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Cytokine and Chemokine Expression in CSF May Differentiate Viral and Autoimmune NMDAR Encephalitis in Children

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Abstract

Childhood encephalitis is a potentially devastating condition with significant morbidity and mortality. Researchers currently lack biomarkers for differentiating infectious encephalitis from those with autoimmune causes which may delay adequate treatment. The authors studied the possibility of using cerebrospinal fluid cytokine and chemokine levels for this purpose. Children admitted to hospital care fulfilling criteria for encephalitis were prospectively included. Children who underwent lumbar puncture but were not classified as central nervous system infections served as controls. Cytokine and chemokine levels in the cerebrospinal fluid obtained upon initial presentation were analyzed using Luminex technology. In children with infectious encephalitis (n = 13), the cerebrospinal fluid displayed markedly elevated mean levels of IL6, IL7, and IL13 as compared to N-methyl-D-aspartate receptor (NMDAR) encephalitis (n = 4) and controls (n = 13). The expression of IL6 appeared to precede that of IL13. Analysis of selected cerebrospinal fluid cytokines may thus allow differential diagnosis of infectious and NMDAR encephalitis already at the initial lumbar puncture and enable immediate therapy.

Keywords

pediatric, immunomodulation, central nervous system, childhood

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Encephalitis in children is a potentially devastating condition with significant morbidity and mortality.[1] The etiology may be infectious or autoimmune and in a considerable proportion remains unknown.[2,3] The understanding of encephalitis caused by autoimmune reactions with autoantibodies directed against neuronal cell-surface receptors, in children mainly the N-methyl-D-aspartate receptor (NMDAR), has developed during the last decade. Clinically, these children may present with combinations of neuropsychiatric symptoms, movement disorders, seizures, speech disturbance and consciousness instability.[4,5]

The presenting symptoms of childhood encephalitis as a whole vary substantially, making early and correct diagnosis a challenge. The commonly used diagnostic tools, that is, traditional cerebrospinal fluid analysis, electroencephalograms (EEG) and brain imaging are useful for detecting encephalitis but have a very low specificity in differentiating etiologies. In addition to an increased number of white blood cells in the cerebrospinal fluid, viral central nervous system infections usually elicit a strong immunological response resulting in the release of proinflammatory cytokines.[6-9] This may thus be useful for diagnosis, but whether cytokines are expressed similarly in autoimmune encephalitis is not known.

In a prospective study of childhood encephalitis ongoing at the authors' hospital, an 11 months old girl was included who initially presented with herpes simplex encephalitis but thereafter deteriorated and was diagnosed with NMDAR encephalitis.[10] At the time of her relapse she was again sampled and the marked difference in cytokine patterns in the cerebrospinal fluid between the 2 episodes generated the hypothesis that autoimmune encephalitis is distinguishable from that of viral origin. If generally applicable, this could offer a novel way of identifying cases of NMDAR encephalitis that can be made immediately upon presentation in the emergency room and that does not rely on antibody detection.

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In this manuscript the authors report a different inflammatory profile in the cerebrospinal fluid of children with NMDAR encephalitis as compared to viral encephalitis. It is seen very early in the clinical course and might therefore prove to be useful to differentiate between the conditions and allow early therapy.

Methods

Cohort

Children aged 28 days to 16 years admitted to the authors' primary and tertiary care hospital in northern Stockholm, between May 2011 and February 2015 who showed (1) signs of cerebral dysfunction either as (I) encephalopathy defined as altered consciousness, personality or behavioral changes lasting for more than 24 hours, or (II) abnormal EEG findings compatible with encephalitis (2) pleocytosis ($\geq$ 6 white blood cells/μL) in cerebrospinal fluid. Children with other verified causes of symptoms were excluded. The diagnosis infectious encephalitis (n = 13) required PCR detection of a virus in the cerebrospinal fluid or serology in the case of tick-borne encephalitis virus. Four children were NMDAR-positive as described below. Children who had a lumbar puncture for other indications were used as controls (n = 13). All cerebrospinal fluid samples were taken at first lumbar puncture and prior to any treatment except for the samples presented in Figure 2 illustrating a temporal development.

