Supplementary Online Content

Leypoldt F, Höftberger R, Titulaer MJ, et al. Investigations on CXCL13 in anti–*N*-methyl-D-aspartate receptor encephalitis: a potential biomarker of treatment response. *JAMA Neurol*. Published online December 1, 2014. doi:10.1001/jamaneurol.2014.2956.

This supplementary material has been provided by the authors to give readers additional information about their work.

eAppendix. Immunohistochemistry of Human Biopsy Samples

Formalin-fixed, paraffin-embedded tissue sections (3-4μm thick) from right frontal biopsy material of a patient with anti-NMDAR encephalitis (case #5[1]) were deparaffinized and the antigen retrieved as reported. Sections were then serially incubated with 0.3% hydrogen peroxide for 15 minutes at room temperature, 10% horse serum in PBS for 1h, and the primary antibody overnight at 4°C. The following primary antibodies were used: polyclonal (goat) antibody CXCL13 (1:15; R&D System, Minneapolis, USA), CD68 (1:5000; activated microglia/macrophages, DAKO, Glostrup, Denmark), CD138 (1:50; plasma cells, plasmablasts, DAKO, Glostrup, Denmark), CD3 (1:100; Leica, Bannockburn, IL), CD8 (1:20; DAKO), CD4 (1:20; Biocare, Concord, CA), and CD20 (1:250; DAKO). The next day, sections were incubated with the appropriate secondary antibody (1:1000) for 1h at room temperature (Vector lab, Burlingham, CA, USA), avidin-biotin-peroxidase for 40 minutes, and visualized with diaminobenzidine (DAB) (Vector lab). Sections were then counterstained with Mayer's hematoxylin.

eReferences

1. Martinez-Hernandez E, Horvath J, Shiloh-Malawsky Y, Sangha N, Martinez-Lage M, Dalmau J. Analysis of complement and plasma cells in the brain of patients with anti-NMDAR encephalitis. Neurology. 2011;77(6):589–593.

eTable. Statistical Analysis of CSF CXCL13 in Initial and Follow-up Samples of Patients With Anti-NMDAR Encephalitis

All patients n=167 first available sample					
General linear model			Post-hoc testing		
	P-value	Effect size°	Group	Mean[&] (95% CI), n	P-Value
Corrected model	**0.001**	0.241			
Age	**0.005**	0.062			
Gender	0.914	0.000			
Maximum mRS 5	0.811	0.000			
Onset-to-sample time	**0.004**	0.065	months 1-2¥	14.9pg/ml (10.6-21.0) n=113	
			months 3-4¥	4.4pg/ml (2.0-9.7) n=21	**p=0.017**
			months 5-12¥	3.5pg/ml (1.9-6.7) n=33	**p=0.0003**
Prodromal symptoms	**0.011**	0.051	prodrome[#]	20.3pg/ml (12.5-33.0) n=67	n/a
			no prodrome[#]	10.0pg/ml (6.2-16.0) n=44	
MRI abnormal	0.448	0.005			
CSF abnormal	0.117	0.020			
Stay in ICU	0.398	0.006			
Tumor found	0.104	0.021			
Prior immuno-suppression[§]	0.302	0.009			
Interaction Prodrome * Tumor	**0.039**	0.034	no tumor, prodrome[#]	16.9pg/ml (9.9-28.9) n=56	p=1.0
			no tumor, no prodrome[#]	11.0pg/ml (6.6-18.4) n=36	
			tumor, prodrome[#]	26.2pg/ml (9.7-70.5) n=20	p=0.45
			tumor, no prodrome[#]	6.4pg/ml (1.7-24.4) n=8	

Patients treated within 90 days of onset n=137 first available sample				
General linear model			Group means	
	P-value	Effect size°	Group	Mean (95% CI), n	P-Value
Limited response at 8 months	**0.003**	0.078	Limited response at 8 months	16.4 pg/ml (10.0-26.8), n=57	n/a
			Favorable response at 8 months	8.6 pg/ml (CI 5.6-13.1), n=80	

