Cytokines in New-Onset Refractory Status Epilepticus Predict Outcomes

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Title: Cytokines in New-Onset Refractory Status Epilepticus (NORSE) predict outcomes

Running head: Cytokine profiles in NORSE patients

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Summary for Social Media

1. Twitter handle

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2. What is the current knowledge on the topic?

Several arguments suggest New-Onset Refractory Status Epilepticus (NORSE) is a disorder of immunity or inflammation, likely post-infectious. Nonetheless, no inflammation biomarker has been validated for patients with NORSE.

3. What question did this study address?

Here, we investigated inflammation using cerebrospinal fluid and serum cytokines/chemokines in patients with NORSE to better understand the pathophysiological mechanisms underlying NORSE and its long-term consequences.

4. What does this study add to our knowledge?

We identified significant differences in serum and cerebrospinal fluid cytokine/chemokine profiles between patients with NORSE and other patients with refractory status epilepticus. Elevated innate immunity-associated pro-inflammatory cytokines in patients with NORSE correlated with worse outcomes at discharge and several months after SE ended.

5. How might this potentially impact on the practice of neurology?

These findings highlight the involvement of innate immunity-related inflammation in NORSE pathogenesis and suggest the importance of anti-inflammatory interventions to improve outcomes.

Abstract

Objective

To investigate inflammation using cerebrospinal fluid (CSF) and serum cytokines/chemokines in patients with New-Onset Refractory Status Epilepticus (NORSE) to better understand the pathophysiology of NORSE and consequences.

Methods

Patients with NORSE (n=61, including n=51 cryptogenic), including its subtype with prior fever known as FIRES (febrile infection-related epilepsy syndrome), were compared to patients with other refractory SE (RSE, n=37), and control patients without SE (n=52). We measured 12 cytokines/chemokines in serum or CSF samples using multiplexed fluorescent bead-based immunoassay detection. Cytokine levels were compared between patients with and without SE, and between the 51 patients with cryptogenic NORSE (cNORSE) and the 47 patients with a known-etiology RSE (NORSE n=10, other RSE n=37), and correlated with outcomes.

Results

A significant increase of IL-6, TNF-α, CXCL8/IL-8, CCL2, MIP-1α and IL-12p70 pro-inflammatory cytokines/chemokines was observed in SE patients compared to patients without SE, in serum and CSF. Serum innate immunity pro-inflammatory cytokines/chemokines (CXCL8, CCL2, MIP-1α) were significantly higher in patients with cNORSE compared to non-cryptogenic RSE. NORSE patients with elevated innate immunity serum and CSF cytokine/chemokine levels had worse outcomes at discharge and several months after SE ended.

Interpretation

We identified significant differences in innate immunity serum and CSF cytokine/chemokine profiles between patients with cNORSE and non-cryptogenic RSE. The elevation of innate immunity pro-inflammatory cytokines in patients with NORSE correlated with worse short- and long-term outcomes. These findings highlight the involvement of innate immunity-related inflammation, including peripherally, and possibly of neutrophil-related immunity in cNORSE pathogenesis and suggest the importance of utilizing specific anti-inflammatory interventions.

Abbreviations: **cNORSE**, cryptogenic New-Onset Refractory Status Epilepticus**; FIRES**, Febrile Infection Related Epilepsy Syndrome; **ICU**, Intensive Care Unit; **NORSE**, New-Onset Refractory Status Epilepticus; **PCA**, Principal Component Analysis; **RSE**, Refractory Status Epilepticus

Introduction

New-Onset Refractory Status Epilepticus (NORSE) is defined as refractory SE (RSE) that occurs in adults or children without active epilepsy and without a clear acute or active structural, toxic or metabolic cause identified in the first few days.[1] Mortality is around 12% in children and even higher in adults (16-27%).[2,3] The majority of patients have long-term neurocognitive and functional disability, often including drug-resistant epilepsy.[3,4] Understanding of the pathophysiological mechanisms underlying NORSE and its long-term consequences is crucial to improve NORSE management and prevent secondary neuronal injury.

Several arguments suggest that NORSE results from a post-infectious process leading to exacerbated cerebral inflammation. First, an abnormal cerebrospinal fluid (CSF) with mild pleocytosis and mildly elevated protein levels is frequently found.[3,5–7] Second, polymorphisms in cytokine-related genes were found in patients with NORSE for whom SE onset was preceded by a febrile illness (a subtype of NORSE known as Febrile-Infection Related Epilepsy Syndrome [FIRES]).[8,9] Third, several studies reported increased serum and/or CSF cytokine levels in patients with NORSE. Specifically, Th1-associated cytokines/chemokines and innate pro-inflammatory cytokines IL-1ß, IL-6 and CXCL8 (previously known as IL-8) were elevated in the CSF of patients with NORSE compared to patients with chronic epilepsy.[8,10–14] A recent quantitative proteomic analysis of CSF samples suggested the involvement of the innate and lymphocyte-mediated immune response in patients with NORSE for whom no etiology was identified despite an extensive workup.[15] There is less evidence for disturbance in serum cytokines in patients with NORSE.[8,11] No study has evaluated the direct association between cytokine/chemokine levels and the outcome of patients with NORSE. However, a decrease in CSF pro-inflammatory cytokine levels was found to be associated with clinical improvement after intrathecal dexamethasone or anakinra therapies.[10,16] The CSF IL-6 and CXCL8 levels were found positively associated to an up-proteomic score that has been suggested as a promising indicator for assessment of the severity of NORSE in a cohort of 11 patients.[15] Pro-inflammatory cytokines may enhance seizure recurrence and subsequent sequelae.[8,17–19] Elevated pro-inflammatory cytokines in young children with febrile SE was correlated with T2 MRI hippocampal hyperintensity and with the development of hippocampal sclerosis.[17]

Patients with NORSE, especially if prolonged and unexplained (i.e. cryptogenic, cNORSE), often empirically receive immunotherapy to treat a presumed underlying inflammatory process in the hope to shorten SE and minimize sequelae. Recently, a large international group of

experts recommended using first-line immunotherapy (corticosteroids, intravenous immunoglobulins or plasma exchange) within the first 72 hours after onset of RSE.[20] Second-line immunotherapies (rituximab, the IL-1 receptor antagonist anakinra, or IL-6 blockers such as tocilizumab) were suggested to be used within one week in cNORSE with inadequate response to first-line immune treatment.[20] Additional investigations are needed to better understand the pathophysiology of cNORSE, especially related to inflammation, and to support the use of any specific immune treatment.

Here, we investigated CSF and serum cytokines/chemokines in 61 patients with NORSE, including 51 patients with cNORSE.

Methods

Study design, setting and participants

This study was approved by the Yale University (NORSE/FIRES biorepository, IRB #1511016840), the Paris Pitié-Salpêtrière Hospital (2012, CPP Paris-VI and 2020, CPP Saint-Louis Hospital), and the INSERM (C16-16-20152482) ethic committees. Patients or relatives were informed and gave their consent. The study was designed and reported in accordance with the STROBE statement. Patients were enrolled in 13 hospitals in the United States, one in Canada and one in France, all between February 2013 and July 2022.

We included patients at least two years old with NORSE (n=61), patients with other forms of refractory SE from known-etiology (RSE, n=37), patients with autoimmune encephalitis without SE (n=12), patients with pharmacoresistant epilepsy who had a seizure within 24 hours before sample collection (n=22), and control patients without epilepsy (n=18; more details below). Serum samples were collected for all patients while CSF samples were collected only for some patients (NORSE n=29; RSE n=14; autoimmune encephalitis n=10; controls n=17). Serum and CSF samples were collected at the same time or within two days of each other for 63 patients (NORSE n=22; RSE n=14; autoimmune encephalitis n=10; controls n=17). These cases were used to compare CSF and serum samples within the same patient. Patients with NORSE or other forms of RSE were grouped as "patients with SE"; while patients with autoimmune encephalitis, pharmacoresistant epilepsy or control patients were grouped as "patients without SE".

