

**MR imaging criteria differentiating asymptomatic PML from new MS lesions during natalizumab
pharmacovigilance**

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ABSTRACT

Objective

Differentiation between progressive multifocal leukoencephalopathy (PML) and new multiple sclerosis (MS) lesions on brain magnetic resonance imaging (MRI) during natalizumab pharmacovigilance in the absence of clinical signs and symptoms is challenging but is of substantial clinical relevance. We aim to define MRI characteristics that can aid in this differentiation.

Methods

Reference and follow-up brain MRI of natalizumab-treated MS patients with asymptomatic PML (n=21), or asymptomatic new MS lesions (n=20) were evaluated with respect to characteristics of newly detected lesions by four blinded raters. We tested the association with PML for each characteristic and constructed a multivariable prediction model which we analyzed using a receiver operating characteristic (ROC) curve.

Results

Presence of punctuate T2 lesions, cortical grey matter involvement, juxtacortical white matter involvement, ill-defined and mixed lesion borders towards both grey and white matter, lesion size of >3cm, and contrast enhancement were all associated with PML. Focal lesion appearance and periventricular localization were associated with new MS lesions. In the multivariable model, punctuate T2 lesions and cortical grey matter involvement predict for PML, while focal lesion appearance and periventricular localization predict for new MS lesions (area under the curve: 0.988, 95% CI: 0.977-1.0, sensitivity: 100%, specificity: 80.6%).

Interpretation

The MRI characteristics of asymptomatic natalizumab-associated PML lesions proved to differ from new MS lesions. This led to a prediction model with a high discriminating power. Careful assessment of the presence of punctuate T2 lesions, cortical grey matter involvement, focal lesion appearance, and periventricular localization, allows an early diagnosis of PML.

INTRODUCTION

Pharmacovigilance of multiple sclerosis (MS) patients includes the detection of disease activity, co-morbidities (eg, vascular pathology) and drug-related adverse events including opportunistic infections. For these purposes, brain MRI plays an essential role, in addition to monitoring of clinical symptoms and paraclinical tests (e.g. cerebrospinal fluid (CSF) testing and blood biomarkers).[1–3]

The introduction of natalizumab, a humanized monoclonal antibody against the α 4-integrin adhesion molecule approved for the treatment of relapsing MS, has further stressed the need for strict pharmacovigilance, including brain MRI monitoring.[4–6] Though clinically highly effective, the use of natalizumab is associated with an increased risk for JC virus (JCV) related diseases such as progressive multifocal leukoencephalopathy (PML), an opportunistic infection of glial and neuronal cells in the central nervous system (CNS) caused by reactivation and replication of the JCV, and granule cell neuronopathy (GCN).[4,7–13]

Early detection of natalizumab-associated PML is clinically relevant because PML patients who are asymptomatic at the time of diagnosis have a better functional outcome and survival compared to those who are symptomatic.[14–16] Several reports have indeed demonstrated that brain MRI may detect natalizumab-associated PML lesions several months before the patient develops symptoms (asymptomatic natalizumab-associated PML lesions).[14–22] Therefore, the use of brain MRI in pharmacovigilance of natalizumab treated MS patients for screening purposes with respect to the early detection of PML in high risk patients has been recommended by recent expert panel guidelines.[23–25]

When a new brain lesion is detected on MRI in clinically stable MS patients during natalizumab treatment, the crucial but also challenging question is whether this is a new asymptomatic PML lesion or a new MS lesion. As JCV DNA is frequently undetectable in the CSF of these asymptomatic PML patients, it is of paramount importance to accurately make this distinction based on MRI findings.[20] On brain MRI, new asymptomatic PML lesions may be rather small and mimic other pathologies, especially MS lesions, as they can share common characteristics.[20,26–28] This allows

for asymptomatic PML lesions to be missed or misinterpreted as MS lesions, with clinical consequences.

