

## Short Communication

## Successful combined targeting of B- and plasma cells in treatment refractory anti-NMDAR encephalitis



Olafur Sveinsson<sup>a,c,\*</sup>, Mathias Granqvist<sup>a,c</sup>, Yngve Forslin<sup>b,d</sup>, Kaj Blennow<sup>e,f</sup>,  
Henrik Zetterberg<sup>e,f,g,h</sup>, Fredrik Piehl<sup>a,c</sup>

<sup>a</sup> Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden

<sup>b</sup> Department of Clinical Science, Intervention and Technology, Karolinska Institutet, Stockholm, Sweden

<sup>c</sup> Department of Neurology, Karolinska University Hospital, Stockholm, Sweden

<sup>d</sup> Department of Radiology Karolinska, University Hospital Stockholm, Sweden

<sup>e</sup> Department of Psychiatry and Neurochemistry, University of Gothenburg, Molndal, Sweden

<sup>f</sup> Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Molndal, Sweden

<sup>g</sup> Department of Molecular Neuroscience, UCL Institute of Neurology, London, United Kingdom

<sup>h</sup> Dementia Research Institute, London, United Kingdom

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## ABSTRACT

We describe an extremely severe case of therapy refractory NMDA receptor encephalitis (NMDAe) in a 26-year-old woman. After rituximab, bilateral oophorectomy, repeated cycles of high dose methylprednisolone and plasma exchange, she received repeated cyclophosphamide, tocilizumab (interleukin-6 inhibitor) and finally bortezomib (plasma cell depleting drug) leading to remission after 204 days in intensive care. Two years after disease onset her cognitive functions are still affected, but slowly improving and the cerebral atrophy has been partly reversed. The cerebrospinal fluid biomarker profile suggests an early synaptic/dendritic process, with subsequent neuroaxonal degeneration motivating aggressive treatment early on.

## 1. Introduction

NMDA receptor encephalitis (NMDAe) is an antibody-mediated autoimmune disorder, first described as a discrete entity by Dalmau and colleagues after observing four female patients with teratoma and neuropsychiatric symptoms (Vitaliani et al., 2005). Subsequently, the target antigen was identified as the NR1 subunit of the NMDA-receptor, which is abundantly expressed in the frontal and hippocampal regions of the brain (Dalmau et al., 2007). NMDAe occurs both as a paraneoplastic and an autoimmune or parainfectious condition, the latter sometimes triggered by herpes simplex encephalitis. NMDAe is likely to be the most common form of the paraneoplastic encephalitides and the most common autoimmune encephalitis second to acute disseminated encephalomyelitis (Granerod et al., 2010). NMDAe has a wide clinical spectrum; from mild to very severe disease, where mortality rates are as high as 20% (Titulaer et al., 2013), but specific treatment algorithms remain to be established. A large proportion of patients do not have focal injuries visible on magnetic resonance imaging (MRI), while others display hyperintense lesions predominately in the hippocampi, but also in other areas. In severe cases global brain atrophy, mainly in fronto-temporal regions, may be prominent. In classical forms of

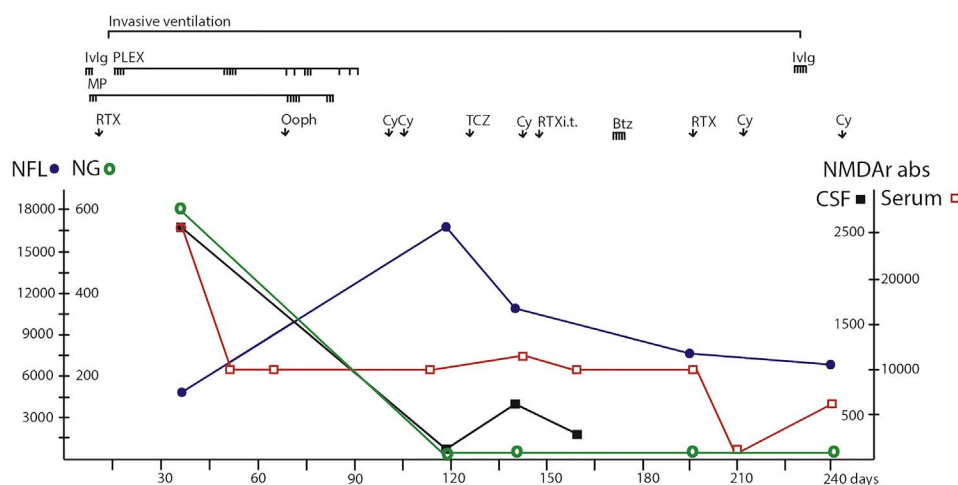
paraneoplastic encephalitides, specific nerve cell populations are targeted, leading to loss of neurons and permanent deficits. In contrast, a remarkable finding in a long-term follow-up of two patients with severe NMDAe was that a pronounced fronto-temporal atrophy during the acute phase of the disease was reversible (Iizuka et al., 2010). The biological basis for this phenomenon is unknown and reports on biomarker profiles in NMDAe are limited but growing (Constantinescu et al., 2016; Liba et al., 2016).

