

# Diagnostic Value of N-methyl-D-aspartate Receptor Antibodies in Women With New-Onset Epilepsy

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**Background:** In women younger than 45 years, a new form of encephalitis associated with ovarian teratoma and presenting with seizures and psychiatric symptoms has been described. Most patients have antibodies to NR1/NR2 heteromers of the N-methyl-D-aspartate receptor (NMDAR).

**Objective:** To assess the frequency and significance of antibodies to NMDAR in otherwise unexplained new-onset epilepsies in young women.

**Design:** Prospective cohort study.

**Setting:** University department of epileptology.

**Patients:** From January 1, 2005, to June 30, 2007, we identified 19 female patients aged 15 to 45 years with unexplained new-onset epilepsy. In addition, we studied 61 cerebrospinal fluid–serum sample pairs from patients with other cryptogenic epilepsies and 11 cerebrospinal fluid–serum sample pairs from surgically treated patients with epilepsy with no evident encephalitic abnormalities.

**Main Outcome Measures:** Antibodies to NMDAR and characteristics of affected patients.

**Results:** Five of the 19 patients had antibodies against NMDAR. These patients had diffuse cerebral dysfunction and seizure origins. Psychiatric symptoms and pleocytosis were significantly associated with this group of patients. The disease course was episodic, in part relapsing-remitting, with full recoveries either spontaneously or after corticosteroid or intravenous immunoglobulin treatments. Only 1 patient had a neoplasm (multiple neuroendocrine tumors that included the ovaries) identified to date. In the control series, one 22-year-old man with a cryptogenic, severely encephalopathic seizure disorder was NMDAR antibody positive, and he also recovered fully.

**Conclusions:** Anti-NMDAR encephalitis accounts for a relevant proportion of otherwise unexplained new-onset epilepsies. Patients harboring NMDAR antibodies usually have prominent psychiatric symptoms and pleocytosis, and they may develop hypoventilation. Anti-NMDAR encephalitis is not always paraneoplastic.

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A NEW TYPE OF ENCEPHALITIS in female patients younger than 45 years has recently been described.<sup>1</sup> The associated syndrome is characterized by an acute organic psychiatric disorder, seizures, dyskinesias, autonomic instability, abnormal cardiac conduction, a decreased level of consciousness, and central hypoventilation, suggesting diffuse brain dysfunction in most patients.<sup>1,2</sup> Many patients

tumor removal and subsequent immunotherapy.<sup>1</sup> This distinguishes this encephalitis from paraneoplastic encephalitis with “well-characterized” onconeural antibodies<sup>5</sup> directed to intracellular antigens.<sup>6</sup>

In a series of 100 patients, 76 had seizures.<sup>7</sup> We, therefore, wondered whether anti-NMDAR antibodies might contribute to the classification and management of otherwise unexplained new-onset epilepsies in young women.

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have an ovarian teratoma, but the disorder may occur without tumor association.<sup>3,4</sup> The target epitopes are contained in NR1/NR2 heteromers of the N-methyl-D-aspartate receptor (NMDAR).<sup>1</sup> Despite severe and potentially lethal symptoms, most patients experience remarkable improvement after

## METHODS

### PATIENTS

We reviewed information from all 847 female inpatients aged 15 to 45 years studied in the Department of Epileptology, University of Bonn, between January 1, 2005, and June 30, 2007. We selected those with unexplained new-onset epilepsy (ie, those who had recurrent seizures starting in the past 5 years with neither an obvious provoking factor nor an apparent

remote origin, such as a brain malformation or tumor, trauma, central nervous system infection, or idiopathic generalized epilepsy).

Studies included medical history, physical examination, interictal electroencephalography (EEG), cerebral magnetic resonance imaging (MRI), tumor search, routine blood and cerebrospinal fluid (CSF) tests (cell count, protein content, oligoclonal bands, albumin CSF to serum ratio, and IgG CSF to serum ratio for the determination of blood-brain barrier disturbance and intrathecal IgG synthesis according to Reiber<sup>8</sup>), and studies for the most common neurotropic agents: varicella-zoster virus in 19 patients (as assessed by polymerase chain reaction [PCR] and antibodies), herpes simplex virus in 18 (as assessed by PCR and antibodies), human herpesvirus 6 in 14 (as assessed by PCR and antibodies), cytomegalovirus in 10 (as assessed by PCR and antibodies), measles virus in 12 (as assessed by PCR and antibodies), enterovirus in 2 (as assessed by PCR and antibodies), spring summer meningoencephalitis in 11 (as assessed by antibodies), *Treponema pallidum* in 10 (as assessed by *Treponema pallidum* particle agglutination assay), and *Borrelia burgdorferi* in 12 (as assessed by antibodies).

