7T MRI in natalizumab-associated PML and ongoing MS disease activity
A case study

ABSTRACT
Objective: To assess the ability of ultra-high-field MRI to distinguish early progressive multifocal leukoencephalopathy (PML) from multiple sclerosis (MS) lesions in a rare case of simultaneous presentation of natalizumab-associated PML and ongoing MS activity.

Methods: Advanced neuroimaging including 1.5T, 3T, and 7T MRI with a spatial resolution of up to 0.08 mm³ was performed.

Results: 7T MRI differentiated between PML-related and MS-related brain damage in vivo. Ring-enhancing MS plaques displayed a central vein, whereas confluent PML lesions were preceded by punctate or milky way-like T2 lesions.

Conclusions: Given the importance of early diagnosis of treatment-associated PML, future systematic studies are warranted to assess the value of highly resolving MRI in differentiating between early PML- and MS-induced brain parenchymal lesions.

GLOSSARY
EDSS = Expanded Disability Status Scale; FLAIR = fluid-attenuated inversion recovery; IRIS = immune reconstitution inflammatory syndrome; MR = magnetic resonance; MS = multiple sclerosis; NAA = N-acetylaspartate; NTZ = natalizumab; PML = progressive multifocal leukoencephalopathy; SWI = susceptibility-weighted imaging; T1W = T1-weighted; T2W = T2-weighted; TE = echo time; TI = inversion time; TR = repetition time.

One adverse effect of effective disease-modifying therapies of multiple sclerosis (MS) such as natalizumab (NTZ) is the development of progressive multifocal leukoencephalopathy (PML).

However, identifying PML lesions at early stages remains difficult in daily clinical routine as their presentation is often similar to that of MS plaques. NTZ-associated PML initially causes subcortical or U-fiber lesions, which are also a hallmark of MS. On the other hand, large edematous MS plaques can mimic expanding PML lesions. Moreover, CSF PCR testing for JC virus DNA has recently been proven imperfect and can further delay PML diagnosis.

We assessed the potential of ultra-high-field MRI at 7T with a spatial resolution of up to 0.08 mm³ to differentiate between early PML and MS-related brain damage in a case of simultaneous presentation of NTZ-associated PML, ongoing MS activity, and immune reconstitution inflammatory syndrome (IRIS).

METHODS
Clinical investigations comprised detailed neurologic examination, MRI at 1.5T, 3T, and 7T, magnetic resonance (MR) spectroscopy at 3T, and longitudinal CSF analysis. Additionally, blood samples were tested for neutralizing antibodies to NTZ. Written informed consent was obtained from the patient for all procedures.

Image acquisition. Clinical routine MRI at 1.5T and 3T included T1-weighted (T1W), T2-weighted (T2W), fluid-attenuated inversion recovery (FLAIR) or double inversion recovery, and diffusion-weighted imaging.

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Funding information and disclosures are provided at the end of the article. Go to Neurology.org/nn for full disclosure forms. The Article Processing Charge was paid by the authors.

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7T MRI was performed using a whole-body MR scanner (Magnetom; Siemens, Erlangen, Germany) equipped with a 24-channel receive head coil (Nova Medical, Wilmington, MA) and a birdcage volume coil used for transmission. The imaging protocol (day 4) included T2*W imaging (echo time [TE] = 25.0 ms; repetition time [TR] = 1600 ms; spatial resolution = 0.2 × 0.2 × 2.0 mm³ and 0.5 × 0.5 × 2.0 mm³), pregadolinium and postgadolinium T1W magnetization-prepared rapid gradient echo (TE = 2.98 ms; TR = 2300 ms; inversion time [TI] = 900 ms; spatial resolution = 1.0 × 1.0 × 1.0 mm³), FLAIR (TE = 90 ms; TR = 16000 ms; TI = 2925.5 ms; spatial resolution = 1.0 × 1.0 × 3.0 mm³), and pregadolinium and postgadolinium T1W volumetric interpolated brain examination.

3T single volume MR spectroscopy was performed using a point resolved spectroscopy sequence (volume of interest = 20 × 20 × 20 mm³, TR/TE = 3000/30 ms, number of averages = 128, acquisition time = 1024 ms).

