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# Bridging the Gap: Tailoring an Approach to Treatment in Febrile Infection-Related Epilepsy Syndrome

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## Abstract

Cytokine profiling prior to immunotherapy is increasingly prevalent in Febrile infection-related epilepsy syndrome (FIRES). In this case, an 18-year-old boy presented with first-onset seizure after a nonspecific febrile illness. He developed super refractory status epilepticus requiring multiple anti-seizure medications and general anesthetic infusions. He was treated with pulsed methylprednisolone, plasma exchange and ketogenic diet. Contrast-enhanced MRI brain

revealed post-ictal changes. EEG showed multifocal ictal runs and generalized periodic epileptiform discharges. Cerebrospinal fluid analysis, autoantibody testing and malignancy screen were unremarkable. Genetic testing revealed variants of uncertain significance (VUS) in the CNKSR2 and OPN1LW genes.

Initial serum and CSF cytokine analyses performed on days 6 and 21 revealed that IL-6, IL-1RA, MCP1, MIP1β and IFNγ were elevated predominantly in the CNS, a profile consistent with cytokine release syndrome. Tofacitinib was initially trialed on day 30 of admission. There was no clinical improvement and IL-6 continued to rise. Tocilizumab was given on day 51 with significant clinical and electrographic response. Anakinra was subsequently trialed from days 99 to 103, as clinical ictal activity re-emerged on weaning anesthetics, but stopped due to poor response.

Serial cytokine profiles showed improvement after 7 doses of tocilizumab. There was corresponding improved seizure control.

This case illustrates how personalized immunomonitoring may be helpful in cases of FIRES, where proinflammatory cytokines are postulated to act in epileptogenesis. There is an emerging role for cytokine profiling and close collaboration with immunologists for the treatment of FIRES. The use of tocilizumab may be considered in FIRES patients with upregulated IL-6.

Febrile infection-related epilepsy syndrome (FIRES) is a rare catastrophic epileptic encephalopathy associated with significant mortality and morbidity.[1] The pathogenesis of FIRES remains unclear and has been postulated to involve a fulminant postinfectious inflammatory process. Previous studies have shown that certain cytokines appear to be upregulated in FIRES, and there may be an emerging role for immunotherapy in treatment of this disease. [2]

Cytokine profiling prior to immunotherapy is increasingly prevalent. However, tailoring multiple immunotherapeutic agents to the evolving immunologic profile is not commonly performed in FIRES. We describe our experience with immunologic profile directed immunotherapy in an 18-year-old boy with FIRES. Through this case, we discuss the role of cytokines in neurological disorders, techniques used in cytokine profiling, and how this may translate to clinical practice with directed immunotherapy.

**Case report**

Initial Presentation

An 18-year-old boy presented with first-onset seizure a week after a nonspecific febrile illness. He subsequently developed super refractory status epilepticus. He received multiple lines of anti-seizure medications, such as levetiracetam, valproic acid, topiramate, perampanel, phenobarbital, gabapentin, clobazam, vigabatrin and rufinamide and several general anesthetic infusions, including midazolam, propofol, thiopentone and ketamine. Despite this, his EEG continued to show generalized periodic epileptiform discharges and multifocal electrographical ictal runs with associated motor manifestations of rhythmic head, eye, shoulder and jaw

movements. He was given a course of pulsed methylprednisolone, plasma exchange and started on ketogenic diet. Contrast-enhanced MRI brain showed diffuse brain swelling and increased signal of the supra- and infra-tentorial gyri, consistent with post-ictal changes. CSF analysis did not reveal any infective etiology. Autoantibody testing and malignancy screening, which included whole body CT and ultrasound of the testes, were unremarkable.

Genetic testing revealed variants of uncertain significance (VUS) in the CNKSR2 and OPN1LW genes. CNKSR2 deletion or mutation has been implicated in epilepsy-aphasia spectrum disorders. However, the mutation found in our patient has not been described to cause status epilepticus.[3]

Cytokine Analysis

Initial serum and CSF cytokine analyses done on days 6 and 21 (referencing the first day of admission as day 1) revealed that IL-6, IL-1RA, MCP1, MIP1β and IFNγ were highly elevated predominantly in the CNS. This was consistent with cytokine release syndrome, whereby activated T cells or NK cells induce activation of macrophages. There was worsening CSF cytokine derangement on day 21 compared to day 6 of admission. This correlated with CRP, ferritin, lactate dehydrogenase (LDH), ALT and AST levels which peaked around that period.