NORSE was defined as refractory SE that occurs in children or adults without active epilepsy and without a clear acute or active structural, toxic or metabolic cause identified in the first couple of days.[1] The subset of patients with NORSE who had a preceding febrile illness (between 24 hours and two weeks before SE onset) were defined as patients with FIRES (n=24).[1] Patients with refractory SE (RSE) and deterioration of previous epilepsy condition (n=16) or those without a medical history of epilepsy and for whom an etiology was found in the first 72 hours (n=21) were defined as patients with other forms of RSE, but not NORSE. This group included patients with tumors (n=11), vascular disorders (n=3), metabolic disorders (n=2), posterior reversible encephalopathy syndrome (n=2), mild brain trauma (n=2), and alcohol abuse (n=1). We excluded patients with post-anoxic SE and patients whose SE was linked to a severe trauma or any intracranial hemorrhage requiring immediate neurosurgery.

The autoimmune encephalitis group included adults with epilepsy but without SE, for whom a diagnosis of definite or probable autoimmune encephalitis was based on recent consensus criteria.[21] A positive staining for antibody against intracellular antigens or synaptic proteins was found in serum or CSF samples of 11 patients out of 12 (NMDAR n=5, GAD65 n=2, CASPR-2 n=2, LGI1 n=1, Hu n=1). Patients with autoimmune encephalitis and RSE were considered as NORSE, but not cNORSE (n=6).

The pharmacoresistant epilepsy group included adults admitted for video-EEG monitoring for a pre-surgical evaluation or for a resection of the epileptic focus. All patients had a seizure within 24 hours before serum collection. No lumbar puncture was performed for these patients.

Control patients were aged at least 18 years and admitted on neurology wards. A clinical neurological evaluation and/or a lumbar puncture were performed for all of them. Patients whose symptoms were linked to an acute pathological condition (trauma, hemorrhage) were excluded as well as patients with an abnormal lumbar puncture (i.e. pleocytosis, increased protein levels, intrathecal synthesis). The diagnoses for those patients were psychogenic disorders, non-inflammatory peripheral neuropathies or healthy volunteers.

Sample collection and processing

Red-top blood tubes were collected and spun down at 1,500 g for 10 minutes to obtain serum. As serum cytokine levels are known to be impacted by delays prior to spinning,[22] we established criteria for the effective use of previously collected samples. Samples had to be spun down

within 3 hours after collection. When the delay prior to spinning was not reported, we processed the samples for cytokine measurement and we used a visual quality criterion: the fluorescence peak had to be tight reflecting a good quality sample (Supplementary Figure 1). Polypropylene tubes with no additive were used for collecting CSF. CSF tubes were spun down at 1,500 g for 10 minutes in order to remove CSF cells and the supernatant were collected. Both serum and CSF samples were stored at -80°C until analysis.

Cytokine measurement

Twelve cytokines/chemokines were measured in serum and CSF samples on a BD LSR Fortessa (Yale University School of Medicine) by multiplexed fluorescent bead-based immunoassay detection (BD Biosciences) according to the manufacturer's instructions. Serum and CSF concentrations of interleukin (IL)-1ß, IL-4, IL-10, IL-12p70, IL-17A and tumor necrosis factor (TNF)-α were measured using the BD Cytometric Bead Array Human Enhanced Sensitivity Master Buffer Kit. Serum and CSF concentrations of IL-6, CXCL8/IL-8, CCL2/MCP-1, CCL3/MIP-1α, G-CSF and VEGF were measured using the BD Cytometric Bead Array Human Soluble Protein Master Buffer Kit. All samples were measured undiluted. Cytokine/chemokine concentrations were interpolated from the corresponding calibration curve. All cytokine/chemokine concentrations were expressed in pg/mL.

Serum and CSF IL-1ß concentrations were also measured for a subset of patients on a Quanterix SP-X imaging and analysis platform (Sorbonne Université, Pitié-Salpêtrière Hospital) using the Human CorPlex Cytokine Panel Array kit (Quanterix). Single-plex bead-based ultra-sensitive immunodetection of IL-17A was performed by digital ELISA using the Simoa (single molecule array) HD-1 analyzer (Quanterix), according to the manufacturer's instructions.

Statistical analysis

Analyses were performed with R Studio (v.1.2.5019) and graphs were computed with GraphPad Prism software 9 (v.9.2.0).

We performed an unsupervised principal component analysis (PCA) to assess the capability of cytokines/chemokines to detect group differences between patients with SE (i.e. NORSE and RSE) and patients without SE (i.e. autoimmune encephalitis, pharmacoresistant epilepsy and controls). The impact of each original variable (cytokine/chemokine) is represented as a vector (arrow). The length of each arrow reflects its contribution in building the PCA and the

orientation of the arrow highlights the direction of increase for the given variable. Cytokine/chemokine levels were then compared between patients with SE and patients without SE with Mann-Whitney tests. Next, we performed Kruskal-Wallis tests to evaluate the impact of subgroups. Post-hoc comparisons were performed by using Dunn tests when appropriate. A second PCA was done to assess the capability of cytokines/chemokines to detect group differences between patients with cNORSE (n=51) and patients with a known-etiology RSE (NORSE n=10 and other forms of RSE n=37). Cytokine/chemokine levels were compared between these two subgroups with Mann-Whitney tests.

Correlation between cytokine/chemokine levels, demographic and clinical data were evaluated by calculating Spearman's rho values and their level of significance. A heat map was created for serum and CSF using GraphPad Prism software. The heat map colors correspond to correlations grading from -1 (negative correlation, blue) to no correlation (white) to 1 (positive correlation, red). The Benjamini-Hochberg test procedure was used to correct for multiple comparisons.[23] All statistical tests were two-sided with a type I error rate of 5%.

Data availability

All anonymized data are available on request.

Results

Study participants

Serum samples were collected for 98 patients with SE (NORSE n=61; RSE n=37) and 52 patients without SE (controls n=18; autoimmune encephalitis n=12; pharmacoresistant epilepsy n=22). CSF samples were collected for 43 patients with SE (NORSE n=29; RSE n=14) and 27 patients without SE (controls n=17; autoimmune encephalitis n=10). Serum and CSF samples were collected at the same time or within two days of each other for 63 patients (NORSE n=22; RSE n=14; autoimmune encephalitis n=10; controls n=17). These cases were used to compare CSF and serum samples within the same patient. All patients were adults except four children or teenagers (9, 12, 15, and 17 years old) in the NORSE group. 39% (24/61) of patients with NORSE presented with a preceding febrile illness before SE onset and therefore qualified as FIRES. Among pediatric patients, three out of four also had FIRES. 77% (47/61) of patients with NORSE presented initially with SE with prominent motor symptoms, including 34 with generalized convulsive SE, nine focal motor SE, two tonic SE and two myoclonic SE.

Demographic data, serum and CSF timing and main CSF results are shown in Table 1. No difference in gender or age was found between subgroups of patients. All the serum and the CSF samples were collected in patients with an ongoing SE for the NORSE and RSE subgroups. Serum samples were collected sooner after SE onset for patients with other forms of RSE compared to patients with NORSE ($p<0.001$). The percentage of patients with CSF pleocytosis was significantly different among subgroups ($p=0.0058$) in contrast to the median CSF protein level ($p=0.86$) (Table 1).

Comparison of serum and CSF cytokine/chemokine levels between patients with SE and patients without SE

To investigate whether the cytokine/chemokine profile is altered in the serum and the CSF from patients with SE, data were analyzed using PCA plots. The results from PCA underlined overall differences between patients with SE and patients without SE, both in the serum (Fig.1A) and in the CSF (Fig.1B). This analysis also revealed the homogeneity of patients without SE and further highlighted parameters most associated with SE, that were, in serum CCL2, G-CSF, IL-6, CXCL8 or IL-8, IL-10, MIP-1α and VEGF; and in CSF, CCL2, G-CSF, IL-1ß, IL-6, CXCL8 and VEGF.