The challenge of diagnosing asymptomatic PML and differentiating asymptomatic PML lesions from new MS pathology has been stressed by a previous study showing that the sensitivity for the detection of asymptomatic PML was only 59.5% with a specificity of 91.7%. [28] In addition, there was moderate agreement among the readers with respect to the detection of asymptomatic PML (Fleiss' kappa of 0.52). [28,29] The two main factors contributing to the low sensitivity appeared to be the inability to detect smaller new asymptomatic PML lesions and the misinterpretation of asymptomatic PML lesions as new focal MS lesions. Thus, the identification of discriminative MRI lesion characteristics that differentiate asymptomatic PML from new MS lesions during pharmacovigilance is of utmost clinical importance. The aim of this study was to identify these MRI lesion characteristics.

PATIENTS AND METHODS

Standard protocol approvals, registrations, and patient consents

Brain imaging of MS patients treated with natalizumab is part of the standard patient care. A waiver was provided by the local institutional review board (IRB), stating that the requirements of the Medical Research Involving Human Subjects Act (WMO) did not apply to this study and that an official IRB approval was not required. We obtained written informed consent from all included patients for the use of the clinical, laboratory and MRI data for research and educational purposes.

Study design and patient selection

In this retrospective follow-up study, we further analyzed data obtained following a previous study, which included asymptomatic natalizumab-associated PML patients with MS, and MS patients either with or without new MS lesions on follow-up brain MRI during natalizumab treatment[28]. The study design, patient selection and patient characteristics of the study groups have been described in the previous study.[28] In short, the asymptomatic PML group comprised patients from the Dutch-Belgian natalizumab-associated PML database as well as patients who were referred to us for second opinion and research purposes. This group included 21 patients, 15 of whom were female. Mean age: 45 years (standard deviation (SD): 11 years); mean natalizumab treatment duration: 44 months (SD: 18 months); median of two new lesions per patient (range 1-5). These patients showed no new concomitant MS lesions on the follow-up MRI. The patients with new MS lesions during natalizumab treatment were selected from our local cohort of natalizumab-treated MS patients. This group included 20 patients, 14 of whom were female. Mean age: 38 years (SD: 8 years); mean natalizumab treatment duration: 11 months (SD: 4 months); median of two new lesions per patient (range 1-6). These patients showed no new concomitant PML lesions on the follow-up MRI. Given the aim of the current study, patients without new lesions on follow-up MRI were not included.

MRI protocols and readers

The detailed information regarding MRI protocols (including at least axial T2/proton density (PD)-weighted or FLAIR images in all patients) used in this study and the clinical experience of the blinded readers who performed the ratings have been described in the previous study.[28] Diffusion weighted images (DWI) were not included in the current study because, although DWI were mostly present in the asymptomatic PML group, these images were not available in the new MS lesion group.

Image analysis

The image analysis for the current study was performed in a second evaluation subsequent to the first image analysis step (to determine for each patient if new lesions were present on the follow-up scan compared to the reference scan, and whether the readers considered the lesions to be suggestive for PML or MS pathology), which was analyzed in the previous study.[28] In the second evaluation, each reader had to score the lesion characteristics of all newly detected lesions according to a specially designed scoring scheme based on previously reported PML and MS lesion characteristics.[21,22,26,30,31] The proposed characteristics were: the lesion distribution (unilobar (lesions confined to one lobe), multilobar (lesions in two or more contiguous lobes) or widespread (lesions in two or more non-contiguous lobes or present in both hemispheres)); hemispherical involvement (unilateral or bilateral); anatomical location (frontal lobe, parietal lobe, occipital lobe, temporal lobe, corpus callosum, thalamus, basal ganglia, brainstem or cerebellum), tissue involvement (juxtacortical white matter, deep white matter, periventricular white matter, cortical grey matter or deep grey matter), lesion appearance (focal, diffuse, confluent irregular or infiltrative), lesion borders towards the white and grey matter (sharp, mixed or ill-defined), lesion size (>3cm or <3cm in diameter), signs of mass effect (present or absent), signs of edema (present or absent), contrast-enhancement (present or absent), intensity on T1, T2, and FLAIR images (hypointense, isointense, hyperintense), and the presence of punctuate T2 lesions (punctiform T2/FLAIR hyperintense lesions in the vicinity of the main PML lesions recently described as “milky

way appearance” and/or punctiform T2/FLAIR hyperintense lesions with a perivascular spread, <5 mm in diameter).