Tofacitinib was commenced at day 30 in view of the elevated IFNγ suggesting increased T and NK cell activity, as well as its superior CNS penetration compared to monoclonal antibodies.. After 3 weeks of therapy, electrographic improvement was inconsistent. IL-6 levels continued

to rise (eTable 1), hence tocilizumab 8mg/kg was added on day 51. Improvement in CRP was observed after day 65 and significant electrographic improvement was noted at day 67 (Figure). The previous bihemispheric independent epileptiform abnormalities which frequently evolved into electrographical ictal runs had become less frequent periodic discharges, with greater lengths of suppression seen. This facilitated the reduction of midazolam and ketamine infusions. Tocilizumab was dosed at 2-weekly intervals for the first 4 doses. Daily subcutaneous anakinra was also given after 4 doses of tocilizumab (from day 99 to 103), because of the re-emergence of organized periodic discharges with concomitant motor manifestations on weaning anesthetic infusions. This was stopped after 5 days due to lack of clinical and electrographic response. Monthly tocilizumab was instituted as maintenance therapy and tofacitinib was stopped on day 103. Plasma IL-6 levels had decreased markedly when checked on day 163.

Serial Cytokine Analysis

Serial cytokine profiling showed improvement and subsequent near-normalization of cytokine levels after 7 doses of tocilizumab were given over 5 months (eTable 1). CRP (peak 267mg/L, normal range 0-10mg/L) and ferritin levels (peak 1322ug/L, normal range: 20-300ug/L) had also normalized with treatment. This was commensurate with improved seizure control. He was weaned off anesthetic infusions completely at day 125 of admission. Currently, at day 249, the patient is in a minimally conscious state and undergoing rehabilitation. His current anti-seizure medication regime, consisting of levetiracetam, phenobarbital, perampanel, rufinamide,

vigabatrin and clobazam, is being gradually down-titrated. The interval of tocilizumab dosing has also been lengthened given the sustained improvement in his cytokine profile.

## Discussion

There is increasing recognition of the role cytokines play in various disease processes.

### What are cytokines?

Cytokines are small molecules involved in intercellular communication and are classified in categories such as interleukins, interferons and tumor necrosis factors. A complex interplay between cytokines mediates the immune response. Cytokine release syndrome (CRS), or "cytokine storm", occurs where a dysregulated immune response is triggered inappropriately. In CRS, pro-inflammatory cytokines and excessive immune cell hyperactivation causes severe systemic inflammatory syndrome and multiorgan dysfunction.[4] C-reactive protein and ferritin are usually elevated, and patients may have cytopenias. IFNγ, IL-1, IL-6, TNF, and IL-18 are often elevated in CRS and thought to have central immunopathologic roles.[4] More recently, CRS was recognized to play a role in severe COVID-19 infection and immune-related adverse events (irAEs) from immune-checkpoint inhibitors. For example, elevated serum IL-6 levels have been found in both patients with severe COVID-19 infection and irAEs, for which tocilizumab, an anti-IL-6 receptor monoclobal antibody, was demonstrated to be efficacious.[5,6]

What is cytokine profiling?

Cytokine profiling can be done with various technologies, including Enzyme-linked immunosorbent assay (ELISA), cytometric bead array (CBA), Luminex and Meso Scale Discovery (MSD). Caution ought to be exercised in the comparisons of results across different methods of profiling, as large inter-laboratory variations exist. Furthermore, each technology has differing test sensitivities and accurate detection ranges.[7]

Cytokine profiling in various disease states can provide valuable insights into disease pathogenesis and potential therapeutic targets. In patients with neuromyelitis optica spectrum disorder (NMOSD), IL-6 was found to be significantly elevated in both serum and CSF, and correlated with disability.[8] It is postulated that IL-6 plays an important role in NMOSD pathogenesis by promoting plasmablast survival, disrupting the blood-brain barrier and stimulating antibody production against aquaporin-4.[8] This provides the basis for use of novel therapies in NMOSD, such as the anti-IL-6 receptor monoclonal antibody satralizumab.

Beyond classically neuro-inflammatory diseases, there is increasing evidence for the role of cytokines in the pathogenesis of epilepsy. It has been shown that seizures can lead to cytokine production. This promotes further inflammatory changes such as the potentiation of free radical species, alterations in glutamatergic neurotransmission and disruption of the blood-brain barrier, which results in the development and progression of epilepsy.[9] Experimental animal models have shown post-ictal IL-1β expression in microglia and astrocytes, and demonstrated that IL-1β enhances neuronal excitability.[10] A meta-analysis of epilepsy patients

has also demonstrated elevated levels of serum IL-6, IL-17 and CSF IL-1β, IL-10 in these patients.[11]

