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CSF Tau Is a Biomarker of Hippocampal Injury in Cryptogenic New-Onset Refractory Status Epilepticus

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ABSTRACT

Objective: Cryptogenic new-onset refractory status epilepticus (cNORSE) is a devastating condition characterized by the de novo onset of status epilepticus with unclear etiology. The identification of relevant early biomarkers in cNORSE is important to elucidate pathophysiology, aid clinical decision-making, and prognosticate outcomes in cNORSE.

Methods: CSF samples were obtained within 7 days of NORSE onset from an adult cNORSE cohort in a national referral center in South Korea. Nineteen patients with cNORSE were studied: 9 were male (47.4%) and the median age was 35.0 [IQR: 27.0–54.3] years. CSF from 21 patients with other neurological diseases (atypical parkinsonism, postural orthostatic hypotension syndrome, epilepsy, and cerebellar ataxia) was used as controls. Proteomic analysis was conducted using the Olink platform, and potential biomarker candidates were correlated with clinical data and MRI findings.

Results: Based on correlation analyses between proteomic data and clinical outcomes, total tau (t-tau) was selected as a potential biomarker. Patients with cNORSE had higher CSF t-tau levels than controls ($p < 0.001$). Early detection of high CSF t-tau was associated with the presence of hippocampal atrophy in the postacute phase of cNORSE ($p = 0.044$). The initial elevation of t-tau levels also correlated with a higher number of anti-seizure medications used ($p = 0.031$) and less improvement in Clinical Assessment Scale in Autoimmune Encephalitis (CASE) scores 1 month after NORSE onset ($p = 0.066$). T-tau levels were correlated with CSF pro-inflammatory cytokines/chemokines and mediators of neuronal damage.

Interpretation: Elevated CSF t-tau levels detected early after cNORSE onset may be a useful marker of initial brain injury and predict subsequent hippocampal atrophy.

Yihui Goh, Yoonhyuk Jang, and Soo Jean Shin contributed equally as the co-first authors.

1 | Introduction

New-onset refractory status epilepticus (NORSE) is characterized by the onset of status epilepticus in patients without prior history of seizures, frequently associated with significant morbidity and mortality [1]. It is often cryptogenic, with no cause found in up to half of cases despite extensive evaluation, including that of underlying neural autoantibodies and malignancy [2]. Cryptogenic NORSE (cNORSE) is currently defined by its clinical presentation, rather than being a specific diagnosis with clear underlying pathophysiology.

There is an emerging need to identify relevant biomarkers in cNORSE to elucidate its pathophysiology and prognosticate outcomes [3]. Recent studies have increasingly implicated cytokine dysregulation as a key factor in the pathophysiology of cNORSE, reflecting central nervous system (CNS) inflammatory activation [4, 5]. Proinflammatory cytokines have shown potential as both a prognostic and predictive marker in cNORSE [4, 5]. The degree of innate cytokine activation also appears to correlate with the extent of MRI lesions in cNORSE [6]. Furthermore, genetic studies indicate that autoinflammatory mechanisms may play a role in cNORSE [7]. These findings emphasize the critical role of inflammation in cNORSE and suggest that biomarkers associated with inflammatory cascades may hold valuable clinical implications. While inflammation is a crucial initial factor, it represents only one aspect of cNORSE pathophysiology. The downstream effects of inflammation, particularly neuronal damage, could also be an important determinant of long-term outcomes [8]. Neuronal loss is a common pathological finding in NORSE patients [9]. Earlier studies have evaluated biomarkers of neuronal damage, such as serum neuron-specific enolase (NSE) and serum neurofilament light chain (sNFL) in patients with status epilepticus [3, 10]. NSE is commonly used to prognosticate hypoxic ischemic encephalopathy [11]. However, its utility in status epilepticus appears to be limited to the diagnosis of status epilepticus [12], as it has not been shown to correlate with SE semiology or duration [13]. sNFL appears to correlate with seizure duration and short-term functional outcomes in patients with status epilepticus [10, 14]. However, these studies have been performed in heterogeneous status epilepticus cohorts of varying etiologies, and not specifically in patients with cNORSE, who may have different underlying patho-mechanisms.

The identification of acute phase biomarkers that reflect neuronal damage, and possibly, prognosticate functional outcomes, represents a gap in the understanding of cNORSE. This study seeks to address this gap by using CSF proteomic analysis of patients with cNORSE. We aim to identify a novel biomarker that not only enhances our understanding of cNORSE pathophysiology, but also offers insights into the early detection of neuronal damage and its relation to patient outcomes.

2 | Materials and Methods

2.1 | Design, Setting, and Participants

This study was approved by the Seoul National University Hospital Institutional Review Board (IRB no. 1402-047-555, 1705-130-856, and 2308-152-1459) and written informed consent was obtained from all patients and/or legal guardians. Seoul National University Hospital (SNUH) enrolls patients who present with possible autoimmune encephalitis into an observational cohort, where neuronal autoantibody testing is performed for all individuals. A retrospective review of consecutive adult (age 18 years and older) NORSE patients enrolled into this cohort between 1 March 2014 and 31 March 2023 was performed. NORSE patients were identified according to consensus definitions, and cryptogenic NORSE was defined as the de novo onset of status epilepticus (SE) refractory to at least 2 appropriately selected and dosed parenteral anti-seizure medications (ASM) including benzodiazepines, without an identifiable structural, toxic or metabolic cause [15]. Thorough evaluation of neuronal autoantibodies, underlying malignancy, genetic causes, and infective etiologies including bacteria, virus, fungal infections, and prion disease was performed in this cohort. The diagnostic evaluations performed in this cohort have been previously described in detail in earlier publications [6, 16].

