Retrospective analysis of NMDA receptor antibodies in encephalitis of unknown origin

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ABSTRACT

Background: Anti-NMDA-receptor (NMDAR) encephalitis is a severe disorder that occurs in association with antibodies to the NR1 subunit of the NMDAR and results in a characteristic syndrome.

Objective: To determine in a single institution setting whether patients previously diagnosed with encephalitis of unknown origin had anti-NMDAR encephalitis.

Methods: Charts of 505 patients aged 18 to 35 years admitted to the intensive care unit (ICU) during a 5-year period were retrospectively reviewed for criteria of encephalitis of unknown etiology. These included encephalitic signs with psychiatric symptoms (agitation, paranoid thoughts, irritability, or hallucinations); seizures; CSF inflammation; and exclusion of viral or bacterial infection. Archived serum and CSF samples of patients fulfilling these criteria were examined for NMDAR antibodies. Follow-up visits allowed the analysis of the natural disease course and estimation of prognosis.

Results: Seven patients (all women) fulfilled the indicated criteria; 6 of them had NMDAR antibodies. Ovarian teratomas were detected in 2 patients, in one 3 years after the onset of encephalitis. Outcome was favorable in all patients. One patient without teratoma improved spontaneously along with disappearance of NMDAR antibodies.

Conclusions: Anti-NMDAR encephalitis represented 1% of all young patients' admissions to the ICU. Six of 7 cases with the indicated clinical criteria had anti-NMDAR encephalitis. NMDAR antibodies should be tested in all patients with encephalitis who fulfill these criteria. *Neurology*® 2010;75:1735–1739

GLOSSARY

ICU = intensive care unit; **IgM** = immunoglobulin M; **NMDAR** = NMDA receptor; **TPO** = thyroid peroxidase.

Anti-NMDA-receptor encephalitis is a severe disorder with characteristic clinical features including psychiatric symptoms, decreased levels of consciousness, hypoventilation, epileptic seizures, and dyskinesias.[1,2] The disease has initially been described in young women with ovarian teratoma, but is also common in women without tumor, and in men and children.[3] Diagnosis is based on highly specific autoantibodies directed against the NR1 subunit of NMDA-type glutamate receptors (NMDAR) in serum or CSF.[2] Before identification of these antibodies, patients presenting with clinical symptoms resembling anti-NMDA-receptor encephalitis were often diagnosed with encephalitis of unknown origin after exclusion of known viral or autoimmune causes.

Given its characteristic disease course, we assumed that a relevant proportion of patients previously diagnosed with encephalitis of unknown origin would have had anti-NMDAR encephalitis. We therefore conducted a retrospective study analyzing archived blood and CSF samples of young patients previously hospitalized at the intensive care unit (ICU) with encephalitis of unknown etiology. Follow-up visits of anti-NMDAR-positive patients allowed us to

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analyze the spontaneous disease course as well as antibody titers and CSF findings in a longitudinal manner.

METHODS **Patients.** From 2,990 patients hospitalized between June 2004 and June 2009 at the ICU, Department of Neurology, Charité University Hospital, Berlin, Germany, charts of all patients between 18 and 35 years of age (n = 505) were screened for the following criteria: 1) encephalitic signs with psychiatric symptoms (agitation, paranoid thoughts, irritability, or hallucinations), 2) seizures, 3) CSF inflammation, and 4) exclusion of viral/bacterial etiology. Archived CSF/serum samples (before any immunotherapy) of patients fulfilling these criteria were retrospectively analyzed for NMDAR antibodies. Seropositive patients were re-examined and tumor staging with abdominal ultrasound and body PET/MRI was performed.

Standard protocol approvals, registrations, and patient consents. The Charité University Hospital ethical committee approved the experiments and all patients gave informed written consent for research and publication.

