RESEARCH ARTICLE OPEN ACCESS

Identification of Distinct Biological Groups of Patients With Cryptogenic NORSE via Inflammatory Profiling

Martin Guillemaud,[1] Mario Chavez,[1] Firas Kobeissy,[2] Annamaria Vezzani,[3] Anthony D. Jimenez,[4] Maysaa Merhi Basha,[5] Ayush Batra,[6] Sophie Demeret,[7] Onome Eka,[8] Krista Eschbach,[9] Brandon Foreman,[10] Nicolas Gaspard,[11,4] Elizabeth E. Gerard,[6] Teneille Emma Gofton,[12] Hiba A. Haider,[13,14] Stephen T. Hantus,[15] Charles L. Howe,[16] Amy Jongeling,[17] Mariel Kalkach-Aparicio,[18] Padmaja Kandula,[19] Karnig Kazazian,[12] Minjee Kim,[6] Yi-Chen Lai,[20] Clémence Marois,[7] Andrew Mellor,[9] Wazim Mohamed,[5] Mikaela Morales,[21] Cederic M. Pimentel,[22] Alexandra M. Ramirez,[10] Claude Steriade,[17] Aaron F. Struck,[18] Olga Taraschenko,[23] Nathan Torcida Sedano,[11] Mark S. Wainwright,[21] Ji Yeoun Yoo,[8] Kevin K.W. Wang,[2] Vincent Navarro,[24] Lawrence J. Hirsch,[4] and Aurélie Hanin[1,25,26,4]

Correspondence
Dr. Hanin
aurelie.hanin@icm-institute.org

Neurol Neuroimmunol Neuroinflamm 2025;12:e200403. doi:10.1212/NXI.0000000000200403

Abstract

Background and Objectives

Emerging evidence suggests that immune dysregulation plays a pivotal role in triggering cryptogenic new-onset refractory status epilepticus (c-NORSE), prompting a consensus on early initiation of immunotherapy. However, despite similar timing of administration, responses to immunotherapies have been varied and unpredictable, suggesting the presence of heterogeneous underlying mechanisms**.** The aim of this study was to identify distinct inflammatory response subtypes in patients with c-NORSE by analyzing their cytokine profiles. Insights into underlying mechanisms were sought to understand the pathophysiology and guide personalized therapies to improve patient outcomes.

Methods

Sixty-two patients with c-NORSE were included. A comprehensive panel of 96 cytokines was analyzed in serum samples. Patients were clustered based on their cytokine profiles using the Louvain algorithm, an unsupervised graph-based clustering method. The identified clusters of patients were compared regarding cytokine levels and clinical features. Protein pathway analysis was used to explore the biological relevance of the inflammatory markers within each cluster. Patients with c-NORSE were compared with control patients (n = 18) and patients with other forms of refractory SE (n = 45).

Results

Compared with controls, patients with c-NORSE exhibited significant differences in 33 cytokines. Pathway analysis revealed dysregulations in chemotaxis and neutrophil recruitment and migration, highlighting the importance of innate immunity in patients with c-NORSE. Within the c-NORSE cohort, 3 clusters of patients emerged: cluster A, lacking specific inflammatory markers; cluster B, with a much stronger innate-immunity cytokine-driven inflammatory response compared with clusters A and C; and cluster C, defined by dysregulated autoimmune processes. Notably, patients in cluster B showed a statistically significant elevation of innate

MORE ONLINE

Supplementary Material

[1]Sorbonne Université, Institut du Cerveau, Paris Brain Institute, ICM, INRIA, Inserm, CNRS, AP-HP, Hôpital de la Pitié-Salpêtrière, France; [2]Department of Neurobiology, Center for Neurotrauma, Multiomics & Biomarkers, Morehouse School of Medicine, Atlanta, GA; [3]Department of Acute Brain and Cardiovascular Injury, Mario Negri Institute for Pharmacological Research, IRCCS, Milan, Italy; [4]Department of Neurology, Comprehensive Epilepsy Center, Yale University School of Medicine, New Haven, CT; [5]Wayne State University School of Medicine, Detroit, MI; [6]Northwestern University, Feinberg School of Medicine, Chicago, IL; [7]Neuro-Intensive Care Unit, AP-HP, Hôpital de la Pitié-Salpêtrière, Sorbonne Université, Paris, France; [8]Icahn School of Medicine at Mount Sinai, New York City; [9]Department of Pediatrics, Section of Neurology, University of Colorado School of Medicine, Children's Hospital Colorado, Aurora, CO; [10]Division of Neurocritical Care, Department of Neurology and Rehabilitation Medicine, University of Cincinnati, OH; [11]Department of Neurology, Hôpital Universitaire de Bruxelles–Hôpital Erasme, Université Libre de Bruxelles, Belgium; [12]Schulich School of Medicine and Dentistry, Western University, London Health Sciences Center, Ontario, Canada; [13]Department of Neurology, University of Chicago, IL; [14]Epilepsy Center, Emory University School of Medicine, Atlanta, GA; [15]Epilepsy Center, Cleveland Clinic, Neurological Institute, Ohio; [16]Department of Neurology, Mayo Clinic, Rochester, MN; [17]Department of Neurology, NYU Langone Medical Center, New York City; [18]University of Wisconsin, Department of Neurology, Madison, WI; [19]Weill Cornell Medicine, Department of Neurology, New York City; [20]Division of Pediatric Critical Care Medicine, Department of Pediatrics, Baylor College of Medicine, Houston, TX; [21]Divison of Pediatric Neurology, Seattle Children's Hospital, University of Washington, Seattle; [22]Neurocritical Care, Emory University, Atlanta, GA; [23]Department of Neurological Sciences, University of Nebraska Medical Center, Omaha, NE; [24]Epilepsy Unit and Department of Clinical Neurophysiology, AP-HP, Hôpital de la Pitié-Salpêtrière, Sorbonne Université, ERN-Epicare, Paris, France; [25]Department of Metabolic Biochemistry, AP-HP, Hôpital de la Pitié-Salpêtrière, Paris, France; and [26]Universite Paris Cité, Faculté de Pharmacie, France.

The Article Processing Charge was funded by the authors.

Abbreviations

ASM = antiseizure medication; **c-NORSE** = cryptogenic new-onset refractory status epilepticus; **FIRES** = febrile infection–related epilepsy syndrome; **HLH** = hemophagocytic lymphohistiocytosis; **ICU** = intensive care unit; **IL-1** = interleukin 1; **IQR** = interquartile range; **JAK-STAT** = Janus kinase signal transducer and activator of transcription; **MOGAD** = myelin oligodendrocyte glycoprotein antibody disease; **PCA** = principal component analysis; **STROBE** = Strengthening the Reporting of Observational Studies in Epidemiology; **TNF** = tumor necrosis factor.

immune–related proinflammatory cytokines associated with leukocyte recruitment and degranulation. By contrast, those in cluster C showed activation of Janus kinase signal transducer and activator of transcription (JAK-STAT) pathways, suggesting autoimmune mechanisms. Patients in clusters B and C demonstrated varied responses to immunotherapies, with cluster C patients showing favorable outcomes after multiple immunotherapies.

Discussion

The identification of distinct inflammatory subgroups in c-NORSE suggests that variations in the underlying immune mechanisms contribute to differential treatment responses. These findings underscore the importance of personalized therapeutic strategies, potentially targeting specific inflammatory pathways, to optimize clinical outcomes in this challenging condition.

Introduction

New-onset refractory status epilepticus (NORSE) is a rare and devastating form of status epilepticus (RSE). NORSE abruptly occurs in adults and children without active epilepsy or preexisting relevant neurologic disorders and with no clear acute or active structural, toxic, or metabolic cause identified within 72 hours.[1] The onset of SE can be preceded by prodromal syndromes, including fever, headaches, or behavioral changes, which, although nonspecific, could guide toward an etiology (e.g., behavioral changes in the case of anti-NMDA receptor encephalitis).[2] If a febrile infection occurs between 2 weeks and 24 hours before the onset of SE, patients qualify as having febrile infection–related epilepsy syndrome (FIRES), a subset of NORSE.[1] The prognosis for NORSE, including FIRES, is often poor, with a high rate of mortality, long-term neurocognitive and functional disabilities, and post-NORSE epilepsy.[2-4]

Although some cases of NORSE can be attributed to proven autoimmune encephalitis, infectious encephalitis, or rare genetic disorders, >70% remain unexplained (cryptogenic NORSE [c-NORSE]).[2,5,6] Emerging evidence suggests that immune dysregulation may trigger the onset and contribute to the adverse consequences of c-NORSE. First, polymorphisms in cytokine-related genes have been identified in cryptogenic FIRES, potentially explaining their susceptibility to specific infections with a predilection to CNS complications.[7] Second, a multiproteomic analysis revealed distinct protein profiles in the CSF of patients with c-NORSE compared with those with anti-NMDA receptor encephalitis, indicating an upregulation of pathways related to innate and lymphocyte-mediated immune response in patients with c-NORSE.[8] These findings suggest different pathogenic mechanisms for c-NORSE and noncryptogenic RSE. Similarly, significant differences in innate immunity serum cytokine/chemokine profiles were identified between patients with c-NORSE and noncryptogenic RSE, with patients with c-NORSE exhibiting significantly higher levels of CXCL8, CCL2, and MIP-1α.[9] Higher levels of proinflammatory cytokines were associated with worse short-term and long-term outcomes. However, serum cytokine profiles were variable and sometimes even normal.[9]

There is a consensus among experts on the early use of immunotherapy to address the presumed inflammatory condition to shorten the duration of SE and minimize long-term sequelae.[10] However, evidence for a beneficial effect and even more evidence to favor certain types of immunologic interventions are still lacking.[10] Disturbances in innate immunity can be managed with immunotherapy regimens that target specific proinflammatory cytokines, such as interleukin 1 (IL-1) receptor antagonist (anakinra) or IL-6 receptor antagonist (tocilizumab), or by using intrathecal dexamethasone to inhibit the production of proinflammatory cytokines within the CNS.[11] Despite similar timing of administration, responses to immunotherapies have been varied and unpredictable, suggesting the presence of heterogeneous underlying mechanisms.[11] Further research is needed to elucidate the heterogeneity of c-NORSE, particularly regarding inflammation profiles, and to help select targeted immune treatments.

