

Full Length Article

Targeted proteomic profiling of serum and CSF reveals CASP-8 as a candidate biomarker in anti-NMDAR encephalitis

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ABSTRACT

Anti-NMDA receptor (NMDAR) encephalitis is an autoimmune disorder of the central nervous system involving both B- and T-cell activation. In this exploratory study, targeted proteomics was used to characterize protein expression profiles in serum and cerebrospinal fluid (CSF) from patients with anti-NMDAR encephalitis. Samples from eight patients and 16 age- and sex-matched symptomatic controls were analyzed in a retrospective case-control design. Protein concentrations in serum and CSF were quantified using Proximity Extension Assay (PEA) technology targeting 182 proteins related to immunity, synaptic regulation, and neuronal maintenance. Linear regression identified seven significantly altered proteins in CSF and three in serum after Bonferroni correction ($p < 0.00027$). In CSF, six proteins associated with immune signaling and neuronal support (ADA, CCL19, CXCL5, BMP-4, FGF-5, CDH3) were decreased, while the protease CASP-8 was elevated. In serum, proteases CTSC and CASP-8 were increased, whereas ADA, a key regulator of purine metabolism and immune signaling, was decreased. Strong to moderate correlations between CSF and serum levels were observed for ADA ($r = 0.606$, $p = 0.013$) and CASP-8 ($r = 0.526$, $p = 0.037$). Longitudinal CSF data from two patients revealed dynamic changes in CXCL5, CCL19, and CASP-8 corresponding to disease activity and treatment response. Overall, these findings revealed distinct yet related proteomic signatures in serum and CSF, suggesting compartment-specific immune responses involving both innate and adaptive pathways. The consistent elevation of CASP-8 highlights its potential as a biomarker of disease activity and warrants further investigation.

1. Introduction

Autoimmune encephalitides (AIE) are a heterogeneous group of immune-mediated disorders affecting the central nervous system (CNS), characterized by a wide spectrum of symptoms, including epileptic seizures, cognitive impairment, and psychiatric disturbances (Uy et al., 2021). AIE can be triggered by factors such as viruses; in particular herpes simplex virus type 1 (HSV-1); or underlying neoplasms expressing neuronal antigens (Graus et al., 2016). However; in many cases; the initiating event driving the immune response remains unidentified. Most patients with AIE exhibit neuronal autoantibodies detectable in cerebrospinal fluid (CSF) or serum; with N-methyl-D-aspartate receptor

(NMDAR) antibodies being the most common (Hébert et al., 2020). Patients with anti-NMDAR encephalitis often respond favorably to immunotherapy; with substantial potential for clinical recovery (Titulaer et al., 2013). Nonetheless; many individuals continue to suffer from long-term sequelae; such as cognitive dysfunction; even after the resolution of acute symptoms (Brenner et al., 2024).

A deeper understanding of the immunological pathways involved in anti-NMDAR encephalitis is important for improving diagnostics, identifying therapeutic targets, and predicting long-term outcomes. Previous biomarker studies in anti-NMDAR encephalitis have largely focused on cytokine measurements using conventional immunoassays (Ciano-Petersen et al., 2022). Elevated CSF levels of inflammatory mediators

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such as CXCL13; CXCL10; IL-10; IL-7; IL-6; IL-17 A; CCL3; CCL20; and IFN- γ have been reported; suggesting combined activation of B- and T-cell pathways (Byun et al., 2016; Leypoldt et al., 2015; Liba et al., 2016; Liu et al., 2020). Of particular interest are IL-6 and IL-17 A, which promote the differentiation of pro-inflammatory Th17 cells and inhibit the development of anti-inflammatory regulatory T cells (Tregs), contributing to the development of autoimmune disease (Byun et al., 2016; Lee, 2018). Serum cytokine profiles similarly show increased levels of IL-6, CXCL13, IL-17 A, IL-2, and IFN- γ (Byun et al., 2016; Ulusoy et al., 2012; Wesselingh et al., 2023). Neuronal damage markers such as neurofilament light chain (NFL) are also elevated during the acute phase of anti-NMDAR encephalitis in serum and CSF, suggesting axonal damage (Constantinescu et al., 2016). To date; one of the most comprehensive proteomics investigations in AIE was conducted by Räuber et al.; who analyzed CSF protein profiles using mass spectrometry in patients with anti-NMDAR; anti-LGI1; and anti-GAD65 encephalitis (Räuber et al., 2023). Their findings revealed distinct protein signatures in AIE patients compared to inflammatory and non-inflammatory controls, including alterations in proteases and proteins involved in synaptic transmission, brain connectivity, and neurodegeneration.

This study aimed to characterize serum as well as CSF protein profiles in patients with anti-NMDAR encephalitis using targeted proteomics, focusing on markers related to inflammation, neuronal signaling, synaptic function, and CNS homeostasis. The primary objective was to identify proteins differentially expressed in anti-NMDAR encephalitis patients compared to matched controls. Secondary aims included replication of previously reported cytokine alterations; elevated CSF levels of CXCL10, IL-6, and IL-17 A (Byun et al., 2016; Chen et al., 2018; Liao et al., 2021; Liba et al., 2016; Liu et al., 2020; C. Zeng et al., 2018; Zou et al., 2020). Finally, we conducted an exploratory analysis of longitudinal protein changes in patients with serial samples.

