ORIGINAL CONTRIBUTION

Failure of Natalizumab to Prevent Relapses in Neuromyelitis Optica

Ingo Kleiter, MD; Kerstin Hellwig, MD; Achim Berthele, MD; Tania Kümpfel, MD; Ralf A. Linker, MD; Hans-Peter Hartung, MD; Friedemann Paul, MD; Orhan Aktas, MD; for the Neuromyelitis Optica Study Group

Objective: To describe first experiences with the integrin inhibitor natalizumab, given to patients with suspected relapsing-remitting multiple sclerosis (MS) who were later diagnosed with aquaporin 4–positive neuromyelitis optica (NMO).

Design: Retrospective case series.

Setting: Neurology departments at tertiary referral centers in Germany.

Patients: Patients with NMO who tested positive for antibodies to aquaporin 4.

Intervention: Treatment with natalizumab.

Main Outcome Measures: Relapses and accumulation of disability.

Results: We identified 5 patients (4 female; median age, 45 years) who were initially diagnosed with MS and treated with natalizumab before diagnosis of NMO was established. Natalizumab was given as escalation therapy after failure of first- or second-line immunomodulatory therapies for MS. During natalizumab therapy (median duration, 8 infusions; range, 2-11 infusions), all 5 patients displayed persisting disease activity; a total of 9 relapses occurred (median duration to relapse, 120 days; range, 45-230 days) after the start of treatment. Four patients had an accumulation of disability and 1 patient died 2 months after cessation of natalizumab treatment.

Conclusions: Our results suggest that natalizumab fails to control disease activity in patients with NMO. Neuromyelitis optica should be considered as a differential diagnosis in patients with suspected MS who are unresponsive to natalizumab therapy.

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NEUROMYELITIS OPTICA (NMO) is a disabling autoimmune central nervous system (CNS) disorder with clinical attacks mainly involving the optic nerves and the spinal cord.[1,2] The detection of a serum antibody to the CNS water channel aquaporin 4 (AQP4) as a highly specific biomarker in most patients with NMO[3-5] has facilitated its distinction from multiple sclerosis (MS), which may be difficult solely on the basis of clinical and neuroradiological findings. Thus, it is conceivable that a substantial number of patients with NMO have been misdiagnosed with MS, in particular prior to the availability of AQP4 antibody testing. While first-line therapy for MS comprises disease-modifying drugs such as interferon beta and glatiramer acetate, NMO usually requires aggressive immunosuppression or a specific B-cell–targeted therapy.[6-10] Treatment options that are beneficial in MS, especially interferon beta, are of no proven efficacy or may even be harmful in NMO.[11-16]

Natalizumab is a monoclonal antibody against the adhesion molecule very late activation antigen 4, an $\alpha 4\beta 1$ integrin expressed on leukocytes, and is approved for treatment escalation in patients with relapsing-remitting MS with breakthrough disease and patients with MS with highly active disease.[17] Interestingly, natalizumab not only reduces the entry of $CD4^+$ and $CD8^+$ T lymphocytes into the CNS but also decreases the number of $CD19^+$ B cells and antibody-producing $CD138^+$ plasma cells in the cerebrospinal fluid (CSF) for at least 6 months after infusion.[18] Thus, interference with B-cell invasion into the CNS may provide an immunological rationale for evaluation of natalizumab in NMO therapy. However, clinical experience on natalizumab application in NMO is lacking to date.

Here, we describe 5 patients who were treated with natalizumab for suspected re-

Author Affiliations are listed at the end of this article.
Group Information: The Neuromyelitis Optica Study Investigators are listed at the end of this article.

lapsing-remitting MS, but were subsequently diagnosed with AQP4-positive NMO after experiencing severe relapses during natalizumab therapy.

METHODS

PATIENTS

To identify patients with NMO who were treated with natalizumab, we used the network of the German Neuromyelitis Optica Study Group (http://www.nemos-net.de). This network is a nationwide open association of neurological centers interested in NMO/NMO spectrum disorders. It collects clinical features of such patients in a retrospective and prospective fashion approved by the institutional review boards of the participating academic centers and in accordance with the German data protection law. At the time of analysis, 153 patients with NMO or NMO spectrum disorders according to the revised 2006 criteria by Wingerchuk et al[19] had been captured. In the present retrospective approach, we included all patients with confirmed NMO and IgG antibodies to AQP4 (AQP4-Abs) who had a history of treatment with natalizumab. We identified 5 patients at 4 university medical centers (Ruhr University Bochum, Ludwig-Maximilians-University Munich, Technische Universität München, Munich, and University Medical Center Regensburg). All patients had initially been diagnosed with relapsing-remitting MS, according to the McDonald criteria revised in 2005,[20] before receiving natalizumab as an escalation therapy after failure of first- or second-line MS therapies.