Laboratory Examinations

Blood and cerebrospinal fluid were prospectively sampled during the acute phase. Routine cerebrospinal fluid analysis including pathogen screening was performed by the Karolinska University Hospital Laboratory. Detection of serum immunoglobulins directed against the NMDAR was performed by either the Department of Clinical immunology, Akademiska Sjukhuset, Uppsala or the Immunology section of the Karolinska University Hospital Laboratory. Both laboratories use a 2-step procedure from EuroImmune (Lübeck, Germany). Briefly, immunofluorescense screening on fixed neuronal rat tissue, followed by detection of specific staining on NMDAR transfected HEK293 cells was used.

Cytokine Analysis

Cerebrospinal fluid obtained during the acute phase was frozen at –70C for later analysis. A custom-made multiplex assay (Bio-Plex Pro, Life Science Bio-Rad, Stockholm, Sweden) was used for the detection of IL1β, IL1RA, IL4, IL6, IL7, IL8, IL10, IL12p70, IL13, IL15, IL17, IFNγ, MCP1, IL18, and MIF. Assays were run according to the manufacturer's instructions using 50 microliters of cerebrospinal fluid. Technical replicates for each sample were used where enough cerebrospinal fluid was available. Data were acquired and validated on a Bio-Plex 200 system and analyzed with Bio-Plex Manager software (Bio-Rad). Standard curves were constructed using 5-point logistic regression. Lower and upper limits of quantification were calculated as the highest and lowest measured reliable standard points. Data points below detection limit were given half the lower limit of quantification, and data points above were given double the upper limit of quantification.

Statistical Analysis

Since the data set did not fulfill normality criteria and due to large differences in variability across groups, nonparametric statistics was applied. Kruskal-Wallis ANOVA was followed by a 2-tailed multiple comparisons test between groups. The authors used a multivariable mixed effects model adjusted to handle heterogeneous variances across groups to control for confounding factors sex, age, and cerebrospinal fluid cells. A P value below .05 was considered significant for all statistical analyses. All values presented in results are means $\pm$ standard deviations.

Results

Demographic characteristics, clinical signs, and symptoms and results of EEG and neuroimaging were recorded and are displayed in table 1.

In children with infectious encephalitis (n = 13), the initial mean cerebrospinal fluid levels of IL6 (1732.3 $\pm$ 1566.5 vs 17.4 $\pm$ 12.7, with no overlapping values between the groups). IL7 (18.2 $\pm$ 12.0 vs 1.3 $\pm$ 1.1) and IL13 (114.2 $\pm$ 83.9 vs 23.6 $\pm$ 42.7) were significantly higher than in patients with NMDAR encephalitis (n = 4; Figure 1 and Table 2). When comparing infectious cases to controls (n = 13), IL1β, IL6, IL7, IL8, IL13, and IL18 reached statistical significance (Figure 1 and Table 2). There were no significant differences between NMDAR encephalitis and control patients.

As an increased expression of cytokines theoretically could be caused by higher cell numbers in cerebrospinal fluid in infectious cases, the authors used a multivariable mixed effects model to study this association as well as for the variables sex and age. No significant relationship could be demonstrated between cerebrospinal fluid pleocytosis, sex, or age and cytokine levels ($P > .45$ for IL6 and $P > .10$ for IL1β). Indeed, in several patients there was a marked cytokine response in the cerebrospinal fluid with low cell numbers, and inversely cerebrospinal fluid samples with high white blood cells but low cytokine responses.

Two of the 4 cases of NMDAR encephalitis in this study initially had herpes simplex encephalitis, developing NMDAR positivity after several weeks. Analysis of the cytokine and chemokine pattern from one of these patients displayed a temporal profile (Figure 2) with an initial response with IL6, IL8, IL10, IL15, IFNγ, and MCP1 during the active herpes simplex encephalitis. After 12 days of treatment a clear response with IL13 and IL18 was detected. NMDAR positivity was evident 21 days post herpes simplex encephalitis, and did not generate any changes in the cytokine/chemokine-pattern. The initial IFNγ response waned, to reach almost normal levels at 30 days. Furthermore, a postinfectious down regulation of IL12 and IL15 was seen. Both Herpes-associated NMDAR cases had lower levels of IL12 and IL15 as compared to naïve NMDAR cases.