Control patients were aged 18 years and above, with CSF testing performed for the evaluation of other neurological conditions. The diagnoses in the control group included epilepsy, atypical parkinsonism, cerebellar ataxia, and postural orthostatic hypotension syndrome.

2.2 | Patient Profiles, Paraclinical Findings, and Outcome Measures

Demographic data, including age, gender, and diagnosis of premorbid neurodegenerative disease, were noted. Clinical information, such as seizure type, duration of intensive care unit (ICU) stay, CSF findings, anti-seizure medication (ASM), and anesthetic infusion use, was included. Brain magnetic resonance imaging (MRI) obtained at the initial phase of NORSE and at 2 months from NORSE onset (or next available interval scan after 2 months) was reviewed by two neurologists (Y.J. and S.H.A.) and evaluated for hippocampal atrophy with Scheltens' scale [17]. Outcomes were measured longitudinally with the Clinical Assessment Scale in Autoimmune Encephalitis (CASE) score [18] and modified Rankin Scale (mRS).

2.3 | Proteomic Analysis

CSF proteomic analysis, which included t-tau, was performed with the Olink Target 96 Neurology and Target 96 Neuro Exploratory panels (Olink Proteomics, Uppsala, Sweden) on CSF samples obtained within 7 days of NORSE onset. 1 μL of each patient's CSF was used per panel. Serum and CSF cytokines/chemokines were also analyzed with the Olink Target 48 Cytokine panels, in 14/19 (73.7%) and 19/19 (100%) of cNORSE patients, respectively. The qPCR readout was processed with Olink NPX Signature (Olink Proteomics, Uppsala, Sweden). Protein concentrations were expressed as Normalized Protein Expression (NPX), Olink's relative protein quantification unit. The limit of detection (LOD) was determined by using negative control samples. Quality control measures were carried out according to Olink's standard protocol.

P-tau 181 was analyzed with the Invitrogen KHO0631 enzyme-linked immunosorbent assay (ELISA) Kit (Invitrogen,

Massachusetts, United States) in all patients with cNORSE and controls, aside from one cNORSE patient with insufficient CSF samples. Sample dilution was not performed.

2.4 | Statistical Analysis

Statistical analysis was performed with R software v4.4.1 (2024; R Team, Vienna, Austria). Categorical variables were analyzed with Fisher's exact test and continuous variables with Mann–Whitney *U* and *t*-tests as appropriate. A *p*-value of <0.05 was considered significant.

In evaluating the relationship of proteomic markers to cytokines/chemokines and development of interval hippocampal atrophy, Spearman's rank correlation and Mann–Whitney U tests were used for continuous and categorical outcomes, respectively. To control for multiple comparisons, the Benjamini–Hochberg correction was applied to adjust the *p*-values for false discovery rate control. A secondary analysis without correction was performed to further explore correlations between proteomic markers and early clinical outcomes.

To evaluate the association between CSF t-tau level and outcomes, the cNORSE study population was divided into two groups based on the median value of t-tau. This was to ensure an equal proportion of patients between groups while avoiding assumptions about the underlying distribution of t-tau levels. Univariate linear and logistic regression analyses were subsequently performed with t-tau groups, as well as other clinical and paraclinical variables, to identify factors associated with cNORSE severity and outcomes. Factors with *p*-values <0.1 in univariate regression were included in multivariate analysis.

3 | Results

We enrolled a total of 19 patients with cNORSE who had CSF collected within 7 days of cNORSE onset (Figure 1), and 21 control patients with other neurological diseases.

