Possible Biomarkers of Chronic Stress Induced Exhaustion - A Longitudinal Study

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Abstract

Background
Vascular endothelial growth factor (VEGF), epidermal growth factor (EGF) and monocyte chemotactic protein-1 (MCP-1) have previously been suggested to be potential biomarkers for chronic stress induced exhaustion. The knowledge about VEGF has increased during the last decades and supports the contention that VEGF plays an important role in stress and depression. There is scarce knowledge on the possible relationship of EGF and MCP-1 in chronic stress and depression. This study further examines the role of VEGF, EGF and MCP-1 in women with chronic stress induced exhaustion and healthy women during a follow-up period of two years.

Methods and Findings
Blood samples were collected from 105 women with chronic stress induced exhaustion on at least 50% sick leave for at least three months, at inclusion (T0), after 12 months (T12) and after 24 months (T24). Blood samples were collected at inclusion (T0) in 116 physically and psychiatrically healthy women. The plasma levels of VEGF, EGF and MCP-1 were analyzed using Biochip Array Technology. Women with chronic stress induced exhaustion had significantly higher plasma levels of VEGF and EGF compared to healthy women at baseline, T12 and at T24. There was no significant difference in plasma levels of MCP-1. Plasma levels of VEGF and EGF decreased significantly in women with chronic stress induced exhaustion during the two years follow-up.

Conclusions
The replicated findings of elevated levels of VEGF and EGF in women with chronic stress induced exhaustion and decreasing plasma levels of VEGF and EGF during the two years follow-up support the contention that VEGF plays an important role in stress and depression. However, further studies are needed to elucidate the possible relationship of EGF and MCP-1 in chronic stress and depression.
Introduction

In the late 1990’s, the number of people on long-term sick leave increased in Sweden, mainly due to stress-induced mental disorders. Many of these patients exhibited persistent fatigue and cognitive problems such as memory loss and lack of concentration. The Swedish National Board of Health and Welfare suggested that the term Exhaustion Disorder (ED) should be used for this fatigue condition apparently caused by prolonged stress without sufficient recovery (2003).

In 2004 the diagnosis ED was added to the Swedish version of the International Classification of Diseases, tenth version (ICD-10). Equivalents to ED in the international literature are chronic burnout [1], clinical burnout [2], stress-related exhaustion [3], job stress related depression [4,5] and possibly neurasthenia [6].

ED may occur after long-term stress of at least six months duration, according to the diagnostic criteria [7]. Characteristic symptoms are mental and physical fatigue, sleep disturbance, emotional problems such as irritability and depressed mood, cognitive problems and reduced stress tolerance (Table 1). The cognitive problems have been substantiated by psychological testing and involve impaired memory and reduced auditory and visual attention [1]. There is some evidence that chronic work-related stress may be associated with regional morphological changes in the brain [8,9]. Symptoms of depression and anxiety are often present at onset, but usually remit long before the fatigue and the cognitive problems [10,11] indicating that ED is a diagnostic entity different from depression.

Table 1. Criteria for Exhaustion Disorder according to the Swedish National Board of Health and Welfare6.

| A. Physical and mental symptoms of exhaustion during at least two weeks. The symptoms have developed in response to one or more identifiable stressors present for at least six months. |
|---|
| B. The clinical picture is dominated by markedly reduced mental energy, as manifested by reduced initiative, lack of endurance, or increased time needed for recovery after mental effort. |
| C. At least four of the following symptoms have been present, nearly every day, during the same 2-week period: |
| 1. Concentration difficulties or impaired memory |
| 2. Markedly reduced capacity to tolerate demands or to work under time pressure |
| 3. Emotional instability or irritability |
| 4. Sleep disturbance |
| 5. Marked fatigability or physical weakness |
| 6. Physical symptoms such as aches and pains, palpitations, gastrointestinal problems, vertigo or increased sensitivity to sound |
| Physical symptoms such as aches and pains, palpitations, gastrointestinal problems, vertigo or increased sensitivity to sound |
| D. The symptoms cause clinically significant distress or impairment in occupational, social or other important respects. |
| E. The symptoms are not due to the direct physiological effects of a substance (e.g., a drug of abuse, a medication) or a physical illness/injury (e.g., hypothyroidism, diabetes, infectious disease). |