2. Method

2.1. Ethical approval

This study was conducted in accordance with the principles of the Declaration of Helsinki. It was approved by the Swedish Ethical Review Authority (Dnr: 2019–03068) for the AIE patients and the Regional Ethics Board of Uppsala (Dnr: 2013/278) for the controls. Written informed consent was obtained for all participants, except for deceased anti-NMDAR encephalitis patients, for whom consent was presumed (in line with the ethical application).

2.2. Study participants

This case-control study included patients with anti-NMDAR encephalitis ($n = 8$) and symptomatic controls investigated for neurological disease without abnormal findings (Controls, $n = 16$). Individuals testing positive for anti-NMDAR antibodies in serum or CSF at Uppsala University Hospital between 2012 and 2020 were invited to participate. Serum and CSF samples were collected during routine clinical care, and medical records were reviewed for diagnostic verification. Eight patients fulfilled the diagnostic criteria for definite anti-NMDAR encephalitis (Graus et al., 2016), all of whom had IgG anti-GluN1 antibodies detected in CSF (fixed CBA, Euroimmun, Lubeck, Germany).

Control subjects were recruited from the Neurology Department at Uppsala University Hospital. These individuals presented with neurological symptoms such as paresthesia (31%), headache (31%), gait disturbance (20%), vertigo (6%), foot drop (6%), and fasciculations (6%), but had no confirmed neurological disease following clinical work-up, and routine CSF analyses were normal. Controls were matched 2:1 to anti-NMDAR encephalitis cases by age and sex as closely as possible.

2.3. Sample collection and handling

Serum and CSF samples from anti-NMDAR encephalitis cases were collected in additive-free tubes, stored at 2–8 °C for up to 7 days, and subsequently frozen at –20 °C. Serum and CSF from the controls were centrifuged and then snap-frozen at –80 °C. All samples underwent a single freeze-thaw cycle and were further aliquoted and randomized into a 96-well plate before analysis.

2.4. Protein quantification via proximity extension assay

Protein levels in serum and CSF were quantified using the Proximity Extension Assay (PEA) technology (OLINK Proteomics, Uppsala, Sweden), which combines an antibody-based immunoassay with quantitative PCR (qPCR). Samples were randomized and processed in parallel in a double-blinded fashion, ensuring even distribution across six 96-well plates to maintain a balanced representation of study groups. Proteomic analyses were conducted at the Clinical Biomarkers Facility, Science for Life Laboratory, Uppsala University, with sample identities and donor information concealed from laboratory personnel. For each protein, a matched antibody pair is conjugated to unique DNA oligonucleotides. Upon dual binding to their target protein, the oligonucleotides hybridize and are extended by DNA polymerase, forming a unique DNA template. The subsequent amplification and quantification using qPCR determine the initial protein concentration in the sample. The protein concentration is expressed as normalized protein expression (NPX) units, represented as arbitrary units on a log₂ scale. Higher NPX values indicate greater protein abundance; a 1 NPX increase corresponds to a doubling in protein concentration (Assarsson et al., 2014; Ebai et al., 2015).

Two OLINK panels were used for proteomic profiling: the Target 96 Inflammation panel (92 proteins related to inflammatory processes), and the Target 96 Neurology panel (92 proteins associated with the function of the nervous system). Two proteins (Beta-NGF and GDNF) were present on both panels; to avoid duplication, only values from the Neurology panel were retained, yielding a final dataset of 182 unique proteins for analysis. All samples were processed according to Olink's quality control procedures. One control CSF sample in the Neurology panel returned no quantitative data and was therefore excluded from analysis. One serum sample and one CSF sample from anti-NMDAR encephalitis cases in the Inflammation panel were flagged during quality control but yielded quantitative data and were retained in the analyses.

2.5. Statistical analysis

All statistical analyses were conducted using NPX values. Although a high proportion of NPX values for some cytokines (e.g., IL-17 A, IL-2) were below the assay's lower limit of detection (LOD), these values were retained for analysis, as they provide meaningful relative quantification. The proportion of NPX values below LOD is stated in the results section when relevant. Group differences in CSF and serum biomarker concentrations between anti-NMDAR encephalitis cases and controls were evaluated using linear regression, both unadjusted (model 0) and adjusted for age and sex (model 1). Bonferroni correction was applied for multiple comparisons; adjusted p -values below 0.00027 (0.05/182) were considered significant.

Principal component analysis (PCA) was performed on pre-selected NPX values of all proteins with a significant group difference (unadjusted p -value <0.05). Protein correlation between CSF and serum was visualized with scatter plots including LOESS curves and linear least squares lines, with values of Pearson correlation coefficient (r) reported. Strengths of correlations were classified according to the British Medical Journal guidelines (Wechsler, 1997). Anti-NMDAR cases were stratified by time from symptom onset to sample collection (<1 month vs. >1 month) and compared to controls using one-way ANOVA with

Tukey's post hoc test for multiple comparisons.

For replication of previous findings, as listed in the last paragraph of the introduction, adjusted significance thresholds were based on the number of comparisons: $0.05/3 = 0.017$.

Graphical visualizations (PCA plots, box plots, and spaghetti plots) were created in GraphPad Prism version 10.4.1 for Mac OS X. All other statistical analyses and figures were computed in R version 4.4.2.

