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Alterations in the serum proteome following electroconvulsive therapy for a major depressive episode: a longitudinal multicenter study

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Figures 1
Tables 2
References 65
Abstract

BACKGROUND: Electroconvulsive therapy (ECT) is the most effective treatment for severe depression, but the biological changes induced by ECT remain poorly understood.

METHODS: This study investigated alterations in blood serum proteins in 309 patients admitted to ECT for a major depressive episode. We analyzed 201 proteins in samples collected at three time-points: just before the first ECT treatment session (T0), within 30 minutes after the first ECT session (T1), and just before the sixth ECT session (T2).

RESULTS: Using statistical models to account for repeated sampling, we identified 152 and 70 significantly (<5% false discovery rate) altered proteins at T1 and T2, respectively. The most pronounced alterations at T1 were transient increased levels of prolactin, myoglobin, and kallikrein-6. However, most proteins had decreased levels at T1, with the largest effects observed for pro-epidermal growth factor, proto-oncogene tyrosine-protein kinase Src, tumor necrosis factor ligand superfamily member 14, sulfotransferase 1A1, early activation antigen CD69, and CD40 ligand. The change of several acutely altered proteins correlated with electric current and pulse frequency in a dose-response-like manner. Over a five-session course of ECT, some acutely altered levels were sustained while others increased, e.g., serine protease 8 and chitinase-3-like protein 1. None of the studied protein biomarkers were associated with clinical response to ECT.

CONCLUSIONS: We report experimental data on alterations in the circulating proteome triggered by ECT in a clinical setting. The findings implicate hormonal signaling, the immune system, apoptotic processes, and more. None of the findings were associated with clinical response to ECT.
Introduction

Depressive disorders affect millions of people every year and incur substantial societal costs (1). Strong evidence supports the use of electroconvulsive therapy (ECT) in severe depression resistant to antidepressants, particularly when psychotic symptoms or suicidal ideation are present (2-4). ECT yielded an overall clinical response rate of 80% in a large Swedish study (5, 6).

Despite the effectiveness and long history of ECT, the mechanism of action remains unknown. A wide array of hypotheses has been suggested, involving hormones, neurotrophic factors, and neurotransmitters (see reviews (7-9)) These hypotheses are based on previous studies demonstrating that ECT results in release of pituitary hormones (10), altered levels of neurotropic factors (11), affects neuroinflammation (12), as well as neurotransmitters such as serotonin, glutamate, GABA, norepinephrine, and neuropeptides (13). If better understood, biochemical changes caused by ECT may reveal novel insights into the pathomechanisms of depression as well as point to new potential pharmacological targets.

Changes in circulating levels of blood proteins offer a window into the biological mechanisms underlying ECT. Previous studies have reported ECT-related changes in a few selected proteins, which include conflicting findings on brain-derived neurotrophic factor (9, 14-16), acutely increased but long-term decreased levels of interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-alpha) suggesting a normalization of depression-related microglial activation (17), and unaltered levels of S100B and neuron-specific enolase suggesting absence of significant neuronal damage (18-20). Yet, only two small studies have explored a broader set of the blood serum proteome in the context of ECT (21, 22). Proteomic techniques have been used to study the pharmacological response of both antidepressants (23, 24) and ketamine (25), and findings from other fields have demonstrated the potential of proteomics to both unravel novel biology (e.g., in Alzheimer’s disease (26)), and to develop clinically viable biomarker panels (e.g., in ovarian cancer (27)).
Although ECT is very effective on average, some patients show sub-optimal response to ECT and biomarkers that predict the outcome of ECT could be leveraged to adapt a personalized treatment strategy. Increased volume in the dentate gyrus (28), differential trajectories in interleukin-8 blood plasma concentrations (29), and common genetic variants (30) have independently been suggested as predictors of clinical response to ECT. However, the predictive accuracy of these suggested biomarkers falls short in comparison with clinical predictors, and more explorative biomarker studies are needed.