In this study, we analyzed a panel of 96 inflammatory biomarkers in 62 patients with c-NORSE to determine whether distinct inflammatory response subtypes can be identified, which may provide insights into the underlying pathophysiologic mechanisms.

Methods

Standard Protocol Approvals, Registrations, and Patient Consents

This study was approved by Yale University (NORSE/FIRES biorepository, institutional review board #1511016840 and #2000031611) and the Paris Pitié-Salpêtrière Hospital (APHP,

COLETTE). Informed consent was obtained from all patients or their legally authorized representatives according to the Declaration of Helsinki. The study was designed and reported following the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) statement. Patients were recruited from 22 hospitals in the United States; 3 in Canada; and one in France, Belgium, and Spain. Data were prospectively extracted from medical records and collected in a REDCap database.

We included 62 patients with c-NORSE, aged at least 2 years, for whom no etiology was found despite an extensive workup, usually including anti-neuronal antibody screening, genetic analysis, and metagenomics. Patients with c-NORSE were compared with patients with an RSE of known etiology (RSE, n = 45) and control patients (n = 18); the latter included healthy controls (n = 7) and patients with noninflammatory peripheral neuropathies (n = 4), idiopathic intracranial hypertension (n = 4), normal pressure hydrocephalus (n = 2), or epilepsy admitted for a psychological disorder (n = 1). The serum specimens were collected and stored according to the standardized procedures.[12]

The functional outcomes of patients with SE have been assessed at intensive care unit (ICU) discharge and within 12 months after SE ended using the Glasgow Outcome Scale Extended, ranging from 1 [death] to 8 [upper good recovery]. Patients who have experienced at least one unprovoked post-NORSE seizure after their initial hospitalization for NORSE were classified as having post-NORSE epilepsy. The follow-up information was collected by the research teams in each center and was reviewed with experts (L.J.H. and N.G.).

Biological Marker Measurement

Ninety-six serum markers were analyzed using Eve Technologies' Human Cytokine 96-Plex Discovery Assay (MilliporeSigma, Burlington, MA). All samples were measured undiluted and in duplicate, with the mean value used for further analysis. Spearman correlations among the 96 biological features were analyzed to identify related variables, resulting in the formation of 3 representative groups of markers using the Ward hierarchical clustering. Each group of markers was characterized by its principal component, reflecting the weighted contribution of each feature within the group (eFigure 1). As a result, 3 new features (i.e., one for each group) were generated for each patient.

Cluster of Patients

Using these new 3 features, a similarity matrix was computed for all patients with c-NORSE, which facilitated the construction of a connectivity graph.[13] Patients were linked with weights ranging from 0 to 1, where the most similar patients (according to the new 3 features) had weights closer to 1, represented by shorter links on the graph. On the contrary, patients with highly dissimilar feature profiles exhibited connectivity weights approaching zero. Clusters of patients with c-NORSE were subsequently identified using the Louvain algorithm, a nonparametric graph-based clustering method.[14] The Louvain method leverages the structure of the patient network to identify communities within the graph. Compared with other graph-based methods, Louvain offers the advantage of being computationally efficient while delivering strong performance across graphs of varying sizes.[15] The literature has shown that using a network-based model combined with the Louvain community detection algorithm rather than classical clustering methods achieves superior results on various biological data sets using similar approaches.[16] To compare patients with c-NORSE and other forms of RSE, the patients with RSE were included in the graph using the same methodology applied to the patients with c-NORSE. Cluster analyses were performed in Python (version 3.10.13).

Prediction Tests

A leave-one-out cross-validation test was performed to evaluate the predictive capacity and robustness of the clustering method. In this test, 1 patient was removed from the data set, the method was retrained on the remaining patients, and the excluded patient was then projected onto the graph. The new cluster assignment of the excluded patient was compared with their original cluster when all patients were included. This process was repeated for each patient, and predictive accuracy was calculated accordingly.

Pathway Analysis

We compared the median values of each marker between c-NORSE and control groups. Markers were considered significantly overexpressed if the ratio between the patients with c-NORSE and control patients was greater than 1.2. These differentially expressed markers were used to define each c-NORSE cluster and identify overexpressed pathways specific to each patient cluster.

To understand the role of the biological features, PathwayStudio (Desktop version v10.0; Elsevier, United Kingdom) was used to analyze protein-protein interaction, molecular functions, and biological and pathologic processes (accessed on 2024/9/10). We identified the 100 most significant pathophysiologic processes for each cluster of patients. We used the Venn diagram tool [17] to visualize the overlap and distinctions among pathways across different patient clusters. Inflammation-related pathways were selected from the cluster-specific pathways, and a SubNetwork Enrichment Analysis algorithm was used to examine protein-protein interactions and associated functions. This algorithm uses the Fisher exact test to detect nonrandom associations between 2 categorical variables that are organized by a specific relationship.[18,19]

Statistical Analysis

All analyses were performed using R Statistical Software (v4.1.2; R Core Team 2021).

Kruskal-Wallis tests were used to compare continuous biological and clinical features among clusters of patients. Post hoc comparisons were performed using Dunn tests when

appropriate. Fisher tests were used to compare categorical variables among clusters of patients. The significance threshold was set at a p value of 0.05.

Data Availability

All data are available on request from the corresponding author.

Results

Study Participants

Serum samples were collected from 62 patients with c-NORSE, with a median age of 31 years (interquartile range [IQR]: 21–60), comprising 49 adults (79%, range 18–87) and 13 children (21%, range 4–17). Forty-one patients (66%) had a previous febrile illness and qualified as FIRES. Serum was collected after a median delay of 12 days after SE onset (IQR: 5–20). Forty-six patients (74%) received at least one first-line immunotherapy before sample collection (steroids n = 43, IV immunoglobulins n = 31, plasma exchange n = 11). Twelve patients (19%) received at least one second-line immunotherapy before collection (anakinra n = 9, tocilizumab n = 5, rituximab n = 2, intrathecal dexamethasone n = 1). Twelve patients (19%) died during ICU stay, with 6 additional deaths within 12 months of SE resolution. Of those alive at the ICU discharge, 30 of 49 (61%) had post-NORSE epilepsy and 4 without recurring seizures did not take antiseizure medications (ASMs).

Among the 45 patients with RSE of known etiology (median age 45 years [IQR: 25–63]), 14 (31%) had previous epilepsy. The causes of SE included autoimmune encephalitis (n = 7), tumoral epilepsy (n = 7), epilepsy with changes in antiseizure medication regimens (n = 7), genetic cause (n = 5), metabolic cause (n = 4), posterior reversible encephalopathy syndrome (n = 4), previous epilepsy without an additional cause identified (n = 3), multiple underlying causes without previous epilepsy (n = 3; 1 with vascular sequelae and alcohol withdrawal; another with acute kidney failure, sepsis, and toxocariasis; and the third one with febrile SE and CACNA1A neurodevelopmental disorder), traumatic brain injury (n = 2), viral encephalitis (n = 2), and nonviral encephalitis (n = 1). Serum samples were collected at a median of 6 days after SE onset (IQR: 2–14). Six patients (13%) died during ICU stay and 8 more within 12 months after SE resolution, including 4 from tumor progression. All patients with RSE were admitted to the ICU, with 35 (78%) receiving anesthetics and 13 (29%) receiving immunotherapy before sample collection. By contrast, none of the 18 control patients was admitted to the ICU or received immunotherapy.

Comparisons of Biological Features Between c-NORSE, RSE, and Control Groups

Serum analysis revealed significant differences among the 3 groups of patients (i.e., c-NORSE, other RSE, and controls) across 35 of the 96 biological features (eTable 1). Initially, we compared the 62 patients with c-NORSE with the 18 controls, identifying significant differences in 33 features. Among these, 29 features were increased while 4 were decreased in patients with c-NORSE (eTable 1). Notably, patients with c-NORSE exhibited higher levels of FLT-3L, IL-6, CXCL8/IL-8, IL-10, and IL-18, suggesting a severe systemic inflammatory response, potentially indicative of a cytokine storm. To distinguish dysregulations specific to c-NORSE from those associated with SE in general, we next compared the 62 patients with c-NORSE with the 45 patients with RSE of known etiology. Ten features showed significant differences, with 9 features, including FLT-3L, CXCL8/IL-8, and granzyme B, being overexpressed in c-NORSE (eTable 1).