3. Results

3.1. Patient characteristics

This retrospective case-control study included samples from 8 anti-NMDAR encephalitis cases [2 males (25.0%), median age: 34.5 years (IQR 16.3–63.5 years)] and 16 controls [3 males (18.8%), median age: 32.5 years (IQR 21.3–62.5 years)]. Clinical characteristics of all anti-NMDAR cases are summarized in Table 1. Seven cases had CSF samples available for testing, and seven cases had serum samples available (Table 1). Among the anti-NMDAR encephalitis cases, one had an ovarian teratoma. Only one anti-NMDAR patient (case 1) had received

Table 1

Demographic and clinical data of anti-NMDAR encephalitis cases. †Sample available for this study. ‡Patient diagnosed with RIS and anti-NMDAR encephalitis simultaneously. §Patient diagnosed with HSV-1 encephalitis six weeks prior. Abbreviations: CBA = Cell-based assay (fixed CBA, Euroimmun, Lubeck, Germany); OCB = oligoclonal bands; NFL = Neurofilament light chain; RIS = Radiologically isolated syndrome; HSV-1 = Herpes simplex virus 1.

Case	Sex	Age	Symptoms at onset	Onset to CSF/serum collection	Sample†	Antibody test result	CSF analysis	EEG	Brain MRI	Treatment prior to sampling	Other
1	F	72	Seizures, cognitive dysfunction, dystonic movements, abnormal speech	> 1 month	CSF	Anti-NMDAR antibody positive in serum and CSF (CBA). Titration not performed.	Normal	Focal slow, focal epileptic	Subdural hematoma (considered unrelated)	IVIG and oral prednisone (3 days prior)	
2	M	16	Seizures, cognitive dysfunction, abnormal speech	> 1 month	Serum + CSF	Anti-NMDAR antibody positive in serum and CSF (CBA). Titration not performed.	Pleiocytosis, elevated protein, OCB+	Focal slow	Normal	None	
3	F	6	Seizures, psychiatric symptoms, abnormal speech	> 1 month	Serum + CSF	Anti-NMDAR antibody positive in serum with titre 1/20, positive in undiluted CSF (CBA).	Normal	General slow, focal slow, focal epileptic	Multifocal enhancing lesions in both grey and white matter	None	
4	F	35	Psychiatric symptoms, cognitive dysfunction	< 1 month	Serum	Anti-NMDAR antibody positive in serum and CSF (CBA). Titration not performed.	Pleiocytosis, OCB+, NFL 2030 ng/L	General slow	Demyelination	None	RIS‡
5	F	73	Psychiatric symptoms, cognitive dysfunction	< 1 month	Serum + CSF	Anti-NMDAR antibody negative in serum, positive with titre 1/16 in CSF (CBA).	Pleiocytosis, elevated protein, OCB+	Focal slow, general slow	Unilateral medial temporal lobe T2 hyperintensity	None	HSV-1§
6	M	34	Psychiatric symptoms, cognitive dysfunction, abnormal speech	< 1 month	Serum + CSF	Anti-NMDAR antibody negative in serum, positive in CSF (CBA). Titration not performed.	Pleiocytosis	Focal slow	Normal	None	
7	F	17	Cognitive dysfunction, psychiatric symptoms, seizures, autonomic instability	< 1 month	Serum + CSF	Anti-NMDAR antibody negative in serum, positive with titre 1/4 in CSF (CBA).	Pleiocytosis	Focal slow	T2 hyperintensity in unilateral cortical lesion (considered postictal)	None	
8	F	38	Cognitive dysfunction, psychiatric symptoms, abnormal speech	< 1 month	Serum + CSF	Anti-NMDAR antibody negative in serum, positive in CSF (CBA). Titration not performed.	Normal	General slow	Normal	None	Ovarian teratoma

immunotherapy before sampling (3 days prior), including intravenous immunoglobulins (IVIG) and oral prednisone.

3.2. Distinct serum and CSF protein profiles in anti-NMDAR encephalitis

Unadjusted linear regression analyses using NPX values (Model 0) identified six CSF proteins that were significantly reduced in anti-NMDAR encephalitis cases (FGF-5, ADA, CXCL5, CDH3, BMP-4, CCL19) compared with controls (Bonferroni-corrected $p < 0.00027$; Table 2), and elevated levels were observed for one CSF protein (CASP-8) (Fig. 1A-H). In serum, the levels of the two proteins CTSC and CASP-8 were significantly increased compared to controls (Table 2; Fig. 2A-D). Adjustment for age and sex (Model 1) yielded similar results (Table 2), except for ADA being identified as significantly reduced in serum when using Model 1.

3.3. Protein profiling of CSF and serum in anti-NMDAR encephalitis: Expression patterns and cross-compartment correlations

Principal component analysis (PCA) of all proteins with significant group differences (CSF proteins $n = 47$, serum proteins $n = 24$; unadjusted $p < 0.05$; Supplementary Table 1) was performed as an exploratory visualization to illustrate the group-level structure of the data. The PCA plots demonstrated a distinct separation between anti-NMDAR encephalitis cases and controls in serum and CSF (Fig. 3A-B), with clearer separation in CSF compared to serum, suggesting compartment-specific alterations.

Hierarchical clustering of CSF biomarkers further identified specific molecular groupings, including a close association between ADA and FGF-5. In serum samples, CASP-8 clustered closely with CTSC, reinforcing their co-alteration in anti-NMDAR encephalitis.

Correlation analyses between protein levels in CSF and serum indicated statistically significant strong to moderate correlations for ADA ($r = 0.606$, $p = 0.013$) and CASP-8 ($r = 0.526$, $p = 0.037$) (Supplementary Fig. 1). No significant correlation was found between CSF and serum levels of CTSC ($p = 0.952$), suggesting that peripheral expression

patterns may only partially reflect CNS system alterations in anti-NMDAR encephalitis. Stratifying anti-NMDAR cases by time from symptom onset to sample collection (<1 month vs. >1 month) revealed significantly higher CASP-8 levels in the CSF of patients with short symptom duration ($p = 0.027$; Supplementary Fig. 2). No other CSF or serum proteins showed significant differences based on timing.