Detection of anti-NMDAR antibodies. Plasmids containing glutamate receptor (type NMDA; subunits NR1/NR1 or NR1/NR2)[2] were transfected into HEK293 cells. Recombinant cells were used side-by-side with untransfected cells, frozen sections of rat hippocampus and cerebellum as substrates for indirect immunofluorescence. Slides were incubated with patient samples at starting dilution of 1:10 (serum) or undiluted (CSF). Quantification of intrathecal antibody synthesis was determined as described.[4] Antibody indices >4 were considered as intrathecal NMDAR-specific antibody synthesis.[4]

Infectious serology and anti-TPO antibodies. Immunoglobulin M (IgM) antibodies against viral/bacterial pathogens were determined in archived serum samples by multiparametric indirect immunofluorescence assay (Respiratory Tract Profile 1, Euroimmun, Lübeck, Germany). Antibodies against thyroid peroxidase (TPO) were determined by anti-TPO-ELISA (immunoglobulin G) (Euroimmun, Lübeck, Germany).

Western blots. Primary hippocampal neurons were generated from mice at embryonic day 16, plated on cover slips, and Western blots of membrane fractions were performed as described.[5] NMDAR-1 was detected by a monoclonal antibody (Synaptic Systems, Göttingen, Germany) using enhanced chemiluminescence. Anti-actin polyclonal antibodies were used for control (Sigma, Deisenhofen, Germany).

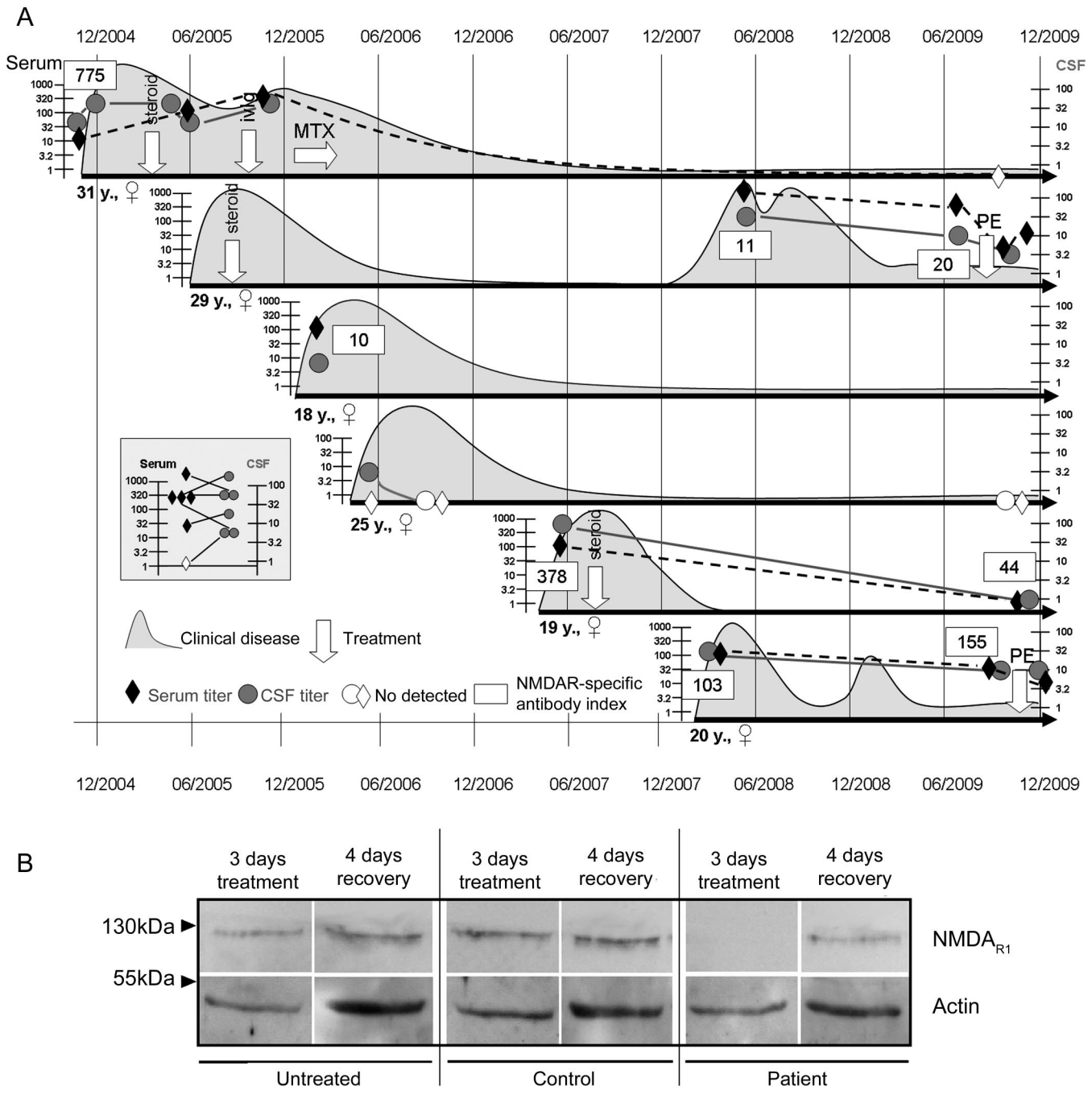
RESULTS **Retrospective analysis of patients with encephalitis.** Seven patients (all women) were identified using the abovementioned clinical criteria. Of these, 6 (median 22.5 years, range 18–31 years) were retrospectively tested positive for anti-NMDAR antibodies (figure, A). Incubation of hippocampal cell cultures with patient serum resulted in downregulation of NMDA receptors (figure, B), demonstrating the pathogenic potential of the antibodies.[6] All patients had initially presented with psychiatric symptoms (table). Prodromal symptoms and dyskinesias were found in 5 patients each. Three patients showed autonomic instability and 4 had brain MRI abnormalities. All showed slow EEG activity and CSF abnormalities. High CSF/serum antibody indices reflected intrathecal synthesis of anti-NMDAR antibodies during acute encephalitis (figure, A). Three patients received supportive treatment only. The other 3 were treated with methylprednisolone; additionally, 1 patient was given IV immunoglobulins. In this patient, therapy was further escalated with weekly methotrexate. No patient had a tumor at presentation.