The serum cytokine/chemokine levels were further compared using Mann-Whitney tests in the 98 patients with SE and 52 patients without SE (Fig.1C-D). In patients with SE compared to those without SE, higher serum levels were seen for pro-inflammatory cytokines involved in the innate immune response such as IL-6 ($p<0.001$), TNF-α ($p=0.022$), CXCL8, ($p<0.001$), MIP-1α ($p=0.0010$) and CCL2 ($p=0.019$). Patients with SE had also significantly elevated levels of IL-12p70 ($p<0.001$) and IL-4 ($p=0.015$), but IL-17A appeared similar ($p=0.12$). Anti-inflammatory cytokine IL-10 was also elevated in the serum of patients with SE ($p<0.001$). The pro-inflammatory cytokine IL-1ß was not consistently elevated in serum from patients with SE ($p=0.11$). Similarly, serum 1L-1ß levels were not significantly more frequently above the detection threshold for patients with SE (21%) compared to patients without SE (10%) ($p=0.075$). Both growth factors, G-CSF and VEGF, were elevated in the serum of patients with SE compared to patients without SE (G-CSF: 0.73 ± 1.75 *vs* 0.13 ± 0.25, $p=0.011$; VEGF: 644.4 ± 796.52 *vs* 94.39 ± 91.95, $p<0.001$) (data not shown).

In the CSF from patients with SE, cytokine alterations were also seen (Fig.1E-F). These appeared to mainly involve the innate immune system with higher IL-6 ($p<0.001$), CXCL8 ($p<0.001$), MIP-1α ($p=0.011$) and CCL2 ($p<0.001$) levels, while IL-17A ($p=0.35$) appeared unchanged in the CSF. The CSF IL-1ß levels were detectable for only five patients, whom all belonged to the group of patients with SE. Nonetheless, patients with SE were not more likely to present detectable CSF IL-1ß levels ($p=0.15$). G-CSF was also elevated in the CSF from patients with SE compared to patients without SE (4.50 ± 12.58 *vs* 0.11 ± 0.19, $p<0.001$) (data not shown).

The CSF/serum ratios of CXCL8, CCL2 and G-CSF were higher for patients with SE compared to patients without SE (CXCL8: 7.49 ± 17.23 *vs* 1.49 ± 2.82, $p=0.033$; CCL2: 53.24 ± 55.49 *vs* 19.84 ± 16.64, $p=0.0078$; G-CSF: 10.34 ± 29.8 *vs* 0.48 ± 1.29, $p<0.001$) (data not shown).

Comparison of serum and CSF cytokine/chemokine levels within subgroups

Serum analysis showed differences among the five subgroups of patients for nine cytokines/chemokines: CCL2, G-CSF, IL-4, IL-6, CXCL8, IL-10, IL-12p70, MIP-1α and VEGF (Table 2). We performed post-hoc comparisons for those nine cytokines/chemokines. Patients with autoimmune encephalitis had higher serum levels of IL-6 ($p=0.016$) compared to patients with pharmacoresistant epilepsy, although their IL-6 serum levels were lower than those of patients with NORSE ($p=0.013$) or other forms of RSE ($p=0.0029$). Patients with NORSE, when compared to controls, showed elevated serum IL-6 ($p<0.001$), CXCL8 ($p<0.001$), IL-10 ($p<0.001$), IL-12p70 ($p=0.0052$) and VEGF ($p=0.013$) levels. In other patients with RSE, elevations were only seen in IL-6 ($p<0.001$) and IL-10 ($p=0.0011$) serum levels compared to controls. Patients with NORSE showed elevated serum CXCL8 ($p<0.001$), CCL2 ($p=0.013$), MIP-1α ($p=0.022$), IL-10 ($p=0.033$) and IL-4 ($p=0.0090$) levels compared to patients with other forms of RSE. No difference was found among the subgroups of patients in serum levels of IL-1ß and IL-17A, evaluated with multiplexed fluorescent bead-based immunoassay. Consequently, the serum levels of IL-1ß and IL-17A were only compared for a subset of patients for whom levels were evaluated with a high-sensitivity method. While their serum levels were above the detection range for all patients, we did not find significant differences between patients with NORSE and patients with other forms of RSE, either for IL-1ß ($p=0.39$) or IL-17A ($p=0.80$).

The same analysis was conducted on the CSF samples and showed differences among the four subgroups of patients for five cytokines: CCL2, G-CSF, IL-6, CXCL8 and MIP-1α (Table 2). Post-hoc comparisons were performed for those five cytokines. Both patients with NORSE and patients with other forms of RSE had higher CSF levels of those five cytokines/chemokines compared to controls (NORSE vs controls: $p<0.001$ for IL-6, CXCL8, MIP-1α and GCSF, $p=0.0043$ for CCL2; and RSE vs controls $p<0.001$ for IL-6, CXCL8, CCL2 and G-CSF, and $p=0.017$ for MIP-1α). Patients with NORSE had higher CSF levels of CCL2 ($p<0.001$) compared to patients with autoimmune encephalitis, whereas patients with other forms of RSE had higher CSF levels of IL-6 ($p=0.0034$), CXCL8 ($p=0.021$), CCL2 ($p<0.001$) and G-CSF ($p=0.029$) compared to patients with autoimmune encephalitis. Patients with autoimmune encephalitis had higher CSF levels of CXCL8 ($p=0.029$) and MIP-1α ($p=0.0026$) compared to controls. In the CSF, only IL-1ß levels evaluated with the high-sensitivity method were found significantly higher for patients with NORSE compared to patients with other forms of RSE ($p=0.016$).

Similarly, differences in the CSF/serum ratios among the four subgroups of patients were found for the CSF/serum ratios of four cytokines: CXCL8, CCL2, MIP-1α and G-CSF. Post-hoc comparisons were performed for those four cytokines (Table 2). Patients with RSE had higher CSF/serum ratios of CXCL8 ($p<0.001$), CCL2 ($p=0.010$), MIP-1α ($p=0.015$) and G-CSF ($p<0.001$) compared to control patients. Patients with NORSE had higher CSF/serum ratios of MIP-1α ($p=0.0035$) and G-CSF ($p=0.016$) compared to control patients. The CSF/serum ratio of CXCL8 was found significantly lower for patients with NORSE compared to patients with RSE ($p=0.018$).

Time delay between SE onset and sample collection

In order to explain if the disturbances of cytokine/chemokine profile in patients with NORSE may be related to confounding factors, we first looked at the correlation between cytokine levels and the time delay between SE onset and sample collection. There was no correlation between cytokine/chemokine serum levels and the time delay between SE onset and sample collection, with the exception of a weak correlation for CXCL8 (rho 0.202, $p=0.046$). Patients for whom CSF collection was delayed after SE onset had lower CCL2 (rho -0.347, $p=0.022$) and VEGF (rho -0.318, $p=0.038$) CSF levels.

Ictal burden and cytokines

We next looked at the daily ictal burden (total amount of time with seizure activity) on the day of the sample collection. The daily ictal burden was reported for 31 patients with NORSE and 24 patients with RSE. No significant difference in the daily ictal burden was found between the two groups (p=0.12). However, there was a positive correlation between the daily ictal burden and serum levels of IL-6 (rho 0.402, p=0.025), CXCL8 (rho 0.383, p=0.033), G-CSF (rho 0.538, p=0.0018) and IL-10 (rho 0.360, p=0.047), specifically for patients with NORSE.

NORSE management and etiology

An etiology was found in 10 out of 61 NORSE cases (16%). The most frequent etiologies were NMDAR (n=4) and GAD65 antibody-associated encephalitis (n=2). Other etiologies were herpes simplex virus 1 encephalitis (n=1), measles encephalitis (n=1), toxocariasis (n=1) and CNS lymphoma (n=1). 57% (34/60) received immune therapy before sample collection: 29 received steroids, 21 received intravenous immunoglobulins and nine had plasma exchanges. No significant difference in cytokine/chemokine levels was found between patients previously treated with immune therapy and other patients (Table 3). Treatment was started on average eight days (+/- 12 days) after SE onset.

Comparison of serum and CSF cytokine/chemokine levels between cNORSE and non-cryptogenic RSE

In order to investigate if cytokine/chemokine profile is altered in patients with cNORSE, we compared the cytokine/chemokine levels between patients with cNORSE (n=51) and patients with NORSE or other RSE for whom an etiology was found ('non-cryptogenic RSE', n=47; including the 10 patients with a non-cNORSE and the 37 patients with other forms of RSE) (Fig.2).