Medical records were retrospectively assessed for disease duration, previous treatments, total number of relapses, exacerbations before, during, and after cessation of natalizumab, disability scored by the Expanded Disability Status Scale,[21] duration until NMO diagnosis, and anti-AQP4 antibody titers. Brain and spinal cord magnetic resonance imaging (MRI) findings during and after therapy with natalizumab were reevaluated for MS- or NMO-typical lesions in the brain and spinal cord, in particular for longitudinally extensive spinal cord lesions extending over 3 vertebral segments. Furthermore, detailed clinical information was obtained with regard to the period of natalizumab treatment, cessation of natalizumab, and diagnosis of NMO. Clinical, radiological, and histopathological features of patient 5 have been described elsewhere in detail.[22]

AQP4 SEROLOGY

A recently described cell-based flow cytometry assay was used for quantification of serum AQP4-Abs by detection of the difference in median fluorescence intensity (ΔMFI).[5]

RESULTS

DEMOGRAPHICS AND CLINICAL FEATURES PRIOR TO NATALIZUMAB THERAPY

We identified 5 patients (4 female, 1 male) who had initially been treated with natalizumab for suspected relapsing-remitting MS but were subsequently diagnosed with AQP4-Abs–positive NMO (**Table**). The median disease duration at initiation of natalizumab was 9 years (range, 4-31 years) and the median age was 45 years (range, 35-56 years). All patients had experienced pronounced disease activity (median, 12 relapses; range, 6-40 relapses) prior to receiving natalizumab. Recurrent optic neuritis was the initial clinical presentation in patients 1, 2, and 4, diplopia and subsequent vomiting and dysphagia in patient 3, and recurrent myelitis in patient 5. All patients had had at least 1 episode of myelitis and 3 patients had had 1 or more unusually severe relapses, including paraparesis during pregnancy (patient 1), brainstem involvement (patient 3), and blindness and coma (patient 5). No patient had a concomitant overt autoimmune disease. The extended medical history included pyelonephritis in patient 2, elevation of transaminase levels during interferon beta therapy in patient 3, and Barrett esophagus, hepatitis C, myocardial infarction, and hypertension in patient 4.

Brain MRI performed prior to starting treatment with natalizumab showed supratentorial lesions in patients 1, 2, and 5 and had normal findings in patient 4 (Table). Patient 3 initially presented with a large bithalamic lesion extending to the mesencephalon and the brainstem. Although the distribution and appearance of the lesions were unspecific according to established criteria,[23] MRI findings were regarded to be consistent with MS by the local evaluating radiologists in 4 of 5 cases. Four patients (2-5) underwent spinal cord MRI before they started receiving natalizumab, which showed longitudinally extensive lesions in the cervical and thoracic myelon in patients 2, 3, and 4 and several small lesions in the thoracic cord in patient 5. Only patients 2 and 4 were suspected to have NMO prior to initiation of natalizumab, but this diagnosis was not favored by the subsequent treating neurologist. Cerebrospinal fluid analysis was performed in all patients. Patient 1 had persistent oligoclonal bands; patient 3 initially tested negative for oligoclonal bands but tested positive 5 years later at diagnosis of NMO. The remaining 3 patients tested positive for oligoclonal bands during relapses but negative during remissions. Testing for AQP4 was not performed when natalizumab therapy was started. For patients 3 and 4, AQP4-Ab testing was not yet available when the initial diagnosis of MS was made. For the other patients, AQP4-Ab serology was not performed because the clinical presentation was initially assessed to be compatible with MS.

Previous treatments included steroids for relapses (n=5) and azathioprine (n=1), interferon beta (n=4), mitoxantrone (n=2), and rituximab (n=1) for long-term therapy. Owing to ongoing disease activity despite previous disease-modifying therapies, all patients were switched to natalizumab therapy (300 mg intravenously every 4 weeks) as escalation therapy.

TREATMENT RESPONSE TO NATALIZUMAB

Natalizumab was given for a median of 8 infusions (range, 2-11 infusions) at monthly intervals except for 1 patient with repeated infections who received only 8 infusions over 10 months (Table). During natalizumab therapy, all 5 patients experienced at least 1 clinical relapse (**Figure**). The median time from the start of natalizumab to the first relapse was 120 days (range, 45-230 days). Two patients had 1 relapse, 2 patients had 2, and another patient had 3 relapses during natalizumab therapy. Re-