### IMMUNOHISTOCHEMICAL ANALYSIS FOR THE DETECTION OF NEURONAL ANTIBODIES

Indirect immunohistochemical analysis was used to screen for autoantibodies in serum and CSF by one of us (C.G.B.). Samples were stored at  $-20^{\circ}\text{C}$  until testing. Testing was performed on sections of brains obtained from 28-day-old male Wistar rats (Charles River WIGA Deutschland, Sulzbach, Germany). Rats were anesthetized and perfused transcardially with 4% paraformaldehyde. The brains were removed and kept for 4 hours in 2% paraformaldehyde, then in 20% sucrose overnight at  $4^{\circ}\text{C}$  and subsequently for approximately 3 days in 30% sucrose. They were slowly frozen over isopentane and liquid ammonia and were stored at  $-80^{\circ}\text{C}$ . Seven-micrometer-thick sections were cut and mounted on glass slides. For use, they were defrosted and washed in 0.1M phosphate-buffered saline (PBS). As a blocking step, sections were incubated for 30 minutes at room temperature in 10% fetal calf serum in wash buffer (Dako, Hamburg, Germany). Patient serum was applied for 3 hours at  $37^{\circ}\text{C}$  at a 1:500 dilution in the same blocking solution; CSF was used undiluted. After thorough washing in PBS, the sections were incubated for 30 minutes at room temperature with biotinylated anti-human IgG (sheep antibody, Amersham Pharmacia Biotech, Uppsala, Sweden) at a dilution of 1:200 in 10% fetal calf serum in wash buffer. After thorough washing in PBS, avidin peroxidase (Sigma-Aldrich Corp, St Louis, Missouri) at a 1:100 dilution was applied for 30 minutes at room temperature. After another thorough washing step in PBS, labeling was visualized with 3,3'-diaminobenzidine-tetrahydrochloride (Sigma-Aldrich Corp). Sections were counterstained with hemalum. Using this assay, antibodies to hippocampal neuropil,<sup>9</sup> voltage-gated potassium channel (VGKC) antibodies at high titers,<sup>10</sup> onconeural antibodies,<sup>11</sup> and glutamic acid decarboxylase (GAD) antibodies<sup>12,13</sup> can be visualized.

### OTHER ANTIBODY TESTS

To confirm positive findings, serum samples were tested by means of radioimmunoprecipitation assay for antibodies to VGKC (A.V.) (values of 100-400 pmol/L were regarded as low positive, and values  $>400$  pmol/L as high positive<sup>10,14,15</sup>), antibodies to anti-thyroid peroxidase (TPO) (run by the Department of Nuclear Medicine, University of Bonn; reference range,  $<40$  U/mL), and antibodies to GAD (commercially performed by Labor Limbach, Heidelberg, Germany; reference range,  $<0.6$  U/mL; "neurologic range,"  $>70$  U/mL). All serum and CSF samples (not only

those positive on the immunohistochemical screening test described previously herein) from the women with new-onset epilepsies were also tested for antibodies to NMDAR using immunocytochemical analysis on cultures of neurons (to demonstrate cell surface epitopes) and cells specifically transfected with NR1/NR2 heteromers, as reported previously.<sup>1</sup>

### CONTROL SERIES

Sixty-one patients (24 females) older than 15 years (mean [SD] age, 55 [16] years) with unexplained new-onset epilepsy ("cryptogenic epilepsies"<sup>16</sup>) (mean [SD] disease duration, 1.6 [1.3] years) presenting in the same period (all of them  $>45$  years old at onset) underwent CSF and serum studies for routine investigation. They formed control group 1.

Eleven patients with epilepsy (4 females) treated surgically for pharmacoresistant epilepsy with noninflammatory histopathologic findings (hippocampal sclerosis, 4 patients; tumor, 5 patients; dysplasia, 1 patient; and nonspecific, 1 patient; patients' mean [SD] age, 46 [9] years) underwent CSF and serum studies. They formed control group 2.

Serum samples from the control patients were tested by means of immunohistochemical analysis (as described previously herein) for antibodies against the neuropil of hippocampus and, if positive, were further studied using specific tests (NMDAR and GAD antibodies); VGKC antibodies were determined in all of them by means of radioimmunoprecipitation assay.

### STATISTICS

For nominal data, Fisher 2-sided exact tests, and for metric data, 2-sided Mann-Whitney tests, were applied (SPSS 14.0; SPSS Inc, Chicago, Illinois).