Table 1. Distribution of Baseline Clinical Characteristics Between Infectious Encephalitis, NMDAR Encephalitis, and Controls.

Pat	Age (m)	Sex	Main presenting symptom	EEG	Cerebrospinal fluid cell count (mono + poly)	CT/MR	Etiology
1	11	F	seizures, fever, vomiting	consistent with enc	26 + 0	pathological	Herpes
2	77	F	headache, fever, vomiting	consistent with enc	8 + 192	na	Enterovirus
3	130	M	fever, headache	consistent with enc	65 + 39	normal	TBE
4	1	M	fever, irritable, encephalopathy more than 24 h	normal	4 + 2	na	Enterovirus
5	92	F	fever, vomiting, headache	consistent with enc	39 + 15	normal	TBE
6	47	F	fever, tired, vomiting, headache	consistent with enc	51 + 66	na	TBE
7	81	F	headache, vomiting	consistent with enc	64 + 34	na	TBE
8	76	M	fever, headache	consistent with enc	7 + 44	na	TBE
9	40	M	headache, vomiting	consistent with enc	22 + 12	normal	TBE
10	153	F	fever, headache, vomiting	consistent with enc	61 + 31	pathological	EBV
11	77	F	headache, stomach-pain, neck-pain	consistent with enc	54 + 22	na	Varicella
12	22	M	irritability	consistent with enc	164 + 318	pathological	Herpes
13	1	M	fever, altered consciousness, encephalopathy more than 24 h	normal	243 + 7	na	Enterovirus
14	94	F	confusion, seizures	consistent with enc	32 + 0	normal	NMDAR
15	156	F	altered behavior, sensations	abnormal EEG	14 + 0	normal	NMDAR
16	12	F	seizures, aggressive behavior	consistent with enc	30 + 2	pathological	NMDAR (same as 1)
17	23	M	altered personality, encephalopathy more than 24 h	normal	14 + 0	na	NMDAR (same as 12)
18	152	M	commotio	na	0 + 0	na	Control
19	14	F	cortical dysplasia	pathological	0 + 0	pathological	Control
20	61	F	febrile seizure	normal	2 + 0	normal	Control
21	13	F	unclear unconsciousness	normal	0 + 0	normal	Control
22	173	F	unclear unconsciousness	normal	0 + 2	normal	Control
23	21	M	febrile seizure	normal	0 + 0	na	Control
24	6	F	upper respiratory infection	na	0 + 0	na	Control
25	2	M	unclear infection	normal	2 + 0	na	Control
26	119	F	unclear infection	normal	0 + 0	na	Control
27	3	M	upper respiratory infection	na	0 + 2	na	Control
28	2	M	upper respiratory infection	na	0 + 0	na	Control
29	11	M	gastroenteritis	normal	0 + 0	na	Control
30	10	F	upper respiratory infection	normal	2 + 0	normal	Control

Abbreviations: EBV, Epstein-Barr Virus; enc, encephalitis; na, not analyzed; NMDAR, N-methyl-D-aspartate receptor; TBE, tick-borne encephalitis.

Discussion

In this article, the authors describe a feasible method of possibly differentiating infectious and NMDAR autoimmune origins of childhood encephalitis already in the acute phase of the disease.

