020). Hence, NfL was quantified to investigate if long-term stress may induce neurodegeneration, GFAP was quantified to investigate if long-term stress may induce astrocytic activation, and p-tau181 was quantified to investigate if long-term stress may induce Alzheimer-like pathological alterations. The aims of this study were (I) to investigate if plasma levels of NfL, GFAP and p-tau181 differ between patients with ED and healthy controls, and (II) to investigate if these differences persist over time.

2. Method

2.1. Study population

This study is part of a longitudinal study conducted at the Institute of Stress Medicine (ISM), which is a specialist outpatient clinic for patients with ED, located in Gothenburg, Sweden. The patients were referred to ISM from primary care units or occupational health care centers, because of plausible ED. All patients included in the study were judged by a senior physician at the clinic to fulfill the criteria for ED (Table 1), and hence diagnosed with ED at their first visit to the clinic (baseline). According to the diagnostic criteria, patients with other medical conditions that plausibly could explain the fatigue were excluded. So were also patients with alcohol or drug abuse and patients with psychiatric illness other than depression and anxiety. Depression and anxiety were assessed using the Primary Care Evaluation of Mental Disorders (PRIME-MD) instrument (Spitzer et al., 1994). Before consulting the physician, the patient completed a one-page PRIME-MD patient questionnaire that covers questions on somatic as well as mental symptoms. Affirmative responses were followed-up by the physician in a structured interview conforming to the criteria of the Diagnostic and Statistical Manual of Mental Disorders, Fourth Revision, for diagnostic assessment of depression and anxiety disorder. The diagnostic procedure has been described in detail previously (Glise et al., 2012). To be included in the study, patients should not have been on sick leave for more than 6 months. The treatment at ISM lasted for approximately 18 months and has previously been described in detail (Glise et al., 2012). In brief, the patients were offered a stress-reduction program, physical exercise, and monthly visits with the physician. Cognitive behavioral group therapy for insomnia, individual psychotherapy, and/or antidepressant medication was offered when needed. Also, communication with the Social Insurance Office and the employer was facilitated. Following treatment at the clinic, all patients that had passed seven years or more since their first visit to the clinic were invited to participate in a follow-up clinical assessment, including assessment of residual stress-related exhaustion. In total, 163 patients agreed to participate in the follow-up clinical assessment. The patients that agreed to participate (included in clinical assessment, n = 163) were significantly older at baseline (mean age 44 years, SD 9.6) than the patients that were eligible, but did not agree to participate or did not answer the invitation (drop-out group, n = 190) (mean age 41 years, SD 9.0, p = 0.003). There were also significantly more women in the participating group (77 %) than in the drop-out group (67 %, p = 0.041). The groups did not differ at baseline

Table 1
Diagnostic criteria for exhaustion disorder according to the National Board of Health and Welfare (2003).

A. Physical and mental symptoms of exhaustion with minimum two weeks duration. The symptoms have developed in response to one or more identifiable stressors which have been present for at least 6 months.
B. Markedly reduced mental energy, which is manifested by reduced initiative, lack of endurance, or increase of time needed for recovery after mental efforts.
C. At least four of the following symptoms have been present most of the day, nearly every day, during the same 2-week period:
   1. Persistent complaints of impaired memory.
   2. Markedly reduced capacity to tolerate demands or to work under time pressure.
   3. Emotional instability or irritability.
   4. Insomnia or hypersomnia.
   5. Persistent complaints of physical weakness or fatigue.
   6. Physical symptoms such as muscular pain, chest pain, palpitations, gastrointestinal problems, vertigo or increased sensitivity to sounds.
D. The symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning.
E. The symptoms are not due to the direct physiological effects of a substance (e.g., a drug of abuse, a medication) or a general medical condition (e.g., hypothyroidism, diabetes, infectious disease).
F. IF. If criteria for major depressive disorder, dysthymic disorder or generalized anxiety disorder are met, exhaustion disorder is set a comorbid condition.

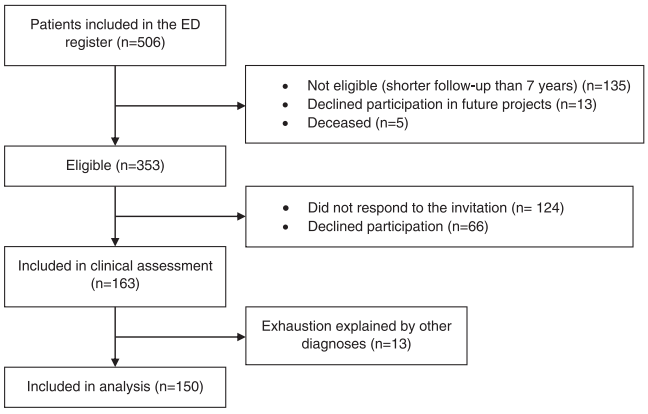

Fig. 1. Flow chart of included patients.

regarding self-reported symptoms of burnout, anxiety, or depression. At the follow-up clinical assessment, 13 of the 163 patients were excluded due to other conditions that could explain the fatigue, 51 patients still fulfilled the diagnostic criteria for ED, and 99 patients no longer fulfilled the criteria for ED and were thus considered recovered (Glise et al., 2020) (see Fig. 1 for flow chart). These 150 patients, which were either fulfilling or not fulfilling the diagnostic criteria for ED at follow-up, were included in the study cohort. The healthy controls were recruited from advertisement in newspapers, in social media, and at the region's intranet. The inclusion criteria for the healthy control group were 1) healthy, and 2) 20–50 years of age. The exclusion criteria were (1) self-rated exhaustion disorder; (2) somatic or psychiatric disease; (3) pregnancy or lactation; (4) overconsumption of alcohol. Hence, the study population consisted of 100 healthy controls, 150 patients with ED at baseline and the same 150 patients at follow-up, 7–12 years later. Blood sampling was not possible in one patient at follow-up, hence the follow up group consisted of 149 former patients at the clinic.