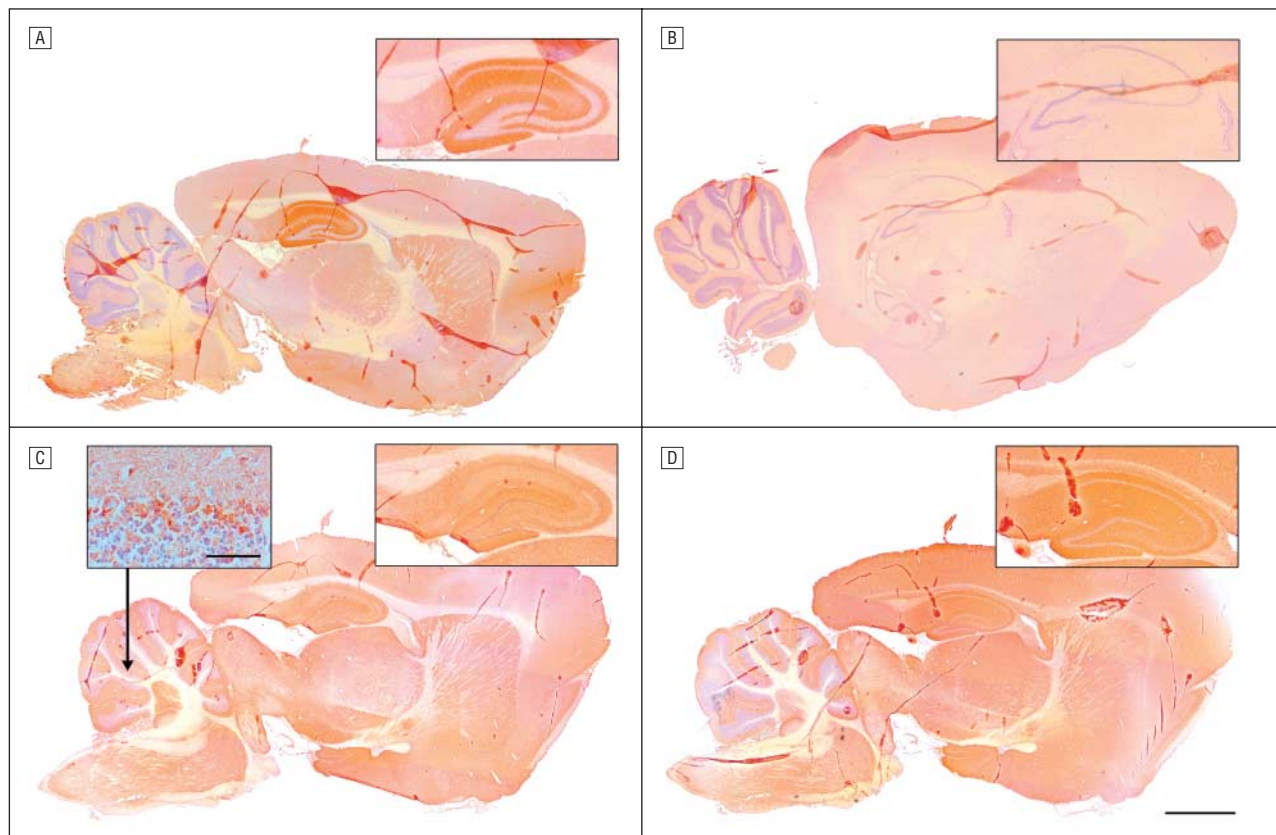
## RESULTS

Nineteen female inpatients aged 15 to 45 years studied during the 30-month period had otherwise unexplained new-onset epilepsy. The remaining 828 female inpatients during this period had chronic epilepsy with a history longer than 5 years (as is commonly found at tertiary care centers), had a distinct lesional epilepsy cause, or were outside the indicated age range. The mean (SD) age of the 19 study patients at assessment was 26 (9) years (range, 16-44 years). At the time of assessment, the mean (SD) disease duration was 1.5 (1.2) years (range, 0.1-4.8 years).