Statistical analysis

Using generalized estimating equations (GEEs) in SPSS statistics software version 22 (IBM Corp., Armonk, NY, USA) we investigated the association of the lesion characteristics with asymptomatic PML or new MS lesions. A logit function was used to link the characteristics of the lesion nested within a patient to the outcome (asymptomatic PML group or new MS lesion group), and an independent correlation structure was chosen as two separate lesions of the same cause within a patient will probably have different lesion characteristics. As the ratings of the current and previous study were performed consecutively, we were unable to include those lesions that were missed by the readers in the first image analysis step (as described in the previous study)[28] in the current analyses. Therefore, the number of rated patients in both groups is not the same for each of the four raters. For each separate lesion characteristic the association with asymptomatic PML or new MS lesions was assessed, results were expressed as an odds ratio (OR) with accompanying 95% confidence interval (CI), and p-value for the test of model effects. An OR greater than 1 indicates an association with asymptomatic PML, conversely an OR less than 1 indicates an association with new MS lesions. For the categorical variables lesion borders towards the white and grey matter, and the lesion dissemination, the categories sharp borders and a widespread lesion dissemination were set as reference category, respectively. Only overall p-values were reported for these variables.

For those lesion characteristics with complete data separation (i.e. in either the PML or the MS group the characteristic is either present or absent for all cases), we tested the association using the Pearson chi-square or Fisher’s exact test. We thereby ignored the repeated scorings within a patient and were unable to calculate an OR.

We built a multivariable prediction model consisting of lesion characteristics predictive for asymptomatic PML or new MS lesions via a forward selection procedure (p-value for entry <0.05).

The model was corrected for the potential confounding patient characteristics age, gender and treatment duration. Using the Receiver Operating Characteristic (ROC) curve, the ability of the model to discriminate between asymptomatic PML and new MS lesions was assessed. The ROC curve was based on all patient ratings of each separate rater combined. When multiple lesions were rated within a patient, the lesion with the highest value of the linear predictor was used for analysis, which is the lesion that is most predictive for PML and would most likely guide treatment decision making. Finally, we calculated a Cohen's kappa between all sets of raters for the lesion characteristics that were significantly associated with asymptomatic PML or new MS lesions in order to test the inter-rater reliability for the scoring of these lesion characteristics. For the categorical characteristics we calculated Fleiss' kappa between the sets of raters. The kappa values were interpreted according to the Landis and Koch scheme: $\kappa < 0.00$, no agreement; $0.00 \leq \kappa \leq 0.20$, slight agreement; $0.21 \leq \kappa \leq 0.40$, fair agreement; $0.41 \leq \kappa \leq 0.60$, moderate agreement; $0.61 \leq \kappa \leq 0.80$, substantial agreement; $0.81 \leq \kappa \leq 1.00$, almost perfect agreement.[29,32]

For all tests, a p-value of <0.05 was considered statistically significant.

RESULTS

Association of the separate lesion characteristics with asymptomatic PML or new MS lesions

We calculated the rating frequencies of all lesion characteristics, the OR's for the association of these lesion characteristics with asymptomatic PML, and the accompanying 95% confidence intervals, and p-values (Table 1).

Presence of punctuate T2 lesions showed a very strong association with asymptomatic PML lesions (OR: 135.6, CI: 18.9-974.7, $p < 0.001$, Figure 1). New lesions with cortical grey matter, or juxtacortical white matter involvement were more likely to be asymptomatic PML lesions than MS lesions (OR: 14.8, CI: 3.6-61.1, $p < 0.001$, and OR: 3.6, CI: 1.2-11.1, $p = 0.023$ respectively, Figure 2). Both ill-defined and mixed borders (compared to the reference category sharp borders) towards the white matter, and towards the grey matter were significantly associated with asymptomatic PML (OR: 9.1, CI: 4.0-20.5, and OR: 6.2, CI: 1.9-20.5, $p < 0.001$ respectively for white matter, and OR: 3.7, CI: 1.6-8.8, and OR: 4.8, CI: 1.6-14.5, $p = 0.001$ respectively for grey matter). Furthermore, presence of contrast-enhancement was significantly associated with asymptomatic PML (OR: 9.5, CI: 1.1-82.2, $p = 0.041$, Figure 2).