Those who consented to participate in the study after having been given a full oral and written description of the study procedure donated blood at baseline and at follow-up, i.e., 7–12 years later. Baseline samples were collected in 2004–2010, and follow-up samples were collected in 2016–2017. Median time between baseline and follow-up were 9 years and 5 months, and mean time between baseline and follow-up were 9 years and 4 months. The healthy controls only donated blood at one timepoint (baseline). Blood samples from the control group were collected in 2017–2018. Blood was drawn in 4 ml K2EDTA tubes (VACUETTE®) and centrifuged at 3500 rpm at + 4 °C for 15 min. The blood plasma was separated in 1 ml aliquots and stored at − 80 °C.

2.2. Protein analysis

Plasma NfL and GFAP concentrations were measured using commercially available kits on a Single molecule array (Simoa) HD-X Analyzer according to instructions from the kit manufacturer (Quanterix, Billerica, MA). Plasma p-tau181 concentrations were measured using an in-house method described previously (Karikari et al., 2020). The measurements were performed in one round of experiments using one batch of reagents by a researcher who was blinded to clinical data. Intra-assay coefficients of variation were below 10 %. One sample with an abnormally high p-tau concentration (300 pg/ml) was considered an outlier and thus excluded from further analysis.

2.3. Ethics

All participants gave their written informed consent before entering the study. This study was performed in accordance with the Declaration of Helsinki and was approved by the Regional Ethical Review Board in Gothenburg, Sweden, which is a part of the Swedish national committee for ethical approval (Dnr 668–15 and 242–15).

2.4. Statistical analyses

Differences in demographic variables between the patients and controls were analyzed using independent samples t-test for continuous variables and Pearson chi-square for dichotomous variables. Differences in demographic variables between two timepoints within the same group were analyzed using paired samples t-test.

Differences in protein concentrations between the ED group at baseline and the control group, between the ED group at follow-up and the control group, and between the group that was recovered and not recovered at follow-up were analyzed using general linear model, adjusting for sex and age. Differences in protein concentrations between the ED group at baseline and the same group at follow-up were analyzed using mixed models adjusting for age, sex, and time. Estimated marginal means for the proteins differ somewhat between the tables due to different adjustments in the models. Data not showing normal distribution were log-transformed before parametric tests. The statistical analyses were performed using the IBM SPSS Statistics version 25.

3. Results

3.1. Baseline characteristics

There were significantly more women in the ED group than in the control group and the ED group was significantly older. Body mass index (BMI) did not differ between the control group and the ED group at baseline, but the ED group had significantly higher BMI at follow-up than at baseline. Marital status did not differ between the groups. The control group had a larger proportion of participants with at least 1 year of university education than the ED group. A significantly larger proportion of the patient group had depression, anxiety, and antidepressant medication compared with the control group. The proportion of patients with depression and anxiety was significantly larger in the ED group at baseline than in the same group at follow-up, while the proportion of patients reporting antidepressant medication did not differ between the two timepoints (Table 2).

3.2. Protein analysis

The plasma levels of NfL were significantly higher in the ED group at baseline than in controls (mean difference 0.167, 95 % CI 0.055–0.279) (Table 3), and significantly lower in the ED group at follow-up than in the same group at baseline (mean difference −0.115, 95 % CI −0.186-(−0.045)) (Table 4). There was no significant difference in plasma levels of NfL between the ED group at follow-up and controls (Table 5).

The plasma levels of GFAP were significantly higher in the ED group at baseline than in controls (mean difference 0.132, 95 % CI 0.008–0.257) (Table 3). There were no significant differences in plasma levels of GFAP between the ED group at follow-up and the same group at baseline (Table 4), nor between the ED group at follow-up and controls (Table 5).

The plasma levels of p-tau181 were significantly higher in the ED group at follow-up than in the same group at baseline (mean difference 0.083, 95 % CI 0.016–0.151) (Table 4). There were no significant differences in plasma levels of p-tau181 between the ED group at baseline and controls (Table 3), nor between the ED group at follow-up and controls (Table 5).

There were no significant differences in biomarker levels at follow-up between the group that had recovered from ED and the group that had not recovered (Supplementary table 1). Likewise, there were no significant differences in biomarker levels at baseline between the group that would go on to recover at follow-up and the group that would not recover (Supplementary table 2).

Table 2
Characteristics of the study population.