### ANTIBODIES

#### New-Onset Epilepsy Without Obvious Origin

Antibodies reacting with the neuropil of hippocampus were identified in 5 of 19 patients' undiluted CSF; the serum samples (at a 1:500 dilution) of these patients were negative by means of immunohistochemical analysis. Specific tests with NR1/NR2 heteromers of the NMDAR confirmed the presence of these antibodies in all the patients' serum and CSF samples. In all cases using equivalent amounts of serum and CSF IgG, the reactivity was substantially higher in CSF, compatible with intrathecal synthesis of anti-NMDAR antibodies (data not shown). **Figure 1** shows the antibody reactivity compared with other antibodies that also immunolabel the neuropil of hippocampus. The NMDAR antibodies predominantly bind to the hippocampus, as previously reported.<sup>1</sup>



**Figure 1.** Indirect immunohistochemical analyses using undiluted cerebrospinal fluid on perfused, sagittally sectioned rat brain. A, Antibodies to *N*-methyl-D-aspartate receptor (patient 4). B, Negative control. C, Glutamic acid decarboxylase antibodies: diffuse labeling pattern plus perisomatic staining of the Purkinje cells extending into adjacent layers (cerebellar inset, bar=50  $\mu$ m). D, Voltage-gated potassium channel antibodies (diffuse staining). The insets (upper right-hand corners) show the hippocampi. Bar=2 mm and is valid for parts A through D.

Of the 14 patients negative for NMDAR antibodies, 3 were positive for GAD antibodies by means of immunohistochemical analysis and radioimmunoprecipitation assay (titers, 100–200 U/mL), all with clinical and neuroradiologic features reminiscent of limbic encephalitis.<sup>13</sup> One patient had MRI-negative extratemporal epilepsy with cognitive impairment and was strongly positive for TPO antibodies (2826 U/L) and at the same time was low positive for VGKC antibodies (143 pmol/L) and very low positive for GAD antibodies (3.2 U/mL on radioimmunoprecipitation assay and negative on immunohistochemical analysis). She responded to corticosteroids suggesting a steroid-responsive encephalopathy associated with autoimmune thyroiditis (SREAT).<sup>17</sup> Another 2 patients also had MRI-negative epilepsies with psychiatric symptoms and elevated anti-TPO antibody titers but were not treated with corticosteroids. Therefore, a diagnosis of SREAT cannot be made. All serum samples were negative for onconeural antibodies. There was no case of viral or paraneoplastic encephalitis.

### Control Cohorts

In the cryptogenic epilepsy control series, antibodies to hippocampal neuropil were found by means of immunohistochemical analysis in 2 patients (CSF in 1 and serum in 1, but only the CSF antibodies were reacting with NMDAR). The sample came from a 22-year-old man with a few weeks of temporal lobe epilepsy (TLE), confusion, delusions, and, finally, stupor. Brain MRI findings, standard CSF vari-

ables, and a tumor search (thoracoabdominal computed tomography [CT]) were unremarkable, but TPO antibody levels were abnormal at 939 U/L. The patient recovered completely while taking high-dose oral corticosteroids. The serum sample positive for antibodies to hippocampal neuropil but negative for NMDAR antibodies came from a 69-year-old man with remote posterior cerebral artery infarction; it remained unclear whether his recent-onset epilepsy was related to this posts ischemic lesion. Other antibodies detected in the cryptogenic epilepsy cohort were reacting with VGKC (6 patients, 5 of whom fulfilled the clinical criteria for limbic encephalitis).<sup>10</sup> The 3 samples with titers greater than 1000 pmol/L reacted on immunohistochemical testing with rat brain neuropil in a diffuse manner, not restricted to the hippocampus; the 3 samples with titers less than 1000 pmol/L were negative by immunohistochemical analysis. In the epilepsy surgery control series, one 29-year-old man with a 7-year history of TLE and a histopathologic diagnosis of hippocampal sclerosis had GAD antibodies (110 U/mL).

### Final Diagnoses and Results of Tumor Searches

In the 6 patients who were positive for NMDAR antibodies (including the male patient from the control series), no alternative diagnosis to “anti-NMDAR encephalitis” could be established. In patient 4, multiple neuroendocrine tumors were already known and treated at manifestation of the neurologic syndrome and subsequent dem-





tent: chest radiograph; abdominal ultrasound; and gynecologic examination, including transvaginal ultrasound; 7 had thoracic and abdominal CT with contrast enhancement and 3 had whole-body positron emission tomography with CT co-registration). No tumors were found.

## CLINICAL FEATURES AND PARACLINICAL FINDINGS

### Disease Duration

The 5 NMDAR antibody-positive patients had a shorter mean (SD) disease duration at assessment than the other 14 young women with recent-onset epilepsies (5 [4] months vs 22 [15] months,  $P=.02$ ).

### The Epilepsies

Semiologic features and interictal EEG findings suggested extratemporal epilepsies in all NMDAR antibody-positive patients, whereas, in the other patients, TLE was diagnosed in 10 patients and extratemporal epilepsy in 4 ( $P=.01$ ).