In the largest study to date, we employed proximity extension assay to investigate changes in circulating levels of 201 unique blood serum proteins triggered by ECT. We also studied proteins in relation to treatment response. By analyzing samples in a repeated measures design, we not only replicate some previous findings but also unravel novel biological processes altered during the course of ECT.
Methods

Patients

Characteristics of the study cohort are presented in Table 1. Study participants (n=309, age range 18–86 years) were patients with a major depressive episode (MDE) scheduled for an index ECT series (3 sessions per week, minimum 6 planned sessions) recruited at 7 hospitals in Sweden (Danderyd, Huddinge, Hudiksvall, Göteborg, Umeå, Uppsala, and Örebro). The study was conducted 2013–2017. All participants provided oral and written informed consent. The study was approved by the Ethical review board in Stockholm, Sweden. Given that the study objective was to investigate within-subject effects of ECT on serum protein levels, we neither included a healthy control group, nor patients with a MDE treated with other modalities (cf. (31, 32)).

Diagnostic assessments

Diagnoses were made by the referring psychiatrists and entered into the Swedish national quality register for ECT (Q-ECT) along with current medications and demographic data (33). For evaluation of baseline depressive symptoms, patients completed the Montgomery Åsberg Depression Rating Scale – Self report (MADRS-S) (34, 35). Clinicians rated the disease severity using the Clinical Global Impression-Severity of Illness scale (CGI-S) (36). Clinicians also completed the CGI-Improvement scale (CGI-I) after the completed ECT series (n=234) (36). Patients were excluded if the Q-ECT was not completed in a timely fashion (within 10 days) (n=12), they received fewer than 6 ECT sessions (n=9), they received ECT for an indication other than MDE (n=24), or they did not provide a T0 sample (n=4). The final analyzed cohort (n=260) included only patients receiving ECT for MDE within the context of (a) major depressive disorder (ICD-10 codes F320, F321, F322, F323, F329, F331, F332, F333, F339, F530); (b) bipolar disorder (F313, F314, F315); (c) another mood disorder (F318, F399, F412, F259) with a pretreatment MADRS-S score ≥20; or (d) patients missing specific indication but where MDE was indicated in free text or implied by a pretreatment MADRS-S score ≥20 (see Table 1). ECT was administered according to Swedish guidelines (37). Unilateral placement is standard, and age-based dosing is used with modifications throughout the treatment series according to clinical
effects and seizure quality. ECT parameters from this study are summarized in Supplementary table 1. In brief, unilateral administration was used for most cases (n=242, 93%) and the most frequent induction agents were thiopental (n=163, 63%) and propofol (n=95, 37%).

Study design

The study was designed to investigate acute and longitudinal effects of ECT. Blood samples were drawn at three different time points (Figure 1a): just before the first treatment session and prior to anesthesia (T0); within 30 minutes after the first treatment (T1); and just before the sixth treatment and prior to anesthesia (T2). Using this design, changes in protein levels from T0 to T1 reflect acute alterations after an ECT session, whereas changes from T0 to T2 reflect longer term changes in the blood serum proteome, occurring during the course of treatment but unrelated to the immediate effects of an ECT administration. Samples were collected in the morning with patients fasting. T2 was sampled prior to the sixth session because six is a frequent minimum number of sessions and this design has been previously employed (21, 31). T2 occurred at least 48 hours after the fifth ECT session. To sample the very last session, one would need to either sample each session or know the total number of sessions in advance; this number is based on an evolving clinical need and thus unknown in clinical practice. All patients in the final cohort hence received at least six ECT sessions (median [range] = 8 [6-23]).