## 2. Case report

A 26-year-old woman of Southeast Asian descent, with no relevant medical family history of autoimmune or psychiatric disease, presented with a rapidly progressing personality disorder, with aggressive behavior, emotional lability, suicidal ideation, visual and auditory hallucinations, insomnia and memory impairment, followed by repeated generalized seizures. Despite initial treatment with antiepileptic drugs (AEDs) she developed status epilepticus and was transferred to neurointensive care. On admission, the CSF analysis revealed a moderate lymphocytic pleocytosis (60 cells/mm<sup>3</sup>), which had risen to 190 cells/mm<sup>3</sup> one week later. Extensive virus and bacterial screens were

\* Corresponding author at: Department of Neurology, Karolinska University Hospital, 171 76 Stockholm, Sweden.

E-mail address: [olafur.sveinsson@karolinska.se](mailto:olafur.sveinsson@karolinska.se) (O. Sveinsson).



**Fig. 1.** Temporal profiles of cerebrospinal fluid neurofilament-light (NFL; pg/mL) and neurogranin (NG; pg/mL) levels (left X axis, filled and open circles, respectively), as well as anti-NMDA receptor cerebrospinal fluid (filled squares) and serum (open squares) antibody titers, respectively (right X axis). The patient needed invasive ventilation due to intractable epileptic seizures early in the disease course in spite of aggressive anti-epileptic and immune modulating drug treatment. The sequential timing of subsequent immune modulation is indicated in the figure. Btz = bortezomib; Cy = cyclophosphamide; Ivlg = intravenous immunoglobulin infusion; MP = methylprednisolone; Ooph = bilateral oophorectomy; PLEX = plasmapheresis; RTX = rituximab; RTX.i.t. = intrathecal rituximab; TCZ = tocilizumab.

negative. Suspecting an autoimmune disorder, the patient began treatment with high dose methylprednisolone six days after admission. Rituximab 1000 mg was then given and plasma exchange was started 13 days after admission, with a total of 4 cycles (18 sessions) over a four-month period. Anti-NMDAR antibodies were subsequently confirmed in serum and CSF (see Fig. 1, for antibody titer progression). Antibody titers remained high despite treatment and undetectable B cells in peripheral blood. No teratoma or tumor was detected with whole body PET or CT. MRI of the pelvis revealed polycystic ovaries, which made it impossible to exclude the presence of a small teratoma. The patient underwent a bilateral oophorectomy (day 62), without signs of teratoma on histopathology. As a next step cyclophosphamide was initiated with a total of 7 cycles (1000 mg per cycle) over four months. The patient remained continuously sedated on propofol, midazolam and morphine with the addition of two to three AEDs but intermittent wake up attempts resulted in increased dyskinesia, and epileptiform and highly characteristic extreme delta-brush activity activity on EEG (Schmitt et al., 2012). The absence of clinical improvement as well as increasing levels of neurofilament-light, a neuroaxonal injury marker measured by enzyme-linked immunosorbent assay (ELISA, UmanDiagnostic, Umea, Sweden) (Fig. 1), motivated additional therapeutic efforts. As a first step tocilizumab, a blocker of interleukin-6 signaling with suggested effects in refractory neuromyelitis optica (Chihara et al., 2011; Araki et al., 2014), was given at a dose of 8 mg/kg at day 119. As the next step 25 mg of rituximab was administered intrathecally at day 133 since CSF flow cytometry revealed a normal proportion (7%) of B-cells despite peripheral blood depletion. Intrathecal B-cell counts fell to 1.5% (3 cells) at follow-up, one month later. Clinically, however, the patient showed no signs of improvement. Follow-up MRI scans taken five months after admission showed a marked progression of atrophy most prominent in fronto-temporal regions (Fig. 2). As a final step bortezomib, a protease inhibitor that results in the depletion of plasma cells, was given as at a dose of 1.3 mg/m<sup>2</sup> mg in 4 repeated weekly injections from day 147. Over the following four weeks, the patient started to display a remarkable improvement considering the time spent in critical care. Seven months after admission, the patient regained sufficient respiratory function to be taken off invasive ventilation. The sedation was weaned without clinical deterioration and a week later, the patient spontaneously opened her eyes. Treatment was resumed with Ivlg and cyclophosphamide at a reduced dose, due to lymphopenia. Over the next months, further neurological improvement occurred, but she also developed a fluctuating very aggressive and violent behavioral disorder, which is well described during the recovery phase of NMDAe, to the point where she became almost unmanageable in somatic care. The patient was discharged to a rehabilitation clinic after 313 days of hospitalization. Since then she has continuously improved and now lives at