### The Neuropsychiatric Syndrome

Four of the 5 NMDAR antibody-positive patients had prominent psychiatric signs and symptoms. Such features were present in only 2 of the antibody-negative patients ( $P=.02$ ). Other neurologic signs were speech dysfunction and a decreased level of consciousness. Features that suggest involvement of subcortical central nervous system structures were present in 2 antibody-positive patients (nystagmus, dyskinesias, dystonia, and hypoventilation) and in 2 antibody-negative patients (dyskinesias). The courses of the 5 antibody-positive patients (mean follow-up, 26 months; range, 15-36 months) are depicted in **Figure 2**. Two patients had a relapsing-remitting course with full recovery between disease episodes. The other 3 patients had only 1 disease episode. Altogether, the 5 patients had 10 disease episodes. Four patients had recovered completely at the most recent follow-up. The remaining patient with left temporal EEG abnormalities improved slowly but had a residual speech problem at the most recent follow-up, which was potentially aggravated by topiramate therapy.<sup>21</sup> For a summary, see **Table 1**.

### Laboratory Findings

These are summarized in **Table 2**. Patients positive for NMDAR antibodies more frequently had an elevated CSF cell count than did antibody-negative patients (5 of 5 vs 2 of 14,  $P=.02$ ).

### Response to Treatment

All NMDAR antibody-positive patients were started on antiepileptic medication in the first 2 months of epilepsy onset. Despite that, patients 2 and 5 had seizure relapses. Patients 2, 3, and 5 received corticosteroids or monthly intravenous immunoglobulins. None relapsed while undergoing immunotherapies. On the other hand, patients 1 and 4 were relapse free without ever receiving immunotreatment.

As many as 5 of 19 young women with otherwise unexplained new-onset epilepsies, identified during a 30-month period in a single institution had anti-NMDAR encephalitis. In 4 of these 5 patients, no tumor was found despite thorough investigations (note, however, that follow-up may have been too short to rule out a tumor in all patients). The same holds true for the additional NMDAR antibody-positive male patient from the cryptogenic epilepsy control series.

All the patients share many similarities with those found in the largest available series.<sup>7</sup> Our patients had a diffuse central nervous system syndrome rather than one with a clear accentuation of temporal lobe dysfunction (compare with limbic encephalitis); none of our patients had temporomedial MRI abnormalities, and there was CSF pleocytosis. The high frequency of acute psychiatric features was striking in these patients, and it distinguished them from antibody-negative patients. Our patients had a favorable outcome, but this did not necessarily depend on tumor removal or immunotherapy, which also confirms previous study findings.<sup>1,3</sup> Neuropathological findings in brain biopsy specimens were without distinctive parenchymal inflammatory changes, as in the previous study.<sup>1</sup>

The present study, however, extends previous observations. To our knowledge, this is the first prospective series of patients with new-onset epilepsy tested for antibody positivity. The single patient with neoplastic disease did not have an ovarian teratoma but she did have multiple neuroendocrine tumors with NMDAR expression. Functional ovarian cysts in this age group are sometimes not easily differentiated from tumor cysts; therefore, follow-up investigations in doubtful cases could be preferable to immediate surgical interventions. In the cryptogenic control series, only 39% were women, which may diminish its power. However, 1 additional NMDAR antibody-positive patient was detected in this control series (and none among the 11 surgically treated patients with well-defined, focal, noninflammatory epilepsy): a 22-year-old man without evidence of tumor but an otherwise typical syndrome (similar to a previously reported male patient).<sup>4</sup> This additional patient also harbored TPO antibodies, and his disorder was initially classified as SREAT.<sup>17</sup> This diagnosis can now be replaced by the more specific diagnosis of anti-NMDAR encephalitis (an example for the expected reclassification of patients with SREAT into more specific immunologic syndromes due to identification of a new diagnostic category with likely uniform pathogenesis<sup>22</sup>). Samples from 7 patients (5 from the core group and 2 from the control groups) contained antibodies to hippocampal neuropil, as evident by immunohistochemical analysis, but only 6 were NMDAR antibody positive. This shows that some antibodies to hippocampal neuropil react with different (unknown) antigens. On the other hand, in the core study group of 19 otherwise unexplained new-onset epilepsies in young females, no NMDAR antibody positivity was missed by immunohistochemical screening using CSF.