Sample collection

Blood samples were drawn in 10 ml serum tubes (Becton, Dickinson and Co), coagulated for 30–60 minutes at room temperature, and subsequently centrifuged for 15 minutes at 2000 x g. Blood serum aliquots were stored locally at the participating hospitals at -20°C for a maximum of 30 days pending transport and storage at -70°C at the Karolinska Institutet Biobank. Nearly all 309 included participants provided samples at all three time points, totaling 914 samples. 13 samples were missing from 11 patients (nT0=4, nT1=4, nT2=5).
Multiplex immunoassays

We analyzed blood serum levels of 201 unique proteins using three Olink Proteomics® panels (Inflammation v.3001, CVD I v.2002, and Oncology I v.4001). These proximity extension assays combine the interaction of two specific antibodies with a real-time quantitative polymerase chain reaction readout. This method allows for multiplex analysis of a large number of assays with low levels of interfering cross-talk (38). Samples were analyzed in two waves (n=329 and n=585). In each wave, samples were randomized across plates (4 plates in wave 1 and 7 plates in wave 2) comprising up to 92 samples per plate in a random distribution of samples from all three time points.

Preprocessing and quality control

Initial preprocessing and quality control were conducted by Olink Proteomics®, delivering data in the normalized protein expression (NPX) format (39). The NPX values represent relative protein abundance on a log2 scale: a one unit increase in NPX corresponds to doubling the absolute concentration of an analyte. Additionally, five clear outlier samples were removed in a combined assessment based on Olink’s internal quality control steps (https://www.olink.com/resources-support/white-papers-from-olink/), principal component analysis scores, and extreme outlier values (NPX < -10 x IQR). Finally, the brain-derived neurotrophic factor (BDNF) assay was excluded due to technical issues.

The inter-panel correlations for assays represented on multiple panels (n=65) were large (median [interquartile range (IQR)] r = 0.94 [0.91, 0.96]). We therefore discarded values from duplicate assays on the panels with most quality control flags. Assays with >30% of values below the limit of detection (39) at ≥2 time points were also excluded. Finally, the waves were combined, and values were scaled with T0 values as reference (mean\(_{\text{T0}}\)=0, standard deviation\(_{\text{T0}}\)=1). The final dataset comprised 180 unique proteins passing quality control in 260 patients (n=773 samples). Supplementary Table 2 lists all studied proteins.
Statistical analyses

The statistical analyses served two main objectives: (1) to estimate changes in protein concentration from baseline (i.e., T0) to T1 and from T0 to T2; and (2) to identify changes in protein concentration indicative of treatment response. To estimate changes from baseline, we employed a generalized least squares (gls) model to estimate the mean value across time for each protein assay. We used an unstructured covariance pattern, i.e., allowing both variance and covariance to be estimated freely across the three measurement points, to account for the longitudinal nature of the data (40). The models were fitted with time, age, sex, and plate number as covariates. The percent change from baseline was estimated by \(100 \times (2^{\beta} – 1)\), where \(\beta\) is the estimate from gls-models (log2-scale) at each time-point. To investigate treatment response, we added an interaction term of treatment response and time. Treatment response was dichotomized from clinicians’ CGI-improvement ratings (see results). \(P\)-values from the gls models were adjusted using the false discovery rate (FDR) (41) where FDR<5% was considered significant. Finally, to test whether specific ECT parameters from the first treatment session influenced the protein levels at T1, we analyzed rank correlations between individual ECT parameters and the estimated mean values at T1 from the gls models.

To explore converging pathways and functions, we annotated the top altered proteins using public databases (gene ontology (GO) (42), PANTHER (43), KEGG pathways, STRING-db (44)). Given the targeted set of proteins and the absence of a control group, we were unable to statistically test for pathway enrichment. All analyses were conducted in R (v.4.1.1) (45) using external packages: nlme (v.3.1), tidyverse (v.1.3.1), and Hmisc (v.4.5). The code is available at github.com/andreasgoteson.
Results

Protein differential abundance analyses

In 260 included individuals with MDE who underwent an index ECT series, we sampled blood serum at three time points to compare protein levels at baseline (T0), 30 minutes after the first ECT session (T1), and just before the sixth ECT session (T2). Out of the 180 included proteins, 152 showed significant (<5% FDR) changes in protein concentration between baseline (T0) and T1, adjusted for relevant covariates (Figure 1b). The +114% increase in prolactin was the largest change observed at T1, along with highly increased levels of myoglobin and kallikrein-6. However, the majority (n=116) of the significantly altered proteins showed decreases at T1, with large effect sizes seen for pro-epidermal growth factor (EGF, -49%), proto-oncogene tyrosine-protein kinase Src (SRC), sulfotransferase 1A1 (SULT1A1), and tumor necrosis factor superfamily member 14 (TNFSF14).