	Control (n = 100)	ED (n = 150)	p-value
Sex			<0.001
- Women, % (n)	52 % (52)	78 % (117)	
- Men, % (n)	48 % (48)	22 % (33)	
Age			
- at baseline, mean (SD)	37 (9.1)	44 (9.6)	< 0.001[a]
- at follow-up, mean (SD)	n.a.	53 (9.7)	< 0.001[b]
Body mass index			
- at baseline, mean (SD)	23.5 (2.8)	24.0 (3.3)	0.186[a]
- at follow-up, mean (SD)	n.a.	25.5 (4.0)	< 0.001[b]
Marital status			0.602
- Married/cohabiting, % (n)	71 % (71)	74 % (111)	
- Not married/cohabiting, % (n)	29 % (29)	26 % (39)	
Education*			0.018
- Higher education, % (n)	89 % (89)	77 % (115)	
- Lower education, % (n)	11% (11)	23 % (34)	
Depression			
- at baseline, % (n)	0 % (0)	72 % (108)	< 0.001[a]
- at follow-up, % (n)	n.a.	21 % (31)	< 0.001[b]
Anxiety			
- at baseline, % (n)	0% (0)	79 % (118)	< 0.001[a]
- at follow-up, % (n)	n.a.	27 % (40)	< 0.001[b]
Antidepressant medication			
- at baseline, % (n)	1 % (1)	29 % (44)	< 0.001[a]
- at follow-up, % (n)	n.a.	28 % (41)	0.764[b]

ED = exhaustion disorder
n = number
SD = standard deviation
*Higher education is defined as at least 1 year of university/college education
[a] Analyzed using independent samples t-test ctrl vs ED baseline
[b] Analyzed using paired samples t-test ED baseline vs ED follow-up

Table 3
Protein concentrations in exhaustion disorder patients at baseline and healthy controls.

	Controls (n = 100)		ED baseline (n = 150)		Difference	
	Mean[a]	SE	Mean[a]	SE	Mean[a]	95 % CI
NfL (pg/ml)[b]	1.734	0.040	1.901	0.039	0.167	0.055 – 0.279
GFAP (pg/ml)[b]	4.633	0.045	4.766	0.043	0.132	0.008 – 0.257
p-tau181 (pg/ml)[b]	2.207	0.036	2.226	0.034	0.019	-0.081 – 0.119

[a] Estimated marginal means adjusted for age and sex.
[b] Log transformed before statistical analyses.

Table 4
Protein concentrations in exhaustion disorder patients at baseline and at follow-up.

	ED baseline (n = 150)		ED follow-up (n = 149 *)		Difference	
	Mean[a]	SE	Mean[a]	SE	Mean[a]	95 % CI
NfL (pg/ml)[b]	2.099	0.037	1.983	0.035	-0.115	-0.186 – (−0.045)
GFAP (pg/ml)[b]	4.858	0.047	4.867	0.044	0.009	-0.076 – 0.094
p-tau181 (pg/ml)[b]	2.239	0.031	2.323	0.029	0.083	0.016 – 0.151

[a] Estimated marginal means adjusted for age, sex and time.
[b] Log transformed before statistical analyses.
*n = 148 for p-tau181

Table 5
Protein concentrations in exhaustion disorder patients at follow-up and healthy controls.

	Controls (n = 100)		ED follow-up (n = 149 *)		Difference	
	Mean[a]	SE	Mean[a]	SE	Mean[a]	95 % CI
NfL (pg/ml)[b]	1.916	0.047	1.920	0.041	0.004	-0.131 – 0.139
GFAP (pg/ml)[b]	4.776	0.052	4.800	0.046	0.024	-0.125 – 0.174
p-tau181 (pg/ml)[b]	2.243	0.040	2.303	0.035	0.060	-0.055 – 0.176

[a] Estimated marginal means adjusted for age and sex.
[b] Log transformed before statistical analyses.
*n = 148 for p-tau181

4. Discussion

The main finding of the study was that the patients suffering from ED had higher plasma levels of NfL than the healthy controls. At follow-up, 7–12 years later, the plasma NfL levels of the ED group no longer differed from those of the control group.

Even though the NfL levels were significantly higher in the patients compared with the controls during the first months of the disease, the mean plasma levels of NfL in the ED group were still within the normal range. In our study, 95 % of the healthy control group had plasma NfL levels < 10 pg/ml, which may be considered a reference value for that age group. The corresponding number for the patient group was 79 %, thus 21 % of the ED group at baseline had NfL levels above 10 pg/ml. The mean value for the ED group at baseline was around 8 pg/ml (Supplementary table 3), whereas plasma NfL levels above 100 pg/ml are found in neurodegenerative disorders such as amyotrophic lateral sclerosis (Ashton et al., 2021). Furthermore, in our study, the plasma NfL levels in the ED group at baseline were on average 1.4-fold higher than the plasma NfL levels in the control group. Comparatively, Alzheimer's disease patients have on average 1.8-fold higher plasma NfL levels than controls (Ashton et al., 2021). The lower levels of plasma NfL in ED than in Alzheimer's disease is likely due to the degree of axonal damage being less severe in ED than in Alzheimer's disease. Hence, although there was a statistically significant difference between patients with ED and healthy controls in our study, our data do not support a massive neurodegeneration in ED. Increased levels of NfL have also been found in another stress-induced disorder, namely post-traumatic stress disorder, with higher NfL levels being associated with more severe PTSD symptoms (Guedes et al., 2021). One possible mechanism for stress-induced neuronal damage and astrocytic activation could be via activation of inflammatory pathways. Chronic psychosocial stress has been associated with increased levels of inflammatory markers (Johnson et al., 2013). Chronic inflammation may lead to neuroaxonal injury, putatively via mechanisms such as oxidative stress, mitochondrial dysfunction, and ion channel dysfunction (Friese et al., 2014).

