

## FULL-LENGTH ORIGINAL RESEARCH

# Fueling the FIRES: Hemophagocytic lymphohistiocytosis in febrile infection-related epilepsy syndrome

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## Summary

**Objectives:** Although secondary hemophagocytic lymphohistiocytosis (HLH) has been reported in children with critical illness of various etiologies, it has not been reported in patients with febrile infection-related epilepsy syndrome (FIRES). We describe a series of patients with concurrent HLH and FIRES in an effort to establish common pathophysiologic abnormalities.

**Methods:** Five patients with FIRES who were assessed for HLH were identified from a neurocritical care database. All were previously healthy and had extensive diagnostic testing. All had clinical deterioration with multiorgan dysfunction prompting HLH screening 20–29 days after hospitalization. Markers for inflammatory dysregulation were assessed in cerebrospinal fluid (CSF) and serum at various time points. Outcomes were assessed 6 months after presentation.

**Results:** Three patients met clinical criteria for secondary HLH. Elevation of specific cytokines/chemokines was variable. CSF neopterin, high mobility group box 1 (HMGB1), and C-X-C motif chemokine ligand 8 (CXCL8) were significantly elevated in all. Interleukin-1 $\beta$  (IL-1 $\beta$ ) and IL-18 were not elevated in any of the samples. Treatment and outcomes were variable.

**Significance:** We describe 3 patients with HLH and FIRES. The co-occurrence of these 2 rare disorders suggests the possibility of a common immune dysregulation phenotype prolonging epileptogenesis. HLH screening in critically ill patients with FIRES may yield a broader understanding of shared inflammatory processes.

## KEYWORDS

febrile infection-related epilepsy syndrome, hemophagocytic lymphohistiocytosis, refractory status epilepticus

## 1 | INTRODUCTION

Febrile infection-related epilepsy syndrome (FIRES) is an epileptic encephalopathy seen in children with refractory

status epilepticus (RSE) after febrile illness.<sup>1–3</sup> The etiology is unknown, although maladaptive activation of the innate immune system is postulated to have a role.<sup>2,3</sup> Seizures are usually refractory to antiseizure drugs (ASDs), anesthetic agents, and immune modulators.<sup>1,2,4</sup> The study of FIRES pathophysiology is stymied by the lack of diagnostic criteria or tests. The diagnosis is one of exclusion usually

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applied late in the disease course when changes in disease markers might be altered by polypharmacy, seizure burden, and complications of critical illness.

The hypothesis regarding FIRES etiology is based on evidence of inflammatory interplay and epileptogenesis extrapolated from status epilepticus (SE) research to FIRES. In genetically predisposed individuals, a non-specific febrile illness triggers the release of inflammatory molecules via innate immune activation and through various mechanisms promotes hyperexcitability.<sup>2,5</sup> Another hyperinflammatory disorder is hemophagocytic lymphohistiocytosis (HLH), which is characterized by uncontrolled activation of histiocyte and lymphocytes resulting in hypercytokinemia and hemophagocytosis.<sup>6,7</sup> HLH is classified as primary due to genetic mutations and secondary due to malignancy, autoinflammatory, infectious, or metabolic conditions.<sup>6</sup> In secondary HLH, the role of innate immune activation via stimulation of Toll-like receptors has been highlighted.<sup>8</sup> Five of 8 criteria are necessary to diagnose secondary HLH (Figure 1).<sup>9</sup> Secondary HLH is usually underrecognized due to lack of criteria specificity,<sup>10</sup> which is why some experts recommend HLH screening in febrile, critically ill patients with multiorgan dysfunction,  $\geq 2$  cytopenias, or splenomegaly.<sup>11</sup>

Although secondary HLH has been reported with infections, malignancies, and autoimmune disorders,<sup>12</sup> it has never been reported in patients with FIRES. We describe our experience with HLH in patients with FIRES and explore a potential pathophysiologic mechanism of secondary systemic and neurologic inflammation.

|                                                                                           |
|-------------------------------------------------------------------------------------------|
| <b>The diagnosis of HLH can be established if either 1 or 2 below is fulfilled</b>        |
| <b>(1) A genetic mutation affecting immune effector function (including familial HLH)</b> |
| <b>(2) Diagnostic criteria for HLH fulfilled (5/8 criteria below)</b>                     |
| <b>Diagnostic Criteria to be evaluated</b>                                                |
| Fever                                                                                     |
| Splenomegaly                                                                              |
| Cytopenias (affecting $\geq 2$ of 3 lineages in the peripheral blood):                    |
| Hemoglobin $< 9$ g/dL (in infants $< 4$ weeks: Hb $< 10$ g/dL)                            |
| Platelets $< 100 \times 10^9/L$                                                           |
| Neutrophils $< 1.0 \times 10^9/L$                                                         |
| Hypertriglyceridemia and/or hypofibrinogenemia:                                           |
| Fasting triglycerides $\geq 265$ mg/dL                                                    |
| Fibrinogen $\leq 1.5$ g/dL                                                                |
| Hemophagocytosis in bone marrow or spleen or lymph nodes                                  |
| No evidence of malignancy                                                                 |
| Low or absent natural killer cell activity                                                |
| Ferritin $\geq 500$ $\mu$ g/L                                                             |
| Soluble CD25 (ie, soluble IL-2 receptor) $\geq 2400$ U/mL                                 |

**FIGURE 1** HLH diagnostic criteria.<sup>9</sup> 2004 Histiocyte Society Criteria specify 5/8 criteria necessary to fulfill a diagnosis of secondary HLH

Key Points

- Elevation of CSF neopterin, HMGB1, and CXCL8 was seen in 5 patients with FIRES
- Central and peripheral inflammatory interplay may promote worsening of epileptogenesis in patients with FIRES
- The co-occurrence of HLH and FIRES suggests a shared inflammatory dysregulation pathway

2 | METHODS

We performed a retrospective analysis of 10 patients with FIRES from 2007 to 2017. Patients were identified from a prospective neurocritical care database. The electronic medical record was used to supplement patient demographics and clinical course information. All had (1) super-refractory status epilepticus (defined as status epilepticus that continues or recurs 24 hours or more after the onset of anesthesia<sup>13</sup>) preceded by a nonspecific febrile illness; (2) an extensive evaluation for infectious, structural, metabolic, and immunologic triggers that did not reveal an etiology for the refractory seizures; and (c) no evidence of chronic disease. Patients were excluded if they had preexistent epilepsy, were younger than 2 years, had a known neurologic disease, or had another diagnosis to explain the clinical presentation. Five children were assessed for HLH.