Table. Patient Characteristics, Treatment, and Clinical Course

Characteristic	Patient No. 1	2	3	4	5
Sex	F	F	F	M	F
Age, y[a]	54	41	45	56	35
Disease duration, y[a]	31	10	5	4	9
Previous treatments (duration, mo)[b]	Steroids for relapses, AZA (152), interferon beta (57)	Steroids for relapses, interferon beta (39)	Steroids for relapses, interferon beta (48)	Steroids for relapses, MIT (18; 72 mg/m^2), RIT (12)	Steroids for relapses, interferon beta (28), MIT (60; 96 mg/m^2), intrathecal steroids (12)
Previous relapses, No.	40	6	6	12	20
EDSS score prior to NAT	4.0 for 3 y	NA	NA	6.0 for 1 y	6.0 for 5 y
Relapses last year, No.[a]	3	3	3	4	3
Natalizumab infusions, No.	8	11	2	8[c]	4
Relapses during NAT, No.[d]	2	3	1	2	1
Relapses in first year after NAT	2	3	0	1	1
EDSS score at start of NAT	4.0	1.0	1.5	6.0	7.5
EDSS score at end of NAT	8.5	3.0	1.5	7.0	9.0
Duration until NMO diagnosis, mo[a]	16	10	2	13	4
AQP4-IgG titer, ΔMFI (time after NAT, mo)	850 (9)	4023 (0)	935 (1), 1618 (4)	2032 (2)	1367 (1)
Cerebral MRI findings	Large symmetric bihemispheric WML before NAT, new supratentorial cystic lesions during NAT	Some unspecific WML before NAT	Initially bithalamic lesion reaching to the mesencephalon, subsequently increasing periventricular WML before NAT; 1 Gd+ lesion after NAT	No lesions before NAT	Periventricular lesions before NAT, large necrotic occipital lobe lesion and brainstem involvement during NAT
Spinal MRI findings	Longitudinal lesion from C2-Th7 after NAT, Gd+	Transient longitudinal cervical/thoracic lesion before NAT, new longitudinal lesions C3-C7 and Th3-Th7 during NAT, Gd+	Longitudinal lesion in the medulla oblongata extending to C2 before NAT; longitudinal lesion Th1-Th3 after NAT, Gd–	Longitudinal lesions Th4-Th6 and C4-C6 before NAT, new longitudinal lesion from Th2-Th6 during NAT, Gd+	Small thoracic lesions before NAT, new longitudinal lesion from C2-Th1 during NAT, Gd+
Subsequent treatments (duration, mo)[b]	Steroids, PE, AZA (18)	PE, RIT (3), CTX (3), RIT + ALEM (12)	RIT (16)	Steroids, PE, RIT (12)	Steroids, PE
EDSS score at last visit (time after NAT, mo)	6.0 (23)	5.5 (18)	2.0 (18)	7.0 (18)	10.0 (2)
Outcome, comments	Previous relapses with severe visual deterioration, relapse with blindness after NAT and PE, relapse free with AZA	After NAT relapsing myelitis, unstable with RIT, CTX, ALEM, responsive to PE (7 courses)	Previous relapse with blindness, allergic reaction to NAT (nAb$^+$), stable under RIT	Suspected NMO prior to start of natalizumab (LETM), stable under RIT	Previous relapse with blindness and coma; artificial ventilation and death due to pneumonia

Abbreviations: ALEM, alemtuzumab; AQP4, aquaporin 4; AZA, azathioprine; CTX, cyclophosphamide; EDSS, Expanded Disability Status Scale; Gd, gadolinium; LETM, longitudinally extending transverse myelitis; MIT, mitoxantrone; MRI, magnetic resonance imaging; NA, not available; nAb, neutralizing antibody; NAT, natalizumab treatment; NMO, neuromyelitis optica; PE, plasma exchange; RIT, rituximab; WML, white matter lesion; ΔMFI, difference in median fluorescence intensity.

[a] Relative to start of NAT.

[b] Treatments appear in chronological order.

[c] Total of 8 infusions during 10 months.

[d] Relapses from first infusion until start of plasma exchange or 4 weeks after last infusion.

lapses were generally severe, with paraparesis or hemiparesis due to myelitis in 4 patients and marked visual deterioration in 2. There was no apparent change in the pattern or severity of relapses compared with disease phases prior to natalizumab therapy. Furthermore, MRI showed new or gadolinium-positive active lesions during relapse in all 5 patients. Four patients had new spinal cord lesions; additionally, patients 1 and 5 showed atypical necrotic cerebral lesions. During natalizumab therapy, clinical disability as measured by the Expanded Disability Status Scale was stable in 1 patient and progressed in 4 patients by 1.0 to 4.5 points. The median Expanded Disability Status Scale score was 4.0 at the start of natalizumab therapy and 7.0 at the end. Patient 3 presented with an allergic reaction (and tested positive for anti-natalizumab-neutralizing antibodies) after the second infusion, and natalizumab therapy was discontinued. Natalizumab therapy was suspended in the other 4 patients because of persisting disease activity, as displayed by relapses and concomitant MRI alterations.

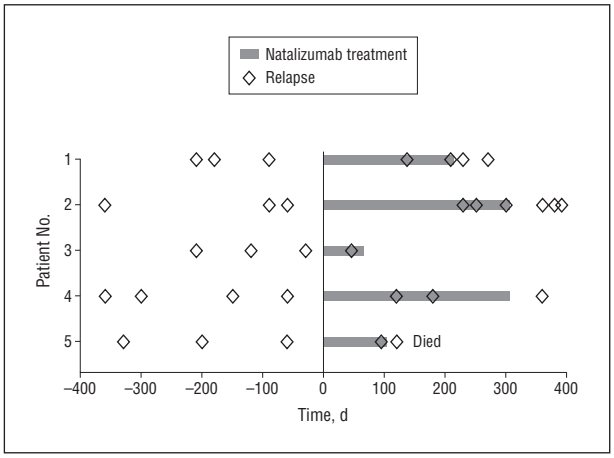

Figure. Relapses in patients with neuromyelitis optica before, during, and after treatment with natalizumab. Shown are all relapses (diamonds) from day –400 to +400 relative to start of medication. Bars depict duration of natalizumab treatment until plasma exchange (patients 1, 2, and 5) or 4 weeks after last infusion (patients 3 and 4).