Clinical outcome and laboratory findings at follow-up. At re-examination, all patients had improved, although 5 of 6 retained clinical deficits. Considering the overall disease course, 3 patients had relapses of encephalitis 6, 12, or 33 months after initial presentation. An ovarian teratoma had been removed in 1 patient in the meantime. Anti-NMDAR antibodies were still detected in serum and CSF of 3 patients. In 2 of them, protracted symptoms and relapses prompted us to perform plasma exchange. In the third patient, low anti-NMDAR-antibody titers were still present despite full clinical recovery. In this patient, however, tumor staging at follow-up revealed a hitherto unknown teratoma. In contrast, antibodies disappeared in 2 patients, 1 with a spontaneous course not receiving any immunomodulatory treatment (CSF and serum) and the other after methotrexate therapy (only serum tested at follow-up). The spontaneous improvement in 1 patient was paralleled by detection of the anti-NMDAR antibody in CSF only, and disappearance in later control samples.

DISCUSSION Our study demonstrates that anti-NMDAR encephalitis is a frequent disorder among young ICU patients with encephalitis of unknown etiology who fulfill the indicated set of criteria. Given that NMDAR antibodies can disappear after the acute phase of the disease, as occurred in patient 4, it is reasonable to speculate that the seventh patient of this series might as well have had anti-NMDAR encephalitis, but antibodies already faded away. Moreover, we might have overlooked other cases due to stringent inclusion criteria.

Follow-up visits exhibited a self-limited disease course in 1 patient without any immunomodulatory treatment. Concomitant with a spontaneous disappearance of antibodies, the patient retained only very slight changes of attention, suggestive of a good clinical prognosis. On the other hand, 1 patient received steroids and IVIg but improved only after methotrexate therapy, together with disappearance of antibodies. This case argues for a benefit of early aggressive immunotherapy, possibly with drug action also in the CNS compartment. To our knowledge, methotrexate has not been used in anti-NMDAR en-

Figure Clinical characteristics, anti-NMDA receptor antibody titers, immunomodulatory treatment, and pathogenic effect of antibodies