6Criterion A-E must be fulfilled to diagnose ED

doi:10.1371/journal.pone.0153924.t001
In order to find biomarkers for ED our research group previously assessed several biological mediators involved in stress response and inflammation in a group of women on prolonged sick leave due to mild mental illness [12]. The study demonstrated significantly elevated plasma concentrations of vascular endothelial growth factor (VEGF), epidermal growth factor (EGF) and monocyte chemotactic protein-1 (MCP-1) in women with prolonged sick leave due to mild mental illness, clinically diagnosed as exhaustion disorder. The levels of EGF were more than two times higher than in the healthy female controls and the levels of VEGF were three times higher, indicating that VEGF and EGF might be potential biomarkers for ED [12].

There is growing evidence that VEGF plays a role in stress and depression. Some studies have shown increased plasma levels of VEGF in patients with depression [13–17]. However, after adjusting for childhood trauma, the association between VEGF and depression were no longer significant according to one of these studies [14].

Another connection between depression and VEGF is the finding that VEGF stimulates the neurogenesis induced by antidepressant medication [18,19]. In rodents, chronic stress has been shown to reduce hippocampal volume in a process involving VEGF [20].

As far as we know, there is scarce knowledge about the possible relationship between EGF, stress and depression. A study on rhesus monkeys found that EGF seems to activate the hypothalamic-pituitary-adrenal axis (HPA axis) through stimulation of corticotropin-releasing hormone (CRH) release from the hypothalamus [21]. Interestingly, previous research has shown that the HPA axis is less sensitive to CRH in patients with ED compared to healthy controls [4,5].

There is also little knowledge about MCP-1 and its possible relationship to stress and depression. A study that examined vascular inflammation in rabbits found that unpredictable chronic mild stress resulted in increased aortic mRNA and protein expression of MCP-1 [22].

In order to further examine the role of plasma levels of VEGF, EGF and MCP-1 in patients with ED, we performed a follow-up study over two years on plasma levels of VEGF, EGF and MCP-1 in a new material of patients with ED and physically and psychiatrically healthy controls.

**Methods**

**Patients with Exhaustion Disorder**

Woman and men with psychiatric conditions (exhaustion disorder, burnout, neurasthenia, anxiety or depression) were selected consecutively from a database containing information about all public employees currently on long term sick leave (at least 50% sick leave for at least three months). This database is handled by one of the largest insurance companies in Sweden, AFA försäkring. Letters with an invitation to participate in the study were sent from the insurance company AFA försäkring. In the letter, individuals were informed that they were welcome to participate in the study if they considered their illness to be work-related. Individuals who did not reject further contact were contacted by the Karolinska Institute research group by telephone for detailed information about the study and for a telephone interview. The individuals lived in the Stockholm area and were 28 to 55 years old.

Individuals who were able to read and write the Swedish language were invited to a computerized Structured Clinical Interview for DSM-IV Axis 1 Disorders and Axis II Personality Disorders (SCID) [23,24] and a subsequent psychiatric and medical assessment performed by an experienced physician.

Those with abuse of alcohol and/or drugs, psychosis, anorexia or bulimia, bipolar disorder, severe personality disorder, serious neurological or endocrine disorder or other serious acute
or chronic illnesses were excluded to ensure that the exhaustion was due to long term stress and no other disorder.

Individuals who fulfilled diagnostic criteria for ED (further called patients) were asked to participate. Diagnostic criteria for ED are shown in table 1. The diagnosis ED was only assessed at baseline.

Healthy controls

The healthy controls were recruited five years after the patients, to serve as reference material to the patients. The Central Bureau of Statistics (SCB) randomly selected 1146 individuals, aged 28–55, from the population with permanent residence in the Stockholm area in the Swedish population register. Invitation letters for participation in the study were sent from SCB to the individuals in 2009. The individuals who agreed to participate were contacted for a first screening by phone. If they claimed to be healthy they were invited to a medical investigation including interview and clinical examination performed by an experienced physician. Individuals with current or previous ED, other types of mental illness, myocardial infarction, stroke or tumor disease were excluded.

Descriptive characteristics

**Educational level.** Educational level was divided into three groups; examination from (1) elementary school (9 years of education), (2) upper secondary school (11–13 years of education) or (3) university (>13 years of education).