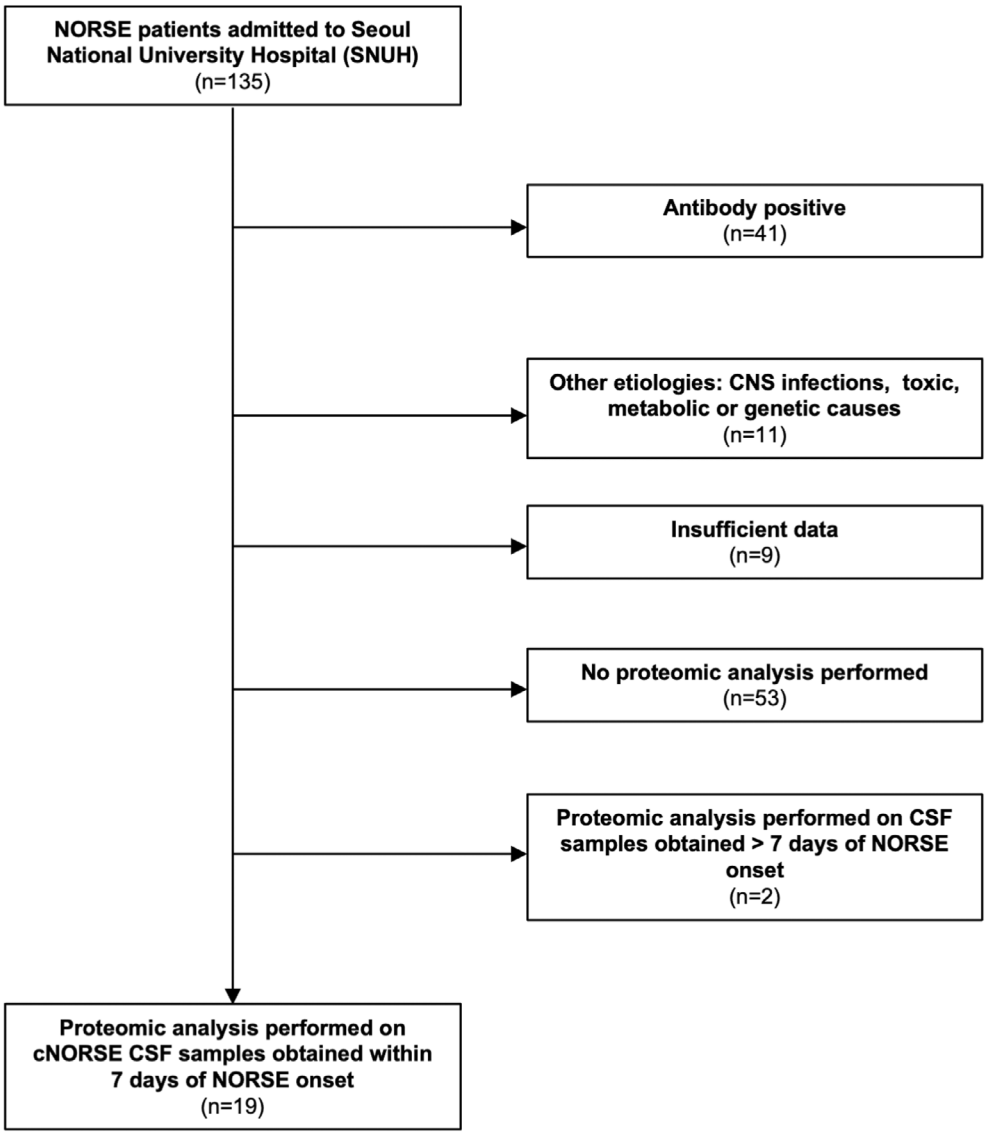

FIGURE 1 | Flowchart illustrating the process of selecting the cNORSE patients in the study cohort.

TABLE 1 | Clinical characteristics of cryptogenic NORSE patients.

Characteristics	Overall ($n=19$)	High Tau group[a] ($n=9$)	Low Tau group ($n=10$)	p
Age at onset, years [IQR]	35.0 [27.0–54.3]	33.0 [27.7–51.9]	38.1 [27.7–53.9]	0.780
Male gender, *n* (%)	9 (47.4)	5 (55.6)	4 (40.0)	0.656
Premorbid mRS [IQR]	0 [0–0]	0 [0–0]	0 [0–0]	0.192
Premorbid neurodegenerative disease, *n* (%)	1 (5.3)	0 (0.0)	1 (10.0)	1.000
mRS at NORSE onset [IQR]	5.0 [5.0–5.0]	5.0 [5.0–5.0]	5.0 [5.0–5.0]	0.399
CASE score at NORSE onset [IQR]	23.0 [21.0–24.0]	23.5 [21.8–24.0]	22.0 [20.0–24.0]	0.472
NORSE type, NCSE, *n* (%)	5 (26.3)	2 (22.2)	3 (30.0)	1.000
CSF				
Days from first seizure to CSF collection [IQR]	2.0 [0.5–4.0]	2.0 [1.0–4.0]	2.0 [0.3–2.8]	0.403
CSF WBC count/μL [IQR]	8.0 [2.3–20.0]	20.0 [11.8–65.8]	2.5 [2.0–3.8]	0.005*
CSF protein, mg/dL [IQR]	49 [33.3–78.7]	68 [30.8–91.8]	41 [37.8–61.9]	0.423
MRI				
Hippocampal atrophy on initial MRI, *n* (%)	4 (21.1)	2 (22.2)	2 (20.0)	1.000
Hippocampal atrophy on postacute phase MRI, *n* (%)	11 (64.7)	8 (88.9)	3 (37.5)	0.049*
Treatment				
Number of anesthetic infusions used during inpatient stay [IQR]	2.0 [1.0–3.0]	2.0 [1.8–3.0]	1.0 [1.0–3.0]	0.583
Duration of anesthetic infusion use [IQR]	23 [6.0–39.0]	37.0 [10.8–48.5]	21.0 [5.0–26.0]	0.136
Number of anti-seizure medications used within first month of NORSE onset, [IQR]	5.0 [4.0–6.0]	6.0 [5.0–7.0]	4.0 [3.0–5.0]	0.017*
Number of anti-seizure medications used during clinical course, [IQR]	6.0 [5.0–7.0]	7.0 [6.0–8.0]	5.0 [4.3–6.0]	0.013*
First-line immunotherapy				
Use of any first line immunotherapy	19 (100.0)	10 (100.0)	10 (100.0)	
Onset to first line immunotherapy, days [IQR]	1.0 [0.5–2.0]	2.0 [1.0–6.0]	1.0 [0.3–1.8]	0.133
Second-line immunotherapy				
Use of any second line immunotherapy, *n* (%)	18 (94.7)	9 (100.0)	9 (90.0)	1.00
Onset to second line immunotherapy, days [IQR]	13.5 [8.0–21.5]	13.0 [11.0–18.0]	16.0 [7.0–22.0]	0.757
Outcomes				
Duration of ICU stay, days [IQR]	28.0 [16.8–45.5]	43.0 [25.0–46.0]	25.0 [15.0–42.0]	0.185
1-month mRS [IQR]	5.0 [5.0–5.0]	5.0 [5.0–5.0]	5.0 [4.3–5.0]	0.095
1-month CASE score [IQR]	21.0 [16.0–24.0]	24.0 [21.0–24.0]	14.0 [9.3–21.3]	0.030*
3-month mRS [IQR]	4.0 [3.0–5.0]	4.0 [4.0–5.0]	4.0 [3.0–5.0]	0.699
3-month CASE score [IQR]	11.0 [5.0–17.0]	11.0 [6.0–15.0]	8.5 [4.5–17.5]	0.642
1-year mRS [IQR]	3.0 [3.0–4.0]	3.0 [3.0–4.0]	3.0 [2.0–4.0]	0.521
1-year CASE score [IQR]	5.5 [3.3–8.0]	6.5 [3.8–7.8]	4.5 [3.3–9.5]	0.796

Abbreviations: CASE, clinical assessment scale for autoimmune encephalitis; mRS, modified Rankin Scale; NCSE, non-convulsive status epilepticus.
[a]High Tau group defined by Tau value ≥ 2.2 NPX.
*$p \leq 0.05$.