The PCA plots did not reveal significant difference between patients with cNORSE and non-cryptogenic RSE, either in the serum (Fig.2A) or in the CSF (Fig.2B). The serum of patients with cNORSE showed an increase of cytokines/chemokines CXCL8 (p<0.001), CCL2 (p=0.0093) and MIP-1α (p=0.016), involved in the innate immune system, compared to patients with non-cryptogenic RSE (Fig.2C-D). The pro-inflammatory cytokine IL-12p70 did not appear significantly different in the serum of patients with cNORSE (p=0.16) compared to patients with non-cryptogenic RSE. In contrast, anti-inflammatory cytokines, IL-4 (p=0.015) and IL-10 (p=0.0026), were elevated in the serum of patients with cNORSE compared to patients with non-cryptogenic RSE.

No significantly statistical difference was found in the CSF between patients with cNORSE and patients with non-cryptogenic RSE (Fig.2E-F).

Outcome data for NORSE and RSE patients

The outcome status at discharge was available for 60 of 61 NORSE cases (98%) and for all 37 patients with other forms of RSE (Table 4). The duration of SE was longer for patients with NORSE compared to patients with other forms of RSE (30 ± 29 *vs* 11 ± 16 days, *p*<0.001). The proportion of patients who expired during hospitalization was higher for NORSE than RSE subgroup (*p*=0.015). However, there was no statistically significant difference in long-term outcomes between the two groups.

Correlation between cytokine/chemokine levels and outcomes

The cluster analysis conducted on the serum concentrations for patients with NORSE revealed positive correlations of the levels of the following cytokines/chemokines with each other: IL-6, CXCL8, CCL2 and MIP-1α from the innate immune system, the growth factors G-CSF and VEGF, and the anti-inflammatory cytokine IL-10 (Fig.3A). A positive correlation was also found between IL-10, IL-12p70, IL-4 and IL-17A whatever their pro- or anti-inflammatory profile. Older patients more frequently had nonconvulsive SE (*p*<0.001), higher serum CXCL8 upon admission (*p*=0.04) and worse outcomes both at ICU discharge (*p*=0.017) and several months after NORSE ended (*p*=0.005). In contrast, patients with FIRES were younger (34 *vs* 49 years old, *p*=0.002), and more frequently had convulsive SE at admission (92% *vs* 68%, p=0.025), cNORSE (96% *vs* 76%, *p*=0.034) and T2 MRI hippocampal hyperintensity (45% *vs* 17%, *p*=0.029). No correlation was found between serum cytokine/chemokine levels and a preceding febrile illness (i.e., FIRES vs non-FIRES cases). Patients with cNORSE were more likely to develop post-NORSE epilepsy (61% vs 13%, *p*=0.015), as were patients with FIRES (80% vs 29%, *p*=0.002) for whom NORSE was cryptogenic in most of cases (23/24, 96%). Those analyses highlighted a correlation between higher serum levels of cytokines/chemokines from the innate immune system and worse outcomes both at ICU discharge (IL-6 *p*=0.001, CXCL8 *p*=0.006, CCL2 *p*=0.001, MIP-1α *p*=0.012, G-CSF *p*=0.033 and VEGF *p*=0.028) and several months after NORSE ended (IL-6 *p*=0.004, CXCL8 *p*=0.005, CCL2 *p*=0.027) (Fig.3A). Specifically, patients with higher cytokine/chemokine levels more frequently had worse outcomes highlighted by a lower GOS-E score (negative correlation).

The CSF cluster analysis for patients with NORSE also revealed a positive correlation between each of the cytokines/chemokines of the innate immune system (IL-6, CXCL8, CCL2 and MIP-1α), the growth factor G-CSF and the anti-inflammatory cytokine IL-10 (Fig.3B). Older patients more frequently had non-convulsive SE (p=0.004) and elevated IL-1ß levels (p=0.013). In contrast, patients with convulsive SE had higher CSF IL-6 (p=0.022), G-CSF (p=0.007) and IL-10 (p=0.031) levels than patients with nonconvulsive SE. No correlation was found between CSF cytokine/chemokine levels, a preceding febrile illness (i.e., FIRES vs non-FIRES cases), NORSE etiology or T2 MRI hippocampal hyperintensity. Nonetheless, there was a correlation between higher MIP-1α levels and worse outcome at ICU discharge (p=0.011), as well as between higher IL-6 (p=0.039), CCL2 (p=0.045), MIP-1α (p=0.040) and G-CSF (p=0.006) levels and worse outcome several months after NORSE ended.

The same analyses were conducted for patients with other forms of RSE (Fig.4). The cluster analysis revealed also a positive correlation between each of the cytokines/chemokines of the innate immune system (IL-6, CXCL8, CCL2 and MIP-1α), the growth factor G-CSF and the anti-inflammatory cytokine IL-10 (Fig.4A). Older patients more frequently had worse outcomes at discharge (p=0.041) and several months after SE ended (p=0.033) (Fig.4A). However, in contrast to patients with NORSE, there was no correlation between the serum levels of IL-6, CXCL8, CCL2, MIP-1α, G-CSF and VEGF and patient outcomes neither at short- nor at long-term.

In the CSF, only IL-6, IL-10 and CCL2 levels correlated with each other (Fig.4B). Similar to patients with NORSE, higher CSF MIP-1α levels correlated with a worse outcome at ICU discharge (p=0.024).

Discussion

In this study, we demonstrated the overproduction of pro-inflammatory cytokines/chemokines in the serum (IL-12p70, IL-6, TNF-α, CXCL8, MIP-1α, and CCL2) and in the CSF (IL-6, CXCL8, MIP-1α and CCL2) of patients with SE compared to patients without SE. Although an increase in IL-6 levels was also observed in patients with autoimmune encephalitis without SE compared to patients with pharmacoresistant epilepsy, the levels were lower compared to all patients with SE. To elucidate the involvement of inflammation specifically in NORSE pathogenesis, we compared cytokine/chemokine levels in patients with NORSE to those of

patients with other forms of RSE. Patients with NORSE had significantly higher serum levels of CXCL8, MIP-1α and CCL2 and significantly higher CSF levels of IL-1ß than those with other forms of RSE. Patients with cNORSE (i.e., no etiology found after extensive evaluation) also had significantly higher serum levels of CXCL8, MIP-1α and CCL2 compared to all non-cryptogenic RSE. In patients with NORSE overall, elevation of serum innate immunity-associated pro-inflammatory cytokines (IL-6, CXCL8, CCL2, MIP-1α) correlated with worse outcomes at ICU discharge and several months after SE ended.

The increase of pro-inflammatory cytokines/chemokines is consistent with findings in animal models of *de novo* SE.[24,25] Activation of the innate immune system, highlighted by the elevation of CSF and serum IL-6 levels in patients with SE, regardless of their SE etiology, could be explained by cytokine production in activated glial cells and blood-brain barrier leakage within minutes after SE onset.[25,26] Increased IL-6 serum levels may also depend on its production by peripheral leukocytes (e.g. macrophages).[27] In our study, serum and CSF IL-6 levels were found correlated with worse outcomes at ICU discharge and several months after NORSE ended. Our data suggest the possible utility of IL-6 blockers such as tocilizumab to prevent SE consequences, regardless of the NORSE etiology. While several single case reports and small case series reported a therapeutic potential of tocilizumab to decrease seizure activity in patients with NORSE,[11,28–30] further studies will be needed to assess the impact of this treatment on long-term outcome.

Increased levels of pro-inflammatory cytokines/chemokines were previously described in pilocarpine-induced seizures and in patients with FIRES and were correlated with subsequent astrogliosis.[12,31–33] The neuronal damage associated with SE in animal models was found to be mediated by CCL2, involved in macrophage recruitment, and IL-1ß production and could therefore potentially be prevented by using anti-IL-1 receptor therapy (e.g., anakinra).[34,35]

Although IL-1ß has been shown to play a critical role in SE and epileptogenesis,[19,36,37] serum IL-1ß was unchanged in our study and no correlation was found between serum and/or CSF IL-1ß levels and the development of post-NORSE epilepsy. This may be related to the timing of sample collection after SE onset, as IL-1ß is reported to be rapidly elevated within the first hours after seizure onset.[24,25,38,39] In fact, serum was collected several days after SE onset, which could preclude seeing the increase of serum IL-1ß levels. However, no significant correlation was found between IL-1ß levels and the latency between SE onset and sample collection in

patients with NORSE. The absence of significant difference may also be related to the difficulty detecting low IL-1ß levels due to its short half-life.