The authors demonstrate that the levels of IL6, IL7, and IL13 are, on average, higher upon presentation to hospital care in children with infectious encephalitis compared to NMDAR encephalitis, suggesting that these cytokines might be useful for differential diagnosis. The analysis of the temporal profile of cytokine expression obtained by repeated lumbar punctures indicates that the combination of IL6 and IL13 may be optimal, representing early and late markers in the infectious cases. A cutoff for IL6 at 100 pg/ml identified all infectious cases and gave no false positives in the NMDA group, whereas a cutoff for IL13 at 100 pg/ml failed to identify 5/13 infectious cases. Thus, in this cohort, IL6-levels over 100 pg/ml *or* IL13-levels over 100 ng/ml identified all infectious cases and may allow sampling at different time-points in infection. The lower levels of cytokines in NMDAR encephalitis is in line with findings from autopsy/biopsy cases where no antibody or complement-mediated tissue injury was seen as well as a low density of inflammatory cells.[11]

Infectious encephalitis is usually caused by viruses, for example, herpes simplex virus, enterovirus, and influenza virus. Elevated white blood cell count in the cerebrospinal fluid is used clinically to confirm a central nervous system infection, but not all patients will have pleocytosis at the time of the lumbar puncture.[3] Elevated cytokine levels in serum and/or cerebrospinal fluid have been described for varying etiologies of infectious encephalitis In children with enteroviral meningitis, IL6 and IL8 were elevated also in children with little pleocytosis, suggesting them as more sensitive markers of ongoing central nervous system infection[6] and possibly markers to differentiate these from autoimmune cases.