3.1 | Characteristics of the cNORSE Cohort

In cNORSE, the median age of onset was 35.0 [IQR: 27.0–54.3] years, and 9 (47.4%) were male. The median premorbid mRS was 0 [0–0]. Only one patient had a premorbid diagnosis of neurodegenerative disease (Parkinson's disease) prior to cNORSE onset. Five (26.3%) presented with non-convulsive status epilepticus. The median mRS and CASE scores at NORSE onset were [5-5] and 23 [21–24], respectively. The median number of days from the first seizure to CSF collection was 2.0 [0.5–4.0]. Six out of 19 patients (31.6%) received immunotherapy prior to CSF proteomic analysis (three patients received steroids prior, two patients received intravenous immunoglobulin (IVIG) prior and one patient received both steroids and IVIG prior to analysis). On follow-up, all patients received first-line immunotherapy and all but one patient received second-line immunotherapy, such as rituximab (17/19, 89.5%) or tocilizumab (12/19, 63.2%). The median duration of ICU stay was 28.0 [16.8–45.5] days. Patients were followed up for a median of 682 [254–1182] days. One-month mRS and CASE scores were 5 [5] and 21 [16–24], respectively. At 3 months, mRS and CASE scores were 4 [3–5] and 11 [5–17], and at 1 year, mRS and CASE scores were 3 [3, 4] and 5.5 [3.3–8.0] (Table 1).

3.2 | Characteristics of the Control Cohort

Controls consisted of patients with other neurological diseases: atypical parkinsonism ($n=5$), postural orthostatic hypotension syndrome ($n=6$), epilepsy ($n=7$), and cerebellar ataxia ($n=3$). The median age of these controls was 40.7 [30.3–52.0] years, and the median disease duration prior to CSF sampling was 1.0 [0.3–1.6] years.

3.3 | Selection of t-Tau as Target Protein

CSF proteins in the Olink panel readouts were evaluated for statistically significant differences (fold change >2, $p<0.05$) in NPX values between patients with cNORSE and controls (See Table S1 for statistically significant readouts). Subsequently, correlation analyses with clinical outcomes performed with multiple comparison correction showed a statistically significant relationship only between MRS at 4 weeks and two proteins, t-tau (MAPT) and nicotinamide/nicotinic acid mononucleotide adenylyltransferase (NMNAT1) (rho $=0.583$, $p=0.035$ and rho $=0.635$, $p=0.014$, respectively). To explore further proteomic associations with hippocampal atrophy, further analysis was performed. An adjusted analysis did not reveal any significant results, but an unadjusted analysis showed that only t-tau had a significant correlation with hippocampal atrophy ($U=12$, $p=0.039$, $r=0.500$). (Figure 2).

Hence, further analysis was done for t-tau in patients with cNORSE and controls. Patients with cNORSE had a much higher median t-tau level (2.134 [1.865–4.857]) compared to control patients (0.552 [0.004–1.277], $p<0.001$). (Figure 2, Table S2). To further evaluate CSF tau phosphorylation in cNORSE, analysis of phosphorylated tau 181 (p-tau 181) was performed. P-tau 181 is a well-known biomarker for various neurodegenerative diseases, such as Alzheimer's disease (AD), and is associated with the progression of pathological changes in AD [19]. However, ELISA testing of p-tau 181 revealed that its level was undetectable in both patients with cNORSE and control samples within our cohort. Thus, t-tau was selected as the target biomarker for further correlation analysis.

3.4 | Radiological Differences Between High and Low Tau Groups

We analyzed the differences between high tau (t-tau value greater or equal to 2.2 NPX, n=9) and low tau (n=10) groups. MRI done at 2 months from cNORSE onset (or next available interval scan after 2 months) was reviewed to assess for hippocampal atrophy in the postacute phase of cNORSE. Seventeen out of the 19 patients with cNORSE (89.5%) had appropriate