We did observe an upregulation of CXCL8 (previously known as IL8), a downstream pro-inflammatory cytokine induced by IL-1ß, in patients with cNORSE.[40] CXCL8 is an innate immunity pro-inflammatory cytokine best known for its role in neutrophil chemotaxis. CXCL8 was suggested to be involved in temporal lobe and pharmacoresistant epilepsy.[17,41,42] The increase of CXCL8 levels in the serum of patients with cNORSE found in our study mirrors the recent findings of a multi-protein analysis study that suggested the involvement of the innate and lymphocyte-mediated immune response in cNORSE pathogenesis.[15] Studies have shown that CXCL8 receptors are expressed by neurons and the acute application of CXCL8 to neurons enhanced neuronal excitability by inducing an intracellular calcium elevation and increasing sodium currents.[43,44] Chronic treatment with MIP-1α, a chemokine involved in macrophage and neutrophil recruitment, was also found able to modulate intracellular calcium dynamics and increase hippocampus NMDA receptor levels.[45] Recent findings showed the activation of CXCL8-CXCR1/2 receptor axis in hippocampal glia and neurons in a murine model of SE and in patients with epilepsy.[46] By modulating neuronal excitability, CXCL8 and CCL3 might enhance SE refractoriness and explain the prolonged SE duration in patients with NORSE. Their deleterious effect could also be explained by modulation of blood-brain barrier permeability and tissue damage. In fact, elevation of CXCL8 and MIP-1α levels can enhance the recruitment of neutrophils and their production of reactive oxygen species.[47] The reactive oxygen species then enhance blood-brain barrier leakage by disturbing tight junctions.[48] Those cellular changes might contribute to the association between elevated CXCL8 and MIP-1α, elevated innate inflammation cytokines and worse outcomes found in our study. That might also explain the correlation previously reported between the CSF CXCL8 levels and the up-proteomic score that has been presented as a promising indicator for the assessment of the severity of NORSE.[15] Those data and our results suggest the possible benefit of anti-CXCL8 therapy to reduce seizure severity and prevent the development of post-NORSE epilepsy and worse outcomes. While anti-CXCL8 therapy has not been reported for the treatment of SE patients, in a mouse model of SE refractory to benzodiazepines, the administration of reparixin, a molecule able to specifically block the activation of CXCR1/CXCR2 by CXCL8, showed a faster SE decay, a reduced incidence of acute symptomatic seizures after SE, and a delayed time to spontaneous seizure onset compared to control animals.[46]

One challenge is determining if cytokine disturbances are driven by the seizures per se or if they are related to the etiology and pathogenesis of NORSE. We suggest that the cytokines measured in the CSF reflect cerebral production triggered by seizures. That might explain why there is no CSF difference between patients with NORSE and patients with RSE who had a similar seizure burden on the day of the sample collection. The cytokines measured in the blood might reflect either a cerebral synthesis with a modulation of blood-brain barrier permeability triggered by seizures, or peripheral synthesis. The lower CSF/serum ratio of CXCL8 added to the similar CSF CXCL8 levels in patients with NORSE compared to patients with RSE might suggest a higher synthesis of this cytokine peripherally in NORSE. Nonetheless, we did not assess the CSF/serum albumin ratios and therefore, we cannot rule out a modulation of the blood-brain barrier permeability. This pro-inflammatory state could not be explained by a delay in sample collection nor by a higher seizure burden for patients with NORSE. We might therefore suggest that patients with NORSE share a common inflammatory pathway that leads to an overproduction of pro-inflammatory cytokine peripherally. The correlation between serum IL-6 and CXCL8 levels with the daily ictal burden for patients with NORSE might highlight a role of those cytokines in NORSE pathogenesis and refractoriness of seizures. However, we cannot rule out with certainty that seizures explain a part of the inflammatory changes present in the serum in patients with NORSE.

In this study, differences in cytokine/chemokine levels were more prominent in the serum than in the CSF of patients with NORSE compared to patients with other forms of RSE. This was also true for patients with cNORSE compared to known-etiology RSE. Moreover, serum cytokine/chemokine levels were more closely associated with outcome than CSF ones. These results suggest that peripheral inflammation may play a pathogenic role in NORSE. Peripheral inflammation has already been found to be involved in seizure onset and duration in the SE pilocarpine model and associated with worse outcome in patients with SE.[49–51] Inhibition of peripheral inflammation led to reduced SE duration and spontaneous seizures in pilocarpine model.[52–54] However, different results have been reported in the kainate post-SE mouse model and in SE induced electrically, suggesting that blood cytokine production could depend on the SE etiology or the type of epilepsy syndrome.[24,50,55] If confirmed, serum inflammation markers such as those we describe here could be used as NORSE biomarkers and provide a new valuable tool to help monitor the disease evolution over time as lumbar puncture is not easily repeated during SE course.

A strength of our study is that we studied cytokines/chemokines in a large international cohort of patients with NORSE and compared these with control patients without epilepsy, with pharmacoresistant epilepsy, with autoimmune encephalitis without SE, and most importantly, with patients with other forms of RSE. In addition, we studied correlation between cytokine/chemokine levels and outcomes both at ICU discharge and several months after SE ended. The fact that specimens were collected several days after SE onset is a limitation, but we did not find a correlation between cytokine/chemokine levels and the time delay between SE onset and sample collection. Further studies would be necessary to compare serum cytokine/chemokine levels from patients with NORSE and patients hospitalized in ICU for a non-neurological acute inflammatory condition to evaluate the specificity of innate immune system disturbances and neutrophils driving inflammatory response in patients with NORSE. We suggest normative thresholds defined as the mean plus 2 standard deviations of the values from control patients for further studies. However, it is worth nothing that control values are dependent on the methods used and are not necessarily generalizable. In addition, further studies are needed to investigate the evolution of cytokine/chemokine levels over disease progression and to look at the impact of immune therapies on cytokine/chemokine levels and multiple outcome measures. Those studies may allow us to conclude whether this inflammation is causing seizure refractoriness, neuronal damage, worse functional outcomes and/or later epilepsy.

In conclusion, this study suggests a role of the innate immune system, and possibly of neutrophils, in NORSE pathogenesis and consequences. It also suggests that immune therapies that target specific aspects of the innate immune system (IL-6 blockers or anti-CXCL8) may be helpful for patients with cNORSE.

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Author contributions

Conception and design of the study: AH, GG, DAH, VN, NG, LJH

Acquisition and analysis of data: AH, JC, KD, YP, MG

Drafting a significant portion of the manuscript or figures: AH, DAH, VN, NG, LJH

Potential Conflicts of Interest

Data availability

Anonymized data will be made available by request from any qualified investigator.

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Figure Legends

Figure 1: Comparison of the serum and the CSF cytokine/chemokine levels in patients with SE and patients without SE.

A and B. The principal component analysis of 12 serum (A) and 10 CSF (B) cytokines/chemokines for patients with SE (blue) and patients without SE (black). The impact of each original variable (cytokine/chemokine) is represented as a vector (arrow). The length of each arrow reflects its contribution in building the PCA and the orientation of the arrow highlights the direction of increase for the given variable. **C**. The repartition of patients for whom serum was collected between those with SE and those without SE. **D**. Comparisons of 10 serum cytokines levels (IL-12p70, IL-17A, IL-4, IL-10, IL-6, IL-1ß, TNF-α, CXCL8, MIP-1α and CCL2) in patients with SE (blue) and patients without SE (black). Serum concentrations were measured by multiplexed fluorescent bead-based immunoassay detection. **E**. The repartition of patients for whom CSF was collected between those with SE and those without SE. **F**. Comparisons of 8 CSF cytokines levels (IL-12p70, IL-17A, IL-10, IL-6, IL-1ß, CXCL8, MIP-1α and CCL2) in patients with SE (blue) and patients without SE (black). CSF concentrations were measured by multiplexed fluorescent bead-based immunoassay detection. Abbreviations: SE, Status Epilepticus; w/o SE, without Status Epilepticus

Figure 2: Comparison of the serum and the CSF cytokine/chemokine levels in patients with cNORSE and patients with RSE for known etiology.