Clinical use of cytokine profiling in FIRES

In FIRES, proinflammatory cytokines such as IL-6, TNF-α, and IL-1β, are postulated to act in neuroexcitability and epileptogenesis.[3] In a comparison of CSF cytokine profiles in pediatric epilepsy patients, FIRES patients were found to have a higher elevation of cytokines compared to patients with febrile status epilepticus and chronic epilepsy, particularly in Th-1 associated cytokines such as IL-6 and TNF-α.[2] When cytokine profiles were compared between paediatric patients with FIRES and those with other inflammatory neurological diseases, FIRES patients were noted to have significantly increased IL-6, IL-8 and CXCL10 levels. These changes were more marked in the CSF than in serum.[10] Jun et al also described a group of adult New Onset Refractory Status Epilepticus (NORSE) patients in whom cytokine analysis was performed, showing highly upregulated CSF IL-6. The majority of this group responded to tocilizumab treatment.[12] Studies in animal models and small numbers of patients indicate that IL-1R signalling is also implicated in initiating the neuroinflammatory cascade.[13] In Clarkson et al's study on FIRES patients, levels of CSF IL-1RA were found to be elevated (similar to our patient). However, these patients demonstrated attenuated inhibition of IL-1R signalling, which suggests a functional deficit in IL-1RA.[14] This provides the pathophysiological basis for treatment of FIRES with targeted immunotherapy.

In our patient, the immunological profile was noted to be markedly variable across different time points and the therapeutic regime was modified accordingly. Unfortunately, given the overlap of treatment periods with tofacitinib and tocilizumab in this case, it is unclear whether the changes in cytokine levels are attributable to one drug or both in tandem. To our knowledge, tailoring of multimodal immunotherapy based on serial cytokine profiling is infrequently performed and mostly restricted to treatment failure in FIRES in case reports.[15]

Furthermore, in most cases of FIRES, CSF and serum cytokine profiling appear concordant,[12] although there have been reports of CNS-restricted IL-6 elevation.[10,15]

In our patient, the cytokine derangement within the first few weeks of presentation was observed predominantly in the CNS, rather than serum. This suggests that CSF cytokine analysis should be considered in FIRES to avoid missing cases of CNS-restricted inflammation. The interplay between inflammation and ictogenesis is complex and additional mechanistic analyses of FIRES pathogenesis are required. Personalized immunomonitoring before and during treatment, with appropriately targeted therapy, may be helpful in FIRES.



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**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*. 04 2018;59(4):739-744. doi:10.1111/epi.14016
2. 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*. 08 2019;60(8):1678-1688. doi:10.1111/epi.16275
3. Damiano JA, Burgess R, Kivity S, et al. Frequency of CNKSR2 mutation in the X-linked epilepsy-aphasia spectrum. *Epilepsia*. 03 2017;58(3):e40-e43. doi:10.1111/epi.13666
4. Fajgenbaum DC, June CH. Cytokine Storm. *N Engl J Med*. 12 03 2020;383(23):2255-2273. doi:10.1056/NEJMra2026131
5. Group RC. Tocilizumab in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform trial. *Lancet*. May 01 2021;397(10285):1637-1645. doi:10.1016/S0140-6736(21)00676-0
6. Tay SH, Toh MMX, Thian YL, et al. Cytokine Release Syndrome in Cancer Patients Receiving Immune Checkpoint Inhibitors: A Case Series of 25 Patients and Review of the Literature. *Front Immunol*. 2022;13:807050. doi:10.3389/fimmu.2022.807050
7. Zhou X, Fragala MS, McElhaney JE, Kuchel GA. Conceptual and methodological issues relevant to cytokine and inflammatory marker measurements in clinical research. *Curr Opin Clin Nutr Metab Care*. Sep 2010;13(5):541-7. doi:10.1097/MCO.0b013e32833cf3bc
8. Fujihara K, Bennett JL, de Seze J, et al. Interleukin-6 in neuromyelitis optica spectrum disorder pathophysiology. *Neurol Neuroimmunol Neuroinflamm*. 09 03 2020;7(5)doi:10.1212/NXI.0000000000000841
9. Kobylarek D, Iwanowski P, Lewandowska Z, et al. Advances in the Potential Biomarkers of Epilepsy. *Front Neurol*. 2019;10:685. doi:10.3389/fneur.2019.00685
10. Sakuma H, Tanuma N, Kuki I, Takahashi Y, Shiomi M, Hayashi M. Intrathecal overproduction of proinflammatory cytokines and chemokines in febrile infection-related refractory status epilepticus. *J Neurol Neurosurg Psychiatry*. Jul 2015;86(7):820-2. doi:10.1136/jnnp-2014-309388
11. de Vries EE, van den Munckhof B, Braun KP, van Royen-Kerkhof A, de Jager W, Jansen FE. Inflammatory mediators in human epilepsy: A systematic review and meta-analysis. *Neurosci Biobehav Rev*. Apr 2016;63:177-90. doi:10.1016/j.neubiorev.2016.02.007
12. Jun JS, Lee ST, Kim R, Chu K, Lee SK. Tocilizumab treatment for new onset refractory status epilepticus. *Ann Neurol*. 12 2018;84(6):940-945. doi:10.1002/ana.25374
13. Vezzani A, Balosso S, Ravizza T. Neuroinflammatory pathways as treatment targets and biomarkers in epilepsy. *Nat Rev Neurol*. 08 2019;15(8):459-472. doi:10.1038/s41582-019-0217-x
14. Clarkson BDS, LaFrance-Corey RG, Kahoud RJ, Farias-Moeller R, Payne ET, Howe CL. Functional deficiency in endogenous interleukin-1 receptor antagonist in patients with febrile infection-related epilepsy syndrome. *Ann Neurol*. 04 2019;85(4):526-537. doi:10.1002/ana.25439
15. Stredny CM, Case S, Sansevere AJ, Son M, Henderson L, Gorman MP. Interleukin-6 Blockade With Tocilizumab in Anakinra-Refractory Febrile Infection-Related Epilepsy Syndrome

