Performance of PML diagnostic criteria in natalizumab-associated PML: data from the Dutch-Belgian cohort

Martijn T. Wijburg, MD,[1,2] Clemens Warnke, MD,[3,4] Frederik Barkhof, MD, PhD,[2,5] Bernard M.J. Uitdehaag, MD, PhD,[1] Joep Killestein, MD, PhD,[1] Mike P. Wattjes MD[2,6]

1. Department of Neurology, Neuroscience Amsterdam, VUmc MS Center Amsterdam, VU University Medical Center, Amsterdam, The Netherlands.
2. Department of Radiology & Nuclear Medicine, Neuroscience Amsterdam, VUmc MS Center Amsterdam, VU University Medical Center, Amsterdam, The Netherlands.
3. Department of Neurology, Medical faculty, University of Düsseldorf, Düsseldorf, Germany.
4. Department of Neurology, Medical Faculty, University of Köln, Köln, Germany.
5. Institutes of Neurology and Healthcare Engineering, UCL, London, UK.
6. Department of Diagnostic and Interventional Neuroradiology, Hannover Medical School, Hannover, Germany.

Word count main text:	1267
Word count abstract:	200
Characters in title	104
Tables:	1
Figures:	1
Online supplementary figures:	1
References:	15

Keywords: progressive multifocal leukoencephalopathy, diagnosis, JC virus, natalizumab, multiple sclerosis

Correspondence:

Martijn T. Wijburg, MD

VUmc MS Center Amsterdam

Departments of Neurology and Radiology & Nuclear Medicine

VU University Medical Center

De Boelelaan 1118

1081 HZ Amsterdam, The Netherlands

Phone: +31-20-444 2834

E-mail: m.wijburg@vumc.nl

ABSTRACT

Objective

To test the current progressive multifocal leukoencephalopathy (PML) diagnostic criteria by applying them to patients previously diagnosed with natalizumab-associated (NTZ) PML in a real-world clinical setting.

Methods

Patients from the Dutch-Belgian NTZ-PML cohort (n=28) were reviewed at the time of first diagnostic work-up and during follow-up, using the PML diagnostic criteria as proposed in a consensus statement from the American Academy of Neurology (AAN).

Results

At first diagnostic work-up, 18 patients (64.3%) met the criteria for high diagnostic certainty for PML ("definite PML" or "probable PML"). During follow-up, this increased to 20 patients (71.4%) as JCV DNA was detected in CSF of two additional patients. Nonetheless, 28.6% of patients were still classified as "possible PML" or "not PML" (6 [21.5%] and 2 [7.1%] patients, respectively) despite a very high suspicion for PML based on lesion evolution and signs of PML-immune reconstitution inflammatory syndrome on MRI, and development of compatible symptoms.

Conclusions

The current case definition of PML has low sensitivity for diagnosis of NTZ-PML in a real-world clinical setting in which MRI is frequently used for PML screening. This may delay diagnosis and appropriate management of PML, and may complicate a valid estimation of PML incidence during NTZ therapy.

INTRODUCTION

Progressive multifocal leukoencephalopathy (PML) may complicate treatment with natalizumab (NTZ) in patients with multiple sclerosis (MS),[1] and early diagnosis improves functional outcome and survival.[2] Criteria for the diagnosis of PML were published in a consensus statement from the American Academy of Neurology (AAN) Neuroinfectious Disease Section, comprising four categories (definite, probable, possible, and not PML) based on the presence of three diagnostic features: clinical symptoms suggestive of PML, imaging findings suggestive of PML, and detection of JC virus DNA in CSF by PCR (CSF JCV PCR).[3]

Enhanced pharmacovigilance by frequent MRI screening in high-risk patients leads to early detection of MRI lesions suggestive of PML, even before the onset of symptoms.[4] In addition, JCV DNA can be undetectable by PCR, especially in those patients with low PML lesion volumes on MRI.[5 6] However, absence of symptoms and a negative PCR hamper the formal diagnosis of PML, even when imaging is highly suggestive of PML. This study aims to investigate the performance of the PML diagnostic criteria in our cohort of NTZ treated patients with PML (NTZ-PML) in a routine clinical pharmacovigilance setting, to determine to what extent these limitations affect sensitivity of these criteria.

METHODS

Study design and patient selection

Patients were recruited from the Dutch-Belgian NTZ-PML cohort, which comprises patients treated at our center (n=5), or referred to us for a second opinion or research purposes (n=23). All were considered to have NTZ-PML by the treating neurologist and the authors based on one of the two following criteria:

(a) A positive JCV PCR in the CSF in combination with lesions on MRI suggestive of PML, irrespective of PML symptoms.