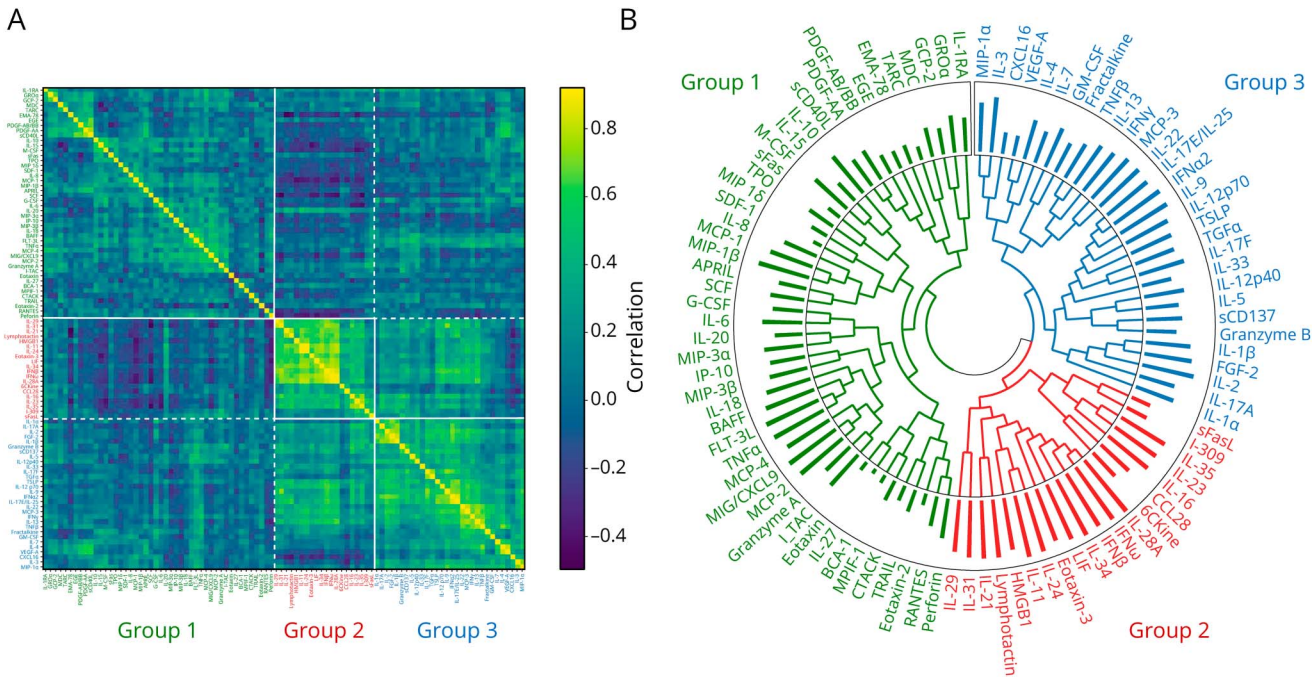
We then investigated whether some cytokine levels were correlated with other cytokines within the c-NORSE cohort. Three distinct groups of biological features were identified through the correlation matrix and Ward hierarchical algorithm in the c-NORSE cohort (Figure 1A). The first group, represented in green (Figure 1B), consisted of 46 features including key innate immunity–related proinflammatory cytokines such as IL-6, CXCL8/IL-8, IP-10, FLT-3L, CXCL9, and tumor necrosis factor (TNF)-α. The second group, shown in red (Figure 1B), included 20 features such as IL-35, IL-21, and IFN-β involved in immunomodulation. The third group, depicted in blue (Figure 1B), comprised 30 features, including IFNγ, IL-2, and IL-17A, which are key mediators of T-cell responses. The full list of features is provided in eTable 2.

Variations across these 3 groups of biological features may reflect distinct underlying pathways. To explore this further, we examined whether patients with c-NORSE could be separated into clusters based on the levels of markers across all 3 groups.

Identification of Clusters of Patients

We generated 3 new features for each patient (i.e., one for each group of biological features) by considering the individual weighted contribution of each feature within the group (eFigure 1 and eTable 2). These new 3 features were then used to construct a connectivity graph of patients (Figure 2A). The Louvain algorithm identified 8 subclusters of patients within the graph, with subcluster sizes ranging from 6 to 11 patients (Figure 2A). There was no significant difference among subclusters in the time to collect biospecimens after SE onset, the number of immunotherapy agents used before serum collection, or the proportion of patients who received at least a first-line or second-line immunotherapy before serum collection. However, significant differences were observed in age ($p = 0.019$) and ASM usage during the hospital stay ($p = 0.046$). Notably, patients from subclusters 0, 6, and 2 were younger (a median of 18.5, 21, and 26 years, respectively) compared with those from subclusters 4 and 3 (a median of 67.5 and 69 years, respectively), with older patients receiving fewer ASMs.

Figure 1 Different Groups of Biological Features



(A) The Spearman correlation matrix identifies 3 distinct groups of biological features. (B) A complete list of the biological features in group 1 (green), group 2 (red), and group 3 (blue) is provided. The graph illustrates the correlation between the various markers, with bars representing the contribution of each marker in the principal component analysis (PCA), which was performed to condense all features within a group into a single composite feature.

By calculating the distance between patients within a subcluster and between subclusters, we were able to group some subclusters, ultimately resulting in 3 main clusters of patients: cluster A (comprising previous subclusters 3 and 4, yielding 13 patients), cluster B (comprising previous subclusters 1 and 5, yielding 16 patients), and cluster C (comprising previous subclusters 0, 2, and 6, yielding 22 patients). Subcluster 7 (11 patients) was centrally positioned among the 3 main clusters (Figure 2A), making it less distinct and more challenging to separate. To minimize potential bias in the analysis, we excluded the patients in this subcluster further labeled as "cluster U" or "undetermined."

When evaluating the methodology's predictive efficacy, the algorithm demonstrated a 96.77% accuracy in identifying the most appropriate cluster (A, B, C, or U) for each patient using a leave-one-out approach. The 2 patients who were misclassified were assigned to cluster U, which shares common characteristics with the other clusters.

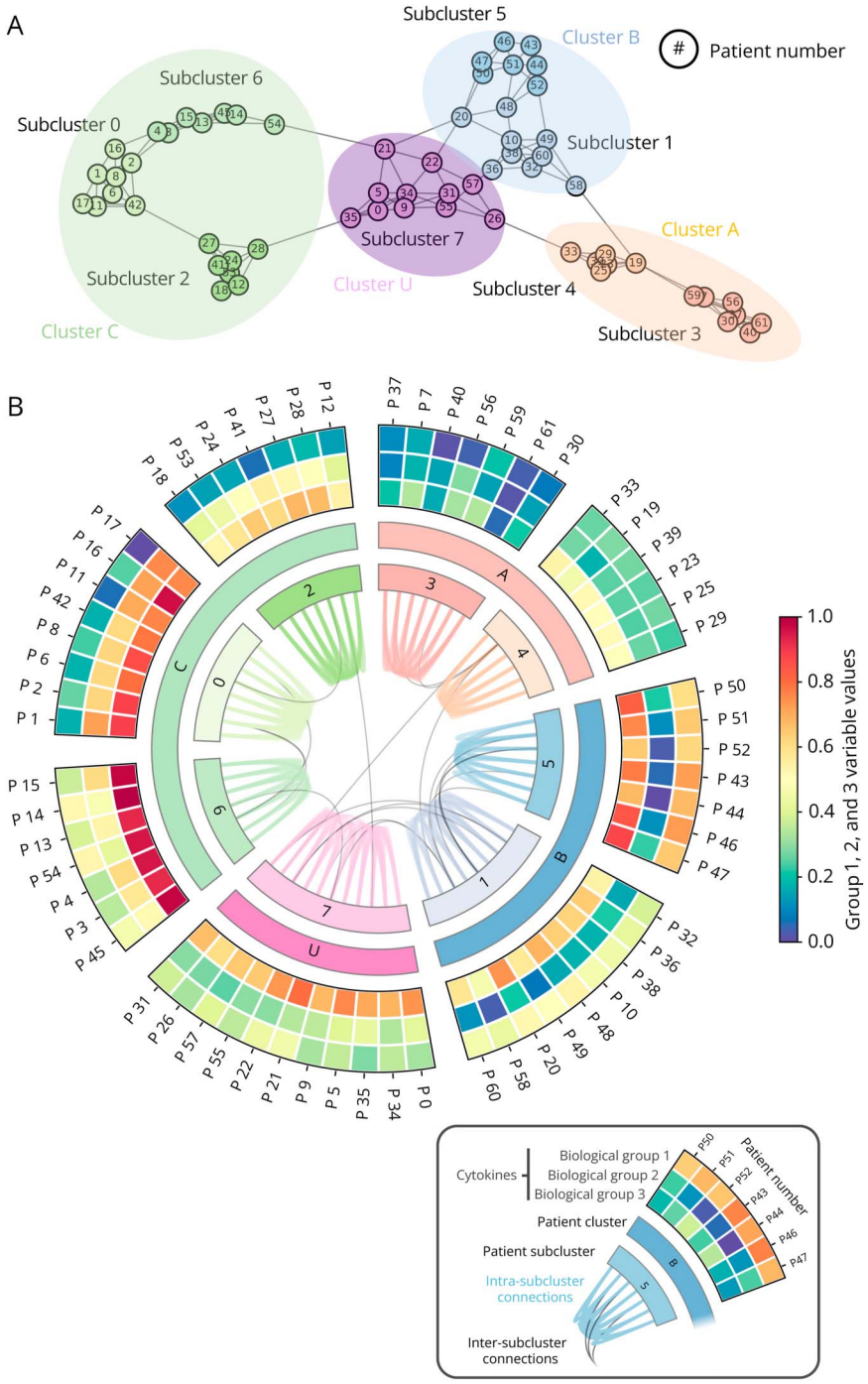
Different Clinical and Paraclinical Features Between Clusters of Patients

Patients from the 3 main clusters A, B, and C were compared to assess whether they also displayed distinct clinical and paraclinical characteristics (Table 1). It is important to note that patients were not clustered based on their hospital location. However, patients in cluster C were significantly younger than those in cluster B ($p = 0.022$) and cluster A ($p < 0.001$), with children being more frequently represented in cluster C than in clusters A and B (odds ratio, OR = 4.4 [1.8–19.4], $p = 0.0012$).