Our institution routinely screens most severely critically ill children with ferritin levels if they have unexplained multiorgan dysfunction, fever, or cytopenias. The sixth patient with FIRES cared for at our institution (Patient 1) had associated multiorgan dysfunction, fever, and cytopenias prompting clinicians to assess her for HLH. All subsequent patients with FIRES (n = 5) have been fully or partially screened for HLH and thus are included in this study.

All 5 patients screened for HLH had further assessment of their inflammatory state. Cerebrospinal fluid (CSF) frozen at  $-80^{\circ}\text{C}$  was assessed for cytokine and chemokine levels a minimum of once in the disease course. Matched serum cytokines and chemokines were measured in 2 patients at least once in the disease course. Cytokine and chemokine analysis was performed at the translational neuroimmunology laboratory at Mayo Clinic. This study was approved by the institutional review boards at both Children's National Health System and Mayo Clinic.

2.1 | FIRES diagnosis—clinical/laboratory features

For a comprehensive list of specific tests performed in each patient, please refer to Table S1.

## 2.2 | HLH diagnosis—clinical/laboratory features

Patients were evaluated for HLH using the 2004 Histiocyte Society Criteria<sup>9</sup> (Figure 1). The presence of 5 or more criteria was considered diagnostic for secondary HLH in the absence of a genetic/molecular diagnosis confirming primary HLH. During the time of HLH screening, supporting clinical features outside of strict HLH criteria such as rash, liver and/or kidney dysfunction, ileus, coagulopathy, and hemodynamic instability were reviewed.

## 2.3 | Outcomes

Outcomes were assessed 6 months after presentation. Outcome analysis included the presence of refractory epilepsy, cognitive state, functional assessment, dependence on medical devices (ie, gastrostomy or tracheostomy with or without ventilator support), and mortality.

## 2.4 | Cytokine and chemokine analysis

CSF was collected from all 5 patients; matched serum was available from 2. CSF controls utilized derive from adult patients with normal pressure hydrocephalus. Serum controls were obtained from healthy adults. Interleukin (IL)-1 $\beta$ , IL-6, IL-10, IL-12p70, and tumor necrosis factor (TNF)- $\alpha$  were measured using an inflammatory cytokine-specific cytometric bead array (#551811, BD Biosciences, San Diego, California). C-C motif chemokine ligand (CCL)-2, CCL5, C-X-C motif chemokine ligand (CXCL)8, CXCL9, and CXCL10 were also measured by cytometric bead array (#552990, BD Biosciences) using an Accuri C6 flow cytometer (BD Biosciences). Limits of detection for the inflammatory cytokine kit were as follows: 7.2 pg/mL IL-1 $\beta$ ; 2.5 pg/mL IL-6; 3.3 pg/mL IL-10; 3.7 pg/mL TNF- $\alpha$ ; and 1.9 pg/mL IL-12p70. Limits of detection for the chemokine kit were as follows: 2.7 pg/mL CCL2; 1.0 pg/mL CCL5; 0.2 pg/mL CXCL8; 2.5 pg/mL CXCL9; and 2.8 pg/mL CXCL10. Quantitation was performed by reference to a standard curve using FCAP Array software (BD Biosciences) and manual analysis in FlowJo 10.1 (Ashland, Oregon). Results are expressed as pg/mL for each analyte.

## 2.5 | Measurement of IL-18, HMGB1, and IL-1RA by ELISA

Serum and CSF levels of IL-18 (#7620, MBL, Woburn, MA), high mobility group box 1 (HMGB1) (#ST51011, IBL International, Hamburg, Germany), IL-1 receptor antagonist (IL-1RA) (#DRA00B, R&D Systems; Minneapolis, Minnesota), and C-reactive protein (CRP) (#DCRP00, R&D Systems) were measured by enzyme-

linked immunosorbent assay (ELISA). Absorbance at 450 nm of the final reaction product was measured with a Spectramax M3 spectrophotometer and quantified by reference to a standard curve. Results are presented as pg per mL for each analyte. The minimum detectable concentrations were as follows: 12.5 pg/mL for serum IL-18, 0.2 ng/mL for serum HMGB1, 6.3 pg/mL for serum IL-1RA, and 10 pg/mL for serum CRP. Minimum detectable concentrations for CSF were not available from the manufacturer.

## 2.6 | Clinical laboratory measurements

CSF and/or serum levels of neopterin, ferritin, soluble CD25, fibrinogen, triglycerides, and all cell number measurements were performed via standard clinical laboratory testing at Children's National Health System.

## 2.7 | Statistics

Wilcoxon rank-sum test was used to compare median CSF neopterin for patients with FIRES to published median CSF neopterin values in the following conditions<sup>14</sup>: acute disorders ( $n = 30$ ), chronic progressive disorders ( $n = 17$ ), febrile exacerbation of chronic static disorders ( $n = 6$ ), and chronic static disorders ( $n = 105$ ). The  $P$  values were adjusted for the number of comparisons using the Sidak method. Data analysis was performed using Stata 14 (College Station, Texas).

# 3 | RESULTS

Patient characteristics are summarized in Table 1. Male-to-female ratio was 3:2. Age ranged from 4 to 16 years (median 4.5, interquartile range [IQR] 4.5-12.5). No patient was immunocompromised at the time of admission or prior. None had a preexisting rheumatologic, autoimmune, or paraneoplastic disorder. Family history was negative for HLH in all patients. The timing of HLH screening ranged from 20 to 29 days after hospitalization (median 23 days, IQR 21-26). All patients continued to have electrographic seizures and/or a background with frequent epileptiform discharges on the ictal-interictal continuum<sup>15</sup> at the time of HLH screening.