home, independent in her activities of daily living and takes no medication besides receiving rituximab every 6 months to reduce the risk of a relapse. During the next two years, MRI showed a substantial recovery in brain volume (Fig. 2). Additional measurements of tissue injury biomarkers on stored CSF revealed (day 42 from admission) high concentrations of neurogranin (Fig. 1) (in house ELISA), a postsynaptic protein enriched in dendritic spines and a marker of synaptic pathology (Portelius et al., 2015). CSF neurogranin concentration decreased thereafter. Total tau (T-tau), phospho-tau (P-tau) and soluble amyloid precursor protein alpha (sAPPa) and beta (sAPPb) concentrations, measured using commercially available immunoassays, were moderately increased early in the disease course (data not shown), with subsequent normalization. Furthermore, the glial markers glial fibrillary acidic protein, S100B and YKL-40 were low throughout the disease course.

### 3. Discussion

Refractory NMDAe constitutes a great clinical challenge, since it is often associated with prolonged intensive care and a high rate of mortality (Titulaer et al., 2013). To the best of our knowledge, the case presented here had the longest uninterrupted ICU period so far published with a relatively beneficial outcome. Despite treatment with immune suppressants such as rituximab and cyclophosphamide, the condition did not improve. Furthermore, the nerve injury marker NFL remained at a moderate to high level and the patient was not improving. Therefore, additional treatment with tocilizumab and bortezomib was given, which led to a clear clinical improvement. It is impossible to attribute the improvement to any single immune suppressant, but it likely represents a combination of several treatments. However, it is clear that the combination of rituximab, administered both in the periphery and directly into the intrathecal compartment, targeting CD20 expressing B cells, and bortezomib that targets plasma cells, on theoretical grounds should provide a strong brake on humoral immunity. This is also in line with recent reports on bortezomib in refractory anti-NMDAe (Behrendt et al., 2016; Scheibe et al., 2017). Still, the number of cases is small and any firm assumptions have to await larger case series. Notably, autoreactive NMDA receptor antibodies remained high in our patient almost two years after disease onset despite clinical improvement, suggesting that autoantibody levels detected by standard clinical measures are not fully reliable for guiding treatment decisions. To our knowledge this is the first time NFL levels have been monitored throughout the disease course. Despite early and aggressive treatment, levels of NFL continued to rise and peaked at four months after admission, three months after the initial surge of anti-NMDAR antibodies. The fact that NFL reflects neuroaxonal degeneration, the most important determinant of permanent