Conversely, new lesions with a focal appearance (as opposed to a diffuse, confluent irregular, or infiltrative appearance), and lesions located in the periventricular white matter were more likely to be new MS lesions instead of asymptomatic PML (OR: 0.026, CI: 0.0081-0.083, $p < 0.001$, and OR: 0.028, CI: 0.0036-0.22, $p = 0.001$ respectively, Figure 3).

Lesion characteristics	Times rated in PML lesions ^a <i>n=105</i>	Times rated in MS lesions ^a <i>n=110</i>	OR for PML ^b	95% CI		P-value ^c
<u>General characteristics:</u>						
Bilateral hemispheric involvement ^d	41	62	0.50	0.15	– 1.6	0.24
Unilobar lesion dissemination ^e	41	29	2.3	0.74	– 7.5	0.30
Multilobar lesion dissemination ^e	23	13	2.9	0.57	– 15.1	0.30
Widespread lesion dissemination	41	68	reference			
Mass effect	1	0	n/a	n/a	– n/a	n/a ^f
Signs of edema	1	2	0.5	0.041	– 6.6	0.62
Lesion size >3 cm ^g	33	0	n/a	n/a	– n/a	<0.001¹
<u>Lesion location:</u>						
Frontal lobe	49	40	1.5	0.54	– 4.3	0.42
Parietal lobe	13	21	0.60	0.20	– 1.8	0.35
Occipital lobe	10	3	3.8	0.78	– 18.1	0.099
Temporal lobe	6	10	0.61	0.17	– 2.1	0.44
Corpus callosum	0	5	n/a	n/a	– n/a	0.060 ²
Thalamus	5	0	n/a	n/a	– n/a	0.026²
Basal ganglia	4	10	0.40	0.077	– 2.0	0.27
Brainstem	12	15	0.82	0.21	– 3.3	0.77
Cerebellum	18	5	4.3	0.89	– 21.3	0.070
<u>Tissue involvement:</u>						
Juxtacortical WM	63	32	3.6	1.2	– 11.1	0.023
Deep WM	47	53	0.82	0.36	– 1.9	0.64
Periventricular WM	1	27	0.028	0.0036	– 0.22	0.001
Cortical GM	23	2	14.8	3.6	– 61.1	< 0.001

Deep GM	13	8	1.8	0.38	–	8.1	0.47
<u>Lesion appearance:</u>							
Focal lesion appearance ^h	47	107	0.026	0.0081	–	0.083	< 0.001
<u>Lesion borders:</u>							
	<i>n=103</i>	<i>n=108</i>					
Ill-defined border toward WM ⁱ	45	13	9.1	4.0	–	20.5	< 0.001
Mixed border toward WM ⁱ	26	11	6.2	1.9	–	20.5	< 0.001
Sharp border toward WM	32	84	reference				
	<i>n=98</i>	<i>n=102</i>					
Ill-defined border toward GM ⁱ	20	10	3.7	1.6	–	8.8	0.001
Mixed border toward GM ⁱ	36	14	4.8	1.6	–	14.5	0.001
Sharp border toward GM	42	78	reference				
<u>Specific PML characteristics:</u>							
Presence of punctuate T2 lesions ^j	56	1	135.6	18.9	–	974.7	< 0.001
<u>MRI sequences and contrast enhancement:</u>							
T1 hypointense ^k	31 (n=74)	47 (n=71)	0.37	0.11	–	1.2	0.107
T2 hyperintense ^k	110 (n=110)	92 (n=93)	n/a	n/a	–	n/a	n/a
FLAIR hyperintense ^k	105 (n=108)	102 (n=103)	2.9	0.25	–	34.5	0.397
Presence of contrast enhancement	23 (n=92)	2 (n=59)	9.5	1.1	–	82.2	0.041

Table 1. Association of all lesion characteristics with asymptomatic PML lesions

PML: progressive multifocal leukoencephalopathy, OR: odds-ratio, CI: confidence interval, WM: white matter, GM: grey matter, n/a: not applicable, FLAIR: fluid attenuated inversion recovery.