A and B. The principal component analysis of 12 serum (A) and 10 CSF (B) cytokines/chemokines for patients with cNORSE (red) and patients with a known-etiology RSE (green). The impact of each original variable (cytokine/chemokine) is represented as a vector (arrow). The length of each arrow reflects its contribution in building the PCA and the orientation of the arrow highlights the direction of increase for the given variable. **C**. The repartition of patients for whom serum was collected between those with cNORSE and those with a known-etiology RSE (non-cryptogenic). **D**. Comparisons of 9 serum cytokines levels (IL-12p70, IL-17A, IL-4, IL-10, IL-6, IL-1ß, CXCL8, MIP-1α and CCL2) in patients with cNORSE (red) and patients with a known-etiology RSE (green). Serum concentrations were measured by multiplexed fluorescent bead-based immunoassay detection for IL-12p70, IL-4, IL-10, IL-6, CXCL8, MIP-1α and CCL2. IL-1ß serum concentrations were measured on a Quanterix SP-X imaging and analysis platform. Single-plex bead-based ultra-sensitive

immunodetection of IL-17A was performed by digital ELISA using the Simoa HD-1 analyzer.
E. The repartition of patients for whom CSF was collected between those with cNORSE and those with a known-etiology RSE (non-cryptogenic). **F**. Comparisons of 6 CSF cytokines levels (IL-17A, IL-6, IL-1ß, CXCL8, MIP-1α and CCL2) in patients with cNORSE (red) and patients with a known-etiology RSE (green). CSF concentrations were measured by multiplexed fluorescent bead-based immunoassay detection for IL-6, CXCL8, MIP-1α and CCL2. IL-1ß CSF concentrations were measured on a Quanterix SP-X imaging and analysis platform. Single-plex bead-based ultra-sensitive immunodetection of IL-17A was performed by digital ELISA using the Simoa HD-1 analyzer.

Abbreviations: NORSE, New-Onset Refractory Status Epilepticus; RSE, Refractory Status Epilepticus

Figure 3: Cluster analysis heat map showing correlations of cytokine/chemokine levels, demographic and clinical data in patients with NORSE.

Figure 3A and 3B show the correlation values for serum (A) and CSF (B) cytokine/chemokine levels with each other and with demographic and clinical data in patients with NORSE. The heat map colors correspond to correlations grading from -1 (negative correlation, blue) to no correlation (white) to 1 (positive correlation, red). * indicates $p<0.05$ with Spearman analysis. The boxes highlight the correlation between the age and biological/clinical data (yellow), the pro-inflammatory state (green), the association between FIRES and biological/clinical parameters (pink) and the prognostic value of cytokines/chemokines (black).

Abbreviations: ASM, number of Anti-Seizure Medications; FIRES, Febrile Infection Related Epilepsy, Syndrome; GOSE; Glasgow Outcome Scale-Extended; Hipp, Hippocampus; NCSE, Non-Convulsive Status Epilepticus; SE, Status Epilepticus; Seq_epilepsy, post-NORSE epilepsy

Figure 4: Cluster analysis heat map showing correlations of cytokine/chemokine levels, demographic and clinical data in patients with other forms of RSE.

Figure 4A and 4B show the correlation values for serum (A) and CSF (B) cytokine/chemokine levels with each other and with demographic and clinical data in patients with other forms of RSE. The heat map colors correspond to correlations grading from -1 (negative correlation, blue) to no correlation (white) to 1 (positive correlation, red). * indicates $p<0.05$ with Spearman analysis.

The boxes highlight the correlation between the age and biological/clinical data (yellow), the pro-inflammatory state (green) and the prognostic value of cytokines/chemokines (black).

Abbreviations: ASM, number of Anti-Seizure Medications; GOSE; Glasgow Outcome Scale-Extended; NCSE, Non-Convulsive Status Epilepticus; SE, Status Epilepticus

A. Serum

B. CSF

A. Serum

PC2 (23.9%) vs PC1 (38.3%)
- cNORSE
- Non-cryptogenic

Loadings: IL-12p70, IL-4, TNF-a, IL-1b, IL-17A, VEGF, IL-10, MIP-1a, CXCL8, G-CSF, CCL2, IL-6

B. CSF

PC2 (14.6%) vs PC1 (32.2%)
- cNORSE
- Non-cryptogenic

Loadings: IL-12p70, MIP-1a, IL-10, IL-6, IL-17A, CCL2, IL-1b, VEGF, G-CSF, CXCL8

C.
- cNORSE n=51
- Non-crypto n=47

D.
[IL12p70] (pg/mL) — cNORSE vs Non-cryptogenic
[IL4] (pg/mL) — cNORSE vs Non-cryptogenic *
[IL10] (pg/mL) — cNORSE vs Non-cryptogenic **
[IL17A] (pg/mL) — cNORSE vs Non-cryptogenic
[IL6] (pg/mL) — cNORSE vs Non-cryptogenic
[CXCL8] (pg/mL) — cNORSE vs Non-cryptogenic ***
[MIP1a] (pg/mL) — cNORSE vs Non-cryptogenic *
[CCL2] (pg/mL) — cNORSE vs Non-cryptogenic **
[IL1b] (pg/mL) — cNORSE vs Non-cryptogenic

E.
- cNORSE n=25
- Non-crypto n=18

F.
[IL17A] (pg/mL) — cNORSE vs Non-cryptogenic
[IL6] (pg/mL) — cNORSE vs Non-cryptogenic
[CXCL8] (pg/mL) — cNORSE vs Non-cryptogenic
[MIP1a] (pg/mL) — cNORSE vs Non-cryptogenic
[CCL2] (pg/mL) — cNORSE vs Non-cryptogenic
[IL1b] (pg/mL) — cNORSE vs Non-cryptogenic

A. Serum

B. CSF

* p-value < 0.05 (Spearman correlation)

Negative corr. ▬ Positive corr.

Age ~ biological/clinical data

Pro-inflammatory state

FIRES ~ biological/clinical data

Cytokines ~ outcome

A. Serum
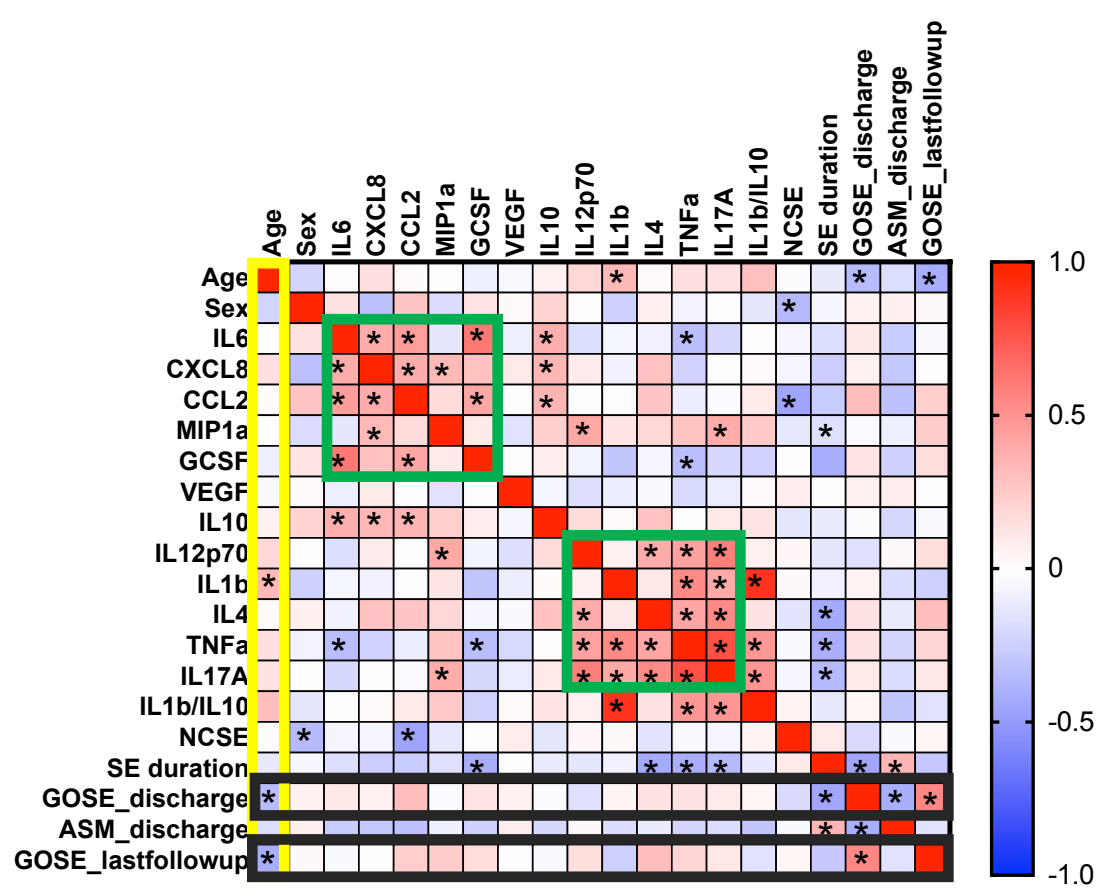
B. CSF

* p-value < 0.05 (Spearman correlation)