Monophasic patients treated within 90 days from onset and follow-up samples available n=35						
Two-way ANOVA			Post-hoc testing[@]			
Variable	Source of variation	P-value	Months after treatment	Limited response, mean [95% CI],n	Favorable response, mean [95% CI],n	Multiplicity-adjusted p-value[@]
CSF CXCL13 in pg/ml in initial and follow-up samples n=35	Months after treatment initiation	**<0.001**	1-2 months	24.7 [5.98-101.8],13	9.00 [2.37-34.1],13	0.23
	Response to therapy	**0.003**	3-6 months	12.7 [4.43-36.11],15	1.69 [0.99-2.88],13	**0.019**
	Interaction	0.36	> 6 months	1.96 [1.09-3.53],18	1 [1-1],9	0.32

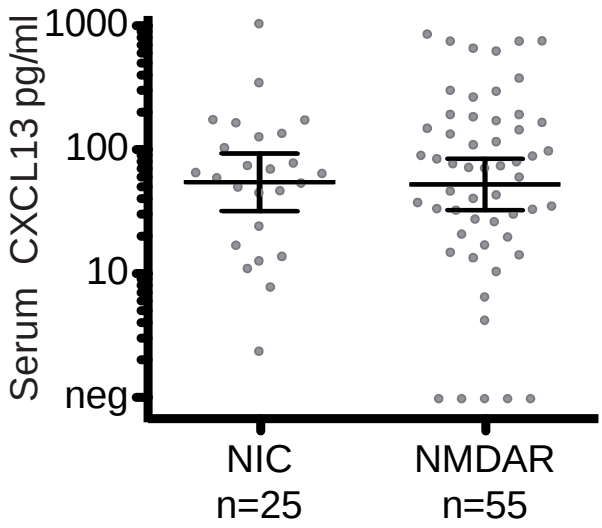
Relapsing patients n=13				
Kruskal-Wallis-Test		Dunn's post-hoc testing		
Variable	P-value	Variable	Mean rank diff.	Multiplicity-adjusted P-value
CSF CXCL13 in pg/ml in initial and follow-up samples n=13	**0.004**	Initial vs. last sample	11.75	**0.044**
		Remission versus last sample	5.61	0.78
		Relapse versus last sample	11.5	**0.027**

[&]Logarithmical mean, recalculated after creation of the model leading to skewed 95% CI of mean intervals. [¥]post hoc testing done using one-way ANOVA with Sidak-Holm post-hoc test

and multiplicity-adjusted p-values of difference to samples from months 1-2. [#]Post-hoc testing done using unpaired t-tests and data from samples from months 1 and 2. [§]To correct for effects of samples acquired before and after initiation of immunosuppression and allow for the immunosuppressive effect on cytokine levels, any immunosuppressive treatment >14 days before sample was included as a factor. °partial eta squared. * Interaction analysis in univariate general linear model. @Post-hoc testing using Sidak-Holm procedure with multiplicity-adjusted p-values. ICU intensive care unit, n/a not applicable.

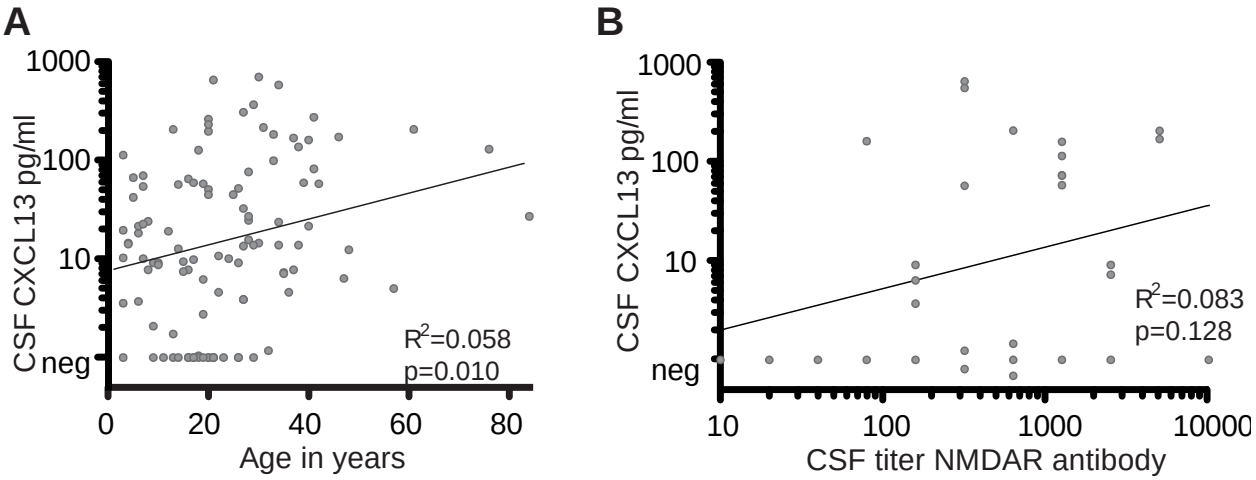
eFigure 1. Serum Concentration of CXCL13 is Not Different Between Anti-NMDAR Encephalitis and Controls

