Reduced vascular endothelial growth factor levels in the cerebrospinal fluid in patients with treatment resistant major depression and the effects of electroconvulsive therapy.

Laura Kranaster a, Kaj Blennow b, c, Henrik Zetterberg b, c, d, e, Alexander Sartorius a

a Department of Psychiatry and Psychotherapy, Central Institute of Mental Health, Medical Faculty Mannheim / Heidelberg University, Mannheim, Germany
b Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, the Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden
c Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden
d Department of Neurodegenerative Disease, UCL Institute of Neurology, London, UK
e UK Dementia Research Institute at UCL, London, UK.

Corresponding author
Laura Kranaster
Department of Psychiatry and Psychotherapy
Central Institute of Mental Health, Medical Faculty Mannheim / Heidelberg University
J5, 68159 Mannheim, Germany
Phone: +49 621 1703-0 - Fax: +49 621 1703-1205 - Mail: laura.kranaster@zi-mannheim.de
ORCID: 0000-0002-8414-0910

Acknowledgments
LK received support by the German Research Foundation (DFG - grant No. KR 4689/3-1).
KB is supported by the Torsten Söderberg foundation, Stockholm, Sweden. HZ is a Wallenberg Academy Fellow supported by grants from the Swedish Research Council (#2018-02532), the European Research Council (#681712) and Swedish State Support for Clinical Research (#ALFGBG-720931).
Abstract

Keywords

ECT; electroconvulsive therapy; vascular endothelial growth factor; depression; affective disorder; cerebrospinal fluid
Introduction

The neurotrophin hypothesis of depression postulates a major contribution of stress-induced decrease in neurotrophin levels to the development and maintenance of depression (Duman, Heninger et al. 1997, Altar 1999, Henn, Vollmayr et al. 2004, Pittenger and Duman 2008). Beneath the most prominent neurotrophin brain-derived neurotrophic factor (BDNF), several lines of evidence are pointing towards an involvement of the vascular endothelial growth factor (VEGF) (Warner-Schmidt and Duman 2008, Nowacka and Obuchowicz 2012, Newton, Fournier et al. 2013). VEGF is widely expressed both centrally and peripherally (Duffy, Bouchier-Hayes et al. 2000–2013). Within the central nervous system (CNS), VEGF plays a role in synaptic transmission (McCloskey, Croll et al. 2005), neurogenesis (Duric and Duman 2013) and memory (Clark-Raymond and Halaris 2013). There are several studies comparing plasma or serum levels of VEGF in patients with depression compared to healthy controls. Albeit some inconsistent findings, meta-analyses found in general higher VEGF levels in patients (Carvalho, Kohler et al. 2015, Sharma, da Costa e Silva et al. 2016). However, it is not known how blood VEGF levels relate to changes in brain VEGF, and there is no study on cerebrospinal fluid (CSF) levels of VEGF in patients with depression as compared with controls. One study showed that CSF VEGF levels in suicide attempters, of whom 79% fulfilled criteria for mood disorder, were lower than in healthy controls (Isung, Ainehband et al. 2012), with suicide attempters having the highest depression scores, as measured on MADRS, had the lowest CSF levels of VEGF. This contrasts other results from serum studies, possibly indicating that serum VEGF levels might reflect on-going central nervous processes in a less valid manner.

Concerning treatment effects, results are even more inconsistent in terms of serum or plasma VEGF levels and antidepressant pharmacotherapy (Deuschle, Gilles et al. 2015, Sharma, da Costa e Silva et al. 2016) or electroconvulsive therapy (ECT) (Minelli, Zanardini et al. 2011, Minelli, Maffioletti et al. 2014, Ryan and McLoughlin 2018). Up to date, there are neither preclinical nor clinical data available about the course of VEGF levels in the CSF during an
antidepressant treatment, such as pharmacotherapy or ECT. However, this seems to be relevant, because it has been shown, that CSF levels of VEGF do not correlate with plasma levels - at least in those mainly depressed suicide attempters (Isung, Aeinehband et al. 2012), which led the authors assume that the lack of correlation between blood and CSF levels might reflect differences in local production of VEGF. In general, CSF represent processes from the central nervous system (CNS) more precisely than blood, especially when products are analyzed that are known to possess ubiquitarian functions within and outside the CNS, such as VEGF.