Three patients fulfilled  $\geq 5$  diagnostic criteria for secondary HLH. The other 2 patients met 3 and 4 criteria but were incompletely screened. Three patients were genetically screened for HLH, and none had a known genetic mutation. Four patients exhibited a maculopapular rash within 2 days of HLH surveillance. Additional clinical features identified during HLH screening included elevation of transaminases in all patients with cholestasis in 3, renal dysfunction in one patient, hemodynamic instability and

TABLE 1 Patient characteristics

| Patient                                     | #1                          | #2                             | #3                              | #4                         | #5                                 |
|---------------------------------------------|-----------------------------|--------------------------------|---------------------------------|----------------------------|------------------------------------|
| Age/gender                                  | 5 y/female                  | 4 y/male                       | 7 y/male                        | 9 y/male                   | 16 y/female                        |
| HD of HLH screening                         | 29                          | 23                             | 22 <sup>a</sup>                 | 20 <sup>a</sup>            | 23                                 |
| HLH—2004 criteria                           |                             |                                |                                 |                            |                                    |
| Fever (Y/N)                                 | Y (> 7 days)                | Y (> 4 days)                   | Y (>7 days)                     | N                          | N                                  |
| Hepato/splenomegaly (Y/N)                   | Y                           | Y                              | Y                               | Y                          | Y                                  |
| Cytopenias $\geq$ 2 cell lines              | Y                           | Y                              | N                               | Y                          | Y                                  |
| Triglycerides $\geq$ 265 mg/dL              | 4989                        | 1744                           | 461                             | 346                        | 621                                |
| Hemophagocytes                              | Low quantity in BM          | Absent in CSF and BM           | Not done                        | Not done                   | Absent in CSF and BM               |
| NK cell activity                            | Low                         | Normal                         | Normal (drawn HD 52)            | Normal                     | Low                                |
| Ferritin >500 ug/L                          | 17 188                      | 1668                           | 1918                            | 378                        | 649                                |
| Soluble CD25 $\geq$ 2400 U/mL               | 5589                        | 7614                           | Not done                        | Not done                   | 10 000                             |
| Genetic screen for HLH                      | Negative                    | Negative                       | Not done                        | Not done                   | Negative                           |
| Multisystem dysfunction                     |                             |                                |                                 |                            |                                    |
| Liver dysfunction                           | Y ( $\uparrow$ AST/ALT)     | Y ( $\uparrow$ AST/ALT/GGT)    | Y ( $\uparrow$ AST/ALT/Bili)    | Y ( $\uparrow$ AST/ALT)    | Y ( $\uparrow$ AST/ALT/Bili/GGT)   |
| AKI w/dialysis                              | N                           | N                              | Y (CRRT)                        | N                          | N                                  |
| Hypotension                                 | N                           | Y                              | Y                               | N                          | Y                                  |
| Ileus                                       | N                           | Y ( $\uparrow$ amylase/lipase) | Y ( $\uparrow$ amylase, lipase) | N                          | Y                                  |
| DIC                                         | N                           | N                              | Y                               | N                          | N                                  |
| Rash (HD)                                   | Y (27)                      | Y (23)                         | N                               | Y (20)                     | Y (21)                             |
| CSF studies/HD                              | WBC0, RBC0, Prt20, Glc106/1 | WBC17, RBC2, Prt32, Glc86/1    | WBC7, RBC0, Prt45, Glc86/1      | WBC8, RBC0, Prt44, Glc70/1 | WBC 5, RBC 0, Prt 37 Glc 63,/1     |
| CSF neopterin (HD) normal range 7-40 nmol/L | 260 nmol/L (20)             | >300 nmol/L (29)               | 122 nmol/L (17)                 | 255 nmol/L (1)             | 296 nmol/L (22)                    |
| Immune modulators (HD)                      |                             |                                |                                 |                            |                                    |
| IVIG/MP                                     | Y (7-11)                    | Y (3-6, 15)                    | Y (3-8)                         | Y (3-8)                    | Y (3-8)                            |
| PLEX                                        | N                           | Y (47-52)                      | Y (11,12,14, 17,19,21)          | Y (9,10,12, 14,16)         | Y (9-13)                           |
| RTX                                         | N                           | Y (58, 72)                     | Y (52, 66)                      | Y (16, 33)                 | Y (17,25,45)                       |
| CTX                                         | N                           | N                              | Y (56,97,126,158)               | N                          | N                                  |
| KD                                          | Y (21-33)                   | Y (12-23 & 39-73)              | Y (17-24)                       | Y (20-56)                  | Y <sup>d</sup> (30-65) IV (80-105) |
| Anakinra                                    | N                           | Y (34-39)                      | Y (26-53)                       | Y (33-63)                  | Y (24-38, 44-126)                  |
| HLH 2004 protocol                           | Y <sup>b</sup> (33-146)     | N                              | N                               | N                          | Y <sup>c</sup> (93-100)            |

(Continues)

**TABLE 1** (Continued)

| Patient                                    | #1                                                     | #2                                                             | #3                                                                                              | #4                                                      | #5                                                                                                    |
|--------------------------------------------|--------------------------------------------------------|----------------------------------------------------------------|-------------------------------------------------------------------------------------------------|---------------------------------------------------------|-------------------------------------------------------------------------------------------------------|
| Outcome<br>(6 months post<br>presentation) | Mild cognitive<br>impairment,<br>independent<br>w/ADLs | Moderate<br>cognitive<br>impairment,<br>participates<br>w/ADLs | Severe cognitive<br>impairment,<br>dependent<br>for ADLs, GT,<br>and tracheostomy-<br>dependent | Mild cognitive<br>impairment,<br>independent<br>w/ADLs. | Severe<br>encephalopathy,<br>dependent for<br>ADLs<br>tracheostomy/<br>ventilator and<br>GT-dependent |

Bold type indicates abnormal. ADLs, activities of daily living; AKI, acute kidney injury; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Bili, bilirubin; BM, bone marrow; CRRT, continuous renal replacement therapy; CTX, cyclophosphamide; DIC, disseminated intravascular coagulation; GGT, gamma-glutamyl transferase; Glc, glucose; GT, gastrostomy tube; Hb, hemoglobin; HD, hospital day; IV, intravenous ketogenic diet; IVIG, intravenous immunoglobulin; KD, ketogenic diet; MP, methylprednisolone; NK, natural killer; PLEX, plasma exchange; Plt, platelets; Prt, protein; RTX, rituximab; WBC, white blood cells.

<sup>a</sup>Incomplete screening.

<sup>b</sup>HLH 2004 chemotherapy protocol: dexamethasone, etoposide + intrathecal methotrexate, and hydrocortisone.

<sup>c</sup>Partial HLH 2004 chemotherapy protocol: dexamethasone 10 mg/m<sup>2</sup>/day.