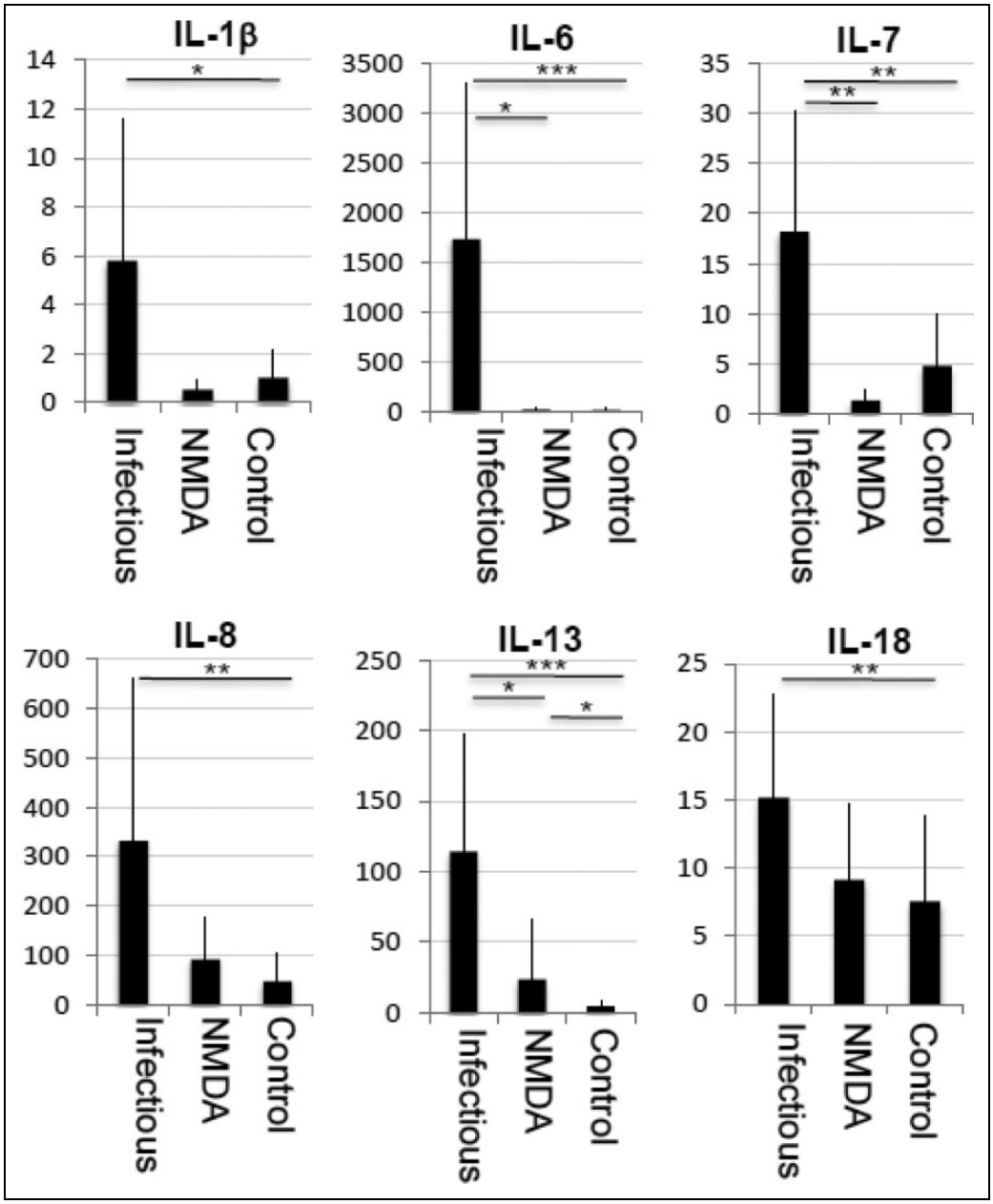

Figure 1. Concentrations of cytokines and chemokines (pg/ml) in cerebrospinal fluid of patients with infectious encephalitis, NMDAR encephalitis and controls. Values are shown as means with error bars showing standard deviations. Levels were significantly higher in infectious cerebrospinal fluid as compared to NMDAR cases for IL6, IL7, and IL13, whereas other cytokines showed a similar trend without reaching significance levels. When infectious cases were compared to controls, IL1β, IL6, IL7, IL8, IL13, and IL18 reached statistical significance. *$P < .05$. **$P < .01$. ***$P < .001$.

Combined forms of infectious and autoimmune encephalitis are also reported. NMDAR antibodies are present in 30% of adult cases with active herpes simplex encephalitis and these antibodies are believed to contribute to the pathogenesis.[12] In some cases anti-NMDAR antibodies may precede the infection, whereas in other they are produced during the acute phase or postinfection.[12,13] In children, the authors and others have reported NMDAR encephalitis appearing about 1 month after herpes simplex encephalitis.[10,13,14] As this may also represent a viral encephalitis relapse, it poses a difficult diagnostic challenge with consequences for the choice of treatment regime. These results suggest the usefulness of cytokine analysis in making this important differential diagnosis.

There are currently no biomarkers that enable differential diagnosis between encephalitis of infectious and of autoimmune origin, although a recent publication has demonstrated increased CXCL 13-levels in NMDAR encephalitis.[15] In childhood viral encephalitis several cytokines such as TNF, IL6, IL8, and IFNγ have been shown to be elevated.[7-9] The cytokine profile in autoimmune encephalitis has not been described in a

Table 2. Levels of Cytokines in Cerebrospinal Fluid of Patients With Infectious Encephalitis, NMDAR Encephalitis, and controls.

	Infectious encephalitis (n = 13)		NMDAR encephalitis (n = 4)		Controls (n = 13)		*P* value ANOVA for group	*P* value post hoc
	Mean	SD	Mean	SD	Mean	SD		
IL1β	5.797	5.760	0.533	0.406	1.010	1.143	.008	inf-NMDAR: > .05 inf-cont: < .05 NMDAR-cont:> .05
IL1ra	57.804	72.934	27.879	11.322	10.159	5.644	.061	Not applicable
IL4	1.421	0.687	0.881	0.790	1.223	0.913	.498	Not applicable
IL6	1732.342	1565.560	17.41	12.791	11.899	15.553	<.001	inf-NMDAR: < .05 inf-cont: < .001 NMDAR-cont:> .05
IL7	18.243	12.0253	1.307	1.160	4.829	5.035	<.001	inf-NMDAR: < .01 inf-cont: < .01 NMDAR-cont:> .05
IL8	331.777	329.517	91.348	85.203	46.425	58.940	.01	inf-NMDAR: < .05 inf-cont: < .01 NMDAR-cont:> .05
IL10	24.919	41.851	5.292	3.477	3.791	1.007	.149	Not applicable
IL12	5.993	5.172	19.813	20.145	9.964	12.853	.124	Not applicable
IL13	114.173	83.870	23.634	42.704	4.292	3.872	<.001	inf-NMDAR: < .05 inf-cont: < .001 NMDAR-cont:> .05
IL15	32.389	30.454	68.199	70.021	42.736	43.093	.345	Not applicable
IL17	8.149	11.931	4.538	4.618	2.950	2.144	.282	Not applicable
IFNγ	147.211	189.184	39.593	40.149	41.447	35.952	.101	Not applicable
MCP1	1418.545	2405.690	275.103	199.903	526.504	511.949	.296	Not applicable
IL18	15.173	7.577	9.139	5.586	7.545	6.236	.024	inf-NMDAR: > .05 inf-cont: < .05 NMDAR-cont:> .05
MIF	177.279	224.983	122.396	98.839	322.137	432.375	.417	Not applicable