Negative corr. ▬ Positive corr.

Age ~ biological/clinical data

Pro-inflammatory state

Cytokines ~ outcome

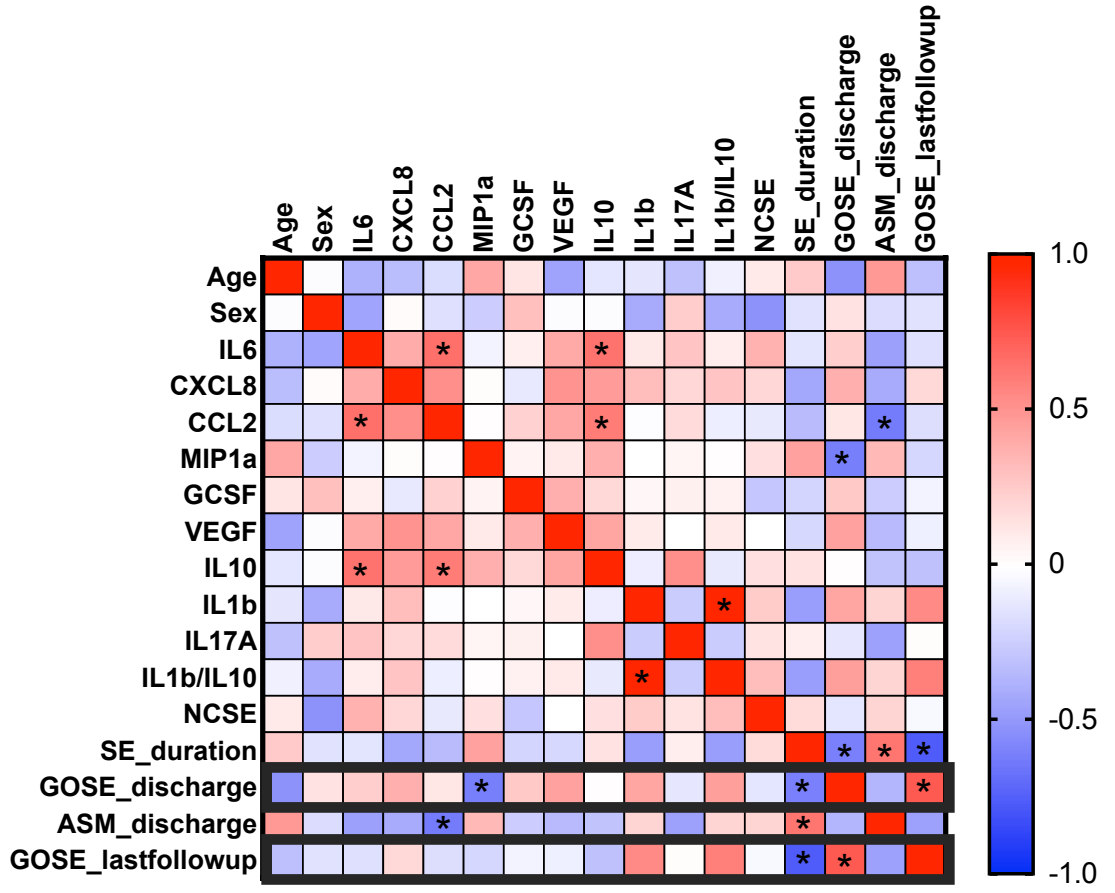|	NORSE Serum n=61 CSF n=29	RSE Serum n=37 CSF n=14	Pharmacoresistant epilepsy Serum n=22	Autoimmune encephalitis Serum n=12 CSF n=10	Controls Serum n=18 CSF n=17	P-value*
Gender, female/male, % female	38/23, 62%	14/23, 38%	12/10, 55%	5/7, 42%	11/7, 61%	0.16
Age, y, median (range)	45 (9 – 87)	53 (8 – 83)	31 (21 – 64)	34 (18 – 77)	41 (23 – 82)	0.06
Timing of serum collection from SE onset, days, median (range)	11 (0 – 127)	2 (0 – 45)	NA	NA	NA	**< 0.001**
Timing of CSF collection from SE onset, days, median (range)	8 (1 – 82)	3 (0 – 45)	NA	NA	NA	0.10
CSF pleocytosis, > 5x10^6 cells/L, n (%)	11 (38%)**	2 (14%)	NA	4 (40%)	0	**0.0058**
CSF protein levels, median (range), mg/dL	40 (17 – 150)	32 (11 – 355)	NA	42 (21 – 116)	36 (22 – 57)	0.86

Table 1: Demographic and biological data of subgroups of patients with and without status epilepticus

Abbreviations: n, numbers; NA, Not Applicable; NORSE, New-Onset Refractory Status Epilepticus; RSE, Refractory Status Epilepticus; y, years

* Kruskal-Wallis test used to compare more than two groups, Mann-Whitney tests to compare two groups, Fisher tests to compare percentages.

** Cryptogenic NORSE n= 9, herpes simplex virus encephalitis 1 n=1; encephalitis with NMDAR antibody-associated encephalitis n=1