In this study, our aims were to analyse the VEGF levels in the CSF in patients with a major depressive episode and compared them with healthy controls and to analyse those CSF levels in patients prior and after a course of ECT. ECT is a highly effective treatment option for severe and treatment-resistant forms of depression (REF for a review?). The complex mode of action of ECT has not yet been fully unravelled, but among other mechanism, it is considered to facilitate neurogenesis, neural plasticity and gray matter growth (Sartorius, Hellweg et al. 2009, Inta, Lima-Ojeda et al. 2013, Rotheneichner, Lange et al. 2014, Sartorius, Demirakca et al. 2016, Sartorius, Demirakca et al. 2018). Additionally, it has been shown that serum or plasma BDNF levels are increased by ECT (Rocha, Dondossola et al. 2016). In terms of VEGF, it has been shown that VEGF is strongly upregulated in the hippocampus by electroconvulsive seizures (ECS) in animal models (Newton, Collier et al. 2003, Altar, Laeng et al. 2004) and has been suggested to represent an important mediator of the neurogenic effect of ECS in the adult [human?] brain (Fournier and Duman 2012).

Based on the available data of suicide attempters, we hypothesize that VEGF CSF level will be lower in severely depressed patients compared to age-matched healthy controls. Additionally, based on the preclinical data of ECS and the clinical data of ECT in patients with depression we assume that ECT might lead to an increase of VEGF levels in the CSF.

**Methods**
Patients

Our prospective study was approved by the ethics committee and all procedures performed in the study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study. The study took place from 2013 to 2015 at the Department of Psychiatry at the Central Institute of Mental Health in Mannheim, Germany. Inclusion criteria were a present depressive episode within the context of a diagnosis of either major depressive disorder or bipolar disorder according to DSM-IV, age above 18 years and the clinical decision for an ECT treatment. Exclusion criteria were substance-related disorders and a lifetime diagnosis of schizophrenia. The Hamilton Depression Rating Scale (HDRS; 21 items version) and Mini-Mental-State Examination (MMSE) were used to assess the severity of depression and cognitive performance, respectively, before and after ECT. A clinical response to treatment was defined as a reduction of at least 50% of the initial HDRS score during the course of ECT.

ECT treatment

Right unilateral brief pulse ECT was performed with a Thymatron IV device (Somatics, LLC. Lake Bluff, IL, USA). S-ketamine (Kranaster, Kammerer-Ciernioch et al. 2011, Hoyer, Kranaster et al. 2014) was used as the anaesthetic substance (~1.0 mg/kg) and succinylcholine was used for muscle relaxation (~1.0 mg/kg). Seizure threshold in all patients was titrated at the initial session and dosing at subsequent treatments was given at least 2.5 times threshold. Seizure monitoring was performed by determining motor convulsive activity with the “cuff” method and electroencephalogram monitoring by the ECT device with bilateral frontomastoid leads. The charge was subsequently increased if patients did not respond clinically or if seizures were insufficient during the ECT course according to established recommendations (American Psychiatric Association 2001). Three ECT sessions per week were performed. All patients started with right unilateral stimulation, in the case of non-response it was possible to switch to bilateral stimulation as a decision of the ECT
supervisor. The patients continued their prior psychotropic medication during the ECT treatment.

Controls

Control samples were from age- and sex-matched patients who sought medical advice because of cognitive impairment without clinical suspicion on depression. Patients were designated as not suffering from Alzheimer’s disease since they had normal CSF T-tau, P-tau and Aβ42 biomarker concentrations (determined using INNOTEST ELISAs, Fujirebio, Ghent, Belgium), according to established cut-offs (ref: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16488378).

Sampling

One day before the first ECT session and between one and seven days after the last ECT session, CSF samples were drawn at around 9:00 am. Lumbar punctures were performed according to standardized procedure. CSF samples were obtained, centrifuged (2000 x g), aliquoted and frozen at -80°C within 30 minutes. The samples were stored at -80°C and were never thawed or refrozen prior to VEGF analysis.

CSF laboratory analyses

CSF levels of VEGF were analyzed using the V-PLEX Plus Human VEGF Kit (MesoScale, Rockville, Maryland). All samples were analyzed in one run, using the same batch of reagents, by Board-certified technicians who were blinded to the clinical data, at the Clinical Neurochemistry Laboratory at Sahlgrenska University Hospital, Mölndal, Sweden. Intra-assay coefficients of variation were below 10%.

Statistical analyses

Statistics were performed using STATA® (StataCorp, Texas 77845, USA, version 11) at a significance level ≤0.05. Normality was examined using the Shapiro-Wilk test, because they
were not normally distributed, non-parametric tests were used. The differences between CSF VEGF levels of patients with depression compared to healthy controls was tested with the Mann-Whitney U test. The impact of ECT on changes in the CSF VEGF concentration was calculated with the Wilcoxon matched-pairs signed-ranks test. The difference of changes in the CSF VEGF concentration during ECT between responders and non-responders was tested with the Mann-Whitney U test. Correlations were tested with the Spearman’s rank correlation. Values below the level of quantification of 1.41 pg/ml (LOQ) were substituted with LOD/2 (Ogden 2010). No corrections for multiple testing were made.