3.4. Replication of cytokine alterations in anti-NMDAR encephalitis: elevated IL-6 in CSF

In an effort to replicate previously reported findings regarding elevated cytokines in anti-NMDAR encephalitis (Byun et al., 2016; Chen et al., 2018; Liao et al., 2021; Liba et al., 2016; Liu et al., 2020; C. Zeng et al., 2018; Zou et al., 2020), we focused on CSF levels of CXCL10, IL-6, and IL-17 A. Following correction for multiple comparisons, only IL-6 was significantly elevated in CSF from anti-NMDAR cases compared with controls ($p = 0.0017$); however, no significant differences were observed for CXCL10 or IL-17 A (Supplementary Fig. 3).

3.5. Longitudinal CSF and serum protein profiles in anti-NMDAR encephalitis: case reports

Serial CSF samples were available for two patients, one of whom also had longitudinal serum samples. Longitudinal data on selected proteins are presented in Fig. 4 for one of the cases: a 17-year-old female (Case 7, Table 1; Fig. 4A-D). The corresponding figure for the second case is presented in Supplementary Fig. 4, together with the rest of the significantly altered proteins from Case 2.

Case 1. A 17-year-old female initially presented with syncope due to second-degree AV block, followed by cognitive decline and daily focal seizures. CSF analysis showed pleocytosis (46 cells/ μ L) and positive anti-NMDAR antibodies. Initial treatment with intravenous methylprednisolone and IVIG led to transient improvement. Tumor screening was negative. CSF levels of CCL19 and CXCL5 were low, whereas CASP-8 and IL-6 were elevated. Two weeks later, she deteriorated clinically, developing status epilepticus and severe dysautonomia, requiring ICU

Table 2

Significantly altered proteins in CSF and serum in anti-NMDAR encephalitis cases compared with controls. Columns 3 and 4 present normalized protein expression (NPX) values (median (Q1-Q3)) for CSF and serum proteins with a significant difference between the anti-NMDAR group and controls. Column 5 presents the p-values from the linear regression model 0, column 6 presents the corresponding p-values from the age- and sex-adjusted regression model 1. *Indicates p-value below Bonferroni corrected p-value cut-off ($p < 0.00027$). Among the 7 CSF proteins presented in this table, CCL19, CXCL5, and CDH3 had 100% values above LOD. CASP-8, FGF-5, ADA, and BMP-4 had more than 80% values above LOD. All serum proteins in this table had 100% of values above LOD.

Protein	Gene	NMDAR NPX	Controls NPX	P-value (Model 0)	P-value (Model 1)
Cerebrospinal fluid					
Fibroblast growth factor 5	FGF-5	1.03 (0.65–1.38)	2.46 (2.20–2.80)	7.8e-07*	2.5e-06*
Adenosine deaminase	ADA	2.69 (1.53–2.78)	4.40 (4.12–4.61)	5.8e-06*	1.4e-05*
C-X-C motif chemokine 5	CXCL5	3.09 (3.02–3.52)	4.58 (4.40–4.79)	7.0e-05*	6.4e-05*
Cadherin-3	CDH3	5.19 (5.08–5.61)	6.31 (6.21–6.37)	8.5e-05*	5.9e-05*
Bone morphogenetic protein 4	BMP-4	2.52 (1.85–3.03)	4.57 (4.37–5.00)	0.0001*	6.2e-05*
Caspase-8	CASP-8	2.92 (1.86–3.13)	1.13 (0.85–1.22)	0.0001*	0.0002*
C-C motif chemokine 19	CCL19	6.80 (6.45–8.42)	9.36 (9.03–9.90)	0.0002*	8.1e-05*
Serum					
Dipeptidyl peptidase 1 (Cathepsin C)	CTSC	5.72 (5.55–5.88)	3.80 (3.49–4.11)	1.7e-09*	3.4e-09*
Caspase-8	CASP-8	5.49 (5.20–6.15)	3.20 (2.84–3.63)	7.5e-06*	2.3e-06*
Adenosine deaminase	ADA	4.50 (4.26–4.69)	5.57 (5.00–5.78)	0.0003	0.0002*