With regard to changes from T0 to T2, 70 proteins showed significantly altered concentrations (Figure 1c). Again, most (n=50) of the significantly altered proteins showed decreased circulating levels at T2, with the largest effect sizes seen for EGF (-33%), SRC, and SULT1A1. Serine protease 8 (+21%) and chitinase-3-like protein 1 (+14%) were the top proteins with increased levels at T2. Table 2 lists the 20 proteins with largest magnitude of change from T0 to T1 and from T0 to T2.

Association with ECT parameters

We next explored the influence of ECT parameters on the magnitude of change at T1 with the hypothesis that a higher fold change would correlate with applied current. Indeed, for most of the proteins with significantly altered levels at T1, we found a significant ($P < 0.05$) correlation with electric current in the expected direction (Figure 1d). Some notable exceptions include kallikrein-6, which increased at T1 but correlated negatively with electric current, and prolactin which only correlated significantly with EEG seizure duration.
**Associations with treatment response**

To identify proteins associated with clinical treatment response, we investigated the effect of response as well as the interaction effect of time and response on T1 and T2 protein levels.

Treatment response was defined by clinicians’ rating of CGI-improvement, where a score of 1–2 (‘very much improved’ and ‘much improved’) was considered ‘response’ (n=180, 79%) and all other records were considered ‘non-response’ (n=51, 21%, CGI-I missing from 30 individuals). The largest effect sizes per term were a negative response estimate for lymphotoxin-alpha (est. (se) = -0.4 (0.14), $P = 0.0035$), a negative interaction effect with parkinson disease protein 7 at T1 (est. (se) = -0.36 (0.16), $P = 0.025$), and a negative interaction effect of oncostatin-M at T2 (est. (se) = -0.28 (0.13), $P = 0.038$). However, none of the associations survived correction for multiple testing (5% FDR). Complete results, as well as estimated response curves, are presented in Supplemental Table 5 and Supplemental Figures 1a-e.

**Functional annotations**

Finally, we annotated proteins with a significant (5% FDR) change of more than ±10% from baseline to functional databases to explore converging functions and pathways (Supplementary Figures 2-6). Most differentially abundant proteins were either intercellular signaling molecules or transmembrane signaling receptors involved in signal transduction, inflammatory response, and apoptotic processes. The biological processes associated with acutely (T0 – T1) altered proteins include cell-cell signaling like immune response and TNF-mediated signaling, proteolysis, and response to mechanical stimulus and hypoxia. By contrast, proteins that changed from T0 to T2 were related to regulatory processes such as regulation of MAPK/ERK cascade and GTPase activity.
Discussion

This study was conducted to understand how ECT affects circulating proteins in a large longitudinal sample of MDE patients (n=260) receiving ECT treatment. We sampled blood serum at the beginning of the first ECT session (T0), 30 minutes after the first session (T1), and at the beginning of the sixth session (T2). We then analyzed 201 unique proteins reflecting a broad set of biological processes. The most pronounced changes include transient increased levels of prolactin, myoglobin, and kallikrein-6, as well as decreased levels of EGF, SRC, TNFSF14, CD69, and CD40L/CD40, which were observed between T0 and T1 and partly sustained to T2. We also analyzed protein levels in relation to clinical treatment response but found no statistically significant associations. Taken together, our findings elucidate numerous biological processes altered by ECT, including pituitary hormone signaling, immune response, apoptotic processes, MAPK/ERK signal transduction, and protease activity.

To our knowledge, there are only two prior small studies investigating changes in the circulating blood serum proteome over an index ECT series. In line with a pilot study (n=12) by Stelzhammer and colleagues (21), we report acute decreased levels of EGF, CD40-L, CD40, MMP-1, MPO, IL1-ra, S100A12, resistin, CXCL10 and CCL4, but found inverse fold change in PGF and SCF, and did not replicate altered levels of IL8 and CXCL9. Ryan and coworkers (22) used two-dimensional difference in gel electrophoresis coupled with mass spectrometry and identified 36 proteins altered by ECT, whereof none was included in the protein panels utilized in this study.