**Current depression and anxiety disorders.** Patients that fulfilled DSM IV criteria for depression at the baseline medical investigation were considered to have depression in addition to ED [25]. Patients that fulfilled DSM IV criteria for panic disorder with or without agoraphobia, social phobia, posttraumatic stress disorder or generalized anxiety disorder were considered to have an anxiety disorder in addition to ED [25].

**Antidepressant medication.** Patients who were on antidepressant medication at the baseline medical investigation were considered to have ongoing treatment with antidepressant medication.

**Vascular disease.** Patients were considered to have vascular disease if they had hypertension, angina pectoris or a history of myocardial infarction, congestive heart failure, or stroke.

Analytical methods

Blood samples were obtained at inclusion (T0), after 12 months (T12) and after 24 months (T24) in patients and only at inclusion in controls. Blood samples were drawn through direct venipuncture from an antecubital vein after 15 minutes of rest. Patients in the present study were initially recruited for an intervention study five years before the controls. Blood sampling routines therefore differed slightly between patients and controls. The controls were instructed to refrain from food or drink for at least 12 hours, except for water, and all blood samples were taken in the morning. However, patients had no restrictions on intake of food or drink and 45% of the blood samples were drawn in the afternoon. Blood samples were collected into tubes containing sodium citrate and immediately centrifuged at 2000g for 20 minutes at room temperature. It was stored in -80°C until analyzed. Plasma levels of VEGF, EGF and MCP-1 from controls and from patients at baseline, after 12 months and after 24 months were analyzed with a commercially available biochip immunoassay system, Randox high sensitivity cytokine array (Randox Laboratories, Antrim, Northern Ireland, United Kingdom) [12]. Briefly, each biochip was coated with antibodies against VEGF, EGF and MCP-1. After 24 hours of incubation (including wash and addition of a secondary antibody) the samples were
analyzed in the Randox investigator. All the values obtained in the present study were within the range of the standard/calibration curve. The samples were not run in duplicates. Plasma levels of VEGF, EGF and MCP-1 from controls and patients were analyzed in different years and therefor in different batches. The T0, T12 and T24 samples from each individual was analyzed in the same batch. The inter, intra-assay variation for the high-sensitive cytokine array was <10% according to manufacturer.

Statistical analysis

STATA/IC 10 was used to perform statistical analyzes. Possible differences in age, educational level, plasma levels of VEGF, EGF and MCP-1 between ED-patients and controls were assessed using Mann-Whitney test, due to skewed data. Basic correlations between time from T0 until T24 and biomarkers (VEGF, EGF and MCP-1) were calculated using Spearman’s correlation. Spearman’s correlation was also used for basic correlations between age and biomarkers (VEGF, EGF and MCP-1).

A median regression analysis model was used to examine associations between outcome variables VEGF, EGF and MCP-1 and antidepressive medication, depression, anxiety, vascular disease or time of blood sampling (morning or afternoon) in patients.

In patients, a logistic regression model for repeated measurements was used to analyze if plasma levels of VEGF and EGF changed over time. Shapiro-Wilk test was used for test of normality. Since the outcomes of VEGF and EGF were strongly skewed the variables were dichotomized and a logistic Generalized Estimation Equation (GEE) model for repeated measurements with an unstructured correlation matrix was used to analyze change over time. The cut-off points were chosen based on the distribution of the variables rather than on clinical experience, since no clinical guidelines for high/low plasma levels exist. The highest plasma level for the controls (39 pg/ml for VEGF and 16 pg/ml for EGF) were chosen cut-off points.

For all statistical tests, p < 0.05 was considered statistically significant.

Ethics

The study was approved by the Regional Ethical Review Board in Stockholm, Sweden, http://www.epn.se/en/start/ d.nr.2004/481-3, 2009/614-32 and 2014/585-31/1. All individuals gave their written informed consent.

Results

Initially both men and women with ED were planned to be included in the study. However, only three men could be recruited. Therefore, we decided to include only women in the study. Inclusion criteria were met by 113 of the patients. After excluding all men (n = 3) and patients who did not leave blood sample (n = 5), 105 female patients with ED were finally included in the study (Fig 1).

A total of 368 healthy controls agreed to participate. Inclusion criteria were met by 166 of the controls. After excluding all men, 116 healthy female controls were finally included in the study (Fig 2).