<sup>d</sup>Variable ketone levels.

ileus in 3 patients, and disseminated intravascular coagulation in 2 patients.

CSF neopterin was sent in all patients as part of the diagnostic work-up for CNS inflammation (reference range 7-40 nmol/L). In our patients with FIRES, the levels ranged from 122 to 300 nmol/L (median 260, IQR 188-297). Median CSF neopterin was higher in comparison with previously published neopterin levels in acute disorders ( $P = 0.0187$ ), chronic progressive disorders ( $P = 0.0036$ ), and static disorders ( $P = 0.0008$ ). There was no significant difference between the median CSF neopterin value of FIRES patients and reported median value for “febrile exacerbation of chronic illnesses.”<sup>14</sup>

Individual cytokine and chemokine measurements are described in detail in Table 2. Figure 2 demonstrates the temporal characteristics of the disease course and treatments employed in each of the 5 patients in this study. Cytokine and chemokine data during the HLH screening phase are demonstrated for 4 patients in Figure 3 (Patient 4 lacked analysis during this phase). Notably, the levels of IL-1 $\beta$  and IL-18 were not elevated in any of the subjects during any of the CSF sampling times and indeed failed to reach detection threshold in many of the patients (Figure 2; Table 2). However, HMGB1 and CXCL8 were substantially elevated in CSF in all patients during the HLH screening phase (Figure 3; Table 2). IL-6 was markedly increased in CSF in 2 patients; CXCL9 was elevated in one subject; CXCL10 was strongly elevated in one patient and more weakly elevated in another 3; and CCL5 was detectably increased in 2 patients during the HLH screening phase (Figure 3; Table 2). Table 2 also indicates that many of these factors were elevated outside of the HLH screening phase. For example, Patient 4, who did not have a sample during the HLH screening phase, exhibited elevated CSF levels of HMGB1, CXCL8, CXCL9, and

CXCL10, as well as exceptionally high levels of CCL5, at a point greater than 90 days from presentation. For the subjects with matched serum and CSF samples, we detected high levels of IL-1RA at several sampling points, consistent with anakinra treatment (Figure 2; Table 2). These patients also exhibited high levels of CRP and IL-6 in serum at several time points (Table 2).

Table 1 summarizes the time course of treatments administered, excluding antiseizure drugs and anesthetics. All patients received immunoglobulin and methylprednisolone early in their disease course for presumed immune-mediated encephalitis. Plasmapheresis was administered in 4. Ketogenic diet (KD) was initiated in all patients at different times during the course of disease and maintained for a variable amount of time. Four patients received rituximab and one received cyclophosphamide. Four patients received anakinra. Outcomes 6 months after FIRES diagnosis were variable. All patients had medically refractory epilepsy. Of the 3 patients diagnosed with HLH, only one patient (Patient 1) was treated with chemotherapy for secondary HLH with central nervous system (CNS) involvement per the 2004 Histiocyte Society recommendations.<sup>9</sup> Within days of treatment initiation, the seizures subsided and encephalopathy markedly improved. At 6-month follow-up, she had mild cognitive impairment but was independent for all activities of daily living (ADLs). One patient (Patient 5) was partially treated for HLH with high-dose dexamethasone. At 6 months, she had severe cognitive impairment necessitating a gastrostomy and tracheostomy with ventilator support and was dependent for ADLs. Patient 2 was not treated for HLH. At 6 months, he was moderately cognitively impaired and participated with ADLs. The 2 patients who did not meet criteria for HLH had different outcomes. Patient 3 was severely cognitively impaired depending on a tracheostomy and gastrostomy as

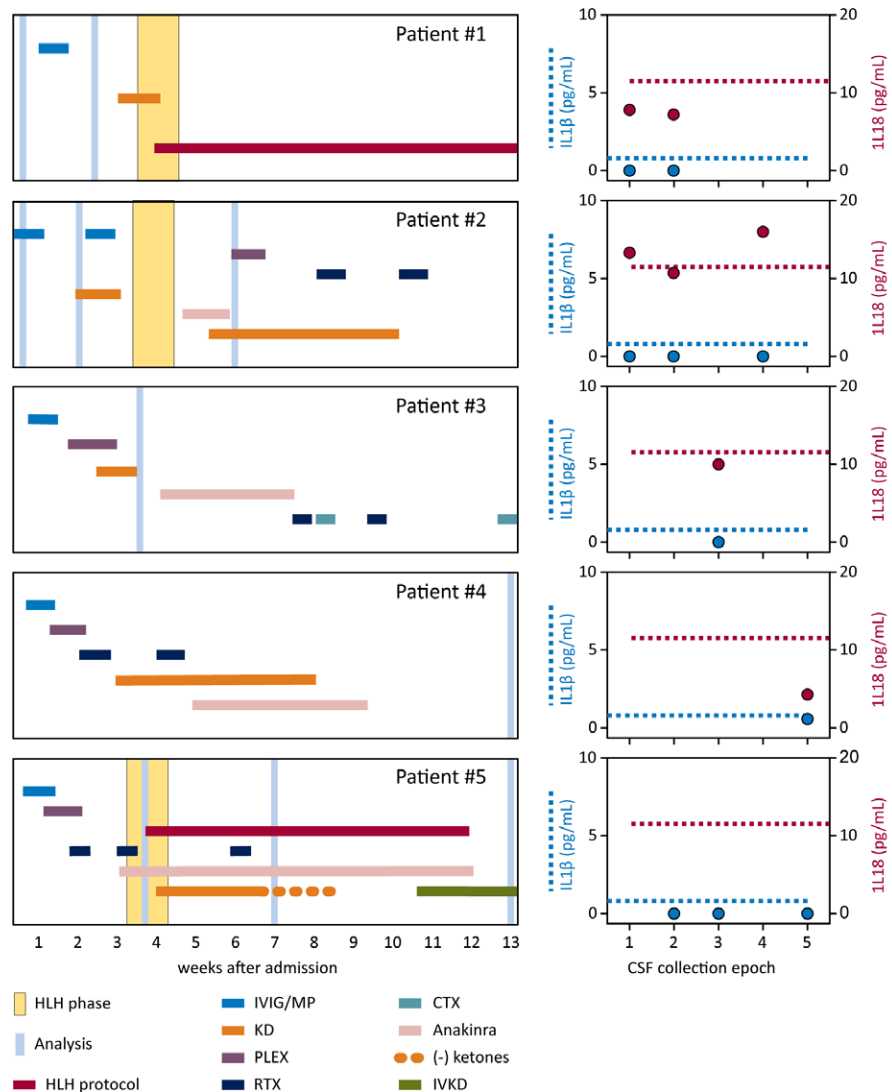
TABLE 2 Serum and spinal fluid cytokine/chemokine analysis