Abbreviations: cont, control; IFNγ, interferon gamma; IL, interleukin; IL1ra, IL1 receptor antagonist; inf, infectious; MCP1, monocyte chemoattractant protein 1; MIF, macrophage migration inhibitory factor; NMDAR, N-methyl-D-aspartate receptor.

similar fashion. This poses a severe clinical limitation since treatment differs between the 2 conditions. In viral encephalitis, immunosuppression may in fact be detrimental as this may cause increased viral replication and subsequent neural damage. In contrast, early treatment with immunosuppression and intravenous immunoglobulins is the therapy of choice in autoimmune encephalitis and the time to treatment is considered an important factor in affecting outcome.[16] Obtaining neuronal antibodies to corroborate an autoimmune etiology takes about 1 week, thereby delaying treatment. Whereas treatment for infectious causes will aim at reducing the viral load (aciklovir, ribavirin, and pleoconaril may all have a positive effect on outcome)[17] the treatment for autoimmune encephalitis aims at reducing the inflammatory response, using, for example, corticosteroids, IVIG, rituximab, or plasmapheresis.[16,18] Using cytokine analysis, these etiologies may be differentiated within hours after admission to the hospital and allow immediate appropriate treatment.

This study has several limitations. First, the number of studied children is low, and for some cytokines significance levels were not reached although absolute values clearly indicated a trend toward differences. Thus, the results of this pilot study need to be corroborated by larger studies. Second, the authors have only included cases of NMDAR autoimmune encephalitis so whether the inflammatory pattern seen in NMDAR encephalitis is specific for this origin or applies to all autoimmune neuroinflammation needs to be studied further. Likewise, whether these differences are also seen in adults must be studied in this population. Third, the definition of encephalitis is often problematic and most studies include cases where no virus is found in the cerebrospinal fluid, labeling them as "probable." To ensure that cases were labeled correctly the authors have in this study only included cases with positive findings in cerebrospinal fluid. Although this significantly reduces the size of the cohort, it increases the specificity of the findings.

The authors thus conclude that analysis of cerebrospinal fluid cytokines is a promising method for differentiating infectious and NMDAR encephalitis already at initial presentation. This could be of high clinical importance as it may direct therapy choices in the acute phase and improve outcome for affected children.