Patients in clusters B and C received more ASMs than those in cluster A ($p = 0.009$ and $p = 0.003$, respectively). However, no differences were observed for anesthetics. In addition, patients in cluster C received more immunotherapies than those in cluster A ($p = 0.01$), with a similar trend observed for cluster B compared with cluster A ($p = 0.087$). This difference could be explained by the higher proportion of white blood cells in the CSF of patients from clusters B and C compared with cluster A ($p = 0.023$). Second-line immunotherapy was also administered more frequently in cluster C compared with cluster A (OR = 2.81 [1.28–7.74], $p = 0.023$), although no significant difference was found between clusters B and C. Notably, in cluster C, 40% of the patients were treated with anakinra and 25% with tocilizumab and/or rituximab. Only a few patients received intrathecal dexamethasone (2 in cluster C and one in cluster B), and none received Janus kinase signal transducer and activator of transcription (JAK-STAT) or anti-TNF therapies.

There were no significant differences across clusters of patients regarding history of autoimmune disorders, prodromes (including psychiatric features), type of SE, or MRI findings. However, outcomes varied notably. Patients in cluster B had worse outcomes at discharge ($p = 0.031$) and

Figure 2 Different Clusters of Patients With c-NORSE



(A) The connectivity graph was extracted from the patients' connectivity matrix, and the 8 subclusters of patients with c-NORSE, identified using the Louvain algorithm, were condensed into 4 clusters of patients. (B) The diagram illustrates the 8 subclusters of patients in the center, followed by the 4 clusters, and finally, the levels of all groups of biological markers (1, 2, and 3), represented by color bars (with reddish colors indicating higher levels). Connections between patients are shown in the center of the circle, where black links represent inter-subcluster connections and colored links indicate intra-subcluster connections. c-NORSE = cryptogenic new-onset refractory status epilepticus.

during follow-up (p = 0.015), as shown by lower Glasgow Outcome Scale Extended scores, than those in cluster C. This suggests that despite similar management strategies, patients in clusters B and C experienced different trajectories of disease severity and recovery, possibly reflecting different underlying responses to the same treatments. Patients in cluster A also had poor outcomes but developed post-NORSE epilepsy less frequently than those in cluster B (38% vs 83%, p = 0.06).

Differences in Biological Feature Levels and Related Pathways Between Clusters of Patients

We next investigated the relative differences in levels of the previously discussed 3 groups of biological features between the 3 clusters of patients. Patients in cluster B exhibited high levels of features associated with groups 1 and 3 (indicated by reddish colors) but low levels of features associated with group 2 (indicated by bluish colors) (Figure 2B). By contrast,

Table 1 Clinical and Paraclinical Features for the Patients Belonging to Clusters A, B, and C

	Cluster A (13 patients)	Cluster B (16 patients)	Cluster C (22 patients)	Intercluster *p* Value[a]
Age, y	69 (35–75)	34 (28–49)	22 (13–31)	<0.001
% Children	1/13	0/16	9/22	0.0038
% Female	10/13	11/16	9/22	0.087
History of autoimmune disorders	2/13	4/16	3/22	0.65
FIRES	6/13	13/16	16/22	0.13
Memory impairments	1/13	1/16	2/22	>0.99
Psychiatric features	3/13	2/16	5/22	0.74
SE duration, d	18 (5–32)	34 (11–51)	12 (6–28)	0.097
Convulsive SE	9/13	15/16	14/22	0.085
ICU duration, d	26 (19–50)	38 (15–87)	31 (12–66)	0.76
CIVAD, #	3 (1–3)	3 (3–4)	3 (2–3)	0.14
ASM, #	4 (4–5)	7 (5–8)	7 (5–9)	0.007
Delay of serum collection, d	14 (12–20)	12 (5–30)	8 (3–18)	0.57
Immunotherapies	2 (1–3)	3 (2–4)	3 (2–4)	0.038
Immunotherapies before serum collection	9/13	10/16	20/22	0.083
Second-line immunotherapies before serum collection	0/13	4/16	6/22	0.086
Number of immunotherapies before serum collection	1 (0–2)	2 (0–3)	2 (1–3)	0.11
Immunotherapies	11/13	15/16	20/22	0.71
Second-line immunotherapy	2/11	7/15	13/20	0.047
Anakinra	1/11	4/15	8/20	0.22
Tocilizumab	0/11	3/15	5/20	0.28
Rituximab	1/11	2/15	5/20	0.61
Intrathecal dexamethasone	0/11	1/15	2/20	>0.99
Last immunotherapy received	—	—	—	—
First-line	9/11	9/15	7/20	0.039
Anakinra	1/11	3/15	3/20	0.88
Tocilizumab	0/11	1/15	3/20	0.54
Rituximab	1/11	1/15	5/20	0.33
Intrathecal dexamethasone	0/11	0/15	2/20	0.50
Ketogenic diet	5/13	5/16	11/22	0.51
CSF WBCs, per µL	3 (1–9)	11 (3–25)	13 (3–27)	0.08
CSF protein, mg/dL	51 (46–62)	43 (36–57)	48 (37–67)	0.73
OCBs	1/9	0/9	1/12	>0.99
MRI temporal T2 HI	6/13	8/16	8/22	0.72
ASM at discharge, #	2 (2–2)	5 (4–6)	3 (3–4)	0.0048
GOS-E score at discharge	3 (1–3)	3 (2–3)	3 (3–5)	0.049
Mortality at discharge	4/13	4/16	2/22	0.24

Continued

Table 1 Clinical and Paraclinical Features for the Patients Belonging to Clusters A, B, and C *(continued)*

	Cluster A (13 patients)	Cluster B (16 patients)	Cluster C (22 patients)	Intercluster *p* Value[a]
GOS-E score at follow-up, 12 mo (higher better)	2 (1–6)	3 (1–4)	6 (4–7)	0.034
Post-NORSE epilepsy at 12 mo	3/8	10/12	12/20	0.11

Abbreviations: ASM = antiseizure medication; CIVAD = continuous anesthetic drug; FIRES = febrile infection–related epilepsy syndrome; GOS-E = Glasgow Outcome Score Extended, HI = hyperintensity; ICU = intensive care unit; OCBs = oligoclonal bands; SE = status epilepticus; WBC = white blood cell.
[a] Kruskal-Wallis tests were used to compare clinical and biological features among clusters of patients. Post hoc comparisons were performed using Dunn tests when appropriate. Fisher tests were used to compare categorical variables among clusters of patients.
Data are represented as median (interquartile range) or ratio.

patients in cluster C displayed high levels of features from groups 2 and 3 but low levels of features from group 1. Patients in cluster A did not show increased levels for any feature groups when compared with other patients with c-NORSE. Meanwhile, patients in the central cluster U demonstrated moderate levels of features from groups 1 and 2 and moderate-to-high levels of features from group 3 (Figure 2B).

To further understand the biological pathways underlying the distinct patient clusters, we examined the functions of the overexpressed markers in patients with c-NORSE compared with nonstatus controls. We analyzed the 100 most significantly upregulated pathways for each cluster of patients with c-NORSE compared with control patients (Figure 3, eTable 3).

A total of 59 pathways were overexpressed across all 3 clusters (A vs controls, B vs controls, and C vs controls), including those related to inflammation, immune response, innate immunity, chemotaxis, and neutrophil recruitment and migration, highlighting the importance of innate immunity in all patients with c-NORSE compared with controls. Of interest, compared with patients belonging to clusters B and C, none of the pathways overexpressed by patients in cluster A were specific to inflammation. However, 16 pathways were specifically overexpressed in cluster B, including those involved in leukocyte recruitment and migration, lymphocyte chemotaxis, degranulation, monocyte infiltration, and hemophagocytic lymphohistiocytosis (HLH) (Figure 3). These findings suggest that although all patients with c-NORSE exhibit innate immune responses, those in cluster B are more prone to experience acute phase inflammatory syndrome, cytokine storm, and rapid immune activation aimed to eliminate pathogens and damaged cells, such as through granzyme B production (Figure 3).

By contrast, cluster C, characterized by high levels of group 2 and 3 features, presents a profile dominated by dysregulated autoimmunity, including abnormal JAK-STAT signaling, and altered B-cell and T-cell function (Figure 3). Dysregulations were even more important for subclusters 6 and 0 within cluster C (Figure 2B).

Comparison With Patients With Other RSE

To determine whether the biological profile can provide insight into the underlying etiology and severity of the disease, particularly when an autoimmune disease is suspected, we further analyzed the biological profiles of patients with RSE and a known etiology (n = 45) (Figure 4A). These patients were projected onto the previously established connectivity graph (Figure 4A).