| Analyte                | IL-1RA   | CRP                    | IL-18      | HMGB1     | IL-6    | IL-1β   | TNF     | IL-12 | IL-10   | CXCL8    | CXCL10     | CCL2     | CXCL9     | CCL5        |
|------------------------|----------|------------------------|------------|-----------|---------|---------|---------|-------|---------|----------|------------|----------|-----------|-------------|
| C CTL <sub>μ</sub> /SD | 102.5/36 | NA                     | 11.5/N=1   | 119/130   | 1.7/0.6 | 0.8/0.4 | 0.1/0.2 | 0/0   | 0.8/0.5 | 20.4/4.8 | 144/80.7   | 809/737  | 16.5/14.3 | 0/0         |
| S CTL <sub>μ</sub> /SD | 366/115  | 2 051 300 <sup>a</sup> | 229.8/45.4 | 4900/2900 | 3.6/1.6 | 1.7/0.8 | 2.2/1.7 | 1/1.6 | 1.6/0.9 | 4.8/1.1  | 158.1/35.9 | 102.1/38 | 74.4/5.2  | 20 080/1277 |
| Patient 1              |          |                        |            |           |         |         |         |       |         |          |            |          |           |             |
| CSF/HD                 |          |                        |            |           |         |         |         |       |         |          |            |          |           |             |
| Sample 1/1             | N/A      | N/A                    | 7.81       | 974       | 9.82    | 0       | 0       | 0     | 0.55    | 115.49   | 1563.5     | 659.3    | 41.6      | 15.6        |
| Sample 2/10            | N/A      | N/A                    | 7.15       | 310       | 5.65    | 0.99    | 0       | 0     | 2.08    | 54.22    | 765.77     | 377.68   | 31.07     | 1.4         |
| Patient 2              |          |                        |            |           |         |         |         |       |         |          |            |          |           |             |
| CSF/HD                 |          |                        |            |           |         |         |         |       |         |          |            |          |           |             |
| Sample 1/1             | N/A      | N/A                    | 13.26      | 340       | 48      | 0       | 0       | 2.97  | 13.1    | 280.2    | 4729.8     | 822.8    | 139.76    | 1.3         |
| Sample 2/7             | N/A      | N/A                    | 10.68      | 1223      | 40.7    | 0       | 0       | 2.45  | 0.03    | 131.98   | 2689.9     | 248.18   | 26.9      | 2.7         |
| Sample 3/42            | N/A      | N/A                    | 15.99      | 817       | 8.56    | 0.55    | 0       | 0     | 1.09    | 73.84    | 724.10     | 286.59   | 22.66     | 5.2         |
| Patient 3              |          |                        |            |           |         |         |         |       |         |          |            |          |           |             |
| CSF/HD                 |          |                        |            |           |         |         |         |       |         |          |            |          |           |             |
| Sample 1/17            | N/A      | N/A                    | 10         | 512       | 7.48    | 0       | 0       | 1.45  | 0       | 47       | 472.28     | 255.78   | 20.44     | 0           |
| Patient 4              |          |                        |            |           |         |         |         |       |         |          |            |          |           |             |
| CSF/HD                 |          |                        |            |           |         |         |         |       |         |          |            |          |           |             |
| Sample 1/92            | 155.5    | N/A                    | 4.5        | 2800      | 1.8     | 0.7     | 0       | 0     | 0.7     | 158.7    | 2237       | 1059.4   | 63.1      | 126.7       |
| Serum                  |          |                        |            |           |         |         |         |       |         |          |            |          |           |             |
| Sample 1/92            | 1396.7   | 26 × 10 <sup>6</sup>   | 387.53     | 6350      | 22.2    | 0       | 0.8     | 0     | 1       | 17.1     | 831.5      | 173.1    | 1003.7    | 25 355.4    |
| Patient 5              |          |                        |            |           |         |         |         |       |         |          |            |          |           |             |
| CSF                    |          |                        |            |           |         |         |         |       |         |          |            |          |           |             |
| Sample 1/22            | 85       | N/A                    | N/A        | 961       | 52.6    | 0       | 0       | 1.1   | 0       | 60.8     | 407        | 757.1    | 46.5      | 0           |
| Sample 2/49            | 12 546   | N/A                    | N/A        | 625       | 189.3   | 0       | 0       | 0     | 0       | 575.1    | 1143.9     | 2992.3   | 63.4      | 6.6         |
| Sample 3/92            | 12 540   | N/A                    | N/A        | 646       | 1.7     | 0.1     | 0.2     | 0     | 0.6     | 119.5    | 662.8      | 1961.7   | 98.2      | 3.4         |
| Serum                  |          |                        |            |           |         |         |         |       |         |          |            |          |           |             |
| Sample 1/22            | 230      | 28 × 10 <sup>6</sup>   | 1350       | 4290      | 1228    | 0       | 0       | 3     | 6.8     | 307.9    | 4315       | 1420.9   | 1536.2    | 615.5       |
| Sample 2/49            | 14 269   | 16 × 10 <sup>6</sup>   | 1489       | 7820      | 17.9    | 0       | 1.2     | 0     | 1.8     | 48       | 657.1      | 670.6    | 710.6     | 25 434      |
| Sample 3/92            | 14 149   | N/A                    | 702.76     | 570       | 3.7     | 0.6     | 0.2     | 0     | 2.0     | 16.3     | 329        | 333.5    | 215.5     | 9867.6      |

CCCTL<sub>μ</sub>/SD, CSF control mean/standard deviation; CCL2, chemokine ligand 2; CCL5, chemokine ligand 5; CRP, C-reactive protein; CXCL10, C-X-C motif chemokine 10; CXCL9, C-X-C motif chemokine 9; HD, hospital day; HMGB1, high mobility group box 1; IL, interleukin; IL-1RA, interleukin 1 receptor activity; SCTL<sub>μ</sub>/SD, serum control mean/standard deviation; TNF, tumor necrosis factor.