DIAGNOSIS OF NMO AND CLINICAL OUTCOME

After natalizumab cessation, NMO was immediately confirmed in most patients (2-5) according to clinical, radiological, and serological criteria[19] and 9 months later in patient 1. This patient had an atypical presentation with extensive bilateral white matter lesions and new cystic cortical lesions. The latter initially were suspected to be due to natalizumab-associated progressive multifocal leukoencephalopathy or other opportunistic viral infections, but repeated CSF and polymerase chain reaction examinations for a variety of viral, fungal, and parasitic pathogens including John Cunningham virus remained negative.

All patients tested positive for AQP4 (patients 2-5 during relapse), with a mean serum titer of 1978 ΔMFI (range, 850-4023 ΔMFI), which was about twice as high as the mean ΔMFI (1013 ΔMFI; range, 67-5604) from a recently published cohort of 52 patients with NMO or NMO spectrum disorders.[24] The highest value was measured in patient 2, who was tested during natalizumab therapy, whereas patient 1 had the lowest value, obtained during remission 9 months after discontinuation of natalizumab therapy.

Simultaneously with cessation of natalizumab, 4 of 5 patients required plasma exchange because of only minor improvement in relapse-associated symptoms achieved with high-dose intravenous steroids. However, all of the 4 patients had further disease activity in the months following plasma exchange and 2 experienced a clinically unfavorable course before NMO-specific immunotherapy was started. Both patients 1 and 5 had severe bilateral optic neuritis leading to blindness, and patient 5 had brainstem involvement and finally died after 2 months because of pneumonia.[22] The 4 surviving patients subsequently received azathioprine (patient 1) or rituximab (patients 2, 3, and 4); 2 of them remained relapse-free thereafter (patients 1 and 3), and patient 4 stabilized. The mean annual relapse rate decreased from 3.2 (range, 3-4) in the year prior to natalizumab therapy and 3.0 (range, 2.4-6.0) during natalizumab therapy to 1.5 (range, 0-3) in the first year after natalizumab therapy. Patient 2 had further relapses despite therapy with rituximab, cyclophosphamide, and alemtuzumab. The Expanded Disability Status Scale at the last visit (median, 18 months after the end of natalizumab) decreased in patient 1, was stable in patients 3 and 4, and increased in patients 2 and 5.

COMMENT

In this study, we retrospectively analyzed the responses of 5 patients with NMO to natalizumab and found that this therapy, established for treatment of breakthrough disease in MS, did not show the anticipated beneficial effect. Our data indicate that relapse frequency was unchanged during natalizumab therapy, and most patients experienced severe exacerbations during and shortly after natalizumab treatment.

Neuromyelitis optica is a relapsing, often disabling disorder with a high mortality rate.[1] Treatment of NMO mainly relies on immunosuppressive therapies such as azathioprine, methotrexate, mycophenolate mofetil, or mitoxantrone,[6,8,11] as well as B-cell depletion with rituximab.[7,10,25] Interferon beta, an immunomodulatory drug used as first-line therapy for relapsing-remitting MS, has been shown to be ineffective,[11] even harmful in some cases.[12-16] Natalizumab is an effective therapy for relapsing-remitting MS, which reduced the annual relapse rate by 69% in the pivotal placebo-controlled study.[17] Recently published subgroup analyses of this phase 3 trial showed that 37% of patients treated with natalizumab, but only 7% of patients with placebo, were free of any detectable disease activity over 2 years, defined by the absence of relapses, sustained disability progression, gadolinium-enhancing lesions, and new or enlarging T2-hyperintense lesions on cranial MRI. Importantly, natalizumab was also effective in patients with highly active MS, defined as at least 2 relapses in the year before study entry and at least 2 gadolinium-enhancing lesions at study entry.[26] In contrast, no reduction in relapse activity was found in our cohort of patients with NMO who were treated with natalizumab for suggested MS. Exacerbations of NMO during natalizumab treatment were severe, and all patients had further relapses shortly after cessation or removal of natalizumab therapy.