Table 2 describes baseline characteristics of patients and controls. Age and educational level did not differ significantly between the groups.

Table 3 shows plasma levels of VEGF, EGF and MCP-1 at baseline. Plasma levels of VEGF and EGF were significantly higher in patients compared to controls. There was no significant difference in MCP-1 between patients and controls. At baseline, plasma levels of VEGF and EGF in patients were widely spread with high standard deviations compared to controls. This is also shown in fig 3.
Table 4 shows that antidepressant medication, depression, anxiety, vascular disease or time of blood sampling (morning or afternoon) did not affect plasma levels of VEGF, EGF or MCP-1 in patients.

The plasma levels of VEGF and EGF decreased significantly with time among patients during the follow-up (p = <0.001 for both VEGF and EGF) (Table 5). However, the plasma levels of VEGF and EGF were still significantly higher in patients, both at 12 months and 24 months after inclusion, compared to controls at baseline (Fig 3).

Spearman’s correlation showed no significant correlation between age and VEGF (Spearman’s rho = -0.072, p = 0.30) or between age and EGF (Spearman’s rho = -0.12, p = 0.093). There was a significant correlation between age and MCP-1 (Spearman’s rho = 0.33, p = <0.001) (data not shown).

Spearman’s correlation showed significantly negative correlation between time (T0 until T24) and VEGF (Spearman’s rho = -0.32, p = <0.001) and EGF (Spearman’s rho = -0.41, p = <0.001) but not for MCP-1 (Spearman’s rho = 0.023, p = 0.77).

**Discussion**

This follow-up study replicates previous findings showing that plasma levels of VEGF and EGF are increased in women with ED compared to healthy controls (Åsberg et al., 2009). In contrast, plasma levels of MCP-1 did not differ between women with ED and healthy controls in our material. Plasma levels of VEGF and EGF decreased significantly during the two year follow-up.

**Plasma levels of VEGF and EGF in ED in comparison with other studies**

In two previous studies by Åsberg et al (2009) and Jonsdottir et al (2009), the levels of VEGF, EGF and MCP-1 in patients with ED were examined. These two studies have reached contradictory results, although materials and methods were similar. Åsberg et al. found that VEGF, EGF and MCP-1 were significantly increased in women with ED compared to healthy women. The study also indicated a “dose-response” effect, since an additional group of women with
Fig 2. Flowchart of inclusion of controls.
doi:10.1371/journal.pone.0153924.g002
occupational stress had levels of VEGF, EGF and MCP-1 that were in between those of women with ED and healthy women. However, Jonsdottir et al, who used a similar group of women and the same analytic method as Åsberg et al, did not find increased plasma levels of VEGF, EGF and MCP-1 in women with ED compared to healthy controls [26].

A reason for different results in the studies could be a dissimilarity between the populations. There might be a difference in severity of ED and in residual confounding among the study populations. A possible confounder such as time from onset of ED to inclusion in study was not measured in any of the studies.

### Plasma levels of VEGF and EGF as biomarkers for ED

Âsberg et al proposed cut-off levels for VEGF, EGF and MCP-1 in plasma for screening and/or diagnostic purpose in ED [12]. Jonsdottir et al opposed to this since they found different levels of VEGF, EGF and MCP-1 using two different analytic techniques on their material [26]. In humans, VEGF family comprises of several growth factors such as VEGF (VEGF-A), VEGF-B, VEGF-C, VEGF-D, and placenta growth factor (PIGF) [27,28]. Unfortunately, the Randox analytic technique used in the present study does not disclose whether only VEGF-A, or also other types of VEGF are detected. Measuring different types of VEGF by different analytic methods could be a reason why Jonsdottir obtained other results.