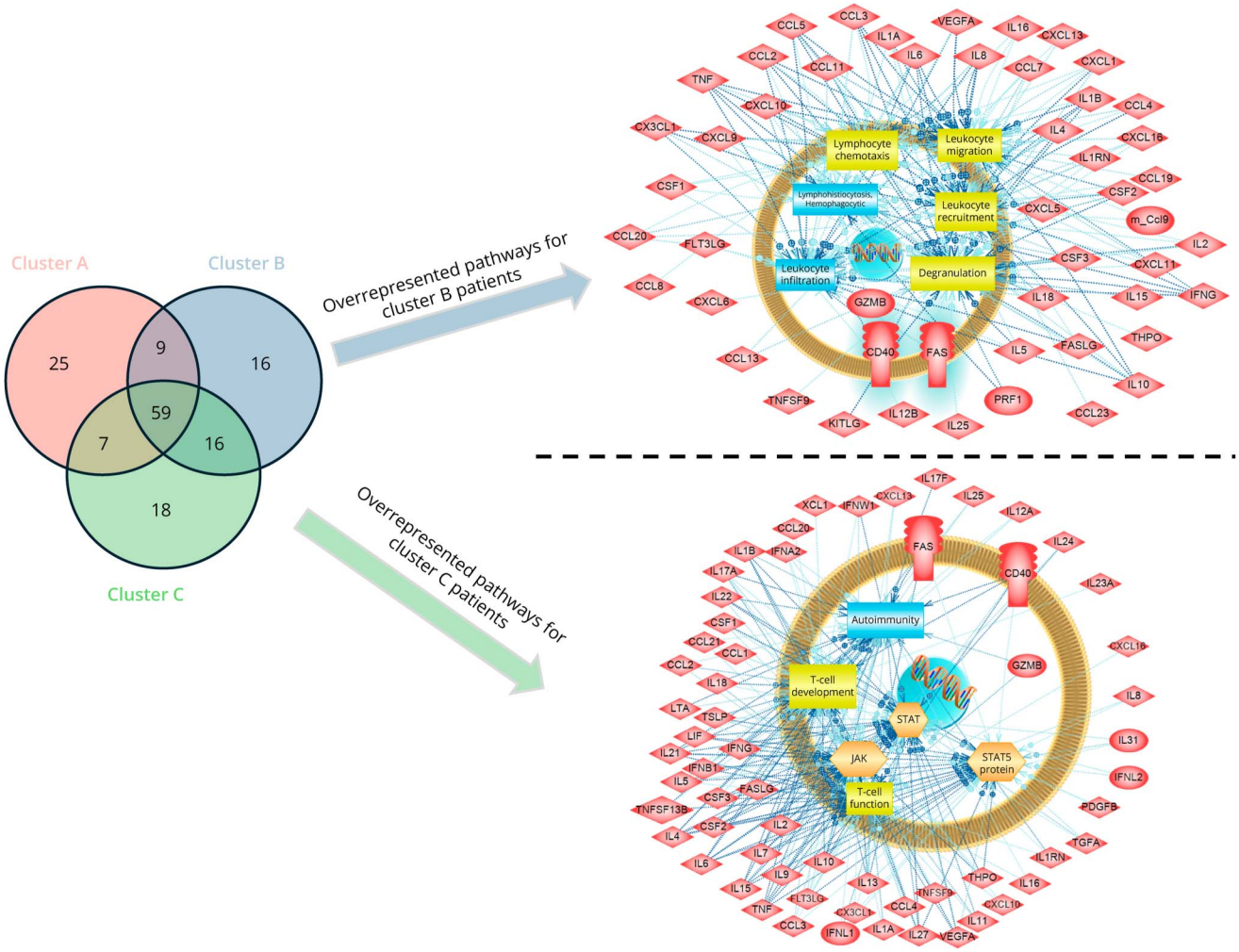
We observed a clear pattern in how patients clustered based on their etiology. Notably, all patients who experienced SE due to preexisting epilepsy with changes in ASM regimens were grouped and showed similarity to patients in subcluster 2 within cluster C, with moderate levels of group 2 and 3 markers and low levels of group 1 markers. Likewise, most patients with SE caused by a brain tumor were grouped and aligned with cluster A.

Notably, 6 of the 7 patients whose SE was attributed to autoimmune encephalitis with identified autoantibodies (NMDAR = 4, GAD65 = 2, myelin oligodendrocyte glycoprotein antibody disease [MOGAD] = 1) were grouped in cluster C, specifically in subclusters 6 and 0 (Figure 4B). By contrast, none of the patients in cluster B had autoimmune encephalitis. Five of the 6 patients in cluster B had worse outcomes at discharge and during follow-up, suggesting more severe disease, as observed for patients with c-NORSE in cluster B.

Discussion

We demonstrated that distinct clusters of patients with c-NORSE can be identified based on serum cytokines/chemokines, potentially revealing different underlying mechanisms of RSE and guiding the use of specific immunotherapies, even when the clinical presentation seems similar. While patients with c-NORSE in all clusters had elevated levels of proinflammatory cytokines related to innate immunity compared with controls and patients with RSE of known etiology, we identified 3 major clusters based on their relative levels of a large number of cytokines/chemokines. The clinical and biological features of each cluster are summarized in Table 2.

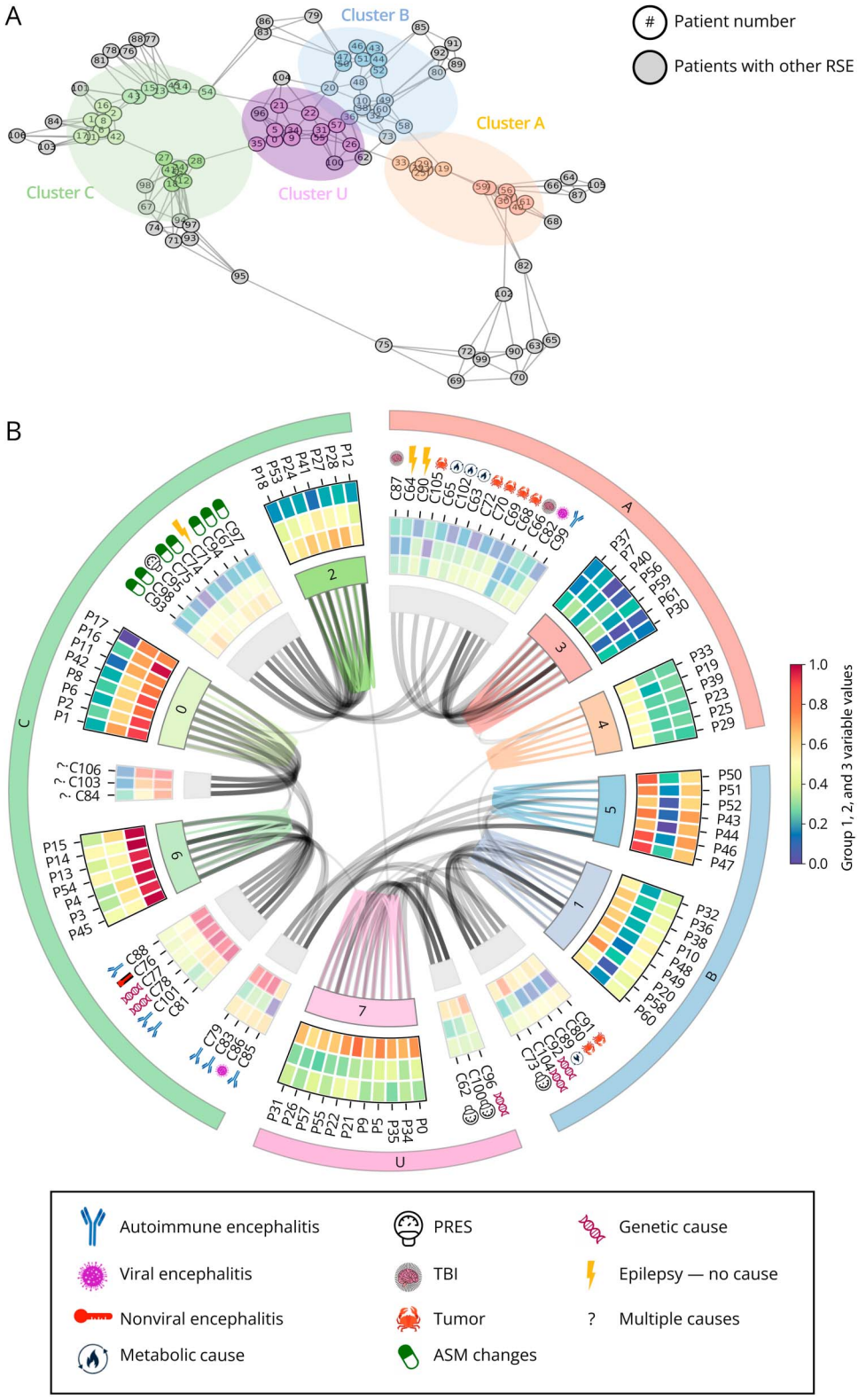
Figure 3 Network Analyses of Protein Interaction and Implicated Biological and Pathologic Processes Involved for the Clusters of Patients With c-NORSE



The Venn diagram highlights the pathways shared by patients in clusters A, B, and C, as well as those unique to specific clusters. A protein pathway analysis was performed, focusing on inflammation-related pathways specific to each cluster. None of the pathways overexpressed in cluster A were related to inflammation. For clusters B and C, cellular processes are represented by yellow rectangles (e.g., leukocyte migration), disease states by blue rectangles (e.g., autoimmunity), and functional pathways by orange hexagons (e.g., JAK). Markers are illustrated in either intracellular or extracellular spaces, based on their expression location. Relationships are shown as lines and arrows. c-NORSE = cryptogenic new-onset refractory status epilepticus.