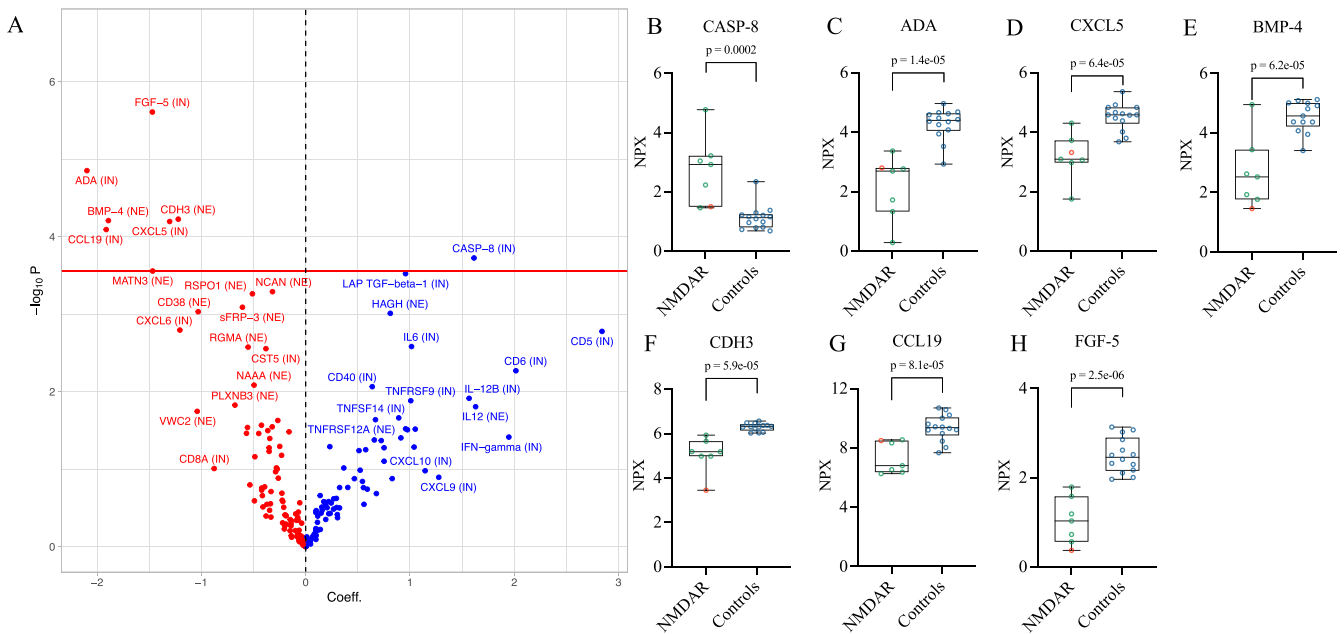


Fig. 1. (A) Volcano plot displaying p -values versus linear regression coefficients (Model 1) for differential protein expression in CSF from patients with anti-NMDAR encephalitis ($n = 7$) compared to controls ($n = 14$). The horizontal red line marks the Bonferroni-corrected significance threshold ($p = 0.00027$). (B–H) Box plots showing NPX values for the seven proteins with a significant difference between cases and controls. Data are presented as box plots representing the median with the box extending from the 25th to 75th percentiles; whiskers indicating minimum and maximum values. Individual cases are represented as green dots, the red dot indicating case 1, who received immunotherapy 3 days prior to sampling, and blue dots representing controls. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

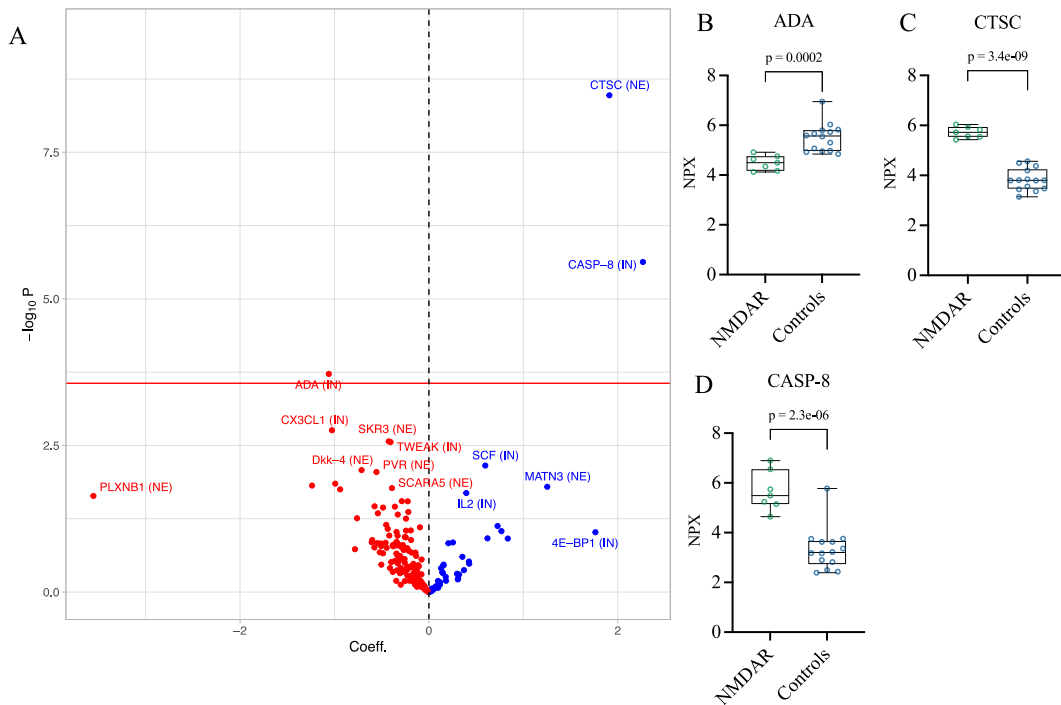


Fig. 2. (A) Volcano plot displaying p -values versus linear regression coefficients (Model 1) for differential protein expression in serum from patients with anti-NMDAR encephalitis ($n = 7$) compared to controls ($n = 14$). The horizontal red line denotes the Bonferroni-corrected significance threshold ($p = 0.00027$). (B–D) Box plots showing NPX values for the three proteins with the most significant differences between cases and controls. Data are presented as box plots representing the median with the box extending from the 25th to 75th percentiles; whiskers indicating minimum and maximum values. Individual cases are represented as green dots and controls as blue dots. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

admission and sedation. During ICU sedation, CSF levels of CCL19 and CXCL5 increased markedly. CASP-8 levels normalized in the second

sample but increased again in the third, and IL-6 peaked before declining. Serum levels of CASP-8 and CTSC remained elevated at both