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### Table 2. Descriptive characteristics of patients and controls.

| Descriptive characteristic | Patients (n = 105) | Controls (n = 116) | P-value |
|----------------------------|-------------------|-------------------|---------|
| Age, mean (standard deviation) | 44.8± (6.9) | 45.3 (7.2) | 0.446 |
| Educational level, median (25%-75%) | 3 (2–3) | 3 (2–3) | 0.203 |
| Percent with antidepressant medication | 45.2± | 0% | - |
| Percent with current depression | 59.6± | 0% | - |
| Percent with anxiety disorders | 17.1% | 0% | - |
| Percent with vascular disease | 14.3% | 0% | - |
| Percent on full time sick-leave | 69.3% | 0% | - |
| Percent on part time sick-leave | 30.7% | 0% | - |

[^3]: 3 missing values
[^b]: 13 missing values
[^c]: 1 missing value
[^d]: 4 missing values

doi:10.1371/journal.pone.0153924.t002

### Table 3. Plasma levels of VEGF, EGF and MCP-1 in patients and controls at baseline.

|                      | Patients (n = 105) | Controls (n = 116) | P-value |
|----------------------|-------------------|-------------------|---------|
| VEGF (pg/ml)         | 50.83 ± (56.43)   | 10.35 (5.03)      | <0.001 |
| EGF (pg/ml)          | 41.43 ± (41.05)   | 2.33 (2.04)       | <0.001 |
| MCP-1 (pg/ml)        | 122.37 ± (55.67)  | 112.49 ± (35.94)  | 0.45    |

[^a]: 7 missing value
[^b]: 8 missing values
[^c]: 1 missing value


doi:10.1371/journal.pone.0153924.t003

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[^1]: Table 2. Descriptive characteristics of patients and controls.
[^2]: Table 3. Plasma levels of VEGF, EGF and MCP-1 in patients and controls at baseline.
In our study we found no correlation between age and levels of VEGF and EGF. This is in line with a previous study of VEGF [29]. However, we did find a correlation between MCP-1 and age, which is not in line with previous studies [30,31].

Before using a biomarker in clinical practice several replications of the results are needed. For VEGF and EGF analyses to be clinically useful, more knowledge on at least four areas is
required. Firstly, an analytic method that distinguishes between different types of VEGF should be used. Secondly, since plasma levels of VEGF and EGF seem to decrease with time, plasma levels of VEGF and EGF may be correlated to time of onset of ED or to severity of symptoms. Thirdly, the effect of body mass index, intake of food or drink before blood sampling and possibly childhood trauma on plasma levels of VEGF and EGF in ED must be further examined. Finally, the potential for adding measures of vascular function should be examined.

Plasma levels of VEGF and EGF as possible mediators of cardiovascular disease

An area for future studies is whether plasma levels of VEGF and EGF are possible mediators of cardiovascular disease in individuals with stress related disorders. Previous research has demonstrated an association between concentrations of VEGF and EGF and hypertension,

### Table 4. Median regression of possible confounders for plasma levels of VEGF, EGF and MCP-1 at baseline (T0) in women with exhaustion disorder.

| VEGFT0        | Coef. | Std. Err. | t    | P>|t|  | 95% Conf. Interval |
|---------------|-------|-----------|------|-----|-------------------|
| Antidepressive medication | 8.06  | 5.96      | 1.35 | 0.18 | -3.78, 19.90      |
| Depression    | -0.33 | 6.91      | -0.05| 0.96 | -14.07, 13.41     |
| Anxiety disorders | -9.54 | 12.05     | -0.79| 0.43 | -33.51, 14.43     |
| Vascular disease | 2.20  | 9.15      | 0.24 | 0.81 | -15.99, 20.39     |
| Time of bloodsampling | -1.22 | 5.98      | -0.20| 0.84 | -13.12, 10.68     |

| EGFT0        | Coef. | Std. Err. | t    | P>|t|  | [95% Conf. Interval] |
|---------------|-------|-----------|------|-----|---------------------|
| Antidepressive medication | 9.17  | 8.44      | 1.09 | 0.28 | -7.62, 25.96        |
| Depression    | -1.06 | 8.19      | -0.13| 0.90 | -17.34, 15.22       |
| Anxiety disorders | -3.30 | 19.31     | -0.17| 0.87 | -41.70, 35.10       |
| Vascular disease | -17.25| 21.04     | -0.82| 0.42 | -59.09, 24.59       |
| Time of bloodsampling | -1.06 | 8.00      | -0.13| 0.90 | -16.97, 14.85       |

| MCP-1T0      | Coef. | Std. Err. | t    | P>|t|  | [95% Conf. Interval] |
|---------------|-------|-----------|------|-----|---------------------|
| Antidepressive medication | 7.77  | 13.09     | 0.59 | 0.55 | -18.25, 33.79      |
| Depression    | 1.85  | 13.83     | 0.13 | 0.89 | -25.64, 29.34      |
| Anxiety disorders | -24.22| 19.71     | -1.23| 0.22 | -63.41, 14.97      |
| Vascular disease | 22.76 | 30.10     | 0.76 | 0.45 | -37.08, 82.60      |
| Time of bloodsampling | 1.42  | 14.05     | 0.10 | 0.92 | -26.52, 29.36      |