(b) In the absence of a positive CSF JCV PCR, the presence of all four following features:

1. a high risk of developing PML, i.e., positive anti-JCV serostatus and NTZ treatment duration >12 months;
2. absence of MS disease activity prior to PML suspicion;
3. MRI lesions highly suggestive of PML with lesion characteristics as previously reported and not suggestive of other diseases,[7-12] as judged by an experienced neuroradiologist (MPW);
4. lesion evolution on follow-up MRI suggestive of PML, including development of immune reconstitution inflammatory syndrome (IRIS), characterized by contrast enhancement within the PML lesion (or outside of the PML lesion following a perivascular pattern) as previously described.[13]

Fulfillment of criterion b is exemplified for one representative case in figure 1.

PML diagnostic criteria

We retrospectively collected data on the presence of symptoms, MRI lesions suggestive of PML,[7-12] CSF JCV PCR results, and histopathological findings. All patients were classified according to the PML

diagnostic criteria (table 1). Classification was performed at first diagnostic work-up for PML, and at the last time point available during the follow-up. Fisher's exact test and one-way ANOVA were used to analyze differences in age, gender and NTZ treatment duration between the diagnostic categories. JCV PCR was performed in different laboratories: NIH (NINDS/NIH, Bethesda, MD, USA) or Focus laboratories (Focus Diagnostics Inc., Cypress, CA, USA) (21 patients), both with a reported current lower limit of detection (LLOD) of 10 copies/ml; Unilabs laboratory, Copenhagen, Denmark (4 patients), current LLOD of 11 copies/ml and three laboratories without a reported LLOD (but with positive PCR results): the Institute for Virology, Heinrich Heine University, Düsseldorf, Germany (1 patients) and two local hospital laboratories (2 patients).

RESULTS

Of the 28 included patients, 17 (60.7%) were female. At first diagnostic work-up for PML, median NTZ treatment duration was 50.5 months (interquartile range (IQR) 36-58), median age was 43.5 years (IQR 40-52), and median interval between MRI and CSF collection was 1 day (IQR 1-5).

Table 1 presents numbers and percentages of patients per diagnostic category, both at first diagnostic work-up and at last follow-up. At first diagnostic work-up 18 (64.3%) patients met the criteria for "definite PML" or "probable PML" according to the PML diagnostic criteria, generally considered as confirmed PML. Nonetheless, at that time, 10 patients who we considered to have PML did not meet these criteria.

Possible case descriptions			**PML diagnostic criteria**		
Clinical features	MRI findings	CSF JCV PCR	Diagnostic category	Number of patients meeting criteria (%)	
				At 1st diagnostic work-up	At last follow-up
+	+	+	Definite	10 (35.7)	18 (64.3)
+	-	+	Probable	0	0
-	+	+		8 (28.6)	2 (7.1)
+	+	- or ND	Possible	5 (17.9)	6 (21.5)
-	-	+		0	0
+	-	-	Not PML	0	0
-	+	-		5 (17.9)	2 (7.1)
-	-	-		0	0

Table 1. Categories of diagnostic certainty for PML diagnosis according to the PML diagnostic criteria,[3] and its application to the Dutch-Belgian NTZ-PML cohort. ND = not determined. Percentages were rounded and may not total to 100%.

Follow-up data were available for at least 6 months after PML diagnosis in all patients. The online supplementary figure presents the changes in diagnostic categories during follow-up based on development of symptoms and the results of repeated CSF JCV PCR. In two patients with a previously negative PCR, the PCR in an additionally collected CSF sample was positive. Nine patients, asymptomatic for PML at first diagnostic workup, developed PML symptoms later on. Eight patients did not exceed the criteria for “possible PML” or “not PML” during follow-up. Seven of them developed PML-IRIS following immune reconstitution. No significant differences were found between the diagnostic categories in terms of age, gender or NTZ treatment duration.

DISCUSSION

Our study demonstrates that the criteria for diagnosis of PML may not be sufficiently sensitive when applied in a real-world clinical setting with early PML lesion detection by MRI. As a result, a significant proportion of patients (10 patients (36%) in our cohort) may have undetectable JCV DNA at first PML suspicion and will not be classified as "definite PML" or "probable PML". This may lead to a certain degree of diagnostic uncertainty, particularly in centers with a low level of experience in PML diagnosis. In this case, this might delay appropriate therapeutic interventions and patient management, possibly leading to an unfavorable outcome. Even during follow-up, a majority of these patients (8 of the 10 in our cohort) may still be categorized as "possible PML" or "not PML", and may thus be registered with the regulatory authorities as 'high suspect cases' rather than 'confirmed cases', impacting official incidence numbers of PML.