Natalizumab inhibits the migration of T and B cells to the CNS and causes a redistribution of lymphocyte subsets in the periphery.[18] Whereas in the CSF the number of $CD19^+$ B cells and $CD138^+$ plasma cells is reduced for at least 6 months after infusion,[18] the absolute number of mature $CD19^+$ B cells is increased in the periphery by approximately 3-fold from month 1 of natalizumab treatment.[27,28] Furthermore, levels of $CD138^+$ plasma cells and, in particular, immature $CD19^+CD10^+$ pre-B cells are elevated in the blood of natalizumab-treated patients.[27] Total peripheral lymphocyte counts are increased as well,[27-29] but only the relative frequencies of B cells increase, whereas frequencies of $CD4^+$ T cells, $CD8^+$ T cells, and $CD16^+CD56^+$ natural killer cells remain unaltered.[28,29]

In recent years it has become clear that NMO is an antibody-mediated disease characterized by the occurrence of pathogenic AQP4-Abs, perivascular deposition of complement and immunoglobulin, and a subsequent

astrocytopathy.[3,30,31] Therefore, one might speculate that persisting or even enhanced disease activity in our patients is a direct cause of a natalizumab-induced increase in the number of peripheral $CD138^+$ plasma cells, which in turn might have caused an increase of circulating AQP4-Abs. Indeed, we found exceptionally high titers of AQP4-Abs during or shortly after natalizumab treatment. Evidence is growing that AQP4-Abs titers correlate with disease activity.[2] In a previous study, the titer of AQP4-Abs strongly correlated with the number of peripheral $CD19^+$ B cells, and breakthrough disease in patients with NMO who were treated with rituximab was associated with an increase in AQP4-Abs,[32] whereas another investigation showed a correlation of disease activity with the number of B cells only.[25] Similarly, a marked increase in AQP4-Abs along with a high relapse rate were found in a patient with NMO who was treated with interferon beta.[16] However, owing to the retrospective design of our study, neither AQP4-Abs titers nor the number of B cells in the peripheral blood were measured before natalizumab therapy.

Alternative immunological mechanisms, which may contribute to therapy failure, include increased B-cell costimulation by activated T cells and enhanced recruitment of eosinophils. Natalizumab was shown to increase the frequency of T cells secreting proinflammatory cytokines such as tumor necrosis factor, interferon γ, and interleukin 17, presumably by sequestration of these cells in the peripheral blood.[33] In particular, the enhanced secretion of interleukin 6 might drive relapses,[29,33] since the interleukin 6 level is increased in the blood and CSF of patients with NMO during exacerbations[34,35] and promotes $CD19^{int}CD27^{high}CD38^{high}CD180^-$ plasmablasts to produce AQP4-Abs.[36] Moreover, natalizumab increases the frequency of peripheral eosinophils,[17] which were implicated in NMO pathogenesis.[37]

Although the patients presented a clinical disease course compatible with NMO, they were initially diagnosed with relapsing-remitting MS, mainly because of the presence of brain lesions. Recently, the traditional concept of NMO as a disease affecting only the optic nerves and the spinal cord was revised, since histopathological and MRI findings demonstrated cerebral involvement in a high proportion of patients. Brain MRI lesions in NMO are usually asymptomatic, show a distribution pattern not compatible with the Barkhof/Tintoré criteria, and sometimes present as tumefactive lesions.[38] The existence of periventricular and callosal lesions does not exclude NMO,[39,40] but extensive symmetric brain parenchymal lesions as seen in patient 1 are more frequent in NMO than in MS, and cloud-like enhancement seems to be a distinctive feature of NMO brain lesions.[41] Similar to patient 1, cystic brain lesions indicating irreversible tissue damage can occur.[41,42] Our data suggest that, even in the presence of white matter brain lesions, a diagnosis of NMO should be considered, particularly in cases with severe optic neuritis, absence of CSF oligoclonal bands,[43] suspicious lesion distribution pattern, and lack of response to otherwise efficient MS therapies. As a cautionary note, our study was not able to exclude the possibility that some patients with NMO similarly misdiagnosed with MS experience a therapeutic benefit from natalizumab. Further studies with a different design will be required to address this issue.

In summary, we present the first evidence that natalizumab is not beneficial in NMO and might even exacerbate disease during or shortly after therapy. Our study emphasizes the distinct pathophysiology of MS and NMO and has clinical implications. Obviously, not all therapeutic approaches used for breakthrough disease in MS such as autologous hematopoietic stem cell transplantation[44] or, in our observation, natalizumab may be beneficial for NMO. Thus, prior to therapy with natalizumab, the diagnosis of MS should be carefully reconfirmed, at least in patients with a primary opticospinal manifestation and unusual brain lesions. We propose testing for AQP4-Abs prior to initiation of natalizumab in all ambiguous cases, although AQP4-Abs might be undetectable in 20% to 30% of patients with NMO. If a patient with MS experiences relapses despite natalizumab therapy, one should consider not only inefficacy of the drug, neutralizing antibodies, or progressive multifocal leukoencephalopathy but also NMO.