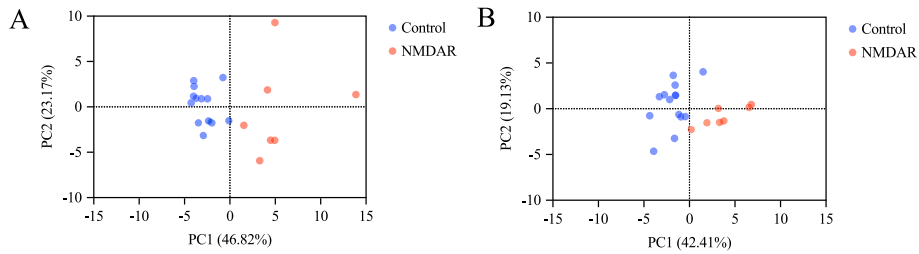


Fig. 3. Fig. 3 Principal component analysis (PCA) based on proteins showing nominal group differences (unadjusted $p < 0.05$). (A) CSF PCA constructed using 47 proteins; each point represents one CSF sample (anti-NMDAR encephalitis, $n = 7$; controls, $n = 14$). (B) Serum PCA constructed using 24 proteins; each point represents one serum sample (anti-NMDAR encephalitis, $n = 7$; controls, $n = 14$).

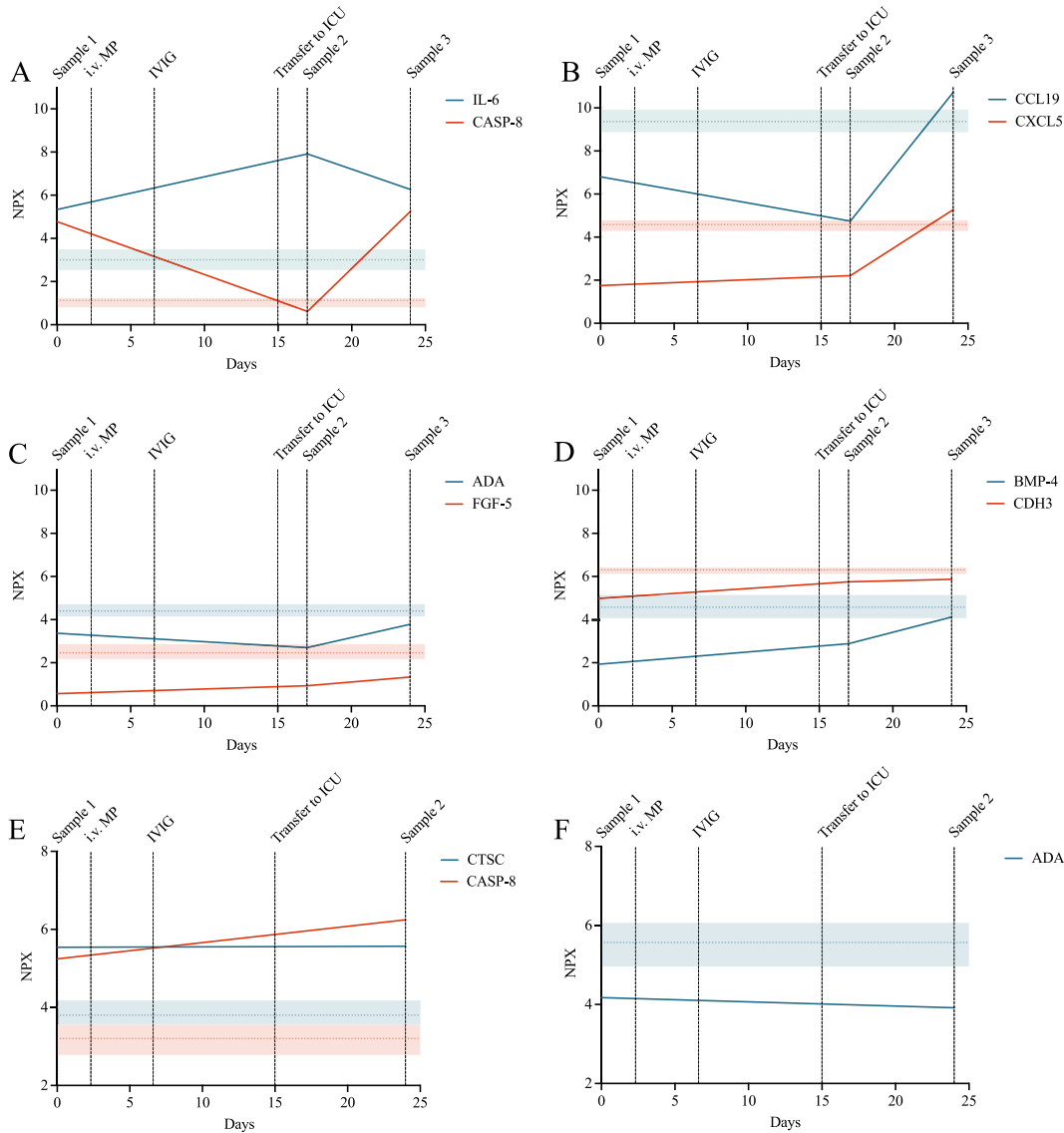


Fig. 4. Longitudinal changes in CSF and serum protein levels in a young female patient (Case 7) with non-paraneoplastic anti-NMDAR encephalitis. Individual longitudinal trajectories of NPX values are shown for CSF IL-6 and CASP-8 (A), CSF CCL19 and CXCL5 (B), CSF ADA and FGF-5 (C), and CSF BMP-4 and CDH3 (D), measured across three time points. Serum NPX values are shown for CTSC and CASP-8 (E), and ADA (F), measured across two time points. Shaded horizontal bands indicate the interquartile range observed in healthy controls ($n = 14$), and dashed horizontal lines indicate the control median. Abbreviations: ICU = intensive care unit; MP = methylprednisolone; IVIG = intravenous immunoglobulins.

available time points, while ADA levels remained decreased. Rituximab (1000 mg) was administered, and clinical improvement followed after 20 days in the ICU. She was discharged after 48 days, seizure-free but with mild cognitive impairment.