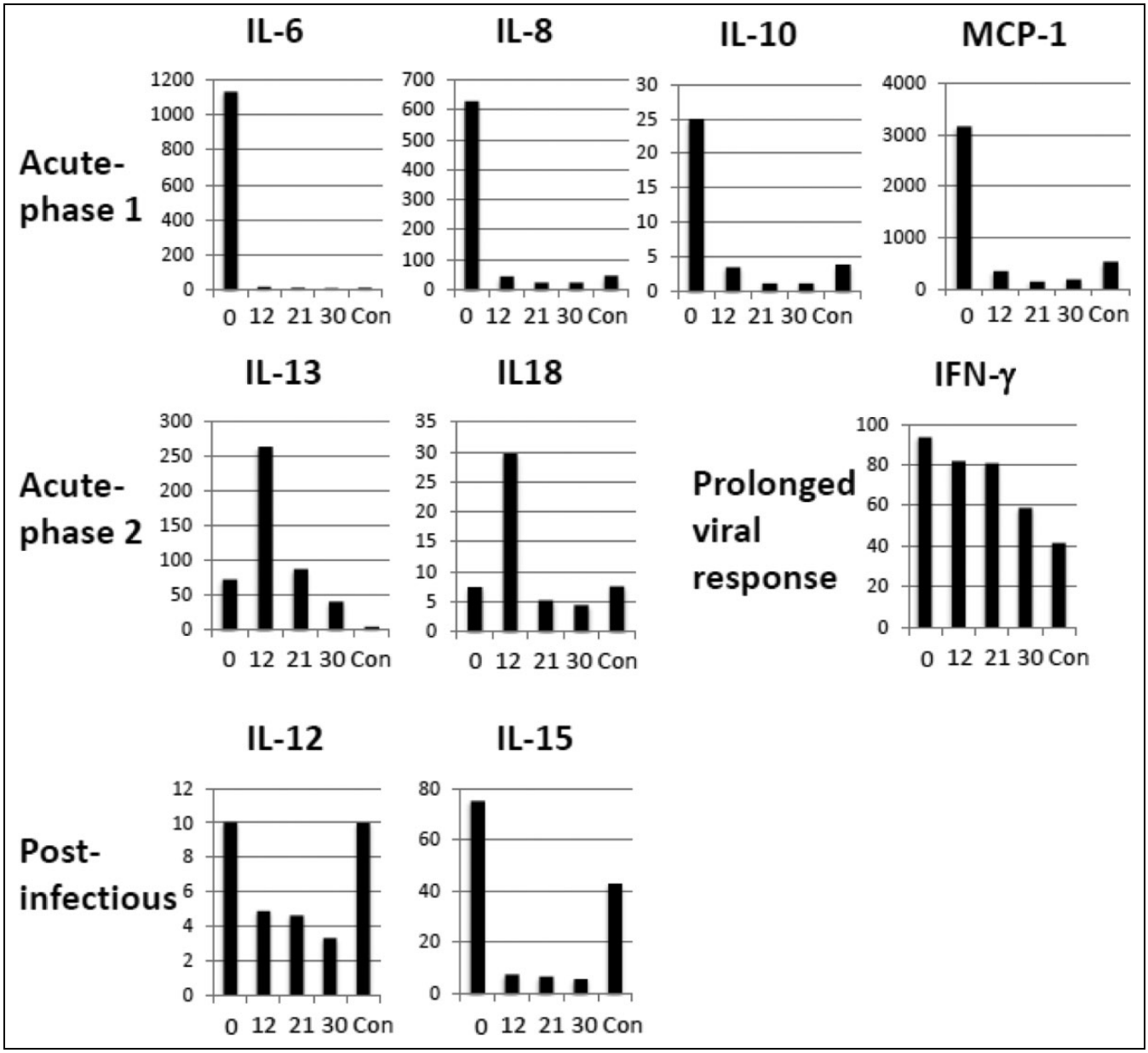

Figure 2. Temporal alterations (days) in concentrations of cytokines and chemokines (pg/ml) in cerebrospinal fluid of a NMDAR encephalitis case compared to controls (Con). Whereas IL6, IL8, IL10, and MCP1 increase in the early acute phase, IL13 and IL18 increase somewhat later. In contrast, IFNγ displays a prolonged pattern and IL12 and IL15 are suppressed following infection. Numbers on x-axis indicate days for lumbar puncture (0-30).

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Author Contributions

SY contributed to study design, patient enrollment, data analysis, statistical analysis, and writing the manuscript. ÅF contributed to study design, patient enrollment, data analysis, statistical analysis, and writing the manuscript. RW acted as principal investigator with study conception and design, patient enrollment, data analysis, statistical analysis, and writing the manuscript.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical Approval

This study was approved by the local ethics committee (Dnr 2010/1206-31/1).