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Author Affiliations: Department of Neurology, University Medical Center Regensburg, Regensburg (Dr Kleiter), Department of Neurology, St Josef-Hospital, Ruhr University Bochum, Bochum (Dr Hellwig), Department of Neurology, Klinikum rechts der Isar, Technische Universität München (Dr Berthele) and Institute for Clinical Neuroimmunology, Ludwig-Maximilians-University (Dr Kümpfel), Munich, Department of Neurology, Friedrich-Alexander University Erlangen, Erlangen (Dr Linker), Multiple Sclerosis Center, Department of Neurology, Medical Faculty, Heinrich-Heine-University Düsseldorf, Düsseldorf (Drs Hartung and Aktas), and NeuroCure Clinical Research and Clinical and Experimental Multiple Sclerosis Research Center, Charité University Medicine, Berlin (Dr Paul), Germany. Dr Kleiter is now with the Department of Neurology, St Josef-Hospital, Ruhr University Bochum.
Correspondence: Ingo Kleiter, MD, Department of Neurology, St Josef-Hospital, Ruhr University Bochum, Gudrunstr 56, 44791 Bochum, Germany (ingo.kleiter@rub.de).
Author Contributions: Drs Paul and Aktas contributed equally to the study. *Study concept and design:* Kleiter, Berthele, Kümpfel, Paul, and Aktas. *Acquisition of data:* Kleiter, Hellwig, Berthele, Kümpfel, and Linker. *Analysis and interpretation of data:* Kleiter, Hellwig, Berthele, Kümpfel, Linker, Hartung, Paul, and Aktas. *Drafting of the manuscript:* Kleiter, Berthele, Kümpfel, Paul, and Aktas. *Critical revision of the manuscript for important intellectual content:* Kleiter, Hellwig, Berthele, Kümpfel, Linker, Hartung, Paul, and Aktas. *Administrative, technical, and material support:* Linker, Hartung, and Paul. *Study supervision:* Kleiter, Berthele, Kümpfel, and Aktas.
Neuromyelitis Optica Study Investigators: The following members of the Neuromyelitis Optica Study (NEMOS) Group have contributed cases with neuromyelitis optica or neuromyelitis optica spectrum diseases to the source data file (alphabetical listing according to site): Ulrich Hofstadt, MD, Department of Neurology, Bay-

reuth Hospital, Bayreuth, Germany; Friedemann Paul, MD, and Klemens Ruprecht, MD, Department of Neurology, Charité University Medicine, Berlin, Germany; Kerstin Hellwig, MD, Department of Neurology, St Josef-Hospital, Ruhr University Bochum, Bochum, Germany; Sabine Niehaus, MD, Department of Neurology, Dortmund Hospital, Dortmund, Germany; Orhan Aktas, MD, Hans-Peter Hartung, MD, Til Menge, MD, and Marius Ringelstein, MD, Department of Neurology, University of Düsseldorf, Düsseldorf, Germany; Ralf Linker, MD, Department of Neurology, Friedrich-Alexander University Erlangen, Erlangen, Germany; Christoph Mayer, MD, and Ulf Ziemann, MD, Department of Neurology, University of Frankfurt, Frankfurt/Main, Germany; Reinhard Reuss, MD, Department of Neurology, University of Giessen, Giessen, Germany; Kersten Guthke, MD, Department of Neurology, Görlitz Hospital, Görlitz, Germany; Frank Hoffmann, MD, and Christian Zentner, MD, Department of Neurology, Martha Maria Hospital, Halle/Saale, Germany; Sven Schippling, MD, Department of Neurology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; Martin Stangel, MD, and Corinna Trebst, MD, Department of Neurology, Hannover Medical School, Hannover, Germany; Sven Jarius, MD, and Brigitte Wildemann, MD, Department of Neurology, University of Heidelberg, Heidelberg, Germany; Barbara Ettrich, MD, Franziska Möller, MD, Florian ThenBergh, and Eva Thomae, MD, Department of Neurology, University of Leipzig, Leipzig, Germany; Achim Berthele, MD, Bernhard Hemmer, MD, and Angela Jochim, MD, Department of Neurology, Technische Universität München, Munich, Germany; Tania Kümpfel, MD, and Hannah Pellkofer, MD, Institute for Clinical Neuroimmunology, Ludwig-Maximilians-University, Munich, Germany; Martin Marziniak, MD, Department of Neurology, University of Münster, Münster, Germany; Tobias Böttcher, MD, Department of Neurology, Neubrandenburg Hospital, Neubrandenburg, Germany; Christian Wilke, MD, Department of Neurology, Helios Clinic Plauen, Plauen, Germany; Ingo Kleiter, MD, and Klemens Angstwurm, MD, Department of Neurology, University Medical Center Regensburg, Regensburg, Germany; Paulus Stefan Rommer, MD, Alexander Winkelmann, MD, and Uwe Zettl, MD, Department of Neurology, University of Rostock, Rostock, Germany; Oliver Neuhaus, MD, Department of Neurology, Sigmaringen Hospital, Sigmaringen, Germany; Jürgen Faiss, MD, and Peter Kern, MD, Department of Neurology, Asklepios Hospital Teupitz, Teupitz, Germany; Arthur Melms, MD, Department of Neurology, University of Tübingen, Tübingen, Germany; Hayrettin Tumani, MD, and Johannes, Brettschneider, MD, Department of Neurology, University of Ulm, Ulm, Germany; Christian Geis, MD, and Christoph Kleinschnitz, MD, Department of Neurology, University of Würzburg, Würzburg, Germany.