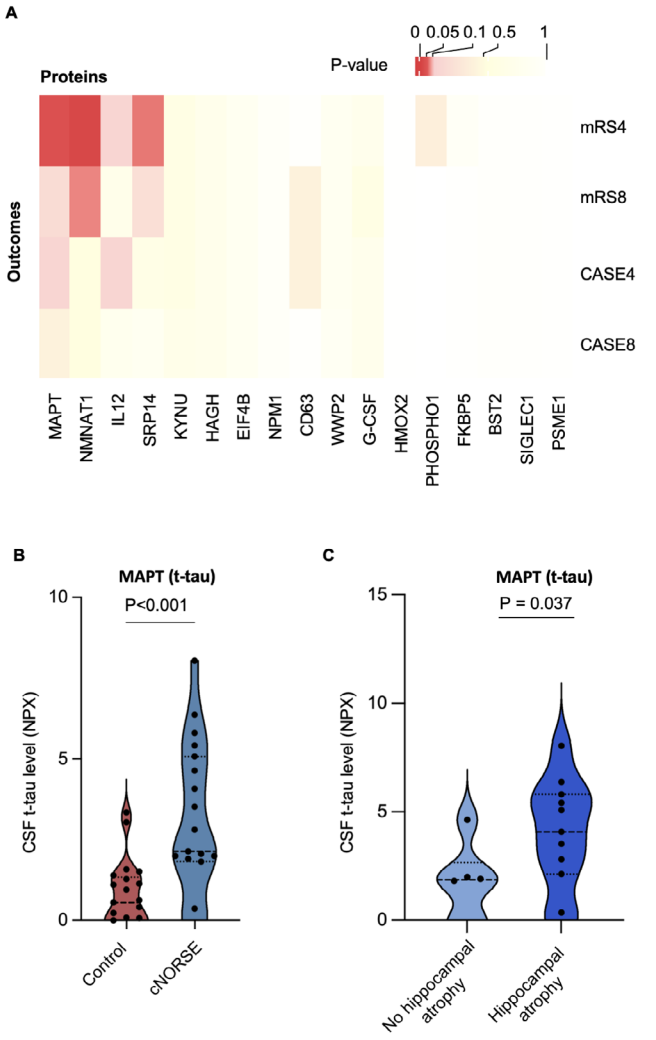

FIGURE 2 | Selection of MAPT (t-tau) as a biomarker. Heatmap representing adjusted correlation analysis between CSF candidate protein levels and clinical outcomes indicated that MAPT (t-tau) and NMNAT1 were potential biomarkers (A). Violin plot of CSF t-tau levels in patients with cNORSE and controls shows the significant elevation of early CSF t-tau in cNORSE compared to controls (B). The dashed lines and dotted lines on the violin plot represent median values and interquartile ranges, respectively. Violin plot of CSF t-tau levels showed a significant difference in patients with hippocampal atrophy compared those without. This did not hold true for NMNAT1 and hence MAPT was chosen as a biomarker for further analyses (C).

available MRI imaging for analysis. A higher proportion of patients with high t-tau had hippocampal atrophy in the postacute phase (88.9%), compared to patients in the low tau group (37.5%). (Table 1, Figure 3).

Univariate regression analysis showed that high CSF t-tau was associated with hippocampal atrophy in the postacute phase (OR = 13.333, 95% CI: 1.405–321.63, $p = 0.044$). (Table 2) Multivariate analysis was not performed as no other factor appeared statistically significant in univariate analysis.

3.5 | Clinical Differences Between High and Low Tau Groups

In the high tau group, there was a higher CSF white blood cell (WBC) count (median 20.0 [11.8–65.8] vs. 2.5 [2.0–3.8] in the low tau group, $p = 0.005$), more anti-seizure medications (ASM) used within the first month of seizure onset (6.0 [5.0–7.0] vs. 4.0 [3.0–5.0], $p = 0.017$) as well as during the entire clinical course (7.0 [6.0–8.0] vs. 5.0 [4.3–6.0], $p = 0.013$) (Table 1, Figure 3). Some patients in the high tau group appeared to have a more refractory clinical course compared to the low tau group, requiring a longer duration to attain seizure freedom over 1 year (Figure 3) Additionally, 1 month CASE scores were higher in the high tau group (24.0 [21.0–24.0] vs. 14.0 [9.3–21.3], $p = 0.030$). However, MRS at 1 month did not reach statistical significance (5.0 [5.0–5.0] in the high tau group vs. 5.0 [3.4–5.0], $p = 0.095$) in this comparison (Table 1).

Subsequently, regression analysis was carried out to examine the factors affecting ASM requirement and changes in CASE score 1 month after NORSE onset (Table 2). Changes in CASE score were calculated by subtracting the CASE score at 1 month from the CASE score at initial presentation, where a negative value denotes an improvement in CASE score. Univariate analysis showed high CSF t-tau was associated with less improvement in CASE score 1 month after onset (β: 5.982, 95% CI: −0.446—12.411, $p = 0.066$). Further multivariate analysis was not performed as there was no other statistically significant variable. CSF WBC count (β: 0.013, 95% CI: −0.001–0.026, $p = 0.059$) and high CSF t-tau (β: 2.333, 95% CI: 0.668–3.999, $p = 0.009$) were associated with the ASM requirement within the first month of NORSE (Table 2). Subsequent multiple linear regression analysis showed that high CSF Tau remained significantly correlated with ASM requirement in the first month (β: 2.206, 95% CI: 0.225–4.187, $p = 0.031$). (Table 3).

3.6 | Associations Between Cytokine/Chemokine Levels and CSF t-Tau

There were no significant associations between serum cytokines/chemokines and CSF t-tau levels in cNORSE patients.