The elevated cytokine levels related to innate immunity align with growing evidence suggesting that RSE onset in patients with c-NORSE may be triggered by dysregulation of innate immunity.[9,20] Increased levels of IL-1β, IL-6, and CXCL8/IL-8 have been frequently observed in these patients compared with controls or patients with epilepsy.[20-22] However, it remains unclear whether this dysregulation is a cause or a consequence of SE. We previously reported higher levels of CXCL8/IL-8, MIP-1α, and CCL2 in the serum of patients with c-NORSE compared with those with RSE of known causes.[9] These findings suggested that inflammation might play a role in driving RSE in patients with c-NORSE and that immunotherapies could help shorten SE duration.[9,23] In support, preclinical studies in rodent models have shown that cytokines such as IL-1β and CXCL8/IL-8 contribute to seizures because blockage of their receptors or respective downstream pathways reduces seizure frequency and shortens SE duration.[24] Moreover, there is increasing evidence implicating innate immunity cytokines in SE refractoriness.[24] In 2022, recommendations for managing patients with NORSE emphasized early immunotherapy, including targeted treatments such as anakinra (IL-1 receptor antagonist) and tocilizumab (IL-6 receptor antagonist) for c-NORSE.[10] Since then, anakinra and tocilizumab have been increasingly used, with up to 70% of patients achieving seizure control within weeks after treatment initiation.[11] The earlier administration of either agent has been associated with shorter SE duration and lower frequency of brain atrophy.[25] However, approximately one-third of patients do not respond to these treatments, possibly because of noninflammatory etiologies or different inflammatory drivers.

Figure 4 Distribution of Patients With a RSE of Known Etiology



4A. Patients with a RSE of known etiology were mapped onto the connectivity graph. 4B. These patients are represented in light colors, with various etiologies indicated by icons, as shown in the legend box. The diagram illustrates the homogeneity of biological profiles within the different etiology groups. RSE = refractory status epilepticus.

In our study, we highlighted that the 3 clusters of pathways shared an upregulation of innate immunity–driven response when compared with control patients, highlighting the importance of innate immunity in NORSE. However, the patients in cluster A showed lower levels of proinflammatory cytokines than those in clusters B and C. Despite ongoing SE,

Table 2 Summary of the Main Clinical and Biological Features for the 3 Clusters of Patients

	Cluster A	Cluster B	Cluster C
Inflammation profile	No inflammation	Innate-immunity cytokine-driven inflammatory response	Dysregulated autoimmunity processes
Overexpressed pathways related to inflammation	None	Leukocyte migration Degranulation HLH	JAK-STAT T-cell function Autoimmunity
Median age (y)	69	34	22
Previous fever (FIRES) (%)	46	81	73
CSF	Normal	Pleocytosis	Pleocytosis
Outcome at last follow-up	Poor	Poor	Good
Post-NORSE epilepsy (%)	38	83	60
Suggested targeted immunotherapy[a]	None	Anakinra, tocilizumab Anti-CXCL8 Anti-TNF Ruxolitinib/tofacitinib	Rituximab[b] Cyclophosphamide[b] Ruxolitinib/tofacitinib[b]

Abbreviations: FIRES = febrile infection–related epilepsy syndrome; HLH = hemophagocytic lymphohistiocytosis; NORSE = new-onset refractory status epilepticus.
[a] The suggestions are preliminary, not based on actual patient responses. Therapeutic decisions should consider a comprehensive evaluation that includes clinical and paraclinical findings.
[b] Specifically for patients in subclusters 0 and 6.

the relative lower levels of cytokine in these patients suggest that cytokine disturbances are not solely a consequence of seizures. These patients were older than those with stronger cytokine dysregulation, which may indicate an age-related decline in immune response.[26] Alternatively, the primary difference may lie in the underlying etiology. Specifically, patients in cluster A may have a non–immune-mediated cause, as evidenced by their lower white blood cell count in the CSF.[27] This aligns with the fact that cluster A was administered fewer immunotherapies. It is important to note that the broad cytokine panel levels were not clinically available, reducing a potential bias in treatment decisions.

We confirmed that patients with c-NORSE, notably those in cluster B, had higher levels of CXCL8/IL-8 compared with those with RSE with established causes. A correlation matrix showed CXCL8/IL-8 levels associated with other key proinflammatory cytokines, including IL6, TNF, CXCL10, and CXCL9. These inflammatory mediators are critically involved in recruiting and activating neutrophils and monocytes, key components of innate immune responses. Overactivation of neutrophil pathways may drive excessive release of granular proteins, enzymes, and reactive oxygen species during degranulation, leading to further neuronal injury.[28] Similarly, overactivation of monocyte pathways may promote excessive tissue remodeling through engraftment of monocyte-derived macrophages, also contributing to neuronal damage.[29] Beyond modulating innate immunity, neutrophils may also promote increased infiltration of adaptive immune cells.[28,30,31] This may explain the higher serum levels of granzyme B in patients with c-NORSE compared with those with RSE of known etiology, independently of a T-cell–driven etiology.[32] The highly dysregulated immune response in group B patients may contribute to prolonged seizures and poor outcomes.[33,34] These patients may benefit from immunotherapies targeting acute inflammation, such as tocilizumab, or those modulating neutrophil activity, such as a CXCL8 inhibitor, or macrophage and monocyte activity, such as anakinra (anti-IL-1) or TNF inhibitor.

Patients in cluster B exhibited overexpression of the HLH pathway, a syndrome characterized by uncontrolled activation of monocytes, macrophages, histiocytes, and lymphocytes, leading to hypercytokinemia and hemophagocytosis. Although primary HLH is related to genetic mutations,[35] it can also arise as a secondary condition triggered by innate immune activation.[36] HLH is often complicated by multiorgan failure.[37] Unfortunately, many of the diagnostic clinical or biological features were not consistently reported in our database or are challenging to interpret because of confounding factors.[38,39] These findings suggest that some of our patients may have developed secondary HLH, potentially contributing to their poor outcomes.[40-43] Recognizing HLH is critical because it could guide the initiation of specific therapies, including immunosuppressive treatments and chemotherapy.[44]

Patients in cluster C exhibited relatively low levels of group 1 markers but increased levels of group 2 and 3 markers. Protein pathway analysis indicated overexpression of JAK-STAT pathways, which regulate transcription of genes involved in immune cell proliferation, differentiation, and survival, with varying outcomes depending on the specific STAT protein activated. We hypothesize that these patients may be experiencing SE associated with an autoimmune disorder,

potentially driven by an unidentified antibody. Notably, STAT5, known to promote autoimmune-mediated neuroinflammation, was overexpressed in these patients.[45] This finding aligns with the discovery of several novel autoantibodies in recent years, highlighting the rapidly evolving field of autoimmune research.[46] Moreover, it may explain why these patients responded favorably to multiple lines of immunotherapy.[47] In addition, clinical similarities between patients in cluster C and those with SE caused by known autoimmune disorders further support this hypothesis. However, screening for novel autoantibodies for c-NORSE has yielded negative results.[48] The SE could also be attributed to cell-mediated encephalitis, given the overexpression of pathways related to T-cell development and function.[49] Given the involvement of the JAK/STAT pathway in this subgroup, targeting these signaling molecules with anti-JAK/STAT therapies, such as ruxolitinib or tofacitinib, could be a promising approach for patients with limited responses to conventional immunotherapies.[50] Ruxolitinib has been tested to reduce hyperinflammation in patients with cytokine storm syndromes[51] and has been found effective in patients with HLH,[52] suggesting its potential relevance for patients in cluster B. However, the use of JAK/STAT inhibitors in SE remains rare. Two studies have highlighted the beneficial effects of ruxolitinib on memory and epilepsy in an animal model of SE.[53,54] In addition, 2 case reports described the use of tofacitinib in patients with FIRES. While the first report described EEG and MRI improvements after 10 days,[50] the second case showed no improvement after treatment initiation.[55] More prospective studies are needed to evaluate the efficacy of these treatments in patients with NORSE.

Our analyses were conducted exclusively on serum samples, and the possibility of concomitant systemic infection cannot be excluded. We prioritized serum over CSF analysis because cytokine profiles in serum exhibited more pronounced changes in patients with c-NORSE compared with those with other RSE,[9] suggesting a significant systemic inflammation component for c-NORSE. In addition, the larger availability of serum samples enabled more detailed analysis compared with the limited number of CSF samples.

However, further studies incorporating CSF analysis would be valuable to assess the comparability of patient subgroups. Longitudinal analysis of serum samples would also help evaluate the consistency of the classifications over the disease course and in relation to treatments received. To achieve these objectives, we are enrolling patients globally into the open NORSE/FIRES biorepository.[12] Despite these efforts, we currently lack sufficient CSF samples or longitudinal serum collections to perform these specific analyses.