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Additional Contributions: Gregory A. Storch, MD, performed polymerase chain reaction analysis of viral pathogens from patient 1.

REFERENCES

1. Wingerchuk DM, Hogancamp WF, O'Brien PC, Weinshenker BG. The clinical course of neuromyelitis optica (Devic's syndrome). *Neurology*. 1999;53(5):1107-1114.
2. Jarius S, Wildemann B. AQP4 antibodies in neuromyelitis optica: diagnostic and pathogenetic relevance. *Nat Rev Neurol*. 2010;6(7):383-392.
3. Lennon VA, Kryzer TJ, Pittock SJ, Verkman AS, Hinson SR. IgG marker of optic-spinal multiple sclerosis binds to the aquaporin-4 water channel. *J Exp Med*. 2005; 202(4):473-477.
4. Paul F, Jarius S, Aktas O, et al. Antibody to aquaporin 4 in the diagnosis of neuromyelitis optica. *PLoS Med*. 2007;4(4):e133.
5. Kalluri SR, Illes Z, Srivastava R, et al. Quantification and functional characterization of antibodies to native aquaporin 4 in neuromyelitis optica. *Arch Neurol*. 2010;67(10):1201-1208.
6. Weinstock-Guttman B, Ramanathan M, Lincoff N, et al. Study of mitoxantrone for the treatment of recurrent neuromyelitis optica (Devic disease). *Arch Neurol*. 2006;63(7):957-963.
7. Jacob A, Weinshenker BG, Violich I, et al. Treatment of neuromyelitis optica with rituximab: retrospective analysis of 25 patients. *Arch Neurol*. 2008;65(11): 1443-1448.
8. Bichuetti DB, Lobato de Oliveira EM, Oliveira DM, Amorin de Souza N, Gabbai AA. Neuromyelitis optica treatment: analysis of 36 patients. *Arch Neurol*. 2010; 67(9):1131-1136.
9. Kim SH, Kim W, Park MS, Sohn EH, Li XF, Kim HJ. Efficacy and safety of mitoxantrone in patients with highly relapsing neuromyelitis optica. *Arch Neurol*. 2011;68(4):473-479.
10. Cree BA, Lamb S, Morgan K, Chen A, Waubant E, Genain C. An open label study of the effects of rituximab in neuromyelitis optica. *Neurology*. 2005;64(7):1270-1272.