### Table 5. Summary Generalized Estimation Equation (GEE) model.

| EGF | Time | OBS | Above cut-off, N(%) | Odds ratio (95% CI) | P-value |
|-----|------|-----|---------------------|---------------------|---------|
|     | 0    | 77  | 52 (68)             | -                   | -       |
|     | 12   | 73  | 36 (49)             | -                   | -       |
|     | 24   | 65  | 19 (29)             | 0.94 (0.91–0.96)⁵   | <0.001  |
| VEGF| 0    | 77  | 31 (40)             | -                   | -       |
|     | 12   | 73  | 18 (25)             | -                   | -       |
|     | 24   | 65  | 6 (9)               | 0.93 (0.90–0.96)⁵   | <0.001  |

⁴Time was assumed to be linear in the GEE model. This assumption was assessed with the NLCHECK command in Stata.

⁵Interprets as: for every year the odds of being above the EGF-cutoff decrease with 6%.

⁶Interprets as: for every year the odds of being above the VEGF-cutoff decrease with 7%.
compared with healthy individuals [32]. A Swedish register study has shown an increased risk of cardiovascular disease in individuals on long term sick-leave due to psychiatric disorder [33]. According to data from the Swedish social insurance office, about half of individuals on long term sick leave due to psychiatric problems suffer from stress related disorders [34]. VEGF and EGF may play a role in increased risk of cardiovascular disease in these individuals.

Strengths and limitations

Replicated findings of a significant elevation of biomarkers for mental illness, as in this study, are rare. The findings are strengthened by the use of a carefully selected and thoroughly examined healthy control group with no current or previous history of mental illness, cardiovascular disorder or cancer.

The main limitations of the study are that controls and patients were collected in different years, that there were some differences in the conditions at blood sampling between the groups and that the plasma levels of VEGF, EGF and MCP-I were analyzed in different batches. These limitations may have affected the results. However, the regression analysis including time of blood sampling (morning or afternoon) could not explain differences in plasma levels of VEGF and EGF. In addition, a previous study on diurnal variation of VEGF, suggests that samples should be drawn after 7 am [29], which was done in both patients and controls in our study. Storage time has not been shown to correlate to the levels of VEGF in plasma stored in -80°C until analyzed [35]. We have not found data from previous studies about sampling factors that could affect the levels of EGF or MCP-1. In our study plasma levels of VEGF and EGF appeared to decrease during follow up, indicating that there is an association that could not only be explained by different sampling methods in controls and patients. Unfortunately, we have no follow up in controls, therefore we do not know if the levels of VEGF, EGF and MCP-1 changes over time in controls.

Other limitations are that the material did not allow for adjustment of nicotine status, hormonal status or body mass index.

Conclusion

The replication of high levels of VEGF and EGF in ED compared to healthy controls, and the decrease in plasma levels of VEGF and EGF within two years, adds important knowledge to the pathophysiology of ED. The study calls for further research in order to develop accurate analytical methods for possible use of VEGF and EGF as biomarkers for ED.

Acknowledgments

We thank research nurse Elisabeth Hollsten for her administrative assistance and blood sampling, PhD Kristina Wahlberg for recruiting patients and data collection, specialized psychologist Aniella Beser and certified biomedical analysist Lisbet Broman for their technical assistance.

Author Contributions

Conceived and designed the experiments: MÅ ÅN. Performed the experiments: MÅ ÅN HW FM. Analyzed the data: JW MÅ RS AN. Contributed reagents/materials/analysis tools: MÅ ÅN HW FM. Wrote the paper: JW MÅ ÅN RS HW FM AN.