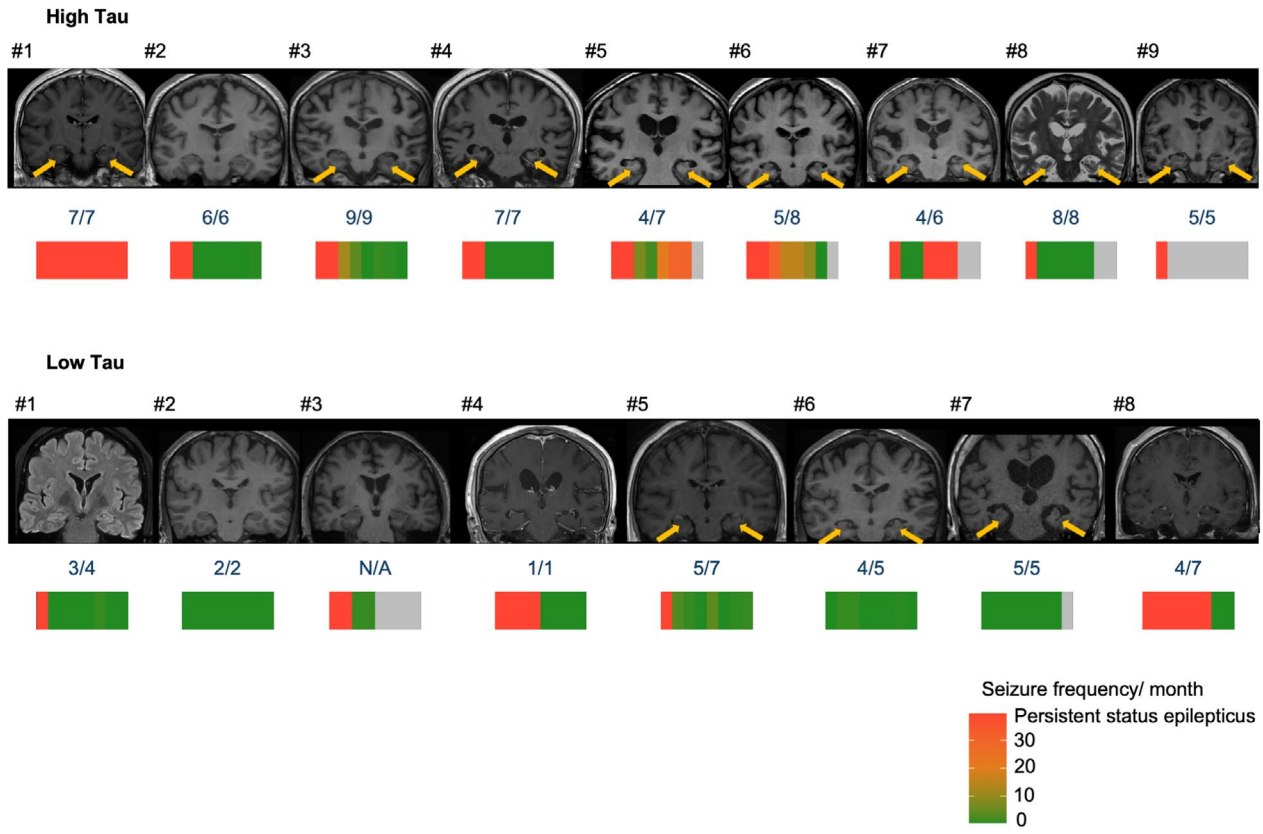

FIGURE 3 | Clinical and radiological differences between patients with high and low t-tau. Coronal images of patients with available interval MRIs at 2 months from NORSE onset (or next available interval scan after 2 months) ($n = 17$) are depicted, with yellow arrows showing the presence of hippocampal atrophy. cNORSE patients with high t-tau had more frequent hippocampal atrophy on interval MRI. The numbers in blue represent the number of anti-seizure medications (ASMs) used within the first month of NORSE onset, followed by the total number of ASMs used during clinical course. Heatmaps show each patient's seizure frequency at 4 weekly intervals for the first year, and missing seizure frequency data are represented in gray. cNORSE patients with high t-tau had more frequent seizures.

However, CSF C-C motif chemokine (CCL)7, CCL13, C-X-C motif chemokine (CXCL) 9, CXCL10, CXCL 11, tumor necrosis factor ligand superfamily member 12 (TNFSF12), lymphotoxin-alpha (LTA), oxidized low-density lipoprotein receptor 1 (OLR1) and hepatocyte growth factor (HGF) were significantly associated with CSF t-tau levels (rho >0.5, $p<0.05$) (Figure 4).

4 | Discussion

In our study, CSF t-tau levels were significantly elevated in patients with cNORSE compared to controls, which included patients with atypical parkinsonism and epilepsy. High CSF t-tau also appeared to correlate with initial cNORSE severity, measured by ASM requirement in the first month and change in CASE score 1 month after onset. Furthermore, the high tau group had a longer duration of anesthetic infusion use, potentially reflecting greater clinical severity, though this difference did not reach statistical significance. Since CSF t-tau levels are a known biomarker of neuronal damage [20, 21], the extent of neuronal damage may be reflected indirectly in these clinical measures. A previous case series has also proposed that CSF t-tau level may be a marker of SE severity, with higher CSF t-tau in refractory SE than in seizures controlled by ASM [22]. However, as this is an uncontrolled study with no defined treatment protocols, ASM usage in this cohort could be affected by physician preference.

T-tau has been used as a biomarker of brain injury in neurodegenerative diseases, such as Alzheimer's disease [23, 24]. It has also been studied as a marker of neuronal damage in traumatic brain injury [25, 26] and ischemic stroke [27], where t-tau levels have been found to correlate with infarct size [28]. The marked increase in CSF t-tau levels in the cNORSE cohort could reflect significant axonal injury that occurs in the acute phase of cNORSE [20], to a much greater extent than that in chronic stable neurological conditions, such as epilepsy or atypical parkinsonism. Higher levels of t-tau in patients with SE compared to patients with epilepsy have also been described in earlier studies, and t-tau levels have been noted to correlate with the duration of seizure [22, 29]. Furthermore, animal models have shown that tau plays an important role in epileptogenesis [30, 31]. In mouse models, tau reduction decreased seizure severity and delayed seizure onset, suggesting that tau reduction could mitigate neuronal overexcitation [32, 33].

However, p-tau 181 levels were undetectable in both cNORSE patients and controls in our cohort. Although seizures have been demonstrated to be associated with tau hyperphosphorylation in animal models [21, 34], and postulated to be linked to neuronal injury causing tau signaling cascade activation [35], p-tau 181 may be specific to neurofibrillary tangle pathology in Alzheimer's dementia [36]. A previous study performed in patients with acute ischemic stroke had similarly shown CSF t-tau elevation, but no change in CSF p-tau 181 [28]. Although tau hyperphosphorylation occurs in seizures and epilepsy, p-tau 181 may be a less significant biomarker in cNORSE.

Aside from t-tau, other potential proteomic biomarkers in our preliminary analysis included NMNAT1. NMNAT1 levels were higher in cNORSE patients compared to controls and were associated with higher 4-week MRS. NMNAT1 is a key enzyme in nicotinamide adenine dinucleotide (NAD^+) biosynthesis and is diffusely expressed in the brain [37, 38]. Earlier studies have shown that

TABLE 2 | Univariate analysis of factors affecting the presence of hippocampal atrophy on postacute phase MRI, change in CASE score 1 month after NORSE onset, and ASM requirement within the first month of NORSE onset. Change in CASE score was calculated by subtracting the CASE score at 1 month from the CASE score at initial presentation, where a negative value denotes an improvement in the CASE score.