Bolded: elevated, N/A: not available. Cytokine/chemokine values in pg/mL.

<sup>a</sup>Control not available.



**FIGURE 2** Cytokine/chemokine analysis respective to disease course and therapies received. IL-1 $\beta$  and IL-18 are depicted simultaneously. Specific cytokine/chemokine values can be found in Table 2. CTX, cyclophosphamide; IVIG/MP, intravenous immunoglobulin/methylprednisolone; IV ketogenic diet; IVKD, HLH chemotherapy protocol<sup>9</sup>; KD, ketogenic diet; PLEX, plasma exchange; RTX, rituximab

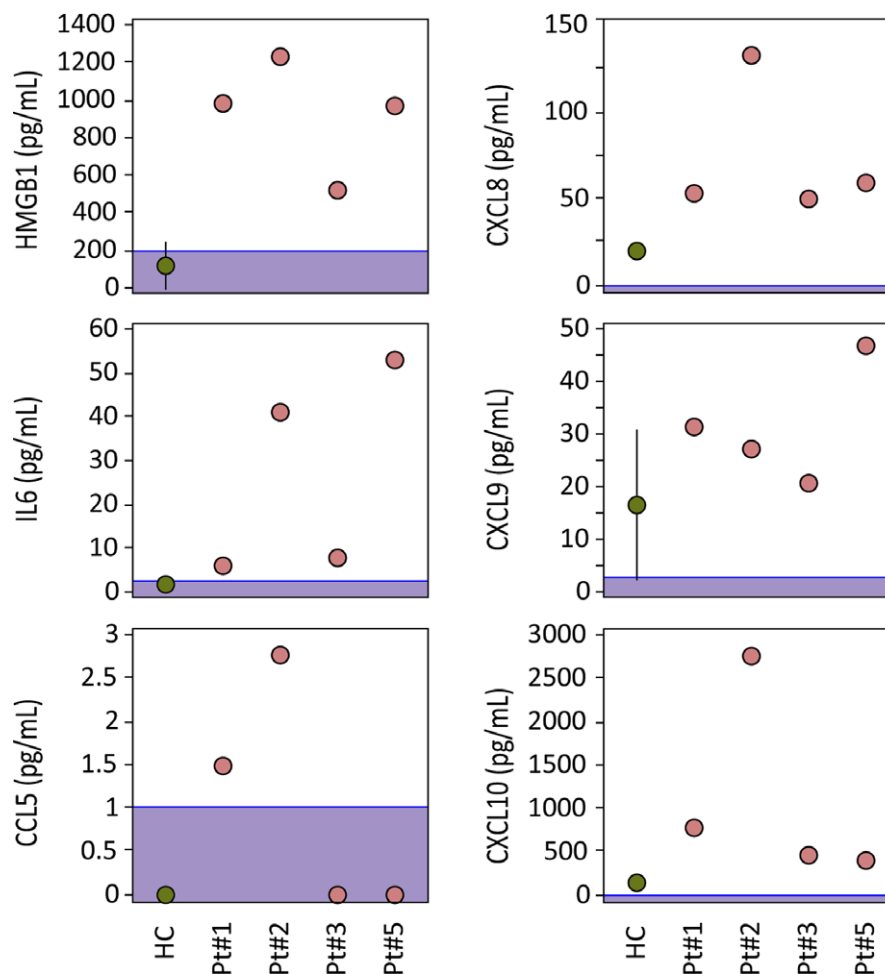
well as dependent for all ADLs. Patient 4 had mild cognitive impairment but was independent for all ADLs.

## 4 | DISCUSSION

FIRES is a catastrophic epileptic encephalopathy of uncertain etiology. The seizures are usually not responsive to antiepileptic medications, anesthetics, and/or typical immune modulating agents. Although innate immune activation is the hypothesized mechanism driving epileptogenesis in the acute period,<sup>2,3,16</sup> a specific etiology for this condition is still unknown. In our center, we observed a pattern of clinical deterioration in FIRES patients 3–4 weeks after presentation. Unexplained fever and multiorgan dysfunction prompted us to evaluate several patients for secondary HLH of which 3 met full criteria. The

concurrence of these 2 rare disorders in these patients brought attention to several associations between the FIRES and HLH. In a comprehensive FIRES literature review, we found several patients with similar clinical deterioration and rash about 4 weeks after illness onset,<sup>1,11,17,18</sup> but none mentioned HLH screening. Although the co-occurrence of systemic inflammatory response syndrome has been reported after status epilepticus,<sup>19</sup> to our knowledge, the coexistence of FIRES and HLH has not been described previously.

We summarize our hypothesized mechanisms for the association between FIRES and secondary HLH observed in our cohort (Figure 4) initiating with a nonspecific febrile illness triggering release of inflammatory molecules. In a genetically predisposed individual, anti-inflammatory mediators are unable to resolve inflammatory responses.<sup>2,20</sup> Activated immune pathways in glial cells, neurons,