Cellular response to stimulus

The most pronounced finding was a transient doubling of serum levels of prolactin, which replicates previous reports (46, 47). Prolactin secreted into the blood stream targets numerous cytokine receptors, thereby regulating various processes in reproduction, metabolism, and immune system regulation (48). In the central nervous system (CNS), prolactin interacts with the dopaminergic system (49) and has various trophic and neuroprotective effects on glial cells (50). The transient
surge in prolactin is likely stress-induced (48), and several other proteins involved in maintaining cellular integrity in response to stress were also altered at T1 (e.g., HSP-27, VIM, SIRT2, CDKN1A). Myoglobin also showed a marked transient increase at T1, most likely caused by muscle contractions during seizure. Myoglobin has been suggested as a biomarker for ECT-related muscle damage (51), but was notably not correlated with EEG seizure time in our data.

**Immune response**

Previous studies have demonstrated that ECT triggers an acute immune response (17) where our study provides higher temporal and molecular specificity. Within 30 minutes after the first ECT session (T1), we detected decreased circulating levels of proteins involved in the early stages of the immune response such as T cell activation: early activation antigen CD69 expressed by naïve T cells, costimulatory markers involved in T cell activation (i.e., CD40, CD40LG, TNFSF14), and increased levels of IL-7 promoting T cell development in bone marrow (52). Notably, IL-6, a proinflammatory cytokine of previous interest in depression research (53), showed only a minor increase at T1 (+4%). Moreover, the hypertensive and inflammatory reaction induced by ECT might cause blood vessels to non-selectively leak plasma components into the tissue. Such leakage could explain the marginally decreased levels of most proteins at T1 compared with T0. Our findings further indicate augmented activity in the FAS signaling apoptotic pathway, which regulates the immune response (54). We detect altered circulating levels of Fas ligand and receptor as well as components of the death-inducing complex (FADD and caspase-8) and effector caspases (caspase-3), most pronounced at T1 but also partly sustained to T2.

**Growth factors**

Another group of findings involve cellular growth regulation, such as EGF signaling that is of vast importance for growth, survival, proliferation, and differentiation of many cell types (including cortical neurons). We found decreased circulating levels of EGF (-49%) together with its receptor (EGF-R) and heterodimers (receptor tyrosine-protein kinases erbB-2 and erbB-4), again most
pronounced at T1 but partly sustained to T2. EGF signaling has been associated with numerous disease processes, including schizophrenia (55) and depression (56, 57). Intracellular mediators of growth signaling are also represented among the top decreased proteins at T1, such as the ubiquitous kinase SRC involved in various signaling pathways, and axin-1 involved in Wnt signaling.

**Longitudinal effects**

Over a five-session course of ECT (i.e., from T0 to T2), some acutely triggered events were sustained but admixed with various processes involved in tissue modulation. The topmost increased protein at T2 was serine protease 8 (+21%), which cleaves and activates epithelial sodium channels (ENaC) and thereby regulates sodium currents that may be altered after a seizure (58). Further, proteins involved in the regulation of the acute immune response (e.g., T-helper 2 cell activity, IL-13 signaling), and apoptosis of inflammatory cells were altered at T2, such as chitinase-3-like protein 1 and pentraxin-related protein PTX3.