	NORSE	RSE	Pharmacoresistant epilepsy	Autoimmune encephalitis	Controls	P-value*
Serum	n=61	n=37	n=22	n=12	n=18	/
IL12p70	**2.56 ± 8.26**	0.71 ± 1.69	0.40 ± 1.38	0.19 ± 0.40	0.18 ± 0.32	**0.0010**
IL17A	0.80 ± 2.73	0.30 ± 1.05	0.18 ± 0.49	**1.07 ± 3.38**	0.090 ± 0.31	0.49
IL4	**1.02 ± 3.45**	0.12 ± 0.51	0.19 ± 0.87	0	0.15 ± 0.43	**0.011**
IL10	**3.78 ± 5.95**	**2.76 ± 6.48**	0.18 ± 0.29	0.52 ± 0.43	0.44 ± 0.95	**< 0.001**
IL6	**179.31 ± 873.68**	**99.70 ± 189.71**	4.84 ± 13.58	**24.08 ± 31.77**	4.05 ± 2.93	**< 0.001**
IL1b[1]	**0.11 ± 0.32**	**0.062 ± 0.20**	**0.051 ± 0.17**	**0.067 ± 0.16**	0	0.31
TNFa	1.25 ± 4.72	0.18 ± 0.50	0.17 ± 0.54	0.073 ± 0.25	0.061 ± 0.16	0.11
CXCL8/IL8	**98.04 ± 153.57**	25.58 ± 23.50	4.60 ± 4.09	15.51 ± 12.81	19.54 ± 22.95	**< 0.001**
MIP-1α	9.26 ± 18.85	2.62 ± 3.47	1.0 ± 1.46	1.92 ± 1.72	4.59 ± 9.65	**< 0.001**
CCL2	74.80 ± 134.55	30.40 ± 36.80	13.64 ± 15.93	18.05 ± 9.85	40.76 ± 57.58	**0.0016**
VEGF	**815.90 ± 927.92**	**366.28 ± 390.15**	58.20 ± 76.37	99.40 ± 120.96	135.29 ± 72.15	**< 0.001**
GCSF	**0.80 ± 1.77**	**0.62 ± 1.74**	0.083 ± 0.31	0.19 ± 0.27	0.15 ± 0.14	**0.0067**
CSF	n=29	n=14	/	n=10	n=17	/
IL12p70[2]	**0.0092 ± 0.050**	0	NA	0	0	0.74
IL17A	0.10 ± 0.33	0.089 ± 0.20	NA	0.060 ± 0.19	0.027 ± 0.070	0.74
IL10	1.26 ± 2.16	1.10 ± 1.64	NA	**0.44 ± 0.80**	0.072 ± 0.10	0.051
IL6	**277.31 ± 654.61**	**159.40 ± 165.17**	NA	15.02 ± 12.99	8.60 ± 14.56	**< 0.001**
IL1b[3]	0.060 ± 0.22	0.15 ± 0.43	NA	0	0	0.35
CXCL8/IL8	**137.13 ± 307.32**	**314.13 ± 688.64**	NA	**26.80 ± 34.58**	6.12 ± 5.17	**< 0.001**
MIP-1α	**2.21 ± 3.68**	**0.65 ± 0.66**	NA	**0.79 ± 0.82**	0.092 ± 0.16	**0.0010**
CCL2	**1069.76 ± 1291.95**	**1179.5 ± 942.74**	NA	220.72 ± 139.75	315.57 ± 249.96	**< 0.001**
VEGF	5.23 ± 12.52	**16.65 ± 23.13**	NA	3.02 ± 5.79	3.50 ± 5.06	0.12
GCSF	**5.54 ± 15.01**	**2.35 ± 4.33**	NA	0.20 ± 0.27	0.052 ± 0.10	**< 0.001**
CSF/Serum	n=22	n=14	/	n=10	n=17	/
IL10	**1.19 ± 1.68**	0.85 ± 1.34	NA	0.64 ± 0.76	0.32 ± 0.39	0.73
IL6	7.42 ± 15.11	2.15 ± 2.00	NA	2.85 ± 4.36	3.97 ± 7.26	0.82
CXCL8/IL8	5.33 ± 10.74	**10.88 ± 24.35**	NA	**3.02 ± 4.51**	0.68 ± 0.56	**0.0056**
MIP-1α	**0.89 ± 1.39**	**0.32 ± 0.34**	NA	**0.76 ± 0.83**	0.037 ± 0.059	**<0.001**
CCL2	49.98 ± 55.21	**58.37 ± 57.60**	NA	18.62 ± 13.90	20.56 ± 18.44	**0.038**
VEGF	0.023 ± 0.059	0.15 ± 0.27	NA	0.061 ± 0.13	0.062 ± 0.13	0.22
GCSF	**15.90 ± 38.84**	**3.06 ± 5.85**	NA	0.97 ± 2.29	0.27 ± 0.52	**0.0039**

Table 2: Cytokines/chemokines concentrations in serum and CSF samples.

Data are expressed as mean ± sd (unit pg/mL). Normative thresholds defined as the mean ±2sd value of the control patients. The concentrations in bold were higher than the mean±2sd value of the control patients.

*Kruskal-Wallis test used to compare the different groups. Results were considered significant when $p<0.05$ (p-value in bold)

[1]Serum IL-1ß levels were detectable for 21% of patients with SE (n=21, NORSE n=14, RSE n=7) and 10% of patients without SE (n=5, pharmacoresistant epilepsy n=3, autoimmune encephalitis n=2, controls n=0). Fisher tests used to compare the percentage of patients with detectable value among the SE and without SE subgroups, $p=0.075$

[2]CSF IL-12p70 level was detectable for only one patient with NORSE. Fisher tests used to compare the percentage of patients with detectable value among the SE and without SE subgroups, $p=1$

[3]CSF IL-1ß levels were detectable for 12% of patients with SE (n=5, NORSE n=3, RSE n=2) and 0% of patients without SE. Fisher tests used to compare the percentage of patients with detectable value among the SE and without SE subgroups, $p=0.15$

Abbreviations: n, numbers; NA, Not Applicable; NORSE, New-Onset Refractory Status Epilepticus; RSE, Refractory Status Epilepticus

	Immunotherapy before sample collection	**No immunotherapy before sample collection**	**P-value***
Blood	n=34 (57%)	n=26 (43%)	
IL12p70	3.90 ± 10.86	0.80 ± 1.55	0.53
IL17A	0.95 ± 3.41	0.62 ± 1.56	0.98
IL4	1.36 ± 4.07	0.63 ± 2.52	0.53
IL10	5.10 ± 7.47	2.17 ± 2.40	0.86
IL6	268.45 ± 1167.40	69.39 ± 92.14	0.98
IL1b	0.11 ± 0.37	0.11 ± 0.27	0.86
TNFa	1.93 ± 6.23	0.38 ± 0.89	0.86
CXCL8/IL8	90.59 ± 150.49	111.37 ± 161.54	0.98
MIP-1α	10.69 ± 22.94	7.71 ± 12.25	0.98
CCL2	77.63 ± 146.53	72.95 ± 122.47	0.98
VEGF	786.80 ± 914.60	868.20 ± 978.94	0.98
GCSF	0.72 ± 1.72	0.93 ± 1.89	0.98
CSF	n=15 (52%)	n=14 (48%)	
IL12p70[1]	0.018 ± 0.069	0	0.98
IL17A	0.15 ± 0.43	0.056 ± 0.16	0.98
IL10	1.52 ± 2.79	0.99 ± 1.21	0.98
IL6	267.61 ± 744.76	287.71 ± 570.44	0.98
IL1b[2]	0	0.12 ± 0.32	0.53
CXCL8/IL8	62.49 ± 70.13	217.10 ± 429.90	0.98
MIP-1α	2.37 ± 3.46	2.04 ± 4.03	0.98
CCL2	847.24 ± 820.57	1308.17 ± 1658.78	0.98
VEGF	1.81 ± 5.54	8.88 ± 16.64	0.80
GCSF	4.72 ± 8.35	6.41 ± 20.21	0.98

Table 3: Cytokine/chemokine concentrations in serum and CSF samples from patients with NORSE according to the previous utilization of immune therapies.

Data are expressed as mean ± sd (unit pg/mL).

*Mann-Whitney test used to compare the different groups. The Benjamini-Hochberg test procedure was used to correct for multiple comparisons. Results were considered significant when $p < 0.05$

[1]CSF IL-12p70 level was detectable for only one patient previously treated with immune therapy. Fisher tests used to compare the percentage of patients with detectable value among the both groups, $p=1$

[2]CSF IL-1ß levels were detectable for only three patients who all were not treated with immune therapy. Fisher tests used to compare the percentage of patients with detectable value among the both subgroups, $p=0.10$

	NORSE	RSE	P-value*
Outcome at discharge	**60/61 (98%)**	**37/37 (100%)**	
Expired (GOS-E=1)	13/60 (22%)	1/37 (3%)	0.015
Unconscious (GOS-E=2)	4/60 (7%)	1/37 (3%)	0.65
Severe disability (GOS-E=3 or 4)	30/60 (50%)	21/37 (57%)	0.54
Moderate disability or better (GOS-E=5 to 8)	13/60 (22%)	14/37 (38%)	0.10
Outcome follow-up	**50/61 (82%)**	**29/37 (78%)**	
Delay after SE ended, days (min-max)	471 (24-901)	259 (6-760)	0.0025
Expired (GOS-E=1)	18/50 (36%)	11/29 (38%)	1
Severe disability (GOS-E=3 or 4)	8/50 (16%)	7/29 (24%)	0.39
Moderate disability (GOS-E=5 or 6)	7/50 (14%)	3/29 (10%)	0.73
Mild persistent disability or full recovery (GOS-E=7 or 8)	17/50 (34%)	8/29 (28%)	0.62

Table 4: Outcome data for NORSE and RSE patients

Abbreviations: GOS-E, Glasgow Outcome Scale-Extended; NORSE, New-Onset Refractory Status Epilepticus; RSE, Refractory Status Epilepticus

* Mann-Whitney tests to compare two groups, Fisher tests to compare percentages.