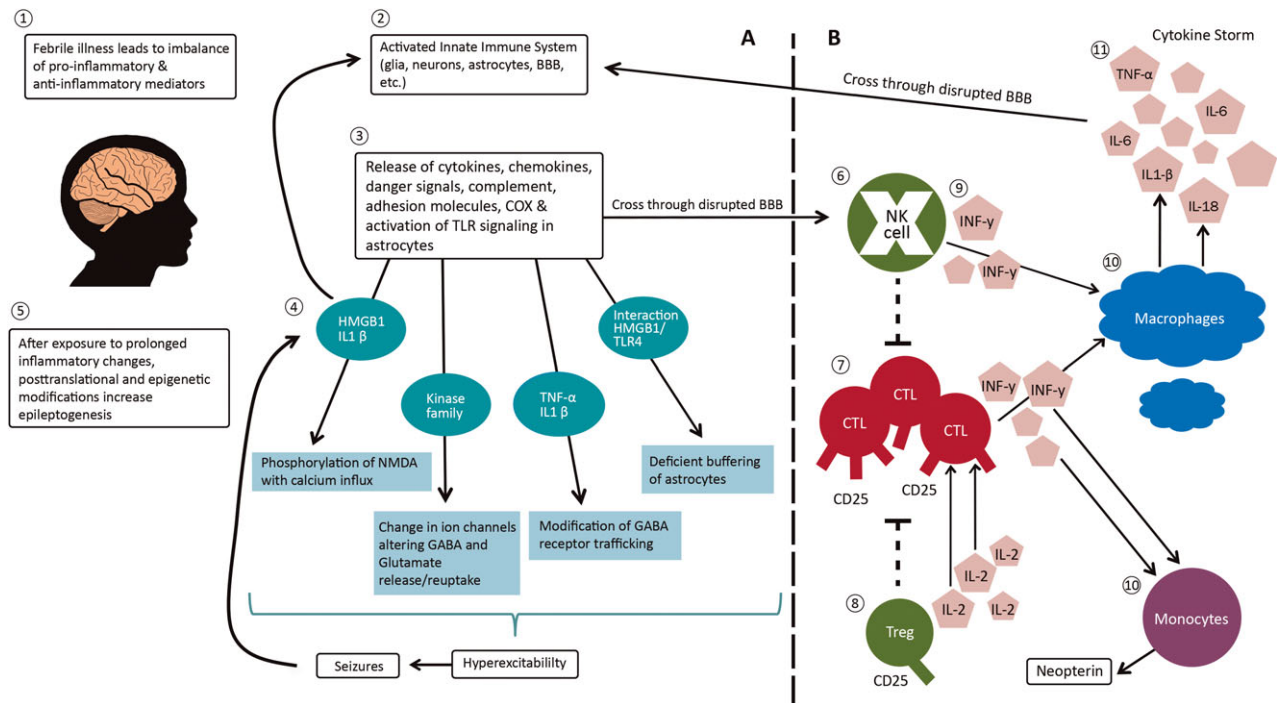
**FIGURE 3** Cytokine/chemokine analysis peri-HLH screening phase. Pt, patient; HC/green dot, healthy control subjects; Pt/Red dots, patient values; Patient 4 lacks cytokine/chemokine analysis during this phase

astrocytes, and cellular components of the blood-brain barrier give rise to an uncontrolled neuroinflammatory cascade marked by production of inflammatory cytokines/chemokines and adhesion molecules that promote infiltration of peripheral immune effectors.<sup>2</sup> A hyperexcitable state develops via (a) *N*-methyl-D-aspartate (NMDA) receptor phosphorylation with neuronal calcium influx<sup>21,22</sup>; (b) ion channel changes leading to alteration of glutamate and  $\gamma$ -aminobutyric acid (GABA) release/reuptake; (c) reduced astrocytic buffering capacity; and (d) glutamate/GABA receptor trafficking modifications.<sup>2,21–23</sup> In parallel, there is activation of gene transcription programs leading to neurogenesis, sprouting, angiogenesis, and chronically dysfunctional astrocytes.<sup>21</sup> Models of SE show that recurrent seizure activity triggers neuroinflammation, thereby perpetuating a cycle of innate immune activation.<sup>24</sup> Put differently, sustained seizures, as those that occur in FIRES, lead to neuroinflammation, higher seizure burden, and further inflammation.

Figure 4B demonstrates secondary HLH pathophysiology, which occurs when natural killer (NK) cells and

T regulatory lymphocytes (Treg) are unable to regulate cytotoxic T lymphocyte (CTL) populations through a variety of genetic and acquired mechanisms. Hyperactivated CTLs secrete large amounts of interferon- $\gamma$  (IFN- $\gamma$ ), thereby stimulating macrophages and inducing hemophagocytosis, culminating in an overwhelming cytokine storm.<sup>8</sup> Secondary HLH can occur via several mechanisms.<sup>7</sup> Through acquired defects in cytotoxicity, there is reduced NK cell function with decreased perforin expression, rendering these cells unable to regulate CTL. This phenomenon can be secondary to preexisting persistently elevated levels of cytokines, including IL-12, IL-18, and IL-6. However, in secondary HLH, there are alternative causes for hyperinflammation and abnormal cytotoxicity is rarely the disease driver.<sup>8</sup>

Animal models have highlighted the role of innate immunity activation via excessive stimulation of Toll-like receptors,<sup>7,25</sup> and HMGB1 has been implicated specifically in the development of macrophage-activating syndrome (MAS), which is classified among the secondary forms of HLH.<sup>26</sup> In our cohort, HMGB1 was elevated in every patient in CSF, but not in serum. IFN- $\gamma$ , a central cytokine in HLH,<sup>7</sup> is also



**FIGURE 4** Proposed mechanism of FIRES and HLH inflammatory interplay. (1) Febrile illness in a genetically predisposed host leads to unbalance of pro- and anti-inflammatory mediators. Inflammatory response is unable to be controlled. (2) The innate immune system is activated. (3) There is release of cytokines (IL-1B, IL-6, TNF- $\alpha$ ), chemokines, danger signals (HMGB1), complement, adhesion molecules, and cyclooxygenase (COX) as well as activation of Toll-like receptor (TLR) signaling in astrocytes.<sup>22</sup> (4) Hyperexcitability occurs through phosphorylation of NMDA with calcium influx, changes in ion channels altering GABA and glutamate release/reuptake, modifications of GABA receptor trafficking, and deficient buffering of astrocytes with inability to reuptake potassium or glutamate.<sup>21,22,24</sup> (5) Recurrent seizures further activate this cycle. With prolonged exposure to neuroinflammation, long-term transcriptional changes occur causing changes in neurogenesis, sprouting, angiogenesis, and chronic astrocyte dysfunction<sup>21,22</sup> contributing to increased epileptogenesis and chronic epilepsy. (6) Meanwhile, an exaggerated TLR receptor activity and marked cytokine crossover occurs via disrupted BBB<sup>20,24</sup> causing NK cell dysfunction. (7) NK cells no longer are able to control the activated CTL pool and become hyperactivated.<sup>8</sup> (8) Treg cells are unable to compete with hyperactivated CTL for the available IL-2 because of upregulation in CD25 in CTL, so Treg numbers decrease.<sup>8</sup> (9) Activated CTLs and NK cells produce massive amounts of IFN- $\gamma$ .<sup>8</sup> (10) IFN- $\gamma$  activates macrophages to hemophagocytose and monocytes to produce neopterin.<sup>29</sup> (11) Macrophages produce proinflammatory cytokine storm<sup>8</sup> which are then able to cross the disrupted BBB to further activate the innate immune system and perpetuate this cycle<sup>20</sup>

associated with the stimulation of monocytes to produce neopterin. Neopterin is a sensitive marker of inflammation<sup>14</sup> that has biochemical and physiologic functions in host defense, but also promotes oxidative stress, which may amplify cytotoxic effects.<sup>27</sup> Neopterin, which is a sensitive and specific marker for HLH<sup>28</sup> correlating with serum ferritin,<sup>29</sup> was markedly elevated in our FIRES cohort, consisting of the finding of increased IFN- $\gamma$  production in these patients and similar to what is observed in HLH.