NfL levels normally increase with age (Khalil et al., 2020). When adjusting for age, we found that the NfL levels had returned to normal levels at follow-up, indicating that the axonal injury seen at baseline do not persist over time. However, many patients still experience problems with memory, stress intolerance, extreme fatigue (Glise et al., 2020), and cognition (Ellbin et al., 2021) at this timepoint. Interestingly, a recent study on astrocytic and neuronal injury after COVID-19 found that plasma levels of NfL and GFAP were increased in severe cases of COVID-19. However, after 6 months the plasma NfL and GFAP levels had normalized, but many patients still experienced symptoms such as fatigue, "brain-fog" and changes in cognition (Kanberg et al., 2021). In multiple sclerosis, NfL levels normalizes in response to effective treatment. NfL is thus considered a marker of ongoing axonal injury (Teunissen and Khalil, 2012) and our data suggest that patients with ED have some degree of ongoing axonal injury in the acute phase of the disease.

At follow-up, the NfL levels are no longer increased, indicating that axons are no longer degrading. However, the axons that were affected in the acute phase of the disease may not recover, and the potential loss of axons may possibly contribute to the sustained symptoms.

A recent study showed that patients with ED have significantly higher plasma concentration of GFAP-positive extracellular vesicles than healthy controls (Wallensten et al., 2021). This is in line with our study, where patients with ED had significantly higher plasma concentration of GFAP than healthy controls. Similar results have been found in animal studies, where chronic mild stress upregulates GFAP immunoreactivity (Du Preez et al., 2021). However, the plasma concentration of GFAP in the ED group at follow-up was not significantly different from neither the patients at baseline nor from controls. Upregulation of GFAP is often used as a marker of reactive astrogliosis, which is a characteristic change in the morphology and function of astrocytes seen in many CNS pathologies including neurodegenerative disorders. Reactive astrogliosis has been proposed to reduce the extent of neurodegeneration and limit the tissue damage in the acute phase of CNS injury. On the other hand, persisting reactive astrogliosis can reduce regeneration and functional recovery at a later stage (Hol and Pekny, 2015). Possibly, this could be a part of the reason why ED patients experience cognitive impairments many years after the onset of the disease.

Long-term stress in midlife has been associated with an increased risk of dementia, especially Alzheimer's disease, later in life (Johansson et al., 2010). While NfL is a general marker of neuronal injury, p-tau181 is a specific marker of Alzheimer's disease that can differentiate Alzheimer's disease from other neurodegenerative disorders. Moreover, plasma p-tau181 increases along the Alzheimer's disease continuum and can be detected at very early stages of the disease (Karikari et al., 2020). We did not find any difference in p-tau181 levels when comparing patients with ED at baseline and healthy controls. However, we found a small but significant increase in plasma p-tau181 in the ED patients at follow-up compared with the same group at baseline, even though we adjusted for age, suggesting that p-tau181 may increase over time in this group. It should be noted, however, that the p-tau181 levels in the ED group at follow-up was not significantly different from those of the control group and far from the plasma p-tau levels seen in Alzheimer's disease (Karikari et al., 2020). The raw p-tau181 levels in our study were around 10 pg/ml (Supplementary table 3), which is in the same range as cognitively unimpaired adults in previous studies (Karikari et al., 2020).

About one third of the patients in our study still fulfilled the diagnostic criteria for ED at the long-term follow-up (Glise et al., 2020). However, we could not detect any differences in biomarker levels between the group that were still exhausted at follow-up compared with the group that were no longer exhausted. Also, there were no differences in biomarker levels at baseline between the group that would go on to recover from ED compared with the group that would still be exhausted 7–12 years later. These data indicate that the long-term ED is not due to increased neurodegeneration in these patients. Moreover, higher levels of these biomarkers at baseline do not predict disease status 7–12 years later. In contrast, both p-tau181 and NfL can be used to predict neurodegeneration and cognitive decline in Alzheimer's disease (Moscoso et al., 2021).