Despite offering new insights into c-NORSE pathophysiology, our study has several limitations. Many of our patients received at least a first-time immunotherapy before serum collection, and 19% received a second-line immunotherapy, which may have affected cytokine levels. However, no significant difference was found in previous immunotherapy use between clusters of patients. The classification has been obtained by using a panel of 96 cytokines that is not readily available in clinical practice and has not yet been certified for patient diagnostics. Another large panel, comprising 71 of the cytokines/chemokines, was recently certified by the US Centers for Medicare & Medicaid Services. However, obtaining timely results can be challenging, particularly for patients hospitalized outside North America. To facilitate clinical application, it is essential to determine the optimal number of features required for accurate patient classification, as we plan to do with additional samples. This would enable the development of a customized cytokine panel that includes the most important markers and could be implemented in hospitals worldwide. In addition, while the Louvain algorithm identified patient clusters based on their similarity patterns, intracluster variability cannot be excluded. Notably, subcluster 2 is likely distinct from subclusters 0 and 6. The similarity between subcluster 2 patients and those with RSE due to ASM regimen changes suggests a potential non-autoimmune mechanism in subcluster 2 patients. Increasing the cohort size will help clarify this question. The suggested immunotherapies in Table 2 are theoretical, not based on actual patient responses. Therefore, therapeutic decisions should always consider a comprehensive evaluation that includes clinical and other paraclinical findings. In addition, most of our control patients were adults, restricting the applicability of the findings to children.

In conclusion, our study highlights distinct inflammatory and autoimmune pathways in subgroups of patients with c-NORSE, emphasizing the need for personalized therapeutic strategies that could lead to more effective treatments and improved patient outcomes.

Acknowledgment

The authors thank Dr. Sara Hocker (Mayo Clinic, Rochester) for her help and patient referrals.

Author Contributions

M. Guillemaud: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data. M. Chavez: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data. F. Kobeissy: drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data. A. Vezzani: analysis or interpretation of data. A.D. Jimenez: major role in the acquisition of data. M. Basha: major role in the acquisition of data. A. Batra: major role in the acquisition of data. S. Demeret: major role in the acquisition of data. O. Eka: major role in the acquisition of data. K. Eschbach: major role in the acquisition of data. B. Foreman: major role in the acquisition of data. N. Gaspard: major role in the acquisition

of data. E.E. Gerard: major role in the acquisition of data. T.E. Gofton: major role in the acquisition of data. H.A. Haider: major role in the acquisition of data. S.T. Hantus: major role in the acquisition of data. C.L. Howe: Analysis or interpretation of data. A. Jongeling: major role in the acquisition of data. M. Kalkach-Aparicio: major role in the acquisition of data. P. Kandula: major role in the acquisition of data. K. Kazazian: major role in the acquisition of data. M. Kim: major role in the acquisition of data. Y.-C. Lai: major role in the acquisition of data; analysis or interpretation of data. C. Marois: major role in the acquisition of data. A. Mellor: major role in the acquisition of data. W. Mohamed: major role in the acquisition of data. M. Morales: major role in the acquisition of data. C.M Pimentel: major role in the acquisition of data. A.M. Ramirez: major role in the acquisition of data. C. Steriade: major role in the acquisition of data. A.F. Struck: major role in the acquisition of data. O. Taraschenko: major role in the acquisition of data. N. Torcida Sedano: major role in the acquisition of data. M.S. Wainwright: major role in the acquisition of data. J.Y. Yoo: major role in the acquisition of data. K.K.W. Wang: major role in the acquisition of data; analysis or interpretation of data. V. Navarro: major role in the acquisition of data; analysis or interpretation of data. L.J. Hirsch: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data. A. Hanin: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data.

Study Funding

The NORSE/FIRES biorepository is supported by the NORSE/FIRES Research Fund at Yale and the NORSE Institute (more information at norseinstitute.org/or email norse@yale.edu).

Disclosure

L.J. Hirsch received support to Yale University for investigator-initiated studies from The Daniel Raymond Wong Neurology Research Fund and the NORSE/FIRES Research Fund at Yale. A. Hanin received postdoctoral grants from the Paratonnerre Association, the Swebilius Foundation, the Servier Institute, and the Philippe Foundation for NORSE-related research. A. Vezzani, K. Eschbach, N. Gaspard, T.E. Gofton, H.A. Haider, C.L. Howe, Y.-C. Lai,O. Taraschenko, L.J. Hirsch, and A. Hanin are members of the Medical and Scientific Advisory Board of the NORSE Institute. K. Eschbach received funding from the friends and family of Finn Mussetter for NORSE and FIRES research. H.A. Haider received in 2020–2022 philanthropic funding from the Neal Nichols Foundation for Status Epilepticus/NORSE Research. V. Navarro received a CURE Epilepsy grant in 2024 for NORSE-related research. The other authors report no competing interests. Go to Neurology.org/NN for full disclosures.

Publication History

Received by *Neurology® Neuroimmunology & Neuroinflammation* January 22, 2025. Accepted in final form March 11, 2025. Submitted and externally peer reviewed. The handling editor was Associate Editor Josep O. Dalmau, MD, PhD, FAAN.

References

1. Hirsch LJ, Gaspard N, van Baalen A, et al. Proposed consensus definitions for new-onset refractory status epilepticus (NORSE), febrile infection-related epilepsy syndrome (FIRES), and related conditions. *Epilepsia*. 2018;59(4):739-744. doi:10.1111/epi.14016
2. Gaspard N, Foreman BP, Alvarez V, et al. New-onset refractory status epilepticus: etiology, clinical features, and outcome. *Neurology*. 2015;85(18):1604-1613. doi:10.1212/WNL.0000000000001940
3. Kramer U, Chi C-S, Lin K-L, et al. Febrile infection-related epilepsy syndrome (FIRES): pathogenesis, treatment, and outcome: a multicenter study on 77 children. *Epilepsia*. 2011;52(11):1956-1965. doi:10.1111/j.1528-1167.2011.03250.x
4. Taraschenko O, Pavuluri S, Schmidt CM, Pulluru YR, Gupta N. Seizure burden and neuropsychological outcomes of new-onset refractory status epilepticus: systematic review. *Front Neurol*. 2023;14:1095061. doi:10.3389/fneur.2023.1095061
5. deCampo D, Xian J, Karlin A, et al. Investigating the genetic contribution in febrile infection-related epilepsy syndrome and refractory status epilepticus. *Front Neurol*. 2023;14:1161161. doi:10.3389/fneur.2023.1161161
6. Wu J, Lan X, Yan L, et al. A retrospective study of 92 children with new-onset refractory status epilepticus. *Epilepsy Behav*. 2021;125:108413. doi:10.1016/j.yebeh.2021.108413
7. Saitoh M, Kobayashi K, Ohmori I, et al. Cytokine-related and sodium channel polymorphism as candidate predisposing factors for childhood encephalopathy FIRES/AERRPS. *J Neurol Sci*. 2016;368:272-276. doi:10.1016/j.jns.2016.07.040
8. Wang D, Wu Y, Pan Y, et al. Multi-proteomic analysis revealed distinct protein profiles in cerebrospinal fluid of patients between anti-NMDAR encephalitis NORSE and cryptogenic NORSE. *Mol Neurobiol*. 2023;60(1):98-115. doi:10.1007/s12035-022-03011-1
9. Hanin A, Cespedes J, Dorgham K, et al. Cytokines in new-onset refractory status epilepticus predict outcomes. *Ann Neurol*. 2023;94(1):75-90. doi:10.1002/ana.26627
10. Wickstrom R, Taraschenko O, Dilena R, et al. International consensus recommendations for management of new onset refractory status epilepticus (NORSE) including febrile infection-related epilepsy syndrome (FIRES): summary and clinical tools. *Epilepsia*. 2022;63(11):2827-2839. doi:10.1111/epi.17391
11. Hanin A, Muscal E, Hirsch LJ. Second-line immunotherapy in new onset refractory status epilepticus. *Epilepsia*. 2024;65(5):1203-1223. doi:10.1111/epi.17933
12. Hanin A, Cespedes J, Pulluru Y, et al. Review and standard operating procedures for collection of biospecimens and analysis of biomarkers in new onset refractory status epilepticus. *Epilepsia*. 2023;64(6):1444-1457. doi:10.1111/epi.17600
13. von Luxburg U. A tutorial on spectral clustering. *Stat Comput*. 2007;17(4):395-416. doi:10.1007/s11222-007-9033-z
14. Blondel VD, Guillaume J-L, Lambiotte R, Lefebvre E. Fast unfolding of communities in large networks. *J Stat Mech*. 2008;2008(10):P10008. doi:10.1088/1742-5468/2008/10/p10008
15. Rahiminejad S, Maurya MR, Subramaniam S. Topological and functional comparison of community detection algorithms in biological networks. *BMC Bioinformatics*. 2019;20(1):212. doi:10.1186/s12859-019-2746-0
16. Kanter I, Yaari G, Kalisky T. Applications of community detection algorithms to large biological datasets. *Methods Mol Biol*. 2021;2243:59-80. doi:10.1007/978-1-0716-1103-6_3
17. bioinfogp.cnb.csic.es/tools/venny/
18. Nikitin A, Egorov S, Daraselia N, Mazo I. Pathway studio--the analysis and navigation of molecular networks. *Bioinformatics*. 2003;19(16):2155-2157. doi:10.1093/bioinformatics/btg290
19. Devaux S, Cizkova D, Quanico J, et al. Proteomic analysis of the spatio-temporal based molecular kinetics of acute spinal cord injury identifies a time- and segment-specific window for effective tissue repair. *Mol Cell Proteomics*. 2016;15(8):2641-2670. doi:10.1074/mcp.M115.057794
20. Kothur K, Bandodkar S, Wienholt L, et al. Etiology is the key determinant of neuroinflammation in epilepsy: elevation of cerebrospinal fluid cytokines and chemokines in febrile infection-related epilepsy syndrome and febrile status epilepticus. *Epilepsia*. 2019;60(8):1678-1688. doi:10.1111/epi.16275
21. Horino A, Kuki I, Inoue T, et al. Intrathecal dexamethasone therapy for febrile infection-related epilepsy syndrome. *Ann Clin Transl Neurol*. 2021;8(3):645-655. doi:10.1002/acn3.51308
22. Lai Y-C, Muscal E, Wells E, et al. Anakinra usage in febrile infection related epilepsy syndrome: an international cohort. *Ann Clin Transl Neurol*. 2020;7(12):2467-2474. doi:10.1002/acn3.51229
23. Vezzani A, Baram TZ. New roles for interleukin-1 Beta in the mechanisms of epilepsy. *Epilepsy Curr*. 2007;7(2):45-50. doi:10.1111/j.1535-7511.2007.00165.x
24. Vezzani A, Di Sapia R, Kebede V, Balosso S, Ravizza T. Neuroimmunology of status epilepticus. *Epilepsy Behav*. 2023;140:109095. doi:10.1016/j.yebeh.2023.109095
25. Hanin A, Jimenez AD, Gopaul M, et al. Trends in management of patients with new-onset refractory status epilepticus (NORSE) from 2016 to 2023: an interim analysis. *Epilepsia*. 2024;65(8):e148-e155. doi:10.1111/epi.18014