References

1. Sandstrom A, Rhodin IN, Lundberg M, Olsson T, Nyberg L (2005) Impaired cognitive performance in patients with chronic burnout syndrome. Biol Psychol 69: 271–279. PMID: 15925030
2. Ekstedt M, Soderstrom M, Akerstedt T (2009) Sleep physiology in recovery from burnout. Biol Psychol 82: 267–273. doi: 10.1016/j.biopsycho.2009.08.006 PMID: 19699775

3. Sandstrom A, Peterson J, Sandstrom E, Lundberg M, Nyström IL, et al. (2011) Cognitive deficits in relation to personality type and hypothalamic-pituitary-adrenal (HPA) axis dysfunction in women with stress-related exhaustion. Scand J Psychol 52: 71–82. doi: 10.1111/j.1467-9450.2010.00844.x PMID: 20964695

4. Wahberg K, Ghatan PH, Modell S, Nygren Å, Ingvar M, et al. (2009) Suppressed neuroendocrine stress response in depressed women on stress-related long-term sick leave: a stable marker potentially suggestive of preexisting vulnerability. Biol Psychiatry 65: 742–747. doi: 10.1016/j.biopsycho.2008.10.035 PMID: 19058782

5. Rydmark I, Wahlberg K, Ghatan PH, Modell S, Nygren Å, et al. (2006) Neuroendocrine, cognitive and structural imaging characteristics of women on longterm sickleave with job stress-induced depression. Biol Psychiatry 60: 867–873. PMID: 16934773

6. Harvey SB, Wessely S, Kuh D, Hotopf M (2009) The relationship between fatigue and psychiatric disorders: evidence for the concept of neurasthenia. J Psychosom Res 66: 445–454. doi: 10.1016/j.jpsycho.2008.12.007 PMID: 19379961

7. Swedish National Board of Health and Welfare. (2003) Utmattningssyndrom. Stressrelaterad psykisk ohälsa (Exhaustion Syndrome. Stress related mental poor health). Stockholm. 2003 (in Swedish).

8. Savic I (2013) Structural Changes of the Brain in Relation to Occupational Stress. Cereb Cortex.

9. Blix E, Perski A, Berglund L, Savic I (2012) Course of mental symptoms in patients with stress-related exhaustion: does sex or age make a difference? BMC Psychiatry 12: 18. doi: 10.1186/1471-244X-12-18 PMID: 22409935

10. Glise K, Ahlborg G Jr., Jonsdottir IH (2012) Long-term Symptoms of Mental Fatigue in Patients with Stress-related Exhaustion Are Not Related to Sex, Age or Initial Burden of Symptoms. European Psychiatry 30: 989.

11. Åsberg M, Nygren A, Leopardi R, Rylander G, Peterson U, et al. (2009) Novel biochemical markers of psychosocial stress in women. PLoS One 4: e3590. doi: 10.1371/journal.pone.0003590 PMID: 19177163

12. Elving B, Buttenschon HN, Foldager L, Poulsen PH, Grynderup MB, et al. (2014) Depression and BMI influences the serum vascular endothelial growth factor level. Int J Neuropsychopharmacol 17: 1409–1417. doi: 10.1017/S1461145714000273 PMID: 24636631

13. Lu S, Peng H, Wang L, Vasish S, Zhang Y, et al. (2013) Elevated specific peripheral cytokines found in major depressive disorder patients with childhood trauma exposure: a cytokine antibody array analysis. Compr Psychiatry 54: 953–961. doi: 10.1016/j.comppsych.2013.03.026 PMID: 23639406

14. Kahl KG, Bens S, Ziegler K, Rudolf S, Kordon A, et al. (2009) Angiogenic factors in patients with major depressive disorder comorbid with borderline personality disorder. Psychoneuroendocrinology 34: 353–357. doi: 10.1016/j.psyneuen.2008.09.016 PMID: 19062198

15. Lee BH, Kim YK (2012) Increased plasma VEGF levels in major depressive or manic episodes in patients with mood disorders. J Affect Disord 136: 181–184. doi: 10.1016/j.jad.2011.07.021 PMID: 21862441

16. Takebayashi M, Hashimoto R, Hisao, K, Tsuchioka M, Kunugi H (2010) Plasma levels of vascular endothelial growth factor and fibroblast growth factor 2 in patients with major depressive disorders. J Neural Transm 117: 1119–1122. doi: 10.1007/s00702-010-0452-1 PMID: 20690032