(A) Disease course, immunomodulatory treatments, and autoantibody titers in patients retrospectively diagnosed with anti-NMDA-receptor encephalitis. Clinical disease is defined as acute onset or relapse of psychiatric symptoms, seizures, disturbed consciousness, and movement disorders. High CSF/serum indices reflect intrathecal synthesis of anti-NMDAR antibodies. Insert: Titers of serum and CSF antibodies were highly variable in all 6 patients at clinical presentation in our hospital. In patient 2, presentation was considered as relapse based on retrospective information about the previous clinical course. Note the different scales of the y axes for serum and CSF. (B) Pathogenic effect of anti-NMDAR antibodies from patient serum. Representative Western blots showing downregulation of NMDA receptors in hippocampal cell cultures after 3 days of incubation with patient serum (dilution 1:100, right), but not with serum of healthy controls (1:100, middle) or in untreated cultures (left). The reduction in NMDA receptors is transient as a further 4 days of recovery (incubation with growth medium only) largely restores the protein expression, demonstrating that antibodies against NMDAR are pathogenic. Actin serves as loading control. IVIg = IV immunoglobulin; MTX = methotrexate; PE = plasma exchange.

cephalitis previously and may therefore be considered as a therapeutic alternative.

Neoplasms occur age-dependently in 9%[3] to 60%[2] of patients, mostly ovarian teratoma. In the current series, tumor detection 3 years after encephalitis in 1 patient with low antibody titers and complete clinical remission underlines the importance of thorough follow-up. In another patient, bilaterally

Neurology 75 November 9, 2010

Table Clinical and demographic data

Symptoms	Patient 1, F, 31 y	Patient 2, F, 29 y	Patient 3, F, 18 y	Patient 4, F, 25 y	Patient 5, F, 19 y	Patient 6, F, 20 y
Prodromal symptoms	NK	Signs of respiratory infection	Subfebrile temperature	Infection of upper respiratory tract, subfebrile temperature	Earache	GI infection 4 weeks before
Psychiatric symptoms	Catatonic-like state, auditory hallucinations	Catatonia, anxiety, confabulation, mutism, flat affect and disorganized thinking, diminished responses to pain, agitation	Delusions, disorganized speech, optic hallucinations, mutism, agitation	Catatonic-like state, suicidal thoughts, anxiety, exhausted, agitation, hallucinations	Anxiety, disorientation, diminished responses to pain, periods of akinesis alternating with agitation	Disorientation, irritability, agitation, screaming, hypoactivity, confusion
Movement disorder	Dyskinesias of hands (complex movements)	Dystonia, orofacial dyskinesias	Orofacial dyskinesias, complex movements with extremities	Orofacial dyskinesias, increased muscle tone	Choreoathetoid movements with extremities	NK
Sleep disorder	Sleep dysfunction	Inversion of sleep pattern	NK	Insomnia	NK	Inversion of sleep pattern
Autonomic dysfunction	NK	Hypoventilation, blood pressure instability	Cardiac dysrhythmia, hypoventilation	NK	Cardiac dysrhythmia, high temperature	NK
Seizures	Partial complex	Generalized tonic-clonic, secondary generalized seizures	Partial complex	Partial complex	Generalized tonic-clonic, status epilepticus	Generalized tonic-clonic
Working diagnosis	Hashimoto encephalopathy	EBV-associated encephalitis	Encephalitis of unknown origin	Encephalitis of unknown origin	Encephalitis of unknown origin	THC-induced psychosis, enterovirus-associated encephalitis
Infectious serology (IgM)	Influenza virus B, *Chlamydia pneumoniae*	*Chlamydia pneumoniae*, *Legionella pneumophila* 12	Influenza virus A and B	Influenza virus A, *Bordetella pertussis*, *Legionella pneumophila* 1, 12	*Legionella pneumophila* 12	*Bordetella pertussis* and parapertussis, *Chlamydia pneumoniae*
Other	Slight hemiparesis, TPO antibodies	Deep venous thrombosis	High levels of serum CK	High levels of serum CK, deep venous thrombosis	High levels of serum CK	TPO antibodies
Diagnostic workup						
CSF at presentation	Lymphocytic pleocytosis (13 leukocytes[a]), oligoclonal bands, protein[b] 14.8 mg/dL	Lymphocytic pleocytosis (63 leukocytes[a]), oligoclonal bands, protein[b] 32.0 mg/dL	Lymphocytic pleocytosis (97 leukocytes[a]), oligoclonal bands, protein[b] 56.4 mg/dL	Lymphocytic pleocytosis (109 leukocytes[a]), oligoclonal bands, protein[b] 73.2 mg/dL	Lymphocytic pleocytosis (13 leukocytes[a]), no oligoclonal bands, protein[b] 39.8 mg/dL	Lymphocytic pleocytosis (234 leukocytes[a]), oligoclonal bands, protein[b] 44.7 mg/dL
CSF follow-up	Not done	Normal	Not done	Oligoclonal bands	Normal	Oligoclonal bands
MRI	Multifocal white matter changes	Periventricular hyperintensity, abnormality parietal/occipital cortex	Slight meningeal contrast enhancement	Normal	Subtle gray matter enhancement in medial temporal lobes and gyrus cinguli	Normal
EEG	Slow activity	Slow activity	Slow activity	Slow activity	Marked slowing and epileptic activity	Intermittent slowing
Tumor	No	No	Yes (teratoma)	No (enlarged ovaries at initial body CT)	Yes (teratoma), at follow-up	No
Initial treatment	Methylprednisolone, IVIg, methotrexate	Prednisolone (without effect)	Supportive care, tumor resection	Supportive care	Methylprednisolone (without effect)	Supportive care
Outcome						
Follow-up, mo	63	58	50	48	37	26
Relapses	After 6 months: agitation, depression, behavioral disinhibition, sleep dysfunction	After 33 months: attention deficit, optical hallucinations, decreased consciousness	No	No	No	After 1 y: optic/tactile hallucinations, manic-depressive episodes
Outcome	Cognitive impairment, deficits of attention, disinhibition, impulsivity	Signs of frontal lobe dysfunction including poor attention and planning	Cognitive impairment, deficits of attention	Memory loss, inattention, emotional lability, mild stable deficits	Complete recovery: no neuropsychological deficit, above-average cognitive functions	Poor attention and planning, impulsivity, behavioral disinhibition