^a Rated by four readers in 41 patients (21 with PML lesion(s) and 20 with new MS lesion(s)), ^b OR>1 is associated with PML lesions, OR<1 is associated with new MS lesions, ^c for test of model effects, ^d compared to “unilateral hemispheric involvement”, ^e compared to “widespread lesion dissemination”, ^f mass effect was rated as present once, resulting in complete data separation, ^g compared to “lesion size <3cm”, ^h compared to “non-focal appearance” (i.e. rated as: diffuse, confluent irregular, or infiltrative appearance), ⁱ compared to “sharp borders”, ^j defined as punctiform T2/FLAIR hyperintense lesions in the vicinity of the main PML lesions recently described as “milky way appearance” and/or punctiform T2/FLAIR hyperintense lesions with a perivascular spread (<5 mm in diameter), ^k compared to isointense/not visible on this sequence.

¹ Calculated by Pearson chi-square and ² calculated by Fisher’s exact test because of complete data separation (i.e. in either the PML or the MS lesion group the characteristic is either present or absent for all cases), and thereby ignoring the repeated scorings within a patient.

Multivariable prediction model of lesion characteristics differentiating asymptomatic PML from new MS lesions

A multivariable model of lesion characteristics predicting a new lesion being either asymptomatic PML or a new MS lesion, was constructed (Table 2). The model was corrected for age and treatment duration.

Presence of punctuate T2 lesions, and cortical grey matter tissue involvement remained significant predictors for asymptomatic PML lesions in the multivariable model (OR:183.2, CI: 11.4-2950.7, $p<0.001$, and OR: 59.8, CI: 8.4-427.6, $p<0.001$). A focal lesion appearance and periventricular white matter tissue involvement remained significant predictors for new MS lesions in the multivariable prediction model (OR: 0.009, CI: 0.0008-0.12, $p<0.001$, and OR: 0.0006, CI: 0.00003-0.0121, $p<0.001$ respectively). The algebraic expression of the model is available as supplementary material.

We calculated a ROC curve for the multivariable model to test the ability of the model to discriminate between asymptomatic PML and new MS lesions (Figure 4). The model showed a very high discriminating power between asymptomatic PML and new MS lesions with an area under the curve (AUC) of 0.988 (95% CI: 0.977-1.0), based on 65 ratings of asymptomatic PML patients and 62 ratings of patients with new MS lesions by the four raters combined. Using a linear predictor threshold of -2.15 corresponds to a sensitivity of 100% with a specificity of 80.6%. Likewise, applying a linear predictor cutoff of 3.04 corresponds to a sensitivity of 86.2% with a specificity of 100%. The ROC curves for each rater separately are available as supplementary material.

Predictive lesion characteristics ^a	OR for PML ^b	95% CI		P-value	
Focal lesion appearance ^c	0.009	0.0008	–	0.12	< 0.001
Periventricular white matter localization	0.0006	0.00003	–	0.0121	< 0.001
Presence of punctuate T2 lesions ^d	183.2	11.4	–	2950.7	< 0.001
Cortical grey matter involvement	59.8	8.4	–	427.6	< 0.001

Table 2. Multivariable prediction model of lesion characteristics differentiating asymptomatic PML from new MS lesions in order of entry in the model.

PML: progressive multifocal leukoencephalopathy, OR: odds-ratio, CI: confidence interval.

^a The model is corrected for age and treatment duration and the lesion characteristics are listed in order of stepwise entering in the model, ^b OR>1 is associated with PML lesions, OR<1 is associated with new MS lesions, ^c Compared to “non-focal appearance” (i.e. rated as: diffuse, confluent irregular, or infiltrative appearance), ^d defined as punctiform T2/FLAIR hyperintense lesions in the vicinity of the main PML lesions recently described as “milky way appearance” and/or punctiform T2/FLAIR hyperintense lesions with a perivascular spread (<5 mm in diameter).