Our study is limited by the use of a retrospective dataset and thus clinical assessment, MRI, PCR procedures, and the number of CSF collections in patients with a negative PCR were not standardized. However, this reflects real world clinical practice in MS pharmacovigilance. The fact that 8 patients never had a positive JCV PCR at the end of follow-up might be considered as a limitation. However, we do consider them to have PML based on the MRI lesion characteristics at baseline and evolution of these lesions during follow-up, only fitting the diagnosis of PML and not suggestive of any other disease according to the NTZ-PML lesion characteristics reported in the literature.[7-13] Furthermore, following immune reconstitution 7 of these 8 patients developed clinical symptoms and MRI findings compatible with PML-IRIS.[13] Finally, all 8 patients had high *a priori* risk for PML (inclusion criterion 1).

It is important to note that false-positive suspicion of PML based on MRI may also negatively impact treatment decisions, such as insufficient immunosuppression in a patient with active MS. There is a need to improve the sensitivity of the PCR analysis (e.g. by enhanced pre-analytic CSF standardization), and of the MRI criteria for PML diagnosis. Furthermore, analysis of intrathecally produced antibody responses to JCV[14 15] may lead to indirect proof of JC viral etiology.

Meanwhile, in conclusion for clinical practice, PML suspicion based on MRI may require repeated CSF collections to confirm PML diagnosis, and if suspicion persists despite repetitively undetectable JCV DNA from CSF, counseling with regards to brain biopsy should be considered. In our view, and in accordance with the principle “primum non nocere”, if presence of JCV DNA as causative agent can neither be proven nor ruled out, patients highly suspected of having PML based on MRI should be treated as PML, in particular if risk factors (e.g. long-term therapy with natalizumab, JCV seropositive) are present. Current PML diagnostic criteria for MS pharmacovigilance purposes should be revised considering these aspects - aspects that only recently became relevant with introducing MRI for screening in patients at risk of developing PML.

Acknowledgments

The authors wish to thank all patients included in the study for agreeing to the use of their MR images and paraclinical data for research and education purposes. In addition, we would like to thank the following physicians for sharing clinical, imaging and paraclinical data on PML patients included in this study: Bob W van Oosten and Chris H Polman (VU University Medical Center, Amsterdam, The Netherlands), Dorine A Siepman and Rogier Hintzen (Erasmus MC, University Medical Center Rotterdam, The Netherlands), Jop Mostert (Rijnstate Hospital, Department of Neurology, Arnhem, The Netherlands), Wibe Moll (Maasstad Hospital, Rotterdam, The Netherlands), Alex EL van Golde (ZGT Hospital, Almelo, The Netherlands), Stephan TFM Frequin (St Antonius Hospital, Nieuwegein, The Netherlands), Paul AD Bouma (Tergooi, Blaricum, Hilversum, The Netherlands), Cristina Tiu (Bucharest, Romania), and Bénédicte Quivron (CH Jolimont, La Louvière, Belgium), Jean Braeckeveldt (Epicura, Baudour, Belgium), Erik van Munster and Jeroen van Eijk (Department of Neurology, Jeroen Bosch Ziekenhuis, 's-Hertogenbosch, The Netherlands), Thea Heersema (Department of Neurology, University Medical Center Groningen, Groningen, The Netherlands), Jaap de Graaf (Isala Hospital, Zwolle, The Netherlands), and Raymond MM Hupperts (Zuyderland Medical Center, Sittard, The Netherlands).

Potential conflicts of interest

MTW does not report any competing interest. CW has received consultancy or speaking fees from Novartis, Bayer, Biogen and Teva. BMJU has received consultancy fees from Biogen Idec, Genzyme, Merck Serono, Novartis, Roche and Teva. FB serves as a consultant for Bayer-Schering Pharma, Sanofi-Aventis, Biogen, Teva, Novartis, Roche, Synthon BV, Genzyme and Jansen Research. JK has received consultancy fees from Merck-Serono, Teva, Biogen, Genzyme and Novartis. MPW has received consultancy fees from Biogen and Roche.

Funding

The MS Center Amsterdam is funded by a program grant (14-358e) from the Stichting voor MS Research (Voorschoten, The Netherlands). CW received support from the Charcot foundation, and the Hertie foundation (P1150063) for this work. FB is supported by NIHR-BRC-UCLH. None of the funders had any role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Author contributions

Conceptualization and design of the study: MTW, CW, JK, and MPW. Acquisition and analysis of data: MTW, JK, FB, BMJU, and MPW. Drafting of a significant portion of the manuscript or figures: MTW, CW, FB, BMJU, JK, and MPW.