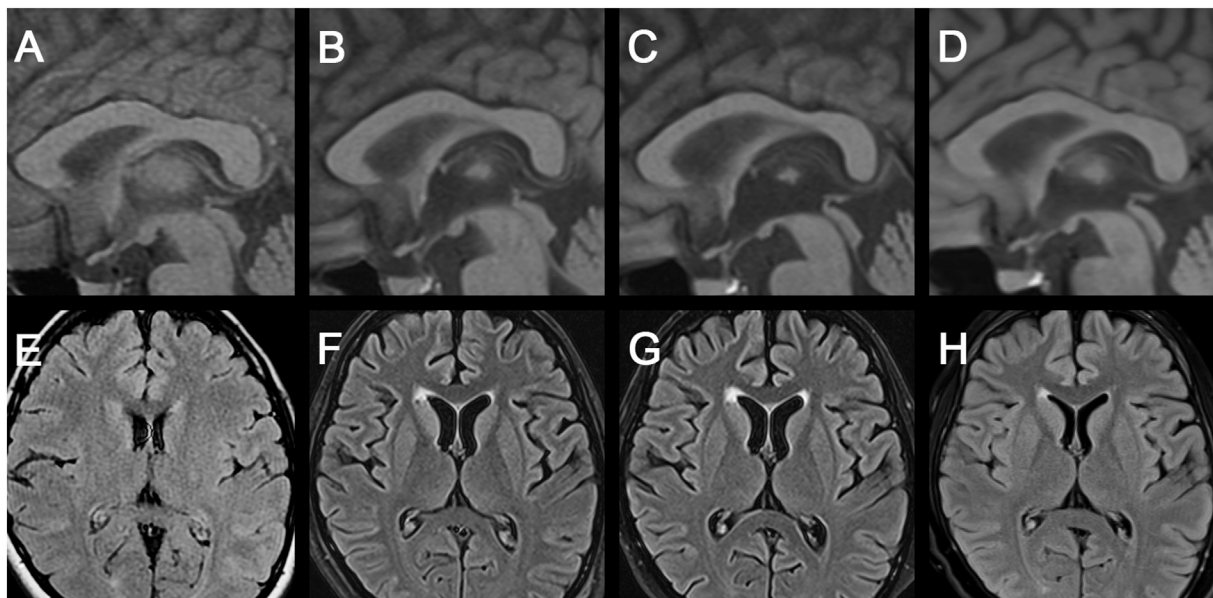


Fig. 2. T<sub>1</sub>-weighted MRI images in the upper row (A–D) show the longitudinal decline of the mid-sagittal corpus callosum area from baseline (A), after 2 months (B) and 4 months (C). Partial recovery of the corpus callosum area can be seen after convalescence, 2 years after onset (D). Corresponding axial FLAIR images in the bottom row (E–H) illustrate an overall increase of the ventricle size and width of sulci as well as increasing size of focal white matter change adjacent to the frontal horns (E–G) and a partial recovery after 2 years (H).

neurological disability in inflammatory conditions such as multiple sclerosis, supports its use also in conditions such as NMDAe (Comabella and Montalban, 2014). It should also be noted that neuropathological examinations of biopsies from three cases of NMDAe revealed little signs of neuroaxonal degeneration in comparison with other forms of autoimmune encephalitis (Bien et al., 2012). Still the levels of NFL recorded here were relatively low given the severity of the condition and in relation to what is observed in for example severe traumatic brain injury (Al Nimer et al., 2015). In contrast to the delayed peak of NFL, especially neurogranin, but also T-tau, P-Tau, sAPPa and sAPPb, peaked early in the disease course.

Taken together, it may be speculated that NMDAe initially leads to predominantly synaptic/dendritic degeneration with limited subsequent secondary nerve cell or long tract neuroaxonal injury, which could explain the relatively good prognosis in spite of the development of pronounced brain atrophy. The mainly fronto-temporal atrophy in this case also fits with the distribution pattern of NMDA receptors (Dalmau et al., 2008). Nevertheless, due to the severity of neurological deficits and substantial mortality seen in severe NMDAe, aggressive immune suppression targeting different aspects of humoral immunity is warranted. Normal CSF concentrations of S100B and YKL-40 suggest limited involvement of astroglial activation, which was an unexpected finding. Further studies are needed to replicate these findings in larger cases series.

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## Conflicts of interest

OS, MG, KB and YF do not report any conflicts of interest. FP has received compensation as chairman of the independent data monitoring committee of Sakura-Sky and Sakura-Star, two phase III trials exploring the effect of SA237 in NMO, where the sponsor is the manufacturer of tocilizumab (Chugai). HZ is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg and has served at advisory boards of Roche Diagnostics, Eli Lilly and Pharmasum Therapeutics.

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