The lesion morphology was subjectively analyzed by a single rater who was not blinded to the diagnosis. A milky way-like lesion was defined as a small lesion on T2*W images (day 4, spatial resolution = 0.2 × 0.2 × 2.0 mm³), typically <3 mm in diameter, within a region of dirty white matter, that clearly shows PML development on a follow-up MRI. Hyperintensities within an extensive PML lesion that were not clearly distinguishable from each other were excluded. Milky way-like lesions were counted on the 7T T2*W images (day 4) with the highest available spatial resolution (0.2 × 0.2 × 2.0 mm³).

CASE REPORT A 47-year-old man had been diagnosed with relapsing-remitting MS in 2010. After switching from interferon β-1a to NTZ, previous clinical and MRI disease activity ceased and twice-yearly MRI did not show any evidence of disease activity until the 37th infusion. Within 3 days after the 37th administration of NTZ, cognitive deficits, uncontrolled emotional expressions, disorientation, and inappropriate behavior developed rapidly. Figure 1
provides a detailed overview of the treatment course and paraclinical findings.

In the initial 1.5T MRI on day 1 immediately after referral to our department we observed PML-suspicious confluent lesions in the cerebellum and the supratentorial bilateral frontal and parietal white matter (figures 1 and 2A). Due to suspected PML, NTZ was discontinued and 5 cycles of plasma exchange started (day 1). PCR testing of CSF was positive for JC virus (155 copies per milliliter), and the Expanded Disability Status Scale (EDSS) score was 4.5.

7T MRI was performed on day 4. The time lapse between the initial scans at 1.5T and at 7T was caused by organizational issues and may limit a comparison between these 2 scans especially regarding the patterns of contrast enhancement. 7T MRI on day 4 revealed different patterns of brain parenchymal damage. First, diffuse rim-like, perilesional contrast enhancement atypical of MS was observed around some PML-suspicious confluent lesions in the bilateral frontoparietal white matter (figure 1). These changes were presumably related to IRIS. Second, 5 ring-enhancing lesions were observed in the right periventricular white matter (figure 2D). Each of these lesions was characterized by a central vein (figure 2C, white arrows) and mild perifocal edema (7T T2*; figure 2C, black asterisks), and one lesion presented signal loss on SWI, e.g., resulting from central iron deposits or severe demyelination. These ring-enhancing lesions were classified as acute MS lesions in accordance with previous reports on characteristic MS lesion morphology imaged by 7T MRI.4–6

Finally, 567 small punctate or milky way-like lesions3 were visualized predominantly in the right centrum semiovale on high-resolution 7T T2W images (figure 2C; figure 3, oval). Of these, only 173 lesions (31%) exhibited a central vein at an ultrahigh in-plane resolution of 0.2 × 0.2 mm² that was

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**Figure 2** 7T MRI can differentiate between early PML and MS lesions

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Two different patterns of brain lesions were observed using 7T MRI: ring-enhancing lesions (black arrows, D) reveal central intralesional veins characteristic for multiple sclerosis (MS) plaques (white arrows, C) and perilesional edema (black asterisks, C) on T2*-weighted imaging. Additionally, small punctate lesions (oval, C) were present on T2*-weighted imaging. These microcystic lesions developed into confluent progressive multifocal leukoencephalopathy (PML) lesions over time (E), and may thus represent earliest PML stages. In contrast, punctate lesions were barely visible on the initial 1.5T T2-weighted images on day 1 (A). Volumetric interpolated brain examination (VIBE)-gad: GDTPA-enhanced 7T VIBE. (A, B) 1.5T MRI (day 1). (C, D) 7T MRI (day 4). (E) 3T fluid-attenuated inversion recovery (day 24).
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25.0 ms; repetition time microcystic PML lesions despite ultrahigh spatial resolution. Sequence parameters: echo time ivascular in appearance (oval) an intralesional central vein was not generally detectable in these focal leukoencephalopathy (PML). Although the lesion distribution pattern was principally perivascular in appearance (oval) an intralesional central vein was not generally detectable in these microcystic PML lesions despite ultrahigh spatial resolution. Sequence parameters: echo time = 25.0 ms; repetition time = 1,600 ms; spatial resolution = (0.2 × 0.2 × 2.0) mm³.