Several questions remain unanswered. Whereas classic paraneoplastic encephalitis results in tissue loss and irre-

**Table 1. Demographic, Clinical, EEG, MRI, and Tumor Search Data for the 6 Anti-NMDAR-Positive Patients**

Patient No.	Age, y	Disease Duration, y	Seizures	EEG			Temporal/Extratemporal Epilepsy	Neurologic Signs and Symptoms	Psychiatric Signs and Symptoms	MRI Findings	Immunotherapy <sup>a</sup>
				Background Rhythm	Slowing	Epileptiform Activity					
1	18	0.3	3 SGTCSs	Alpha	Bitemp theta	Gen spike-wave paroxysms	Extratemporal	None	None	Normal	None
2	22	0.7	R-sided motor SPS, SGTCS	Alpha	L hem theta-delta	None	Extratemporal	Aphasia	Depression, hallucinations	Nonspecific hyperintense WM spots	Corticosteroid pulses, 3-5 g of IV-MP/mo IVIG, 2 g/kg bw/mo
3	24	0.3	CPS, R-sided motor SPS	Alpha	L temp periodic delta	None	Extratemporal	Aphasia, Babinski sign R side positive, hypoventilation	Pathologic crying and laughter, grimacing	Normal	Corticosteroid pulse, 2.5 g of IV-MP, followed by 100 mg of oral prednisone/day for 1½ mo; thereafter, IVIG, 0.4 g/kg bw/mo
4	44	0.1	CPS	Alpha	R hem delta	L temp sharp waves	Extratemporal (+temporal?)	Disturbed vision, dysesthesia L side of body	Delusions, agitation, depression	Normal	None
5	15	0.9	Head version to the R, SGTCS	Theta	Gen theta	R par spikes; subsequently, R hem status	Extratemporal	Stupor, rotatory/downbeat nystagmus, facial dyskinesias, hypoventilation	Paranoia, hallucinations, pathologic crying and laughter	Status-related regressive bilateral cortical signal increase	2nd episode: corticosteroid pulses, 10 g of IV-MP/mo, slow oral corticosteroid tapering 3rd episode: 100 mg/d of slow oral prednisone, tapering
Male patient from control series	22	0.5	CPS	Theta	Gen theta	None	Temporal + extratemporal	Aphasia, mild hemiparesis R side	Lability of mood, pathologic laughter, agitation at night, confusion	Nonspecific hyperintense WM spots	Oral prednisone, start with 150 mg/d, plus azathioprine, 150 mg; prednisone tapered down over 1½ y

Abbreviations: Bitemp, bitemporal; bw, body weight; CPS, complex partial seizure; EEG, electroencephalography; gen, generalized; hem, hemispheric; IVIG, intravenous immunoglobulin; IV-MP, intravenous methylprednisolone; L, left; MP, methylprednisolone; MRI, magnetic resonance imaging; NMDAR, *N*-methyl-D-aspartate receptor; par, parietal; R, right; SGTCS, secondarily generalized tonic-clonic seizure; SPS, simple partial seizure; status, status epilepticus; temp, temporal; WM, white matter.

<sup>a</sup>For the temporal details, see Figure 2.

**Table 2. Laboratory Results for the 6 Anti-NMDAR-Positive Patients**

Patient No.	Autoantibodies							Standard CSF Variables				
	CSF		Serum					WBC Count, $\mu$ L	Protein, g/dL	OCB	BBB Disturbance	Intrathecal IgG Synthesis
	IHC Antibodies to CSF Undiluted	NR1/NR2 Antibodies (CSF Titer)	IHC Antibodies to Serum 1:500 Diluted	NR1/NR2 Antibodies (Serum Titer)	VGKC, pmol/L	Onconeural, ravo blot	TPO, U/mL					
1	Hippocampal neuropil	1:40	Negative	1:50	Negative	Negative	ND	6	0.027	ND	None	None
2	Hippocampal neuropil	1:80	Negative	1:400	Negative	Negative	0	21	0.027	Negative	None	None
3	Hippocampal neuropil	1:40	Negative	1:200	Negative	Negative	13	21	0.051	Positive	None	None
4	Hippocampal neuropil	1:160	Negative	1:800	Negative	Negative	15	6	0.029	Positive	None	None
5	Hippocampal and diffuse neuropil	1:20	Hippocampal and diffuse neuropil	1:50	Negative	Negative	0	40	0.031	Positive	None	Yes
Male patient from control series	Hippocampal neuropil	1:160	Negative	1:400	Negative	Negative	939	1	0.037	ND	None	None