Previous studies aiming to find biological alterations in ED/burnout have been inconclusive. Most studies have focused on the hypothalamus-pituitary-adrenal (HPA) axis, where the results are conflicting, showing increased, decreased, as well as no difference in cortisol levels and other measures of activation of the HPA axis in patients with ED/burnout compared with controls (Jonsdottir and Sjors Dahlman, 2019). Studies on other hormones, including thyroid hormones, prolactin, and growth hormone, as well as studies regarding immune function, and the growth factors EGF and VEGF, show the same conflicting results (Jonsdottir and Sjors Dahlman, 2019). However, lower levels of brain-derived neurotrophic factor (BDNF) have been found in patients with ED/burnout compared with healthy controls in several studies (He et al., 2017; Onen Sertoz et al., 2008; Sjors Dahlman et al., 2019). BDNF is a neurotrophic factor, which promotes hippocampal adult neurogenesis (Lee et al., 2002). BDNF has also been proposed to render neuronal cells resilient to neurodegeneration (Colucci-D'Amato et al., 2020). Stress reduces BDNF expression in the hippocampus (Smith et al., 1995) and inhibits hippocampal adult neurogenesis (Gould et al., 1997). Stress-induced reduction of hippocampal adult neurogenesis has been proposed to be the biological and cellular basis of altered brain plasticity resulting in stress-related syndromes like burnout (Eriksson and Wallin, 2004). These studies, together with the results from the present study, suggest that patients with ED may have increased neurodegeneration and decreased neurogenesis, which may contribute to the symptomatology of ED.

Furthermore, several imaging studies have shown cerebral changes in patients with ED compared with controls. Amygdala has been found to be enlarged, the caudate volumes decreased, and the mesial frontal cortex thinner in patients with ED compared with healthy controls (Savic, 2015). Moreover, patients with ED have reductions in the gray matter volumes of the anterior cingulate cortex and the dorsolateral prefrontal cortex, and reduced volumes of caudate and putamen (Blix et al., 2013). A longitudinal study found that patients with ED have enlarged amygdala volumes, reduced caudate volumes, and reduced thickness in the prefrontal cortex and superior temporal gyrus. After 1–2 years the thinning of the prefrontal cortex and reduction of the caudate volume had normalized, while the amygdala enlargement and the superior temporal gyrus thinning remained (Savic et al., 2018). Also functional alterations have been found in ED. Patients with ED have an altered functional connectivity between the amygdala and the medial prefrontal cortex (Golkar et al., 2014; Jovanovic et al., 2011) and a reduced functional activity in the prefrontal cortex (Skau et al., 2021). Taken together, these cerebral changes have raised the question whether the brain has been injured in ED and, in that case, if the injury is reversible or permanent. A recent study on TBI showed similar results to our study; blood levels of NfL and GFAP were increased compared with controls, while there was no change in tau. Moreover, the NfL levels in mild TBI were in the same range as in our ED cohort (Shahim et al., 2020), suggesting that the extent of neuronal injury in ED may be comparative to the extent of neuronal injury in mild TBI. Supportively, patients with ED and mild TBI show many similarities. Both patient groups show altered functional activity in the frontal cortex during cognitive testing compared with controls, and the experimental procedure resulted in reduced mental energy in both patient groups, but not in controls. Moreover, both patient groups report the same degree of mental fatigue in daily life, and the mental fatigue levels in the patient groups are significantly higher than in the control group (Skau et al., 2019, 2021). However, while the NfL levels in our study had normalized after 7–12 years, the NfL levels in mild TBI were still significantly increased compared with controls after 5 years (Shahim et al., 2020). These results suggest that patients with ED initially have a neuronal injury comparative to that in mild TBI, but there is no permanent neurodegeneration in ED.

4.1. Methodological considerations

A major strength of this study is its longitudinal design and relatively large number of patients. A limitation is that we have not controlled for the potential influence of medication on plasma protein levels. The main reason is that the healthy control group were not on any medication. However, within the patient group, the plasma levels of the studied proteins did not differ between users and nonusers of SSRIs, SNRIs, or other antidepressants. Another possible confounder is previous history of TBI and/or concussion. Previous history of TBI and/or concussion was assessed using self-reported data from the follow-up clinical assessment. Out of the 150 patients with ED, 41 patients had a previous history of concussion, 108 patients had no previous history of concussion, and for 1 patient this information was missing. This information was not assessed for the controls; therefore, this possible confounder could not

be adjusted for in the models. However, to investigate whether the observed differences in NfL and GFAP were due to previous history of concussion, we compared the biomarker levels between the patients with previous concussion and the patients without previous concussion and found no differences between the groups. It should also be mentioned that although GFAP is predominantly expressed by astrocytes of the CNS, GFAP can also be expressed by other cell types (Uhlen et al., 2015), (https://www.proteinatlas.org/ENSG00000131095-GFAP/single+cell+type). Blood samples from the control group were collected in 2017–2018, from the ED group at baseline in 2004–2010, and from the ED group at follow-up in 2016–2017. Hence, the blood samples from the ED group at baseline were stored several years longer than the blood samples from the other groups. However, the blood samples were stored at − 80 °C (in accordance with (O'Bryant et al., 2015)), and had never been thawed before the protein analysis, reducing the risk of protein degradation. Finally, the patients in this study have been referred to a specialist clinic for ED. Hence it is possible that they represent more severe cases of ED compared with patients with ED in primary care. Thus, the results of this study should be validated in a more heterogenous cohort of patients preferably in a primary care setting.

5. Conclusion

In conclusion, here we show that plasma levels of NfL and GFAP were increased in patients with ED during the first months of the disease, indicative of axonal and glial pathophysiological processes, but had normalized at long-term follow-up.