Inter-rater agreement on lesion characteristics

The inter-rater agreement on the scoring of all significant lesion characteristics was calculated (Table 3). The agreement is presented as the median and the range of Cohen's kappa between all sets of two raters.

Of the lesion characteristics included in the multivariable model a focal lesion appearance, periventricular white matter localization, and punctuate T2 lesions showed a moderate inter-rater agreement (median κ : 0.56, 0.59, and 0.57 respectively), and cortical grey matter showed a fair inter-rater agreement (median κ : 0.22).

Amongst the other lesion characteristics that were associated with asymptomatic PML, juxtacortical white matter involvement and presence of contrast enhancement showed a substantial inter-rater agreement, lesion size showed a moderate inter-rater agreement, and lesion borders towards white and grey matter showed just a slight inter-rater agreement.

Lesion characteristics	Median kappa between sets of raters	Range of kappa's		
<u>General characteristics:</u>				
Lesion size >3 cm	0.52	0.18	–	0.70
<u>Tissue involvement:</u>				
Juxtacortical WM	0.72	0.44	–	0.87
Periventricular WM	0.59	0.47	–	0.84
Cortical GM	0.22	0.13	–	0.35
<u>Lesion appearance:</u>				
Focal lesion appearance	0.56	0.38	–	0.82
<u>Lesion borders:</u>				
Lesion border toward WM	0.19	-0.24	–	0.66
Lesion border toward GM	0.091	-0.092	–	0.25
<u>Specific PML characteristics:</u>				
Presence of punctuate T2 lesions	0.57	0.49	–	0.67
<u>MRI sequences and contrast enhancement:</u>				
Presence of contrast enhancement	0.78	0.55	–	0.87

Table 3. Inter-rater agreement for all significant lesion characteristics, expressed as median and range of kappa's between all sets of raters.

PML: progressive multifocal leukoencephalopathy, WM: white matter, GM: grey matter, n/a: not applicable, FLAIR: fluid attenuated inversion recovery.

DISCUSSION

The present study showed that in the absence of clinical signs and symptoms it is possible to differentiate PML lesions from new MS lesions based on careful assessment of lesion characteristics on MRI in MS patients treated with natalizumab. A recent study by Hodel and colleagues comparing natalizumab-treated MS patients without new lesions to patients with asymptomatic natalizumab-associated PML, described useful MRI features predictive for asymptomatic PML lesions (DWI hyperintensity and U fiber involvement).[33] In our study we tried to make one important step further by simulating the real-life pharmacovigilance setting with reference MRI scans and aimed to directly compare the lesion characteristics of new asymptomatic PML lesions and new MS lesions in natalizumab-treated MS patients. We describe rather conclusive MRI lesion characteristics which can aid in differentiating asymptomatic PML lesions from new MS lesions in a pharmacovigilance setting of natalizumab-treated MS patients, thereby potentially increasing the chance of an early and specific diagnosis of asymptomatic PML based on brain MRI.

The presence of punctuate T2 lesions showed a very strong association with asymptomatic PML lesions. In previous studies, punctiform T2/FLAIR hyperintense lesions in the vicinity of the main PML lesions, also described as “milky way appearance” when being present in high numbers,[22] showed to be common in both symptomatic and asymptomatic natalizumab-associated PML.[21,22,30,33] Punctiform T2 lesions are also seen further away from the main PML lesion, even in the hemisphere contralateral to the main PML lesion, and can have a perivascular distribution pattern suggestive of perivascular inflammation.[22,26,30] In clinical practice, it can be difficult to differentiate between punctiform T2 lesions in the vicinity of the main PML lesion and punctiform T2 lesions with a perivascular spread. In addition, it is likely that there is an overlap in these two types of punctuate lesions and they may share similar characteristics in terms of underlying pathology.[34,35] Therefore, we did not differentiate between these two types of punctuate T2 lesions and considered the presence of any punctuate T2 lesions as one characteristic. In line with these results, a recent study showed that these punctuate T2 lesions are frequently visible in various stages of natalizumab-

associated PML as well as in PML due to other conditions, while clinically isolated syndrome and MS patients do not show these punctuate T2 lesions.[36] Given the compiling evidence from our study in addition to the previous studies, the presence of punctuate T2 lesions seems to be one of most important imaging features when differentiating asymptomatic PML lesions from new MS lesions and the presence of this feature could lead to a specific diagnosis of asymptomatic PML.