**Results**

We included 12 patients into the study, who underwent a completed course of ECT and additionally lumbar puncture before and after. In five samples, VEGF levels were below the limit of quantification of 1.41 pg/ml (in one patient in both samples and two other patients in the baseline samples and in one other patient in the sample after ECT). We included 20 healthy controls for baseline comparison and in one sample, the VEGF level was below the limit of quantification. Demographic and clinical features are shown in Table 1. In summary, the groups were similar in terms of age (p=0.44) and gender distribution (p=0.72).

*Differences between patients and controls and possible moderators*

The patients with depression showed lower mean VEGF levels in the CSF than healthy controls (1.98 pg/ml ±1.09 vs. 2.72 pg/ml ±1.33; z =2.1; p=0.041). In our sample, CSF VEGF levels were only associated with age in the group of controls (p=0.004), but not in the group of patients (p=0.72). CSF VEGF was not associated with sex (p=0.36) in both group and not with bipolarity (p=0.26), duration of illness (p=0.57), duration of current episode (p=0.97) and MMSE at baseline (p=0.90) in the group of the patients.

*Differences before and after ECT and correlation with numbers of ECT sessions, time intervals and other ECT parameters*
CSF VEGF concentration at baseline (1.97 pg/ml ±1.09) and after the complete ECT treatment (1.94 pg/ml ±0.88) did not differ from each other (p=0.78, r=0.02). The changes from baseline to final levels were not associated with the number of individual single ECT sessions that were performed in each patient (p=0.49). The CSF VEGF changes during ECT was neither affected by amount of days between the both CSF samples (p=0.83), nor between the amount of days between the last ECT session and the second CSF sample (p=0.28). The VEGF levels of the group of patients, who were switched to bilateral stimulation during the course of ECT did not differ from those who were treated solely unilateral (p=0.41).

CSF VEGF levels and antidepressant efficacy

The changes of VEGF levels during ECT were not associated with change of the extent of depressive symptoms during ECT (p=0.56). The group of responders and remitters to the treatment did not differ in terms of changes of VEGF concentration during ECT to non-responders (n=2; p=0.99). The baseline levels of CSF VEGF were neither associated with the reduction of depressive psychopathology during ECT (p=0.19), nor the therapeutic outcome, measured by response (p=0.45) or remission (p=0.51).

Discussion

This is the first study that investigates VEGF CSF levels in patients with depression compared with controls and that also compared CSF VEGF levels before and after the antidepressant treatment of a course of ECT.

As expected from previous data in suicide attempters, who were mostly diagnosed with a depressive episode (Isung, Aeinehband et al. 2012), the VEGF CSF levels in our clinical sample of patients with treatment-resistant depression were lower than the levels of the age- and sex-matched controls. Importantly, this contrasts findings from previous studies based on measuring VEGD in serum or plasma, which found that VEGF levels in patients with depression often were higher than in controls. Because the CSF measurements likely reflects brain pathophysiology better than blood, it can be assumed that VEGF studies from serum or
plasma are less valid in terms of measuring parameters that reflect on-going CNS processes, especially when taking into account that VEGF contains pleiotropic properties in the periphery (Bates, Beazley-Long et al. 2018) and in the CNS (Ruiz de Almodovar, Lambrechts et al. 2009, Nowacka and Obuchowicz 2012). From a neurobiological view, it seems indeed more plausible, that levels of VEGF as a neurotrophic factor is downregulated in depression, similar to the dynamics of BDNF (Polyakova, Stuke et al. 2015). Unfortunately, we cannot provide data from serum levels in parallel, thus the question of a relationship between CSF and serum levels must remain unanswered. However, at least in the study from Isung and colleagues, no such a correlation was found (Isung, Aeinehband et al. 2012). Another explanation for the discrepant finding of CSF and serum studies is that in both, in the study from Isung and colleagues and in our current study the patients with depression belonged to a subgroup of the severely ill patients, that either tried to commit suicide or were considered as treatment-resistant. In the serum studies the population was more mixed concerning extent of depressive symptoms, status of treatment-resistance and suicidality (Carvalho, Kohler et al. 2015, Sharma, da Costa e Silva et al. 2016).

In contrast to our hypothesis, which was mainly based on preclinical (Segi-Nishida 2011, Elfving and Wegener 2012) and clinical data (Minelli, Zanardini et al. 2011, Minelli, Maffioletti et al. 2014, Ryan and McLoughlin 2018) that VEGF could mediate the effects of electroconvulsive treatment, we did not find differences in the CSF VEGF levels prior and after treatment. Additionally, no associations could be detected with either number of individual ECT sessions or when comparing group differences between responders and non-responders and between remitters and non-remitters. Beneath a statistical power problem or no effect of ECT on CSF VEGF levels at all as reasons for our negative findings, the timing of the second sample might have been suboptimal. It has been shown, that serum VEGF levels that were taken before the treatment did not differ from those in samples that were the day after the end of ECT, but were lower compared to samples that were taken one month after the end of ECT (Minelli, Zanardini et al. 2011). This finding of an on-going phase of re-equilibrating after ECT
already been shown in preclinical and clinical studies for BDNF (Sartorius, Hellweg et al. 2009, Bumb, Aksay et al. 2015).