26. Holtzclaw B. Altered febrile responses in older adults: a systematic review. *Innovation in Aging*. 2020;4(Supplement_1):224. doi:10.1093/geroni/igaa057.722
27. Barry E, Hauser WA. Pleocytosis after status epilepticus. *Arch Neurol*. 1994;51(2):190-193. doi:10.1001/archneur.1994.00540140100019
28. Mayadas TN, Cullere X, Lowell CA. The multifaceted functions of neutrophils. *Annu Rev Pathol*. 2014;9:181-218. doi:10.1146/annurev-pathol-020712-164023
29. Varvel NH, Neher JJ, Bosch A, et al. Infiltrating monocytes promote brain inflammation and exacerbate neuronal damage after status epilepticus. *Proc Natl Acad Sci U S A*. 2016;113(38):E5665-E5674. doi:10.1073/pnas.1604263113
30. Mantovani A, Cassatella MA, Costantini C, Jaillon S. Neutrophils in the activation and regulation of innate and adaptive immunity. *Nat Rev Immunol*. 2011;11(8):519-531. doi:10.1038/nri3024
31. Minns D, Smith KJ, Hardisty G, Rossi AG, Gwyer Findlay E. The outcome of neutrophil-T cell contact differs depending on activation status of both cell types. *Front Immunol*. 2021;12:633486. doi:10.3389/fimmu.2021.633486.
32. Hay ZLZ, Slansky JE. Granzymes: the molecular executors of immune-mediated cytotoxicity. *Int J Mol Sci*. 2022;23(3):1833. doi:10.3390/ijms23031833
33. Vezzani A, French J, Bartfai T, Baram TZ. The role of inflammation in epilepsy. *Nat Rev Neurol*. 2011;7(1):31-40. doi:10.1038/nrneurol.2010.178
34. Di Sapia R, Zimmer TS, Kebede V, et al. CXCL1-CXCR1/2 signaling is induced in human temporal lobe epilepsy and contributes to seizures in a murine model of acquired epilepsy. *Neurobiol Dis*. 2021;158:105468. doi:10.1016/j.nbd.2021.105468
35. Freeman HR, Ramanan AV. Review of haemophagocytic lymphohistiocytosis. *Arch Dis Child*. 2011;96(7):688-693. doi:10.1136/adc.2009.176610
36. Brisse E, Wouters CH, Matthys P. Advances in the pathogenesis of primary and secondary haemophagocytic lymphohistiocytosis: differences and similarities. *Br J Haematol*. 2016;174(2):203-217. doi:10.1111/bjh.14147
37. Lovisari F, Terzi V, Lippi MG, Brioschi PR, Fumagalli R. Hemophagocytic lymphohistiocytosis complicated by multiorgan failure: a case report. *Medicine (Baltimore)*. 2017;96(50):e9198. doi:10.1097/MD.0000000000009198
38. Henter J-I, Horne A, Aricó M, et al. HLH-2004: diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer*. 2007;48(2):124-131. doi:10.1002/pbc.21039
39. Witenko CJ, Littlefield AJ, Abedian S, An A, Barie PS, Berger K. The safety of continuous infusion propofol in mechanically ventilated adults with coronavirus disease 2019. *Ann Pharmacother*. 2022;56(1):5-15. doi:10.1177/10600280211017315
40. Haanpää A, Kämppi L, Kantonen J, Myllykangas L, Laakso SM, Forss N. Hemophagocytic lymphohistiocytosis in an adult patient with super-refractory status epilepticus. *Epilepsia Open*. 2024;9(5):1962-1967.
41. Chen Q-T, Chen M-H, Lin Y-K, et al. Case report: hemophagocytic lymphohistiocytosis complicated by multiple organ dysfunction syndrome following aseptic encephalitis. *Front Immunol*. 2023;14:1296575. doi:10.3389/fimmu.2023.1296575
42. Meenakshi-Sundaram S, Sankaranarayanan M, Jeyaraman M, Ayyappan C, Karthik SN, Pandi S. Super refractory status in a case of Febrile Infection-Related Epilepsy Syndrome due to hemophagocytic lymphocytic histiocytosis. *Epilepsia Open*. 2021;6(1):22-27. doi:10.1002/epi4.12454
43. Farias-Moeller R, LaFrance-Corey R, Bartolini L, et al. Fueling the FIRES: hemophagocytic lymphohistiocytosis in febrile infection-related epilepsy syndrome. *Epilepsia*. 2018;59(9):1753-1763. doi:10.1111/epi.14524
44. Hines M, Bhatt N, Talano J-AM. Diagnosis, treatment, and management of hemophagocytic lymphohistiocytosis in the critical care unit. *Crit Care Pediatr Immunocompromised Hematology/Oncology Patient*. 2019:159-182. doi:10.1007/978-3-030-01322-6_9
45. Monaghan KL, Aesoph D, Ammer AG, et al. Tetramerization of STAT5 promotes autoimmune-mediated neuroinflammation. *Proc Natl Acad Sci USA*. 2021;118(52):e2116256118. doi:10.1073/pnas.2116256118
46. Prüss H. Autoantibodies in neurological disease. *Nat Rev Immunol*. 2021;21(12):798-813. doi:10.1038/s41577-021-00543-w
47. Zhang Y, Zhang X, Wang H, Liu L. New-onset refractory status epilepticus caused by autoimmune encephalitis may have a better prognosis than when due to other causes. *Acta Neurol Scand*. 2024;2024(1):6817696. doi:10.1155/2024/6817696
48. Eisele A, Schwager M, Bögli SY, et al. The role of neuronal antibodies in cryptogenic new onset refractory status epilepticus. *Epilepsia*. 2023;64(12):e229-e236. doi:10.1111/epi.17755
49. Bien CG, Bauer J. T-cells in human encephalitis. *Neuromolecular Med*. 2005;7(3):243-253. doi:10.1385/NMM:7:3:243
50. Jang Y, Lee W-J, Lee HS, Chu K, Lee SK, Lee S-T. Tofacitinib treatment for refractory autoimmune encephalitis. *Epilepsia*. 2021;62(4):e53-e59. doi:10.1111/epi.16848
51. Keenan C, Albeituni S, Nichols KE, Hines M. JAK inhibitors in cytokine storm syndromes. *Adv Exp Med Biol*. 2024;1448:583-600. doi:10.1007/978-3-031-59815-9_39
52. Keenan C, Nichols KE, Albeituni S. Use of the JAK inhibitor ruxolitinib in the treatment of hemophagocytic lymphohistiocytosis. *Front Immunol*. 2021;12:614704. doi:10.3389/fimmu.2021.614704
53. Carrel A, Napoli E, Hixson K, et al. Ruxolitinib-dependent reduction of seizure load and duration is accompanied by spatial memory improvement in the rat pilocarpine model of temporal lobe epilepsy. *Neurotherapeutics*. 2025;22(2):e00506. doi:10.1016/j.neurot.2024.e00506
54. Grabenstatter HL, Del Angel YC, Carlsen J, et al. The effect of STAT3 inhibition on status epilepticus and subsequent spontaneous seizures in the pilocarpine model of acquired epilepsy. *Neurobiol Dis*. 2014;62:73-85. doi:10.1016/j.nbd.2013.09.003
55. Goh Y, Tay SH, Yeo LLL, Rathakrishnan R. Bridging the gap: tailoring an approach to treatment in febrile infection-related epilepsy syndrome. *Neurology*. 2023;100(24):1151-1155. doi:10.1212/wnl.0000000000207068