References

1. Fowler A, Stodberg T, Eriksson M, Wickstrom R. Long-term outcomes of acute encephalitis in childhood. *Pediatrics*. 2010; 126(4):e828-835.
2. Kolski H, Ford-Jones EL, Richardson S, et al. Etiology of acute childhood encephalitis at The Hospital for Sick Children, Toronto, 1994-1995. *Clin Infect Dis*. 1998;26(2):398-409.
3. Fowler A, Stodberg T, Eriksson M, Wickstrom R. Childhood encephalitis in Sweden: etiology, clinical presentation and outcome. *Eur J Paediatr Neurol*. 2008;12(6):484-490.
4. Irani SR, Bera K, Waters P, et al. N-methyl-D-aspartate antibody encephalitis: temporal progression of clinical and paraclinical observations in a predominantly non-paraneoplastic disorder of both sexes. *Brain*. 2010;133(pt 6):1655-1667.
5. Armangue T, Titulaer MJ, Malaga I, et al. Pediatric anti-N-methyl-D-aspartate receptor encephalitis-clinical analysis and novel findings in a series of 20 patients. *J Pediatr*. 2013;162(4):850-856.
6. Wang SM, Lei HY, Su LY, et al. Cerebrospinal fluid cytokines in enterovirus 71 brain stem encephalitis and echovirus meningitis infections of varying severity. *Clin Microbiol Infect*. 2007;13(7): 677-682.
7. Hasegawa S, Matsushige T, Inoue H, Shirabe K, Fukano R, Ichiyama T. Serum and cerebrospinal fluid cytokine profile of patients with 2009 pandemic H1N1 influenza virus-associated encephalopathy. *Cytokine*. 2011;54(2):167-172.
8. Ichiyama T, Ito Y, Kubota M, Yamazaki T, Nakamura K, Furukawa S. Serum and cerebrospinal fluid levels of cytokines in acute encephalopathy associated with human herpesvirus-6 infection. *Brain Dev*. 2009;31(10):731-738.
9. Sato M, Hosoya M, Honzumi K, et al. Cytokine and cellular inflammatory sequence in enteroviral meningitis. *Pediatrics*. 2003;112(5):1103-1107.
10. Wickstrom R, Fowler A, Cooray G, Karlsson-Parra A, Grillner P. Viral triggering of anti-NMDA receptor encephalitis in a child—an important cause for disease relapse. *Eur J Paediatr Neurol*. 2014;18(4):543-546.
11. Bien CG, Vincent A, Barnett MH, et al. Immunopathology of autoantibody-associated encephalitides: clues for pathogenesis. *Brain*. 2012;135(pt 5):1622-1638.
12. Pruss H, Finke C, Holtje M, et al. N-methyl-D-aspartate receptor antibodies in herpes simplex encephalitis. *Ann Neurol*. 2012; 72(6):902-911.
13. Leypoldt F, Titulaer MJ, Aguilar E, et al. Herpes simplex virus-1 encephalitis can trigger anti-NMDA receptor encephalitis: case report. *Neurology*. 2013;81(18):1637-1639.
14. Hacohen Y, Deiva K, Pettingill P, et al. N-methyl-D-aspartate receptor antibodies in post-herpes simplex virus encephalitis neurological relapse. *Move Disord*. 2014;29(1):90-96.
15. Leypoldt F, Hoftberger R, Titulaer MJ, et al. Investigations on CXCL13 in anti-N-methyl-D-aspartate receptor encephalitis: a potential biomarker of treatment response. *JAMA Neurol*. 2015; 72(2):180-186.
16. Wright S, Hacohen Y, Jacobson L, et al. N-methyl-D-aspartate receptor antibody-mediated neurological disease: results of a UK-based surveillance study in children. *Arch Dis Child*. 2015; 100(6):521-526.
17. Thompson C, Kneen R, Riordan A, Kelly D, Pollard AJ. Encephalitis in children. *Arch Dis Child*. 2012;97(2):150-161.
18. Titulaer MJ, McCracken L, Gabilondo I, et al. Treatment and prognostic factors for long-term outcome in patients with anti-NMDA receptor encephalitis: an observational cohort study. *Lancet Neurol*. 2013;12(2):157-165.