Case 2. A 73-year-old female presented with psychosis, cognitive dysfunction, and incongruent speech. She had recovered from a diagnosis of HSV-1 encephalitis six weeks earlier. CSF analysis showed mild pleocytosis (8 cells/ μ L), positive oligoclonal bands, and positive anti-

NMDAR antibodies. She received two courses of IVIG, three weeks apart, due to persistent psychosis. Two weeks later, she was discharged, and at five-week follow-up, she reported full clinical recovery. Serial CSF samples showed initially decreased levels of CCL19 and CXCL5, which normalized after treatment (Supplementary Fig. 4). CASP-8 and IL-6 were elevated at both time points.

4. Discussion

This study provides novel insights into the immunopathology of anti-NMDAR encephalitis through a targeted proteomics approach in matched CSF and serum samples. We identified distinct protein signatures involving both innate and adaptive immune pathways, as well as markers of synaptic regulation and glial activation.

Several of the most significantly downregulated CSF proteins, fibroblast growth factor 5 (FGF-5), cadherin-3 (CDH3), and bone morphogenetic protein 4 (BMP-4), are involved in synaptic maintenance and neurodevelopmental signaling. FGF-5 promotes neuronal survival and differentiation (Lindholm et al., 1994); CDH3 contributes to synaptic structure and integrity (Suzuki and Takeichi, 2008); and BMP-4 regulates neural stem cell activity in the adult hippocampus (Mira et al., 2010). Their coordinated downregulation may reflect impaired neurotrophic signaling and synaptic plasticity; potentially contributing to the cognitive deficits observed in anti-NMDAR encephalitis (Brenner et al., 2024). These findings are consistent with previous proteomic studies showing dysregulation of synaptic and axonal proteins in autoimmune encephalitis (Rauber et al., 2023). Further longitudinal studies are needed to assess whether these proteins could serve as prognostic biomarkers.

We also observed decreased CSF levels of adenosine deaminase (ADA), C—C motif chemokine 19 (CCL19), and C-X-C motif chemokine 5 (CXCL5). While ADA is typically elevated in neuroinflammatory conditions, due to T-cell activation (Handa et al., 2025); its reduction here may reflect a compensatory shift toward less immune activation via elevated extracellular adenosine. CCL19 is a pro-inflammatory chemokine; which, through binding to the CCR7 receptor; is crucial for the recruitment of antigen-presenting dendritic cells and naïve T-cells to secondary lymphoid organs. In contrast to our results; CCL19 is increased in the CSF of patients with multiple sclerosis (MS); (Pashenkov et al., 2003); and in previous case reports of anti-NMDAR encephalitis (Kothur et al., 2017). However; this discrepancy may reflect the small size of our study; potential differences in disease stage; timing of sample collection; and possible methodological limitations of the PEA platform. Nevertheless; it is possible that transient downregulation of CCL19 reflects a compensatory reduction in immune recruitment pathways; with levels rising again during periods of clinical deterioration; as observed in one patient. Similarly; CXCL5; which primarily attracts neutrophils and contributes to tissue repair (Li et al., 2024), was also reduced, suggesting that immune regulatory processes may be active at the time of sampling, with levels potentially increasing during acute disease activity.

Caspase-8 (CASP-8) was consistently elevated in both CSF and serum, highlighting its potential as a biomarker of CNS and systemic immune activation in anti-NMDAR encephalitis. A previous study using the same PEA methodology did not detect elevated CSF CASP-8 levels in patients with multiple sclerosis (Burman et al., 2023). Beyond its canonical role in apoptosis; CASP-8 is increasingly recognized as a regulator of microglial inflammasome activation and neuroinflammation (Zhang et al., 2018; W. Zhang et al., 2024b). Dysregulation of CASP-8 has been implicated in multiple sclerosis (Kim et al., 2022); and may similarly contribute to neuronal injury in anti-NMDAR encephalitis (Rahman et al., 2023). This interpretation aligns with recent PET imaging studies demonstrating microglial activation in autoimmune encephalitis (M. Zhang et al., 2024a); and supports the rationale for exploring microglia-targeted immunotherapies; such as fingolimod (Wesselingh et al., 2019).

Importantly, longitudinal analysis in one severely ill patient

suggested that CSF CASP-8 levels initially decreased following first-line immunotherapy with methylprednisolone and IVIG, which may reflect transient suppression of inflammatory activity. However, CSF CASP-8 levels subsequently rose again during clinical deterioration, indicating a possible association with disease progression and immune activation over time. Although these observations are based on a single case and require validation in larger cohorts, they highlight the potential of CASP-8 as a biomarker for monitoring treatment response and disease activity in anti-NMDAR encephalitis.