REFERENCES

1. Tan CS, Koralnik IJ. Progressive multifocal leukoencephalopathy and other disorders caused by JC virus: clinical features and pathogenesis. *Lancet Neurol* 2010;9:425-37.
2. Dong-Si T, Richman S, Wattjes MP, et al. Outcome and survival of asymptomatic PML in natalizumab-treated MS patients. *Ann Clin Transl Neurol* 2014;1(10):755-64.
3. Berger JR, Aksamit AJ, Clifford DB, et al. PML diagnostic criteria: consensus statement from the AAN Neuroinfectious Disease Section. *Neurology* 2013;80(15):1430-38.
4. Phan-Ba R, Lommers E, Tshibanda L, et al. MRI preclinical detection and asymptomatic course of a progressive multifocal leucoencephalopathy (PML) under natalizumab therapy. *J Neurol Neurosurg Psychiatry* 2012;83:224-26.
5. Wijburg MT, Kleerekooper I, Lissenberg-Witte BI, et al. Association of Progressive Multifocal Leukoencephalopathy Lesion Volume With JC Virus Polymerase Chain Reaction Results in Cerebrospinal Fluid of Natalizumab-Treated Patients With Multiple Sclerosis. *JAMA Neurol* 2018.
6. Kuhle J, Gosert R, Buhler R, et al. Management and outcome of CSF-JC virus PCR-negative PML in a natalizumab-treated patient with MS. *Neurology* 2011;77:2010-16.
7. Wijburg MT, Witte BI, Vennegoor A, et al. MRI criteria differentiating asymptomatic PML from new MS lesions during natalizumab pharmacovigilance. *J Neurol Neurosurg Psychiatry* 2016;87(10):1138-45.
8. Wattjes MP, Vennegoor A, Steenwijk MD, et al. MRI pattern in asymptomatic natalizumab-associated PML. *J Neurol Neurosurg Psychiatry* 2015;86(7):793-8.
9. Hodel J, Darchis C, Outteryck O, et al. Punctate pattern: A promising imaging marker for the diagnosis of natalizumab-associated PML. *Neurology* 2016;86(16):1516-23.
10. Hodel J, Outteryck O, Dubron C, et al. Asymptomatic Progressive Multifocal Leukoencephalopathy Associated with Natalizumab: Diagnostic Precision with MR Imaging. *Radiology* 2016;278(3):863-72.
11. Carra-Dalliere C, Menjot de Champfleur N, Deverdun J, et al. Use of quantitative susceptibility mapping (QSM) in progressive multifocal leukoencephalopathy. (0150-9861 (Print)).
12. Yousry TA, Pelletier D, Cadavid D, et al. Magnetic resonance imaging pattern in natalizumab-associated progressive multifocal leukoencephalopathy. *Ann Neurol* 2012;72(5):779-87.
13. Wattjes MP, Wijburg MT, Vennegoor A, et al. MRI characteristics of early PML-IRIS after natalizumab treatment in patients with MS. *J Neurol Neurosurg Psychiatry* 2015;87(8):879-84.
14. Warnke C, Wijburg MT, Hartung HP, et al. Application of the CSF JCV antibody index to early natalizumab-associated progressive multifocal leukoencephalopathy. *J Neurol Neurosurg Psychiatry* 2017;264(6):1155-64.
15. Warnke C, von Geldern G, Markwerth P, et al. Cerebrospinal fluid JC virus antibody index for diagnosis of natalizumab-associated progressive multifocal leukoencephalopathy. *AnnNeurol* 2014;76(6):792-801.

FIGURE LEGENDS

Figure 1.

Title: MR imaging of a NTZ-PML patient included in this study based on inclusion criterion b.

Legend: Axial fluid attenuation inversion recovery (FLAIR; left column), T2-weighted (middle column) and contrast-enhanced T1-weighted (right column) images of a patient with a high risk for PML development (positive anti-JC virus serostatus and long-term natalizumab treatment (>48 infusions)), no MS disease activity during natalizumab treatment, and lesions on MRI highly suggestive of PML. Multiple FLAIR and T2 hyperintense lesions subcortical, cortical and in the deep white matter, without enhancement after gadolinium (D – F; closed arrowheads). In retrospect, one lesion was already visible four months earlier (A – C; closed arrowhead). CSF was collected three times: on the day of MRI D – F, and one week and one month later. In all samples JC virus PCR was negative (local hospital lab and Focus laboratories (Focus Diagnostics Inc., Cypress, CA, USA)). Natalizumab was stopped and plasma exchange was performed. Two and a half months later, the patient's condition deteriorated with a left sided paralysis and lowered consciousness. Follow-up MRI (G – I) showed progression of the existing lesions and development of new lesions (closed arrowheads), with a patchy contrast enhancement in lesion borders (open arrowheads), in accordance with immune reconstitution inflammatory syndrome (PML-IRIS). The patient met the criteria for "possible PML" according to the PML diagnostic criteria at this stage.