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Author contributions

CH contributed to the conception and design of the study, analyzed and interpreted the data, and wrote the original draft of the manuscript. HZ contributed to the planning of the analysis, interpretation of data and reviewed and edited the manuscript. AS contributed to data collection, reviewed, and edited the manuscript. KB contributed to the interpretation of data and reviewed the manuscript. IJ contributed to the design of the study, interpretation of data and reviewed and edited the manuscript. All authors have read and approved the final version of the manuscript.

Conflict of interests

HZ has served at scientific advisory boards and/or as a consultant for Abbvie, Alector, Annexon, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Pinteon Therapeutics, Red Abbey Labs, Passage Bio, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). KB has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, BioArctic, Biogen, JOMDD/Shimadzu. Julius Clinical, Lilly, MagQu, Novartis, Ono Pharma, Pharmatrophix, Prothena, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program, outside the work presented in this paper. CH, AS and IJ declare no competing interests.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psyneuen.2022.105929.

References

Abdelhak, A., Foschi, M., Abu-Rumeileh, S., Yue, J.K., D'Anna, L., Huss, A., Oeckl, P., Ludolph, A.C., Kuhle, J., Petzold, A., Manley, G.T., Green, A.J., Otto, M., Tumani, H., 2022. Blood GFAP as an emerging biomarker in brain and spinal cord disorders. Nat. Rev. Neurol.

Ashton, N.J., Janelidze, S., Al Khleifat, A., Leuzy, A., van der Ende, E.L., Karikari, T.K., Benedet, A.L., Pascoal, T.A., Lleo, A., Parnetti, L., Galimberti, D., Bonanni, L., Pilotto, A., Padovani, A., Lycke, J., Novakova, L., Axelsson, M., Velayudhan, L., Rabinovici, G.D., Miller, B., Pariante, C., Nikkheslat, N., Resnick, S.M., Thambisetty, M., Scholl, M., Fernandez-Eulate, G., Gil-Bea, F.J., Lopez de Munain, A., Al-Chalabi, A., Rosa-Neto, P., Strydom, A., Svenningsson, P., Stomrud, E., Santillo, A., Aarsland, D., van Swieten, J.C., Palmqvist, S., Zetterberg, H., Blennow, K., Hye, A., Hansson, O., 2021. A multicentre validation study of the diagnostic value of plasma neurofilament light. Nat. Commun. 12, 3400.

Blennow, K., Hampel, H., 2003. CSF markers for incipient Alzheimer's disease. Lancet Neurol. 2, 605–613.

Blix, E., Perski, A., Berglund, H., Savic, I., 2013. Long-term occupational stress is associated with regional reductions in brain tissue volumes. PLOS One 8, e64065.

Colucci-D'Amato, L., Speranza, L., Volpicelli, F., 2020. Neurotrophic factor BDNF, physiological functions and therapeutic potential in depression, neurodegeneration and brain cancer. Int. J. Mol. Sci. 21.

Du Preez, A., Onorato, D., Eiben, I., Musaelyan, K., Egeland, M., Zunszain, P.A., Fernandes, C., Thuret, S., Pariante, C.M., 2021. Chronic stress followed by social isolation promotes depressive-like behaviour, alters microglial and astrocyte biology and reduces hippocampal neurogenesis in male mice. Brain Behav., Immun. 91, 24–47.

Ellbin, S., Jonsdottir, I.H., Eckerstrom, C., Eckerstrom, M., 2021. Self-reported cognitive impairment and daily life functioning 7-12 years after seeking care for stress-related exhaustion. Scand. J. Psychol.

Eriksson, P.S., Wallin, L., 2004. Functional consequences of stress-related suppression of adult hippocampal neurogenesis – a novel hypothesis on the neurobiology of burnout. Acta Neurol. Scand. 110, 275–280.

Försäkringskassan [The Swedish Social Insurance Agency], 2020. Sjukfrånvaro i psykiatriska diagnoser [Sick leave due to psychiatric diagnoses] (in Swedish).

Friese, M.A., Schattling, B., Fugger, L., 2014. Mechanisms of neurodegeneration and axonal dysfunction in multiple sclerosis. Nat. Rev. Neurol. 10, 225–238.

Gisslen, M., Price, R.W., Andreasson, U., Norgren, N., Nilsson, S., Hagberg, L., Fuchs, D., Spudich, S., Blennow, K., Zetterberg, H., 2016. Plasma concentration of the neurofilament light protein (NFL) is a biomarker of CNS injury in HIV infection: a cross-sectional study. EBioMedicine 3, 135–140.

Glise, K., Ahlborg, G., Jonsdottir, I.H., 2012. Course of mental symptoms in patients with stress-related exhaustion: does sex or age make a difference? BMC Psychiatry 12, 18.

Glise, K., Wiegner, L., Jonsdottir, I.H., 2020. Long-term follow-up of residual symptoms in patients treated for stress-related exhaustion. BMC Psychol. 8, 26.

Golkar, A., Johansson, E., Kasahara, M., Osika, W., Perski, A., Savic, I., 2014. The influence of work-related chronic stress on the regulation of emotion and on functional connectivity in the brain. PLOS One 9, e104550.

Gould, E., McEwen, B.S., Tanapat, P., Galea, L.A., Fuchs, E., 1997. Neurogenesis in the dentate gyrus of the adult tree shrew is regulated by psychosocial stress and NMDA receptor activation. J. Neurosci. 17, 2492–2498.

