Failure of Natalizumab to Prevent Relapses in Neuromyelitis Optica

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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. The detection of a serum antibody to the CNS water channel aquaporin 4 (AQP4) as a highly specific biomarker in most patients with NMO 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. Treatment options that are beneficial in MS, especially interferon beta, are of no proven efficacy or may even be harmful in NMO.

Natalizumab is a monoclonal antibody against the adhesion molecule very late activation antigen 4, an α4β1 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. 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. 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-
lapses 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, 133 patients with NMO or NMO spectrum disorders according to the revised 2006 criteria by Wingerchuk et al. 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, 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, 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-like 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 were described elsewhere in detail.

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).

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 thalamic lesion extending to the mesencephalon and the brainstem. Although the distribution and appearance of the lesions were unspecific according to established criteria, 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 spinal cord 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-
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.

### Table. Patient Characteristics, Treatment, and Clinical Course

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patient No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Sex</td>
<td>F</td>
</tr>
<tr>
<td>Age, y.a</td>
<td>54</td>
</tr>
<tr>
<td>Disease duration, y.a</td>
<td>31</td>
</tr>
<tr>
<td>Previous treatments (duration, mo)b</td>
<td>Steroids for relapses, AZA (152), interferon beta (57)</td>
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<tr>
<td>Previous relapses, No.</td>
<td>40</td>
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<tr>
<td>EDSS score prior to NAT</td>
<td>4.0 for 3 y</td>
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<tr>
<td>Relapses last year, No.c</td>
<td>3</td>
</tr>
<tr>
<td>Natalizumab infusions, No.</td>
<td>8</td>
</tr>
<tr>
<td>Relapses during NAT, No.d</td>
<td>2</td>
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<tr>
<td>Relapses in first year after NAT</td>
<td>12</td>
</tr>
<tr>
<td>EDSS score at start of NAT</td>
<td>4.0</td>
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<tr>
<td>EDSS score at end of NAT</td>
<td>8.5</td>
</tr>
<tr>
<td>Duration until NMO diagnosis, moa</td>
<td>16</td>
</tr>
<tr>
<td>AQP4-IgG titer, ΔMFI (time after NAT, mo)</td>
<td>850 (9)</td>
</tr>
<tr>
<td>Cerebral MRI findings</td>
<td>Large symmetric bitemispheric WML before NAT, new supratentorial cystic lesions during NAT</td>
</tr>
<tr>
<td>Spinal MRI findings</td>
<td>Longitudinal lesion from C2-Th7 after NAT, Gd +</td>
</tr>
<tr>
<td>Subsequent treatments (duration, mo)b</td>
<td>Steroids, PE, AZA (18)</td>
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<tr>
<td>EDSS score at last visit (time after NAT, mo)</td>
<td>6.0 (23)</td>
</tr>
<tr>
<td>Outcome, comments</td>
<td>Previous relapses with severe visual deterioration, relapse with blindness after NAT and PE, relapse free with AZA</td>
</tr>
</tbody>
</table>

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.

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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\textsuperscript{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 MFI (1013 MFI), which was about twice as high as the mean MFI (1013 MFI; range, 67-5604) from a recently published cohort of 52 patients with NMO or NMO spectrum disorders.\textsuperscript{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.\textsuperscript{25} The 4 surviving patients subsequently received azathioprine (patient 1) or rituximab (patients 2, 3, and 4); 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.

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.\textsuperscript{1} Treatment of NMO mainly relies on immunosuppressive therapies such as azathioprine, methotrexate, mycophenolate mofetil, or mitoxantrone,\textsuperscript{6,8,11} as well as B-cell depletion with rituximab.\textsuperscript{7,10,25} Interferon beta, an immunomodulatory drug used as first-line therapy for relapsing-remitting MS, has been shown to be ineffective,\textsuperscript{11} even harmful in some cases.\textsuperscript{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.\textsuperscript{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.\textsuperscript{18} 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.\textsuperscript{18} Whereas in the CSF the number of CD19\textsuperscript{+} B cells and CD138\textsuperscript{+} plasma cells is reduced for at least 6 months after infusion,\textsuperscript{18} the absolute number of mature CD19\textsuperscript{+} B cells is increased in the periphery by approximately 3-fold from month 1 of natalizumab treatment.\textsuperscript{27,28} Furthermore, levels of CD138\textsuperscript{+} plasma cells and, in particular, immature CD19\textsuperscript{+} CD10\textsuperscript{+} pre-B cells are elevated in the blood of natalizumab-treated patients.\textsuperscript{27} Total peripheral lymphocyte counts are increased as well,\textsuperscript{27-30} but only the relative frequencies of B cells increase, whereas frequencies of CD4\textsuperscript{+} T cells, CD8\textsuperscript{+} T cells, and CD16 CD56\textsuperscript{+} natural killer cells remain unaltered.\textsuperscript{28,30}

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. 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. 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, whereas another investigation showed a correlation of disease activity with the number of B cells only. 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. 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 gamma, and interleukin 17, presumably by sequestration of these cells in the peripheral blood. In particular, the enhanced secretion of interleukin 6 might drive relapses, since the interleukin 6 level is increased in the blood and CSF of patients with NMO during exacerbations and promotes CD19^+CD27^+CD38^highCD180^- plasmablasts to produce AQP4-Abs. Moreover, natalizumab increases the frequency of peripheral eosinophils, which were implicated in NMO pathogenesis.

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/Tintore criteria, and sometimes present as tumefactive lesions. The existence of periventricular and callosal lesions does not exclude NMO, 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. Similar to patient 1, cystic brain lesions indicating irreversible tissue damage can occur. 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, 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 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 optico-spinal 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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**Additional Contributions:** Gregory A. Storch, MD, performed polymerase chain reaction analysis of viral pathogens from patient 1.

**REFERENCES**