**Biomarkers of response to ECT**

None of the tested serum proteins were significantly associated with treatment response after correction for multiple testing. We recognize two major limitations to this null finding. First, ECT is an efficacious treatment for MDE (79% response rate in our sample), leaving few observations for modelling non-response. Second, the included proteins were targeted for inflammatory, cardiovascular, and oncological disease processes and do not specifically reflect CNS processes. A few of the included proteins have distinct roles in the CNS, where notable mentions are: kallikrein-6, which increased at T1 (+49%) and is a serine protease with activity against, e.g., amyloid precursor protein (59) and alpha synuclein (60, 61); sulfotransferase 1A1, highly decreased at T1 (-37%) and sustained to T2, which catalyzes sulfate conjugation of many chemical compounds including neurotransmitters (62); Parkinson disease protein 7, which has various neuroprotective properties (63) and showed a differential temporal trajectory in responders and non-responders (did not pass 5% FDR); and glial-derived neurotrophic factor, a neurotrophic factor mainly for dopaminergic
neurons (64), which interestingly was high in responders and low in non-responders at T2 (Supplementary Figure 1). As it stands, protein biomarkers for clinical response to ECT are not ready for clinical use. Future studies are encouraged.

Limitations

The strengths of this study include the well-powered repeated measures design and the experimental method covering a broad set of the blood serum proteome. Several limitations merit consideration. First, the large sample size required a nationwide multicenter sample collection, which might have introduced batch effects by unmeasured procedural deviations across sites. Second, there are limitations inherent to the study design. The acute effects of ECT were estimated at sampling 30 minutes after the first session (T1). This time window might be too short to capture some biochemical processes (e.g., IL-6 increases only 60 minutes after a stimulus in rodents (65)). Further, ECT requires both general anesthesia and paralysis. Thus, the T1 effects should be interpreted as reflecting the full ECT administration, not just the specific effects of applied current. Anesthesia, muscle relaxants, and seizure-related effects would not have directly influenced the effects at T2 because samples were drawn prior to anesthesia at both T0 and T2. A third consideration concerns clinical data collected at baseline, which was comprehensive in terms of psychiatric morbidity but less so for somatic conditions (e.g., body mass index was missing). Fourth, there is risk of confounding by site regarding the correlation analyses between ECT parameters and magnitude of change at T1. Finally, it is for ethical reasons not possible to recruit a comparison cohort of severely depressed patients that have blood drawn at the same intervals without receiving any treatment. This is a limitation and we can therefore not formally prove that our results are due to the ECT. It is, however, unlikely that the pronounced changes in serum protein concentrations that we found would spontaneously occur over short time in depressed patients.
Conclusion

In the largest longitudinal study of ECT-related alterations in the blood serum proteome to date, we found profound acute effects triggered by one ECT session with findings related to signal transduction such as hormonal signaling and inflammatory response, apoptotic processes, and proteolysis. Over a five-session course of ECT, the acute lower levels of several intercellular signaling molecules were sustained, together with altered levels of some proteins involved in regulatory processes. These findings add to the literature of peripheral effects associated with ECT. To further our understanding of the biological mechanism of ECT, future studies are encouraged to investigate CNS-specific plasma biomarkers (e.g., neurofilament light chain) and/or biomarkers from CNS tissues (e.g., cerebrospinal fluid).

Disclosures

M.L. declares that he has received lecture honoraria from Lundbeck pharmaceutical. A.J. is currently employed at RegSmart Life Science, SE 75237 Uppsala, Sweden. Erik Joas is currently employed at IQVIA, SE-431 44, Mölndal, Sweden. J.H.L is currently employed at AstraZeneca, SE-43150 Mölndal, Sweden. The contribution to the study from A.J, E.J, and J.H.L was made before their current employments. The other authors report no biomedical financial interests or potential conflicts of interest.

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References


Stockholm: Gothia fortbildning.


Tables

Table 1. Baseline characteristics

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<th>Overall (N=260)</th>
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<tr>
<td>Total sample, n (%)</td>
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<td></td>
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<tr>
<td>T0</td>
<td>260 (100.0%)</td>
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<tr>
<td>T1</td>
<td>257 (98.8%)</td>
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<tr>
<td>T2</td>
<td>256 (98.5%)</td>
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<tr>
<td>Total number of treatment sessions, median (range)</td>
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<tr>
<td>Age, median (IQR)</td>
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<td>Indication, n (%)</td>
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<td>Bipolar disorder, depressive episode (F313, F314, F315)</td>
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<td>MDE (F320, F321, F322, F323, F329, F331, F332, F333, F339, F530)</td>
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<td>Disease severity, median (IQR)</td>
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<td>CGI-S,pre-treatment</td>
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<td>CGI-I</td>
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<td>Treatment response (CGI-I ≤ 2), n (%)</td>
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<td>Medication at baseline, n (%)</td>
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Table 2. Top 20 proteins with largest fold change at T1 and T2, respectively.