Figure 3 Punctate PML lesions visualized by highly resolving T2*-weighted imaging at 7T (day 4)

7T T2*-weighted images delineate even miniscule punctate lesions in early progressive multifocal leukoencephalopathy (PML). Although the lesion distribution pattern was principally perivascular in appearance (oval) an intralesional central vein was not generally detectable in these microcystic PML lesions despite ultrahigh spatial resolution. Sequence parameters: echo time = 25.0 ms; repetition time = 1,600 ms; spatial resolution = (0.2 × 0.2 × 2.0) mm³.

much smaller than a typical vein within the center of an MS lesion. Contrast-enhanced 7T T1W imaging showed corresponding areas of punctate contrast enhancement that were not fully detectable in the initial MRI scans at 1.5T (figure 2).

Due to suspected IRIS, we administered corticosteroid pulse therapy on day 4 when the patient presented with clinical worsening. 7T and 3T MRI on day 11 visualized decreasing perilesional IRIS-like contrast enhancement. Notably, the size of these contrast-enhancing PML lesions, which had probably been recognized by the immune system, remained stable, whereas PML lesions without peripheral contrast enhancement expanded significantly (figure 1). Accordingly, some of these PML lesions presented with restricted diffusion at their edges (data not shown) as revealed by 3T MRI, which has been suggested as a sign of ongoing PML.2,7,8

In response, we initiated antiviral therapy (mirtazapine, melfloquine, ribavirin, amantadine),1 but clinical symptoms worsened (EDSS 8.5), and the patient developed complex partial seizures. Concurrently, PCR on CSF revealed a high number of JC virus DNA copies (7,186 copies per milliliter), and the JC virus antibody index was 5.43 on day 19.

Next, 7T MRI and 3T MRI including proton spectroscopy were performed on day 24. As expected, contrast enhancement of all ring-enhancing MS lesions ceased after high-dose corticosteroid therapy, and the MS lesion size remained stable, whereas PML lesions continued to grow centrifugally (figure 1). Notably, punctate or milky way–like lesions that were detectable on the initial 7T MRI in the right hemisphere had amalgamated into one large, confluent PML lesion during corticosteroid therapy (figure 2E). Proton spectroscopy in a PML lesion and an exemplary MS lesion of almost the same age (figure e-1 at Neurology.org/nn) revealed significantly reduced N-acetylaspartate (NAA), choline, and creatine levels and elevated lipids in the center of the PML lesion indicating ongoing necrosis. In contrast, the MS lesion presented with only moderately decreased levels of NAA. Hence, PML may cause destructive brain tissue damage much more rapidly than MS-related inflammation.

MRI follow-up (1.5T) at day 34 did not reveal new or enlarging PML lesions (figure 1), clinical symptoms began to improve (EDSS 6.0), and JC virus DNA in CSF decreased to 828 copies per milliliter. Neutralizing antibodies against NTZ were not detectable.

DISCUSSION In this rare case of simultaneous NTZ-associated PML, IRIS, and MS relapse, 7T MRI yielded 2 main findings. First, high-resolution T2*W imaging distinguished between MS and PML brain lesions by the presence or absence of a characteristic central intraleisonal vein, which is a hallmark of MS,4,6 but only rarely detectable in lesions of other origin (e.g., neuromyelitis optica,4 Susac syndrome,5 asymptomatic white matter lesions6). In our case, a central vessel was only infrequently visualized within milky way–like PML lesions, and these vessels were much smaller than the veins that are typically observable within MS lesions. Quantification of the milky way lesion count was potentially influenced by (1) a misclassification of small MS lesions or Virchow-Robin spaces as milky way lesions, (2) motion artifacts, or (3) a small gap between the superior and the inferior T2*W image acquisition.