17. Schmidt HD, Duman RS (2007) The role of neurotrophic factors in adult hippocampal neurogenesis, antidepressant treatments and animal models of depressive-like behavior. Behav Pharmacol 18: 391–418. PMID: 17762509

18. Segi-Nishida E, Warner-Schmidt JL, Duman RS (2008) Electroconvulsive seizure and VEGF increase the proliferation of neural stem-like cells in rat hippocampus. Proc Natl Acad Sci U S A 105: 11352–11357. doi: 10.1073/pnas.0710858105 PMID: 18682560

19. Heine VM, Zareno J, Maslman S, Joels M, Lucassen PJ (2005) Chronic stress in the adult dentate gyrus reduces cell proliferation near the vasculature and VEGF and Flk-1 protein expression. Eur J Neurosci 21: 1304–1314. PMID: 15813940

20. Lugger A, Calogero AE, Kalogeras K, Gallucci WT, Gold PW, et al. (1988) Interaction of epidermal growth factor with the hypothalamic-pituitary-adrenal axis: potential physiologic relevance. J Clin Endocrinol Metab 66: 334–337. PMID: 2828409

21. Lu XT, Liu YF, Zhao L, Li WJ, Yang RX, et al. (2013) Chronic psychological stress induces vascular inflammation in rabbits. Stress 16: 87–98. doi: 10.3109/10253890.2012.676696 PMID: 22428781
23. First MB G M, Spitzer RL, Williams JBW, Benjamin LS (1997a) Structured Clinical Interview for DSM-IV Axis II Personality Disorders (SCID-II). Washington DC, American Psychiatric Press
24. First MB S R, Gibbon M, Williams JBW (1997b) Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I). Washington DC, American Psychiatric Press.
25. DSM-IV (2000) Diagnostic and statistical manual of mental disorders. 4. Washington DC: American Psychiatric Association.
26. Jonsdottir IH, Hagg DA, Gliis 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. doi: 10.1371/journal.pone.0007659 PMID: 1988340
27. Holmes DI, Zachary I (2005) The vascular endothelial growth factor (VEGF) family: angiogenic factors in health and disease. Genome Biol 6: 209. PMID: 15693956
28. Shibuya M, Claesson-Welsh L (2006) Signal transduction by VEGF receptors in regulation of angiogenesis and lymphangiogenesis. Exp Cell Res 312: 549–560. PMID: 16336962
29. Hetland ML, Christensen IJ, Lottenburger T, Johansen JS, Svendsen MN, et al. (2008) Circulating VEGF as a biological marker in patients with rheumatoid arthritis? Preanalytical and biological variability in healthy persons and in patients. Dis Markers 24: 1–10. PMID: 18057530
30. Kleiner G, Marcuzzi A, Zanin V, Monasta L, Zauli G (2013) Cytokine levels in the serum of healthy subjects. Mediators Inflamm 2013: 434010. doi: 10.1155/2013/434010 PMID: 23533306
31. Arakelyan A, Petkova J, Hermanova Z, Boyajyan A, Luk J, et al. (2005) Serum levels of the MCP-1 chemokine in patients with ischemic stroke and myocardial infarction. Mediators Inflamm 2005: 175–179. PMID: 16106105
32. Mirhafiz SR, Mohebati M, Feiz Disfani M, Saberi Karimian M, Ebrahimi M, et al. (2014) An imbalance in serum concentrations of inflammatory and anti-inflammatory cytokines in hypertension. J Am Soc Hypertens 8: 614–623. doi: 10.1016/j.jsh.2014.05.007 PMID: 25224864
33. Bryngelson A, Asberg M, Nygren A, Jensen I, Mittendorfer-Rutz E (2013) All-Cause and Cause-Specific Mortality after Long-Term Sickness Absence for Psychiatric Disorders: A Prospective Cohort Study. PLoS One 8: e67887. PMID: 23940764
34. The Swedish Social Insurance Agency (2014) Social Insurance Report 2014:4.
35. Isung J, Mobarrez F, Nordstrom P, Asberg M, Jokinen J (2012) Low plasma vascular endothelial growth factor (VEGF) associated with completed suicide. World J Biol Psychiatry 13: 468–473. doi: 10.3109/15622975.2011.624549 PMID: 22098148