The mechanisms by which a systemic inflammatory response activates neuroinflammation are multifactorial, but disruption in the blood-brain barrier (BBB) that promotes “leakage” of peripheral cytokines and danger signals into the CNS may be of paramount importance.<sup>20,23,30</sup> Furthermore, altered BBB permeability may facilitate trafficking of innate and adaptive immune cells into the CNS,<sup>20</sup> triggering further activation of the brain-resident innate immune system, resulting in epileptogenesis and brain injury. A better understanding of systemic and intrathecal

inflammatory changes in patients with FIRES will potentially allow us to identify the factors that may contribute to this self-perpetuating mechanism.

To better understand the processes that may underlie inflammatory epileptogenesis, we measured cytokine and chemokine levels in our patients with FIRES who were screened for HLH. Elevation of specific cytokines and chemokines in our cohort was highly variable, and there were discrepancies between levels measured in serum versus CSF. HMGB1, IL-6, CXCL8, CXCL9, and CXCL10 were found to be elevated in most patients, but the pattern of changes in these factors relative to the development of HLH were not consistent across subjects. IL-1 receptor type 1 (and IL-18) is the current focus of anakinra therapy and in models of FIRES.<sup>18</sup> Neither IL-1 $\beta$  nor IL-18 was elevated in CSF in our patients (Figure 3), which could explain the lack of response to anakinra. The overexpression of other chemokines and cytokines as well as HMGB1 may be relevant to inflammatory changes in this patient

cohort. Alternatively anakinra was started too late in the disease process and thus unable to mitigate an already evolving inflammatory response. Not surprisingly, elevated levels of cytokines and chemokines largely declined over time, presumably due to improvement in seizure control, fever reduction, therapeutic interventions, and subsiding critical illness, among other factors. Similar to our cohort, an analysis of cytokine and chemokine expression in 14 patients with FIRES by Sakuma et al<sup>31</sup> demonstrated upregulation of several factors, including IL-6, CXCL8, and CXCL10 in CSF. HMGB1 was not tested in that study, and the timing of sample collection after presentation varied greatly.

Treatment for secondary HLH is more standardized than treatment for FIRES, but there exists controversy regarding the administration of chemotherapy to all patients who meet criteria.<sup>12</sup> In our cohort, only Patient 1 received chemotherapy treatment for secondary HLH. The decision behind administering such an aggressive treatment course was related to her markedly elevated ferritin. Ferritin levels higher than 10 000 ng/mL are 90% sensitive and 96% specific for HLH.<sup>32</sup> In this case, HLH appeared to develop as a complication of FIRES, as her ferritin level was only 354 ng/mL early in her course (Day 17), subsequently increasing to 17 188 ng/mL (Day 29). After receiving systemic and intrathecal chemotherapy, this patient exhibited rapid resolution of seizures with marked improvement in encephalopathy. We hypothesize that chemotherapy received for HLH halted an intrathecal inflammatory response that may have been driving the FIRES-related epileptogenesis. This regimen is not without side effects,<sup>33</sup> and the decision to administer chemotherapy needs to be carefully weighed in critically ill patients with FIRES who meet criteria for HLH.

We acknowledge several limitations of this small study. Not all patients were comprehensively screened. While one patient (Patient 4) did not have HLH, Patient 3 met 4/5 criteria necessary for diagnosis. It is possible that if comprehensive screening had been performed, he would have fully met criteria. Furthermore, the five children with FIRES (cared for previous to Patient 1) who were not screened for HLH had associated supporting clinical features outside HLH criteria such as multiorgan dysfunction (4/5), rash (3/5), ileus (4/5), and hemodynamic instability (4/5). It is possible that HLH incidence in our cohort is underestimated because patients were not screened. An additional limiting factor is the lack of specificity of secondary HLH criteria given that many of these features are frequently seen in critically ill patients with sepsis or systemic inflammatory response syndrome.<sup>6,11</sup> Another limitation in the analysis of cytokine and chemokine levels in this study is the heterogeneity of samples relative to disease stage and the lack of matched serum samples for comparison in 3 patients. While every effort was made to process the samples quickly and under identical conditions, there are doubtless variations in sample stability that may

impact detection of extremely sensitive factors such as IL-1 $\beta$ .<sup>18</sup> Likewise, the determination of control ranges for all of the factors is challenged by the unavailability of CSF from healthy control pediatric donors. From a mechanistic standpoint, it is currently unknown whether the changes we observed are a cause or an effect of the underlying seizures. How the levels of these factors relate to the multiple treatments administered and to ongoing seizure burden in each individual patient at the time of collection is also unclear.

## 5 | CONCLUSION

Febrile infection-related epilepsy syndrome is a rare disorder of unknown etiology and without clear diagnostic criteria. The co-occurrence of hemophagocytic lymphohistiocytosis in three patients with FIRES patients suggests the possibility of a common immune dysregulation phenotype, with inflammatory mediator cross talk between the CNS and the periphery serving as one mechanism underlying acute epileptogenesis in these patients. We note CSF neopterin to be markedly elevated in comparison with other CNS inflammatory conditions, and further studies are required to evaluate whether or not it can be used as an early biomarker. We recommend maintaining a high index of suspicion for secondary HLH in patients with FIRES, especially in the presence of other features associated with HLH, such as cholestasis, elevated transaminases and lactate dehydrogenase, rash, lymphadenopathy, and coagulopathy.<sup>11</sup> The decision to administer chemotherapy should always be made with detailed scrutiny of clinical and laboratory data, disease severity stratification, and close collaboration with expert consultants. We support collaborations via national and international databases to collectively gain more experience in understanding and managing these challenging patients.

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## DISCLOSURES

None of the authors has any potential conflict of interests to disclose. The authors confirm that we have read the journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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