The major limitation of this study is of course the small sample size, but the difficulty in recruiting severely depressed patients who agree to undergo lumbar puncture twice for research purposes only in addition to a course of ECT should not be underestimated. It could not be excluded that an insufficient statistical power led to the negative finding of no ECT-induced VEGF rise. However, it was possible to detect changes in other systems in that small sample (Kranaster, Aksay et al. 2016, Kranaster, Hoyer et al. 2017, Hoyer, Sartorius et al. 2018, Kranaster, Hoyer et al. 2018) and at least a strong effect of ECT on VEGF appears unlikely. Anyway, our results are preliminary until a larger replication study might verify them. Other limitations are that we can neither provide data from serum levels nor data from samples that were taken several weeks after the ECT, which might have added much value to our work, and that the control groups was not completely healthy and not well-characterised according to medication. With regard to pharmacotherapy as another potentially confounding factor, patients continued some of their psychotropic medication (antidepressants, antipsychotics, benzodiazepines, lithium) during the ECT treatment, which reflects real world conditions. Medication in each patient was at least kept constant during the ECT course. Finally, we cannot exclude the possible influence of the repeated anaesthesia and muscle relaxant treatment (Stelzhammer, Guest et al. 2013) and repeated lumbar punctures on our results.

In conclusion, we could show for the first time that CSF VEGF concentrations are lower in a clinical sample of patients with treatment-resistant depression compared with matched controls. Additionally, no change in CSF VEGF levels during a course of ECT could be detected in our sample.

**Conflict of interest**

KB has served as a consultant or at advisory boards for Alector, Alzheon, CogRx, Biogen, Lilly, Novartis and Roche Diagnostics, all unrelated to the work presented in this paper. HZ has served at scientific advisory boards for Roche Diagnostics, Samunmed, Wave and
CogRx, and has given lectures in Alzecure-sponsored meetings, all unrelated to the work presented in this paper. KB and HZ are co-founders of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg also unrelated to the work presented in this paper. The other authors declare that they have no conflict of interest.
References


Duffy, A., D. Boucher-Hayes and J. Harney (2000–2013). Vascular endothelial growth factor (VEGF) and its role in non-endothelial cells: Autocrine signalling by VEGF. Austin (TX), Landes Bioscience, Madame Curie Bioscience Database [Internet].


### Tables

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of included subjects</td>
<td>12</td>
<td>20</td>
</tr>
<tr>
<td>Age (mean) ± SD in years</td>
<td>59.0 ± 21.9</td>
<td>63.9 ±13.1</td>
</tr>
<tr>
<td>Sex female/male n/n (in %)</td>
<td>7/5 (58.3/41.7)</td>
<td>9/11 (45.0/55.0)</td>
</tr>
<tr>
<td>Type of depression: unipolar/bipolar n/n (in %)</td>
<td>9/3 (75.0/25.0)</td>
<td>-</td>
</tr>
<tr>
<td>HDRS, sum score</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Baseline HDRS, mean ± SD</td>
<td>29.9 ± 6.6</td>
<td>-</td>
</tr>
<tr>
<td>Final HDRS, mean ± SD</td>
<td>9.0 ± 5.2</td>
<td>-</td>
</tr>
<tr>
<td>Mean change</td>
<td>-20.9 (p&lt;0.001)</td>
<td>-</td>
</tr>
<tr>
<td>Numbers of ECT sessions, mean ± SD</td>
<td>10.6 ±5.0</td>
<td>-</td>
</tr>
<tr>
<td>Switch to bilateral stimulation n (in %)</td>
<td>3 (25)</td>
<td>-</td>
</tr>
<tr>
<td>Responders/Non-responders* n/n (in %)</td>
<td>10/2 (83.4/16.7)</td>
<td>-</td>
</tr>
<tr>
<td>Baseline MMSE, mean ± SD</td>
<td>26.1 ± 4.3</td>
<td>-</td>
</tr>
<tr>
<td>CSF VEGF levels</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Baseline , mean ± SD</td>
<td>1.97 ±1.09</td>
<td>2.72 ±1.33</td>
</tr>
<tr>
<td>Final HDRS, mean ± SD</td>
<td>1.94 ±0.88</td>
<td>-</td>
</tr>
<tr>
<td>Mean change</td>
<td>-0.10 ±0.52</td>
<td>(p=0.78)</td>
</tr>
</tbody>
</table>

Table 1: Demographic and clinical features of the patients.

* Response is defined as a reduction of at least 50% in symptoms, measured by the HDRS.