Abbreviations: CK = creatine kinase; EBV = Epstein-Barr virus; GI = gastrointestinal; IgM = immunoglobulin M; IVIg = IV immunoglobulin; NK = not known; THC = tetrahydrocannabinol; TPO = thyroid peroxidase.

[a] Normal $<5/\mu$L.

[b] Normal <45 mg/dL.

enlarged ovaries were found during encephalitis while body MRI was normal after clinical remission 2 years later. Whether this represents an effective antitumor immune response is unclear, but this has been reported in other paraneoplastic disorders.[7]

In patients without tumors, the mechanisms by which anti-NMDAR antibodies are induced remain unclear. Recently, serologic evidence of acute *Mycoplasma pneumoniae* infection has been associated with anti-NMDAR encephalitis.[8] Interestingly, all patients of this study showed IgM antibodies against respiratory tract pathogens at disease onset (table). Anti-TPO antibodies were detected in serum samples of 2 patients; 1 had initially been diagnosed with Hashimoto encephalopathy. Likewise, concomitant immune reactions against intracellular proteins have also been described in other forms of limbic encephalitis.[9] Levels of serum creatine kinase were elevated in 50% of patients. This observation is of particular interest since some patients with anti-NMDAR encephalitis were initially diagnosed with neuroleptic malignant syndrome based on elevated creatine kinase. Finally, our study demonstrates that analysis of CSF is always required in addition to serum as exclusive intrathecal synthesis of anti-NMDAR antibodies exists.

DISCLOSURE

Dr. Prüss reports no disclosures. Dr. Dalmau has received royalties from a patent re: Ma2 autoantibody test and has patents pending re: NMDA and $GABA_B$ receptor autoantibody tests (license fee payments received from EUROIMMUN AG); and receives research support from funding from EUROIMMUN AG and the NIH/NCI [RO1CA107192 (PI) and RO1CA89054-06A2 (PI)]. Dr. Harms, Dr. Höltje, and Dr. Ahnert-Hilger report no disclosures. K. Borowski is a full-time employee of EUROIMMUN AG. Dr. Stoecker is a full-time employee of and holds stock in EUROIMMUN AG. Dr. Wandinger is a full-time employee of and holds stock in EUROIMMUN AG.

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