	Hippocampal atrophy on interval MRI		Change in CASE score 1 month after NORSE onset		Maximum number of ASM required within the first month of NORSE onset	
Factors	**OR (95% CI)**	***p***	**β (95% CI)**	***p***	**β (95% CI)**	***p***
Age at onset	1.022 (0.971, 1.092)	0.438	−0.045 (−0.217, 0.126)	0.578	−0.014 (−0.066, 0.038)	0.582
Male gender	1.667 (0.218, 15.978)	0.629	1.518 (−5.786, 8.822)	0.661	0.111 (−1.962, 2.185)	0.911
Premorbid MRS	0.613 (0.056, 6.609)	0.648	−0.962 (−3.600, 1.677)	0.445	−0.547 (−1.319, 0.226)	0.153
CASE score at NORSE onset	1.329 (0.968, 2.442)	0.203	0.001 (−0.891, 0.894)	0.998	0.171 (−0.108, 0.450)	0.208
NORSE type	0.750 (0.085, 7.503)	0.794	−2.300 (−9.966, 5.366)	0.528	−0.200 (−2.513, 2.113)	0.857
CSF WBC count	1.106 (0.954, 1.283)	0.180	0.027 (−0.019, 0.073)	0.229	0.013 (−0.001, 0.026)	0.059*
Hippocampal atrophy on initial MRI	1.875 (0.177, 43.977)	0.625	−2.500 (−10.667, 5.667)	0.520	−0.571 (−3.048, 1.905)	0.631
Onset to first line immunotherapy	1.288 (0.938, 2.139)	0.184	0.682 (−0.487, 1.850)	0.230	0.250 (−0.104, 0.603)	0.154
Onset to second line immunotherapy	1.014 (0.969, 1.114)	0.639	−0.022 (−0.169, 0.124)	0.746	0.015 (−0.025, 0.056)	0.432
High CSF Tau	13.333 (1.405, 321.63)	0.044**	5.982 (−0.446, 12.411)	0.066*	2.333 (0.668, 3.999)	0.009**

*$p \leq 0.1$.
**$p \leq 0.05$.

NMNAT1 inhibits neuronal degeneration and modulates oxidative stress [39, 40]. Its potential neuroprotective effects via various signaling pathways have led to its investigation as a therapeutic target in ischemic stroke, hypoxic ischemic injury, and tauopathies [40–44]. NMNAT1 may be released in response to neuronal injury, as suggested by ischemia-induced increases observed in a mouse model of ischemic stroke [44]. However, its function in cNORSE is unclear; in our study, patients with higher NMNAT1 levels had worse 4-week MRS outcomes, which might indicate a compensatory but inadequate response to neuronal injury.

TABLE 3 | Multiple linear regression for factors affecting the maximum number of ASM required within the first month of NORSE onset.

	Maximum number of ASM required within first month of NORSE onset	
Factors	**β (95% CI)**	***p***
CSF WBC count	0.002 (−0.012, 0.016)	0.778
High CSF Tau	2.206 (0.225, 4.187)	0.031**

**$p \leq 0.05$.

Although our data did not demonstrate an association of NMNAT1 with hippocampal atrophy, it represents a potential biomarker and therapeutic target that can be further investigated in cNORSE.

We also explored the associations between t-tau and cytokine/chemokines in this study. Previous studies have examined potential associations in other neurological conditions, such as Alzheimer's disease [45], and multiple sclerosis, where complement activation and CXCL9 have been implicated in neurodegeneration [46]. In NORSE, elevations in certain cytokines have been shown to correlate with worse clinical outcomes and cerebral volume loss, though the mechanism is unclear [4, 6]. Our previous study in cNORSE patients showed that a greater extent of cerebral atrophy on MRI was correlated with increased CSF cytokines (e.g., IL-6, MIP-1α, IL-1β, TNF-α, and CXCL9) [6]. In this cohort, both CSF Th1 (CXCL9, CXCL10, CXCL11) and Th2 chemokines (CCL7, CCL13) appear to be associated with high t-tau levels, which suggests that neuronal damage in cNORSE is not limited to a single inflammatory pathway. However, these associations do not establish causation; elevated cytokine/chemokines may instead reflect a secondary response to neuronal damage, as seen in animal models of brain and spinal cord injury [47, 48] Other inflammatory mediators (LTA, TNFSF12,