Grossi, G., Santell, B., 2009. Quasi-experimental evaluation of a stress management programme for female county and municipal employees on long-term sick leave due to work-related psychological complaints. J. Rehabil. Med. 41, 632–638.

Guedes, V.A., Lai, C., Devoto, C., Edwards, K.A., Mithani, S., Sass, D., Vorn, R., Qu, B.X., Rusch, H.L., Martin, C.A., Walker, W.C., Wilde, E.A., Diaz-Arrastia, R., Gill, J.M., Kenney, K., 2021. Extracellular vesicle proteins and microRNAs are linked to chronic post-traumatic stress disorder symptoms in service members and veterans with mild traumatic brain injury. Front. Pharmacol. 12, 745348.

He, S.C., Zhang, Y.Y., Zhan, J.Y., Wang, C., Du, X.D., Yin, G.Z., Cao, B., Ning, Y.P., Soares, J.C., Zhang, X.Y., 2017. Burnout and cognitive impairment: associated with serum BDNF in a Chinese Han population. Psychoneuroendocrinology 77, 236–243.

Hol, E.M., Pekny, M., 2015. Glial fibrillary acidic protein (GFAP) and the astrocyte intermediate filament system in diseases of the central nervous system. Curr. Opin. Cell Biol. 32, 121–130.

Johansson, L., Guo, X., Waern, M., Ostling, S., Gustafson, D., Bengtsson, C., Skoog, I., 2010. Midlife psychological stress and risk of dementia: a 35-year longitudinal population study. Brain 133, 2217–2224.

Johnson, T.V., Abbasi, A., Master, V.A., 2013. Systematic review of the evidence of a relationship between chronic psychosocial stress and C-reactive protein. Mol. Diagn. Ther. 17, 147–164.

Jonsdottir, I.H., Sjors Dahlman, A., 2019. Mechanisms in endocrinology: endocrine and immunological aspects of burnout: a narrative review. Eur. J. Endocrinol. 180, R147–R158.

Jonsdottir, I.H., Hagg, D.A., Glise, K., Ekman, R., 2009. Monocyte chemotactic protein-1 (MCP-1) and growth factors called into question as markers of prolonged psychosocial stress. PLOS One 4, e7659.

Jovanovic, H., Perski, A., Berglund, H., Savic, I., 2011. Chronic stress is linked to 5-HT (1A) receptor changes and functional disintegration of the limbic networks. Neuroimage 55, 1178–1188.

Kanberg, N., Simren, J., Eden, A., Andersson, L.M., Nilsson, S., Ashton, N.J., Sundvall, P. D., Nellgard, B., Blennow, K., Zetterberg, H., Gisslen, M., 2021. Neurochemical signs of astrocytic and neuronal injury in acute COVID-19 normalizes during long-term follow-up. EBioMedicine 70, 103512.

Karikari, T.K., Pascoal, T.A., Ashton, N.J., Janelidze, S., Benedet, A.L., Rodriguez, J.L., Chamoun, M., Savard, M., Kang, M.S., Therriault, J., Scholl, M., Massarweh, G., Soucy, J.P., Hoglund, K., Brinkmalm, G., Mattsson, N., Palmqvist, S., Gauthier, S., Stomrud, E., Zetterberg, H., Hansson, O., Rosa-Neto, P., Blennow, K., 2020. Blood phosphorylated tau 181 as a biomarker for Alzheimer's disease: a diagnostic performance and prediction modelling study using data from four prospective cohorts. Lancet Neurol. 19, 422–433.

Khalil, M., Pirpamer, L., Hofer, E., Voortman, M.M., Barro, C., Leppert, D., Benkert, P., Ropele, S., Enzinger, C., Fazekas, F., Schmidt, R., Kuhle, J., 2020. Serum neurofilament light levels in normal aging and their association with morphologic brain changes. Nat. Commun. 11, 812.

Lee, J., Duan, W., Mattson, M.P., 2002. Evidence that brain-derived neurotrophic factor is required for basal neurogenesis and mediates, in part, the enhancement of neurogenesis by dietary restriction in the hippocampus of adult mice. J. Neurochem. 82, 1367–1375.

Middeldorp, J., Hol, E.M., 2011. GFAP in health and disease. Prog. Neurobiol. 93, 421–443.

Moscoso, A., Grothe, M.J., Ashton, N.J., Karikari, T.K., Lantero Rodriguez, J., Snellman, A., Suarez-Calvet, M., Blennow, K., Zetterberg, H., Scholl, M., Alzheimer's Disease Neuroimaging, I., 2021. Longitudinal associations of blood phosphorylated Tau181 and neurofilament light chain with neurodegeneration in Alzheimer Disease. JAMA Neurol. 78, 396–406.

National Board of Health and Welfare, 2003. Utmattningssyndrom: Stressrelaterad psykisk ohälsa [Exhaustion Syndrome: Stress related mental illness] (in Swedish). Bjurner och Bruno AB Stockholm, Sweden.