Supplemental figure legends:



Serum CXCL13 levels of patients with anti-NMDAR encephalitis (NMDAR) are not significantly different from those of controls with non-inflammatory conditions (NID). Data is presented as logarithmic mean and 95% CI. CXCL13 concentrations below 1pg/ml are depicted as negative (neg).

eFigure 2. CSF CXCL13 Correlates With Age But Not With CSF Titer of NMDAR Antibodies

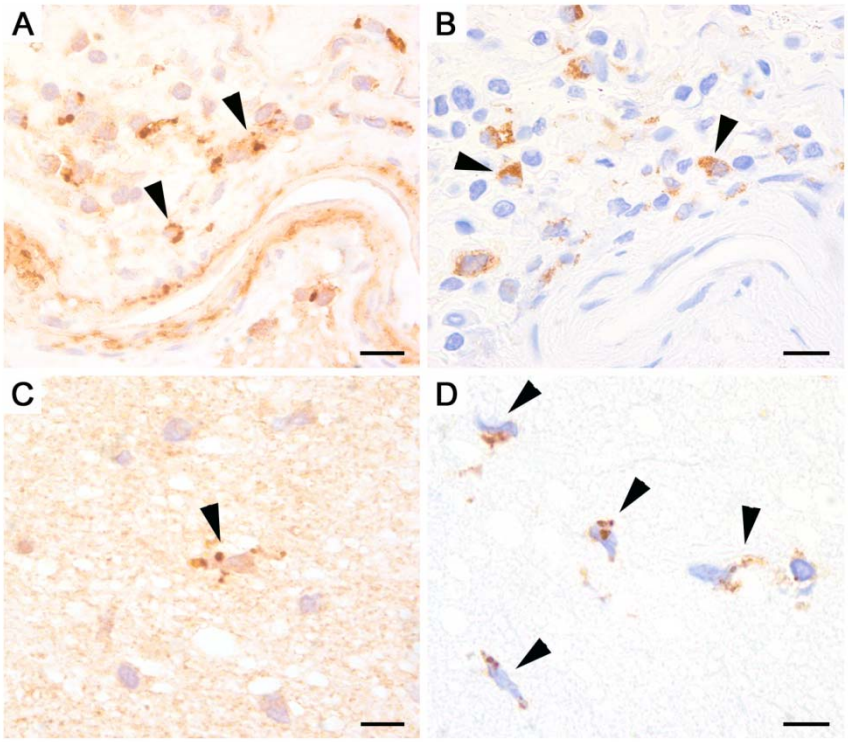


A) CSF CXCL13 measured in samples from patients with anti-NMDAR encephalitis, showing patients' age in years at time of diagnosis. Samples obtained during months 1-2.

B) CSF CXCL13 concentration plotted against the CSF NMDAR antibody titer in the first available CSF sample of patients with monophasic anti-NMDAR encephalitis (n=30) shows lack of significant correlation.

Pearson's R^2 and significance p indicated. CSF CXCL13 concentrations below 1pg/ml are depicted as negative (neg).

eFigure 3. Perivascular Macrophages and Activated Microglia Express CXCL13



A-B) Brain biopsy of a patient with anti-NMDAR encephalitis showing perivascular infiltrates expressing CXCL13 (**A**, arrow heads); these infiltrates were mainly composed of monocytes and macrophages (**B,** arrow heads indicate CD68 expressing monocytes and macrophages).

C-D) CXCL13 was also expressed by activated microglia in the brain parenchyma (**C**, CXCL13, arrow head shows a microglial cell; **D**, CD68, arrow heads show microglial cells). No complement deposition was observed (Case #5[1]). Scale bar A-B 14 µm, C-D 10 µm.