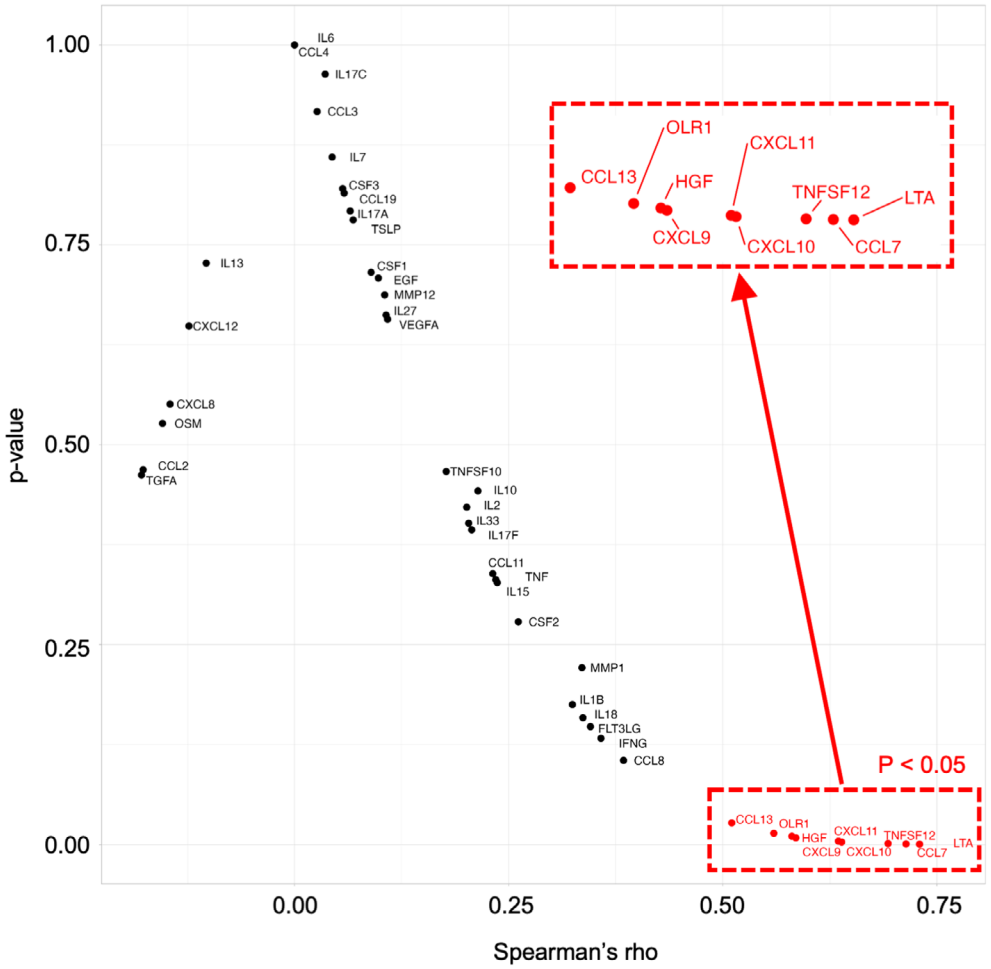

FIGURE 4 | Correlation analysis between CSF cytokines/chemokines and CSF t-tau in cNORSE patients. Scatter plot depicting Spearman's rho on the *x*-axis and corresponding *p*-value on the *y*-axis for each CSF cytokine/chemokine. The red dotted box contains cytokines/chemokines with $p < 0.05$ and rho > 0.5, which are enlarged on the top right panel for greater clarity.

OLR1 and HGF) associated with t-tau in our study have been previously implicated in neurodegeneration and responses to neuronal damage [49–52].

Previous studies evaluating markers of neuronal injury were conducted in SE patients of varying etiologies, and some of these markers appeared to be more useful in the diagnosis of SE, rather than the prognostication of SE outcomes [12, 13]. Our study utilized proteomic analysis to identify a potential biomarker related to early neuronal damage, CSF t-tau, and subsequently examined the utility of CSF t-tau levels with relation to clinical and radiological outcomes in cNORSE patients. The strengths of our study include the selection of a patient cohort limited to cNORSE patients, all of whom underwent comprehensive etiological evaluations. Additionally, the timeframe for CSF proteomic analysis was standardized, as the pathophysiology of different phases of cNORSE may be distinct.

There is a critical need to identify and evaluate biomarkers in cNORSE, which can provide important prognostic information and help identify patients who may benefit from more aggressive interventions [53]. Early detection of elevated t-tau in patients with cNORSE can help identify patients at higher risk of greater clinical severity and subsequent hippocampal atrophy. This may facilitate family discussions to prepare for adverse outcomes and inform therapeutic decisions. Earlier or more intensive immunotherapy regimes could potentially mitigate risks of immune-mediated neuronal damage and improve outcomes in these patients [6]. This is analogous to the emerging use of sNFL in multiple sclerosis, where it assists in identifying patients at high risk of disability worsening for treatment with high-efficacy disease modifying treatment [54, 55].

However, our study has several limitations. There is a relatively small number of patients in this study, and hence it may be underpowered to detect the association between CSF tau and long-term outcomes (mRS and CASE scores). Further studies with repeated longitudinal testing of potential CSF biomarkers in cNORSE could provide more insights into prognostication. Additionally, only cNORSE patients and control patients without neuroinflammatory disorders were compared in this study. Potentially, comparisons with seropositive autoimmune encephalitis patients presenting with status epilepticus and patients with other neuroinflammatory disorders can further elucidate differences in immunological mechanisms driving hippocampal atrophy.

5 | Conclusion

CSF t-tau levels performed early after cNORSE onset may be a useful marker of initial disease severity and predict subsequent hippocampal atrophy. Further studies are required to determine if earlier and more aggressive immunotherapy in cNORSE patients with high CSF t-tau can improve long-term clinical and cognitive outcomes.

Author Contributions

Y.G., Y.J. and S.J.S. analyzed the data, wrote the initial draft of the manuscript, and revised the manuscript. Y.J. and S.H.A. acquired and interpreted clinical data. S.J.S. performed the proteomic analysis. S.Y.M. contributed to sample preparation. S.-T.L. conceptualized the study, interpreted, and analyzed the clinical data, revised the manuscript, and supervised the study. S.-T.L., K.C. and S.K.L. collected clinical data. K.C. and S.K.L. supervised the study.

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Ethics Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Conflicts of Interest

Dr. S-T. Lee reports personal fees from Roche/Genentech (steering committee) and Advanced Neural Technologies (advisory board), and grants from GC Pharma and Celltrion, outside the submitted work. The remaining authors have no conflicts of interest.

Data Availability Statement

Anonymized data that support the findings of this study are available from the corresponding author upon reasonable request of any qualified investigator for purposes of replicating procedures and results. If such data are used for publication, its methods should be communicated, and internationally recognized authorship rules must be applied.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.