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<tr>
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<th>Estimate 1</th>
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### Serum proteomics in ECT for major depressive episode

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Figure legend

Figure 1. Overview of study design and protein differential abundance analysis. a Study participants were recruited for an index ECT series (2-3 weekly sessions) to treat MDE. Information on depression severity (MADRS-S and CGI-S) was collected at baseline, and after the completed ECT series (CGI-I). Samples were drawn at the beginning of the first treatment session (T0), 30 minutes after the first treatment session (T1), and at the beginning of the sixth treatment session (T2). Patients continued the index ECT series between T1 and T2. b Volcano plot showing fold changes (log2) and P-values from the generalized least squares models at T1 and T2, respectively. The top altered protein assays are labelled. c Boxplots showing the 10 top assays with proteins levels at each time point. d Heatmap of rank correlations between ECT parameters (including age) and the fold changes at T1 for the top 30 altered protein assays (dots indicate P < 0.05).

Abbreviations (alphabetic order): CASP-3 Caspase-3, CASP-8 Caspase-8, CCL3 C-C motif chemokine 3, CDKN1A Cyclin-dependent kinase inhibitor 1, CD40-L CD40 ligand, CD69 Early activation antigen CD69, CGI-S/I Clinical Global Impression Severity/Improvement, ECP Eosinophil cationic protein, ECT Electroconvulsive therapy, EGF Pro-epidermal growth factor, EGFR EGF receptor, FADD FAS-associated death domain protein, FAS Tumor necrosis factor receptor superfamily member 6, hK11 Kallikrein-11, HSP 27 Heat shock protein beta-1, IL-17RB Interleukin 17 receptor beta, IL-18R1 IL-18 receptor 1, KLK6 Kallikrein-6, LEP Leptin, LOX-1 Oxidized low-density lipoprotein receptor 1, MADRS-S Montgomery Åsberg Depression Rating Scale Self Report, MB Myoglobin, MPA Myeloperoxidase, MYD88 Myeloid differentiation primary response protein MyD88, NT-3 Neurotrophin-3, PAR-1 Proteinase-activated receptor 1, PECAM-1 Platelet endothelial cell adhesion molecule, PRL Prolactin, PRSS8 Serine protease 8, PTX3 Pentraxin-related protein PTX3, SIRT2 NAD-dependent protein deacetylase sirtuin-2, SRC Proto-oncogene tyrosine-protein kinase Src, ST1A1 Sulphotransferase 1A1, S100A12 Protein S100-A12, t-PA Tissue-type plasminogen activator, TNFSF14 Tumor necrosis factor ligand superfamily member 14, T0/T1/T2 Sample time points, uPAR Urokinase plasminogen activator surface receptor, VIM Vimentin
A

1st ECT session

6th ECT ses.

Last ECT ses.

MADRS-S

CGI-S CGI-I

Sample

2 wks

0-4 wks

30 min

B

-log10(p-value) vs Fold change (log2)

PRSS8

TNFSF14

EGF

ST1A1

CD69

PAR-1

t-PA

T0

T1

T2

C

z-score vs Time point

T0

T1

T2

D

rho

-0.4

-0.2

0.0

0.2

0.4

CD40

t-PA

CD40-L

ST1A1

LOX-1

AXIN1

ECP

MPO

MYD88

FADD

CD69

CASP-3

SIRT2

HSP 27

SRC

KLK6

TNFSF14

PRSS8

EGF

CCL3

NT-3

hK11

S100A12

VIM

CASP-8

uPAR

PAR-1

PECAM-1

CDKN1A

PRL

MB

Pulse width (ms)

Frequency (Hz)

Duration (s)

Current (mA)

Electric charge (mC)

EEG seizure dur (s)

Age