(FIRES). *Child Neurol Open*. 2020 Jan-Dec 2020;7:2329048X20979253.
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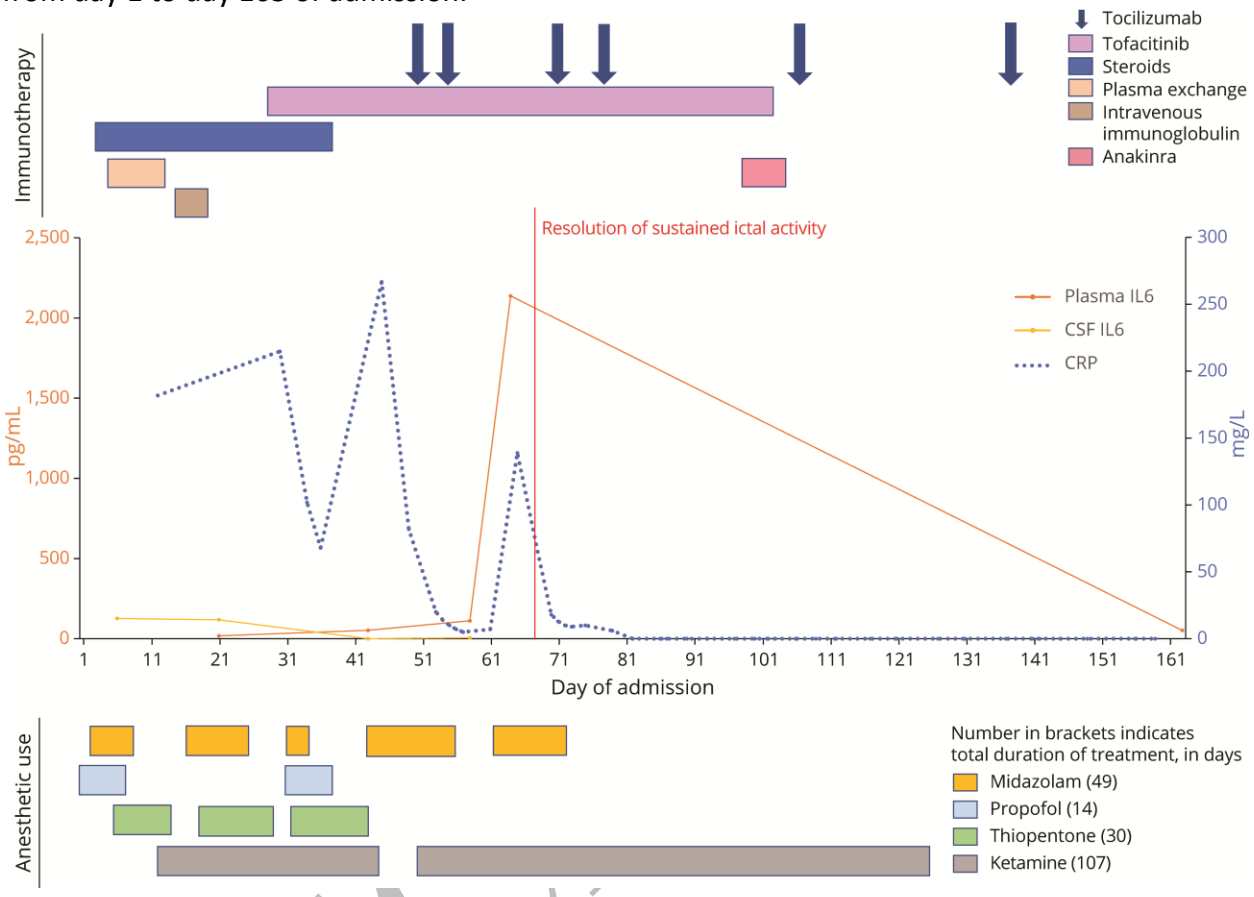
**Figures**

Figure: Immunotherapy and anesthetic use with relation to IL-6 (serum and CSF) and CRP levels from day 1 to day 163 of admission.



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