In serum, CASP-8 clustered with cathepsin C (CTSC), a neutrophil-expressed enzyme (Chitsamankhun et al., 2024); suggesting coordinated systemic proteolytic activity (W. Zhang et al., 2024b). Elevated serum CASP-8 has also been reported in severe stroke and sepsis; consistent with a role in systemic inflammation and apoptosis (Lorente et al., 2022a; Lorente et al., 2022b). While CASP-8 was elevated in both compartments, CTSC likely reflects peripheral immune activation. These findings are consistent with prior reports of increased protease activity and elevated neutrophil-to-lymphocyte ratio in anti-NMDAR encephalitis (Rauber et al., 2023; Shu et al., 2018; Z. Zeng et al., 2019). Targeting excessive protease activity; e.g.; with CTSC inhibitors (Chitsamankhun et al., 2024), may represent a novel adjunctive therapeutic strategy in anti-NMDAR encephalitis.

Although serum biomarkers are attractive due to easier accessibility, our study found limited correlation between serum and CSF protein levels. Only ADA and CASP-8 showed significant cross-compartment associations, similar to prior studies (Martinez-Hernandez et al., 2011), highlighting the challenge of using peripheral markers as surrogates for CNS inflammation in anti-NMDAR encephalitis and emphasizes the continued importance of CSF-biomarkers when feasible.

Consistent with earlier studies, we confirmed elevated CSF IL-6 levels (Byun et al., 2016; Chen et al., 2018; C. Zeng et al., 2018; Zou et al., 2020); reinforcing its role as a key pro-inflammatory cytokine and potential mediator of Th17/Treg imbalance in AIE. However; we did not replicate previously reported increases in CXCL10 or IL-17 A (Byun et al., 2016; Liao et al., 2021; Liba et al., 2016; Liu et al., 2020; C. Zeng et al., 2018), which may reflect differences in sample size, disease stage, patient populations, or assay sensitivity between studies. These discrepancies highlight the complexity of cytokine signaling in anti-NMDAR encephalitis and the need for larger, longitudinal studies to define dynamic immune profiles across disease stages.

Despite the limited sample size, our longitudinal data provide additional support for the clinical relevance of CXCL5, CCL19, and CASP-8. In both cases, these markers tracked with disease activity and treatment response, suggesting potential utility in disease monitoring. However, treatment intensification and evolving disease stage are tightly intertwined, making it difficult to distinguish spontaneous disease dynamics from pharmacological effects in such a small sample. Even though the specific biomarkers studied vary across reports, similar protein profile abnormalities have been observed in previous longitudinal case descriptions (Liba et al., 2016; Liu et al., 2020; Omae et al., 2018). Notably, CSF CASP-8 levels varied depending on the duration of symptoms before sampling, further emphasizing the importance of temporal information in biomarker interpretation. Together, our findings suggest that CASP-8 and CTSC may be promising candidate biomarkers of systemic immune dysregulation in anti-NMDAR encephalitis, and warrant validation in larger, prospective studies.

4.1. Limitations and strengths

The study has some limitations that should be acknowledged. The modest sample size limits generalizability, although this was partially mitigated by carefully matching each case with two age- and sex-matched controls. Given the exploratory nature of the study and the small sample size, we chose a conservative Bonferroni correction, and therefore, the risk of type II error is substantial. Retrospective data collection restricted access to several clinical details, including accurate

functional outcomes and precise symptom-onset timing. Because data extraction relied on routine healthcare records rather than prospective, protocol-driven assessments, there was considerable variability between patients, and some measurements, such as neurofilament light chain, were not available for all individuals. Consequently, correlation analyses with established markers of disease severity or functional outcomes were not performed. Additionally, delayed freezing of anti-NMDAR encephalitis samples collected within routine health care may have influenced protein stability; however, pre-analytical quality markers (Huang et al., 2021) did not differ between groups, and low within-group variability suggests minimal impact. The absence of an independent replication cohort and inflammatory controls means that findings should be regarded as exploratory and hypothesis-generating.

Despite these limitations, this study has several important strengths. The parallel analysis of matched serum and CSF samples using a high-sensitivity, multiplex proteomics platform enabled a broad and compartment-specific characterization of immune and neuro-inflammatory signatures. This approach allowed us to identify both shared and distinct protein alterations across compartments, providing novel insights into CNS and peripheral immune responses in anti-NMDAR encephalitis. Several candidate biomarkers, particularly CASP-8 and CTSC, emerged as promising targets for future validation.

5. Conclusion

This study reveals a distinct, compartment-specific immunopathological profile in anti-NMDAR encephalitis, highlighting the complex interplay between central and peripheral immune responses. Through targeted proteomics of matched CSF and serum samples, we identified protein signatures associated with microglial activation, synaptic dysfunction, and systemic protease activity. The dynamic behavior of markers such as CASP-8 and CCL19 suggests potential utility for disease monitoring. Future studies with larger cohorts, longitudinal sampling using harmonized pre-analytical protocols, careful consideration of sample timing relative to treatment, and inclusion of inflammatory controls are needed to confirm these findings and to explore the clinical relevance of targeting glial or protease-mediated pathways in anti-NMDAR encephalitis.

CRedit authorship contribution statement

Sonja Kosek: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Anna Wiberg:** Writing – review & editing, Data curation. **Barbro Persson:** Writing – review & editing, Data curation. **Katja Gabrysch:** Writing – review & editing, Methodology, Formal analysis. **Joachim Burman:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization. **Anna Rostedt Punga:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization.

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Declaration of competing interest

The authors declare that they have no financial or other conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jneuroim.2026.578927>.

Data availability

The primary OLINK data is available from the corresponding author upon reasonable request. The clinical datasets analyzed during the current study are not publicly available due to the GDPR legislation, but are available from the corresponding author upon reasonable request.

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