Second, punctate or microcystic lesions were distinctly visualized at 7T on day 4, however, not fully detectable on the initial 1.5T MRI scans. Some of these milky way–like lesions were associated with simultaneous gadolinium enhancement. During follow-up, these lesions rapidly formed large confluent plaques characteristic for PML. The interpretation of these findings remains highly speculative.7–9 On the one hand, punctate gadolinium-enhancing lesion pattern has been associated with IRIS, suggesting that these alterations highlight an overwhelming immune response, presumably within perivascular spaces.8 On the other hand, punctate or milky way–like lesions have also been described as an early manifestation of PML, indicating areas of active JC virus replication.9 Our own observations support the hypothesis of ongoing JC virus replication in punctate lesions since high-dose corticosteroid treatment triggered punctate lesion evolution, and increased CSF JC virus load up to 40-fold. The increase in
JC virus load is, however, also explicable by plasma exchange, as reported recently. An early diagnosis of treatment-associated PML is crucial. Our case study emphasizes the need for future studies investigating the value of high-resolution MRI to differentiate early PML from MS-associated brain parenchymal lesions.

**AUTHOR CONTRIBUTIONS**

Tim Sinnecker: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, acquisition of data, study supervision. Jalal Orthman: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, acquisition of data, study supervision. Marc Kühl: drafting/revising the manuscript, analysis or interpretation of data, acquisition of data. Ralf Mekle: drafting/revising the manuscript, analysis or interpretation of data, acquisition of data. Inga Selbig: drafting/revising the manuscript, contribution of viral reagents/tools/patients. Thoralf Niendorf: drafting/revising the manuscript, setting up the MR hardware for the study. Annett Kunkel: drafting/revising the manuscript, acquisition of data. Peter Wuerfel: drafting/revising the manuscript, study supervision. Peter Kern: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, contribution of viral reagents/tools/patients, acquisition of data. Friedemann Paul: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, data supervision, obtaining funding. Jens Faiss: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, acquisition of data, study supervision. Juergen Faiss: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, acquisition of data, study supervision. Jens Wuerfel: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, acquisition of data, study supervision.

**ACKNOWLEDGMENT**

The authors thank Antje Els for technical support.

**STUDY FUNDING**

Supported by the German Research Foundation (DFG Exc 257 to F.P.) and by the German Ministry of Education and Research (Competence Network Multiple Sclerosis KKNMS to F.P. and J.W.), and a research grant from the Guthy Jackson Charitable Foundation/National Multiple Sclerosis Society of the USA.

**DISCLOSURE**

T. Sinnecker received travel funding from Bayer, Teva, Novartis, and Genzyme. J. Orthman, M. Kühl, R. Mekle, and I. Selbig report no disclosures. T. Niendorf received travel funding and/or speaker honoraria from Siemens Healthcare, Erlangen, Germany; is the founder and CEO of MRI.Tools GmbH; and received research support from Siemens Healthcare, Erlangen, Germany, and Helmholtz A. Kunkel, P. Wienecke, and P. Kern report no disclosures. F. Paul is on the steering committee for Novartis OCTIMS Study and MedImmune; received speaker honoraria and travel grants from Bayer, Novartis, Biogen Idec, Teva, Sanofi-Aventis/Genzyme, and Merck Serono; is an academic editor for PLoS ONE; is an associate editor for *Neurology*: *Neuroimmunology & Neuroinflammation*; has consulted for SanofiGenzyme, Biogen Idec, and MedImmune; and received research support from Bayer, Novartis, Biogen Idec, Teva, Sanofi-Aventis/Genzyme, Merck Serono, German Research Council, Werth Stiftung of the City of Cologne, German Ministry of Education and Research, Arthur Arnstein Foundation Berlin, Guthy Jackson Charitable Foundation, and National Multiple Sclerosis Society of the USA. J. Faiss received travel funding and/or speaker honoraria from Bayer, Novartis, Biogen, Genzyme, Boehringer, MerckSerono, and Teva; and is on the editorial board for *Fortschritte Neurologie und Psychiatrie*. J. Wuerfel served on the advisory board for Novartis and Biogen Idec; received travel funding and/or speaker honoraria from Novartis, Bayer, and Biogen; is the CEO of MIAC AG; and received research support from German Ministry of Education and Research, Germany Ministry of Economy, and University Medicine Göttingen. Go to Neurology.org/nn for full disclosures.

Received May 15, 2015. Accepted in final form September 10, 2015.

**REFERENCES**


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7T MRI in natalizumab-associated PML and ongoing MS disease activity: A case study
Tim Sinnecker, Jalal Othman, Marc Kühl, et al.
*Neurol Neuroimmunol Neuroinflamm* 2015;2;
DOI 10.1212/NXI.0000000000000171

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