11. Papeix C, Vidal JS, de Seze J, et al. Immunosuppressive therapy is more effective than interferon in neuromyelitis optica. *Mult Scler*. 2007;13(2):256-259.
12. Warabi Y, Matsumoto Y, Hayashi H. Interferon beta-1b exacerbates multiple sclerosis with severe optic nerve and spinal cord demyelination. *J Neurol Sci*. 2007; 252(1):57-61.
13. Shimizu Y, Yokoyama K, Misu T, et al. Development of extensive brain lesions following interferon beta therapy in relapsing neuromyelitis optica and longitudinally extensive myelitis. *J Neurol*. 2008;255(2):305-307.
14. Tanaka M, Tanaka K, Komori M. Interferon-beta(1b) treatment in neuromyelitis optica. *Eur Neurol*. 2009;62(3):167-170.
15. Shimizu J, Hatanaka Y, Hasegawa M, et al. IFNβ-1b may severely exacerbate Japanese optic-spinal MS in neuromyelitis optica spectrum. *Neurology*. 2010;75 (16):1423-1427.
16. Palace J, Leite MI, Nairne A, Vincent A. Interferon beta treatment in neuromyelitis optica: increase in relapses and aquaporin 4 antibody titers. *Arch Neurol*. 2010;67(8):1016-1017.
17. Polman CH, O'Connor PW, Havrdova E, et al; AFFIRM Investigators. A randomized, placebo-controlled trial of natalizumab for relapsing multiple sclerosis. *N Engl J Med*. 2006;354(9):899-910.
18. Stüve O, Marra CM, Jerome KR, et al. Immune surveillance in multiple sclerosis patients treated with natalizumab. *Ann Neurol*. 2006;59(5):743-747.
19. Wingerchuk DM, Lennon VA, Pittock SJ, Lucchinetti CF, Weinshenker BG. Revised diagnostic criteria for neuromyelitis optica. *Neurology*. 2006;66(10): 1485-1489.
20. Polman CH, Reingold SC, Edan G, et al. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria." *Ann Neurol*. 2005;58(6):840-846.
21. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology*. 1983;33(11):1444-1452.
22. Lee DH, Metz I, Berthele A, et al. Supraspinal demyelinating lesions in neuromyelitis optica display a typical astrocyte pathology. *Neuropathol Appl Neurobiol*. 2010;36(7):685-687.
23. Barkhof F, Filippi M, Miller DH, et al. Comparison of MRI criteria at first presentation to predict conversion to clinically definite multiple sclerosis. *Brain*. 1997; 120(pt 11):2059-2069.
24. Sellner J, Cepok S, Kalluri SR, et al. Aquaporin 4 antibody positive central nervous system autoimmunity and multiple sclerosis are characterized by a distinct profile of antibodies to herpes viruses. *Neurochem Int*. 2010;57(6):662-667.
25. Pellkofer HL, Krumbholz M, Berthele A, et al. Long-term follow-up of patients with neuromyelitis optica after repeated therapy with rituximab. *Neurology*. 2011; 76(15):1310-1315.
26. Havrdova E, Galetta S, Hutchinson M, et al. Effect of natalizumab on clinical and radiological disease activity in multiple sclerosis: a retrospective analysis of the Natalizumab Safety and Efficacy in Relapsing-Remitting Multiple Sclerosis (AFFIRM) study. *Lancet Neurol*. 2009;8(3):254-260.
27. Krumbholz M, Meinl I, Kümpfel T, Hohlfeld R, Meinl E. Natalizumab disproportionately increases circulating pre-B and B cells in multiple sclerosis. *Neurology*. 2008;71(17):1350-1354.
28. Rinaldi L, Rinaldi F, Perini P, et al. No evidence of JC virus reactivation in natalizumab treated multiple sclerosis patients: an 18 month follow-up study. *J Neurol Neurosurg Psychiatry*. 2010;81(12):1345-1350.
29. Wehner NG, Gasper C, Shopp G, et al. Immunotoxicity profile of natalizumab. *J Immunotoxicol*. 2009;6(2):115-129.
30. Misu T, Fujihara K, Kakita A, et al. Loss of aquaporin 4 in lesions of neuromyelitis optica: distinction from multiple sclerosis. *Brain*. 2007;130(pt 5):1224-1234.
31. Bradl M, Misu T, Takahashi T, et al. Neuromyelitis optica: pathogenicity of patient immunoglobulin in vivo. *Ann Neurol*. 2009;66(5):630-643.
32. Jarius S, Aboul-Enein F, Waters P, et al. Antibody to aquaporin-4 in the long-term course of neuromyelitis optica. *Brain*. 2008;131(pt 11):3072-3080.
33. Kivisäkk P, Healy BC, Viglietta V, et al. Natalizumab treatment is associated with peripheral sequestration of proinflammatory T cells. *Neurology*. 2009;72(22): 1922-1930.
34. Yanagawa K, Kawachi I, Toyoshima Y, et al. Pathologic and immunologic profiles of a limited form of neuromyelitis optica with myelitis. *Neurology*. 2009; 73(20):1628-1637.
35. Uzawa A, Mori M, Arai K, et al. Cytokine and chemokine profiles in neuromyelitis optica: significance of interleukin-6. *Mult Scler*. 2010;16(12):1443-1452.
36. Chihara N, Aranami T, Sato W, et al. Interleukin 6 signaling promotes anti-aquaporin 4 autoantibody production from plasmablasts in neuromyelitis optica. *Proc Natl Acad Sci U S A*. 2011;108(9):3701-3706.
37. Correale J, Fiol M. Activation of humoral immunity and eosinophils in neuromyelitis optica. *Neurology*. 2004;63(12):2363-2370.
38. Pittock SJ, Lennon VA, Krecke K, Wingerchuk DM, Lucchinetti CF, Weinshenker BG. Brain abnormalities in neuromyelitis optica. *Arch Neurol*. 2006;63(3):390-396.
39. Pittock SJ, Weinshenker BG, Lucchinetti CF, Wingerchuk DM, Corboy JR, Lennon VA. Neuromyelitis optica brain lesions localized at sites of high aquaporin 4 expression. *Arch Neurol*. 2006;63(7):964-968.
40. Nakamura M, Misu T, Fujihara K, et al. Occurrence of acute large and edematous callosal lesions in neuromyelitis optica. *Mult Scler*. 2009;15(6):695-700.
41. Ito S, Mori M, Makino T, Hayakawa S, Kuwabara S. "Cloud-like enhancement" is a magnetic resonance imaging abnormality specific to neuromyelitis optica. *Ann Neurol*. 2009;66(3):425-428.
42. Saiki S, Ueno Y, Moritani T, et al. Extensive hemispheric lesions with radiological evidence of blood-brain barrier integrity in a patient with neuromyelitis optica. *J Neurol Sci*. 2009;284(1-2):217-219.
43. Bergamaschi R, Tonietti S, Franciotta D, et al. Oligoclonal bands in Devic's neuromyelitis optica and multiple sclerosis: differences in repeated cerebrospinal fluid examinations. *Mult Scler*. 2004;10(1):2-4.
44. Matiello M, Pittock SJ, Porrata L, Weinshenker BG. Failure of autologous hematopoietic stem cell transplantation to prevent relapse of neuromyelitis optica [published online March 14, 2011]. *Arch Neurol*. doi:10.1001/archneurol.2011.38.