Abbreviations: BBB, blood-brain barrier (according to Reiber<sup>8</sup>); CSF, cerebrospinal fluid; IHC, indirect immunohistochemical analysis of sections of perfused rat brain; Intrathecal IgG synthesis, intrathecal IgG synthesis (according to Reiber<sup>8</sup>); ND, not done; NMDAR, *N*-methyl-D-aspartate receptor; OCB, unmatched oligoclonal bands in CSF; protein, protein content (reference range, <0.050 g/dL); ravo blot, immuno-dot-blot for well-characterized onconeural antibodies (see the "Methods" section); TPO, thyroid peroxidase (reference range, <40 U/mL); VGKC, voltage-gated potassium channel (reference range, <100 pmol/L; low positive, 100-400 pmol/L; high positive, >400 pmol/L); WBC, white blood cell (reference range,  $\leq 5/\mu$ L).

SI conversion factors: To convert protein to grams per liter, multiply by 10.0; WBC count to  $\times 10^9/L$ , multiply by 0.001.

versible dysfunction due to T-cell cytotoxicity,<sup>11</sup> an antibody-mediated pathogenesis seems plausible in anti-NMDAR encephalitis.<sup>7</sup> An “NMDA hypofunction hypothesis” may explain the clinical features.<sup>3,23</sup> The demonstration of neuronlike tumor cells expressing NMDAR subunits constitutes a potential (but, given the nonparaneoplastic patients, not necessary) antigenic prerequisite for generation of the antibodies. Myasthenia gravis and Lambert-Eaton myasthenic syndrome have similarities with the described disorder. Both occur in the paraneoplastic and nonparaneoplastic forms, and both are associated with antibodies to cell membrane antigens, which are involved in signal transduction.<sup>24</sup> In these disorders, too, a nondestructive (at least reversible) disease process seems to predominate. Finally, patients with myasthenia gravis and Lambert-Eaton myasthenic syndrome benefit from immunotreatment that modulates or suppresses B-cell function.<sup>25</sup> Similarly, VGKC antibodies in limbic encephalitis have been proposed to exert a functional and, thereby, potentially reversible effect on limbic neurons.<sup>10</sup> It is interesting that, even in the apparently nonparaneoplastic NMDAR antibody-related syndromes described herein, all the patients were younger than 45 years and predominantly female. This contrasts with patients who have VGKC antibodies associated with limbic encephalitis or Morvan syndrome, whose median age at the onset of symptoms is approximately 60 years, with a male to female ratio of approximately 3 to 2 (A.V., unpublished data, 2008).

Finally, there is an emerging group of patients with paraneoplastic and nonparaneoplastic autoimmune encephalitis in association with antibodies to the neurophil of hippocampus.<sup>9,26</sup> The target antigens are unknown, but by immunohistochemical analysis, some of these antibodies closely resemble NMDAR or VGKC antibodies.<sup>27</sup> Therefore, confirmation using specific tests (NR1/NR2 heteromers of the NMDAR and VGKC for immunoprecipitation) is required for appropriate diagnosis. Further investigations of the epidemiologic features, the pathogenesis, and the effective treatment of this disorder are required.

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**Author Contributions:** All authors had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. *Study concept and design:* Elger and Bien. *Acquisition of data:* Niehusmann, Dalmau, Rudlowski, Vincent, Rossi, and Bien. *Analysis and interpretation of data:* Niehusmann, Dalmau, Elger, Rossi, and Bien. *Drafting of the manuscript:* Niehusmann and Bien. *Critical revision of the manuscript for important intellectual content:* Dalmau, Vincent, Elger, Rossi, and Bien. *Statistical analysis:* Bien. *Administrative, technical, and material support:* Dalmau, Rossi, and Bien. *Study supervision:* Dalmau, Rudlowski, Vincent, Elger, Rossi, and Bien.