O'Bryant, S.E., Gupta, V., Henriksen, K., Edwards, M., Jeromin, A., Lista, S., Bazenet, C., Soares, H., Lovestone, S., Hampel, H., Montine, T., Blennow, K., Foroud, T., Carrillo, M., Graff-Radford, N., Laske, C., Breteler, M., Shaw, L., Trojanowski, J.Q., Schupf, N., Rissman, R.A., Fagan, A.M., Oberoi, P., Umek, R., Weiner, M.W., Grammas, P., Posner, H., Martins, R., Star, B., groups, Bw, 2015. Guidelines for the standardization of preanalytic variables for blood-based biomarker studies in Alzheimer's disease research. Alzheimers Dement. (11), 549–560.

Onen Sertoz, O., Tolga Binbay, I., Koylu, E., Noyan, A., Yildirim, E., Elbi Mete, H, 2008. The role of BDNF and HPA axis in the neurobiology of burnout syndrome. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 32, 1459–1465.

Rosengren, L.E., Karlsson, J.E., Karlsson, J.O., Persson, L.I., Wikkelso, C., 1996. Patients with amyotrophic lateral sclerosis and other neurodegenerative diseases have increased levels of neurofilament protein in CSF. J. Neurochem. 67, 2013–2018.

Savic, I., 2015. Structural changes of the brain in relation to occupational stress. Cereb. Cortex 25, 1554–1564.

Savic, I., Perski, A., Osika, W., 2018. MRI shows that exhaustion syndrome due to chronic occupational stress is associated with partially reversible cerebral changes. Cereb. Cortex 28, 894–906.

Shahim, P., Politis, A., van der Merwe, A., Moore, B., Ekanayake, V., Lippa, S.M., Chou, Y.Y., Pham, D.L., Butman, J.A., Diaz-Arrastia, R., Zetterberg, H., Blennow, K., Gill, J.M., Brody, D.L., Chan, L., 2020. Time course and diagnostic utility of NfL, tau, GFAP, and UCH-L1 in subacute and chronic TBI. Neurology (95), e623–e636.

Sjors Dahlman, A., Blennow, K., Zetterberg, H., Glise, K., Jonsdottir, I.H., 2019. Growth factors and neurotrophins in patients with stress-related exhaustion disorder. Psychoneuroendocrinology 109, 104415.

Skau, S., Bunketorp-Kall, L., Kuhn, H.G., Johansson, B., 2019. Mental fatigue and functional near-infrared spectroscopy (fNIRS) - based assessment of cognitive performance after mild traumatic brain injury. Front. Hum. Neurosci. 13, 145.

Skau, S., Jonsdottir, I.H., Sjors Dahlman, A., Johansson, B., Kuhn, H.G., 2021. Exhaustion disorder and altered brain activity in frontal cortex detected with fNIRS. Stress 24, 64–75.

Skillback, T., Farahmand, B.Y., Rosen, C., Mattsson, N., Nagga, K., Kilander, L., Religa, D., Wimo, A., Winblad, B., Schott, J.M., Blennow, K., Eriksdotter, M., Zetterberg, H., 2015. Cerebrospinal fluid tau and amyloid-beta1-42 in patients with dementia. Brain 138, 2716–2731.

Smith, M.A., Makino, S., Kvetnansky, R., Post, R.M., 1995. Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. J. Neurosci. 15, 1768–1777.

Spitzer, R.L., Williams, J.B., Kroenke, K., Linzer, M., deGruy 3rd, F.V., Hahn, S.R., Brody, D., Johnson, J.G., 1994. Utility of a new procedure for diagnosing mental disorders in primary care. The PRIME-MD 1000 study. JAMA 272, 1749–1756.

Stenlund, T., Nordin, M., Jarvholm, L.S., 2012. Effects of rehabilitation programmes for patients on long-term sick leave for burnout: a 3-year follow-up of the REST study. J. Rehabil. Med. 44, 684–690.

Teunissen, C.E., Khalil, M., 2012. Neurofilaments as biomarkers in multiple sclerosis. Mult. Scler. 18, 552–556.

Uhlen, M., Fagerberg, L., Hallstrom, B.M., Lindskog, C., Oksvold, P., Mardinoglu, A., Sivertsson, A., Kampf, C., Sjostedt, E., Asplund, A., Olsson, I., Edlund, K., Lundberg, E., Navani, S., Szigyarto, C.A., Odeberg, J., Djureinovic, D., Takanen, J.O., Hober, S., Alm, T., Edqvist, P.H., Berling, H., Tegel, H., Mulder, J., Rockberg, J., Nilsson, P., Schwenk, J.M., Hamsten, M., von Feilitzen, K., Forsberg, M., Persson, L., Johansson, F., Zwahlen, M., von Heijne, G., Nielsen, J., Ponten, F., 2015. Proteomics. tissue-based map of the human proteome. Science 347, 1260419.

Wallensten, J., Nager, A., Asberg, M., Borg, K., Beser, A., Wilczek, A., Mobarrez, F., 2021. Leakage of astrocyte-derived extracellular vesicles in stress-induced exhaustion disorder: a cross-sectional study. Sci. Rep. (11), 2009.

Weingarten, M.D., Lockwood, A.H., Hwo, S.Y., Kirschner, M.W., 1975. A protein factor essential for microtubule assembly. Proc. Natl. Acad. Sci. USA 72, 1858–1862.