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## REFERENCES

1. Dalmau J, Tüzün E, Wu HY, et al. Paraneoplastic anti-N-methyl-D-aspartate receptor encephalitis associated with ovarian teratoma. *Ann Neurol*. 2007;61(1):25-36.
2. Sansing LH, Tüzün E, Ko MW, Baccon J, Lynch DR, Dalmau J. A patient with encephalitis associated with NMDA receptor antibodies. *Nat Clin Pract Neurol*. 2007;3(5):291-296.
3. Izuka T, Sakai F, Ide T, et al. Anti-NMDA receptor encephalitis in Japan: long-term outcome without tumor removal. *Neurology*. 2008;70(7):504-511.
4. Novillo-López ME, Rossi JE, Dalmau J, Masjuan J. Treatment-responsive subacute limbic encephalitis and NMDA receptor antibodies in a man. *Neurology*. 2008;70(9):728-729.
5. Graus F, Delattre JY, Antoine JC, et al. Recommended diagnostic criteria for paraneoplastic neurological syndromes. *J Neurol Neurosurg Psychiatry*. 2004;75(8):1135-1140.
6. Tüzün E, Dalmau J. Limbic encephalitis and variants: classification, diagnosis and treatment. *Neurologist*. 2007;13(5):261-271.
7. Dalmau J, Gleichman AJ, Hughes EG, et al. Anti-NMDA-receptor encephalitis. *Lancet Neurol*. 2008;7(12):1091-1108.
8. Reiber H. The discrimination between different blood-CSF barrier dysfunctions and inflammatory reactions of the CNS by a recent evaluation graph for the protein profile of cerebrospinal fluid. *J Neurol*. 1980;224(2):89-99.
9. Ances BM, Vitaliani R, Taylor RA, et al. Treatment-responsive limbic encephalitis identified by neurophil antibodies. *Brain*. 2005;128(pt 8):1764-1777.
10. Vincent A, Buckley C, Schott JM, et al. Potassium channel antibody-associated encephalopathy. *Brain*. 2004;127(pt 3):701-712.
11. Dalmau J, Rosenfeld MR. Paraneoplastic syndromes of the CNS. *Lancet Neurol*. 2008;7(4):327-340.
12. Solimena M, Folli F, Denis Donini S, et al. Autoantibodies to glutamic acid decarboxylase in a patient with stiff-man syndrome, epilepsy, and type I diabetes mellitus. *N Engl J Med*. 1988;318(16):1012-1020.
13. Saiz A, Blanco Y, Sabater L, et al. Spectrum of neurological syndromes associated with glutamic acid decarboxylase antibodies: diagnostic clues for this association. *Brain*. 2008;131(pt 10):2553-2563.
14. Hart IK, Waters C, Vincent A, et al. Autoantibodies detected to expressed K<sup>+</sup> channels are implicated in neuromyotonia. *Ann Neurol*. 1997;41(2):238-246.
15. McKnight K, Jiang Y, Hart Y, et al. Serum antibodies in epilepsy and seizure-associated disorders. *Neurology*. 2005;65(11):1730-1736.
16. Commission on Classification and Terminology of the International League Against Epilepsy. Proposal for revised classification of epilepsies and epileptic syndromes. *Epilepsia*. 1989;30(4):389-399.
17. Castillo P, Woodruff B, Caselli R, et al. Steroid-responsive encephalopathy associated with autoimmune thyroiditis. *Arch Neurol*. 2006;63(2):197-202.
18. Bauer J, Elger CE, Hans VH, et al. Astrocytes are a specific immunological target in Rasmussen's encephalitis. *Ann Neurol*. 2007;62(1):67-80.
19. Furlong MA, Fanburg-Smith JC, Miettinen M. The morphologic spectrum of hibernoma. *Am J Surg Pathol*. 2001;25(6):809-814.
20. Bien CG, Urbach H, Schramm J, et al. Limbic encephalitis as a precipitating event in adult-onset temporal lobe epilepsy. *Neurology*. 2007;69(12):1236-1244.
21. Mula M, Trimble MR, Thompson P, Sander JW. Topiramate and word-finding difficulties in patients with epilepsy. *Neurology*. 2003;60(7):1104-1107.
22. Moccillan R, Walterfang M, Velakoulis D. Hashimoto's encephalopathy: epidemiology, pathogenesis and management. *CNS Drugs*. 2007;21(10):799-811.
23. Stone JM, Morrison PD, Pilowsky LS. Glutamate and dopamine dysregulation in schizophrenia. *J Psychopharmacol*. 2007;21(4):440-452.
24. Vincent A, McConville J, Farrugia ME, et al. Antibodies in myasthenia gravis and related disorders. *Ann NY Acad Sci*. 2003;998:324-335.
25. Buckley C, Vincent A. Autoimmune channelopathies. *Nat Clin Pract Neurol*. 2005;1(1):22-33.
26. Bataller L, Kleopa KA, Wu GF, Rossi JE, Rosenfeld MR, Dalmau J. Autoimmune limbic encephalitis in 39 patients: immunophenotypes and outcomes. *J Neurol Neurosurg Psychiatry*. 2007;78(4):381-385.
27. Graus F, Saiz A, Lai M, et al. Neuronal surface antigen antibodies in limbic encephalitis: clinical-immunologic associations. *Neurology*. 2008;71(12):930-936.