Even though MS lesions are known to occur in the juxtacortical white and cortical grey matter (juxtacortical lesions have even been incorporated in the revised MS diagnostic criteria),[31] cortical grey matter and juxtacortical white matter lesion involvement were significantly associated with asymptomatic PML instead of new MS lesions in our study. Indeed, the recent study by Hodel and colleagues nicely showed that a subcortical lesion location, involving the U fibers, was predictive for asymptomatic natalizumab-associated PML.[33] In contrast, a focal lesion appearance, and lesions located in the periventricular white matter were strongly associated with new MS lesions instead of asymptomatic PML, which is not surprising since this type of lesion is one of the well know stereotype MS lesions included in the MRI diagnostic criteria for MS.[31] In our study, we found no significant difference in lesion intensity on T1, T2, or FLAIR images between asymptomatic PML and new MS lesions. However, the presence of contrast-enhancement was significantly associated with asymptomatic PML. This might be due to the fact that the strong treatment effect of natalizumab prevents blood brain barrier breakdown, and therefore new MS lesions during natalizumab treatment are less likely to show any contrast-enhancement. Thus, contrast-enhancing lesion during natalizumab-treatment are more likely inflammatory PML lesions, a phenomenon seen in approximately 30% of the patients at PML diagnosis.[17,21,22,30,37]

In previous reports, PML lesions have been described as having ill-defined borders towards the white matter and sharp borders towards the grey matter.[22] Interestingly, in our data ill-defined and mixed borders compared to sharp borders towards both the white, and the grey matter were significantly associated with asymptomatic PML. This might be due to the fact that the previously

reported observation of sharp borders towards the grey matter is mainly based on research in symptomatic PML patients, whom might show more extensive demyelination in the juxtacortical white matter compared to asymptomatic PML patients, thereby creating a more visible contrast between the grey matter and the lesion.[22]

A lesion size of >3cm was frequently rated in the asymptomatic PML lesions and in none of the new MS lesions. Because of the complete data separation in this lesion characteristics, we tested the association via the Pearson chi-square test, which showed a significant association ($p < 0.001$).

Although, we thereby erroneously ignore the repeated scorings within a patient, and are unable to calculate an OR, it does indicate that this lesion characteristic is highly relevant. In previous reports it has also been demonstrated that symptomatic PML lesions are often larger than 3 cm in diameter as opposed to most focal MS lesions.[22,38] Although the Fisher's exact test gives a significant association of the thalamic and corpus callosal lesion localization with asymptomatic PML and new MS lesions respectively, the small amount of ratings of the presence of these lesion characteristics calls into question the true meaning of these results. In fact, this might very well be the result of the fact that these tests disregard the multiple ratings within each patient and consider each rating as a single patient.

Ultimately, four lesion characteristics made the $p < 0.05$ threshold in the forward selection procedure of the multivariable prediction model, which was corrected for age and treatment duration (table 2). The presence of a focal lesion appearance and periventricular localization remained significant in this multivariable model and predict for new MS lesions. Punctuate T2 lesions and cortical grey matter involvement significantly predict for asymptomatic PML in our multivariable model. When tested in this dataset using an ROC curve, the model showed a very high discrimination power with an AUC of 0.988 and a sensitivity and specificity of 100% and 80.6% respectively when the threshold is chosen for maximum sensitivity (Figure 4).

The inter-rater agreement for the scoring of the lesion characteristics in the multivariable model was moderate for focal lesion appearance, periventricular white matter localization, and punctuate T2 lesions. However, the raters showed a markedly lower (fair) agreement on the scoring of cortical grey matter involvement, indicating that this lesion characteristic is more difficult to score and thus there might be need for dedicated training on the lesion characteristics (for instance via e-learning programs such as <https://ms-pml.org/>).

A limitation of our study is that we had to use GEEs to correct for multiple ratings within a patient. Unfortunately this type of analysis precludes the testing of characteristics with complete data separation (such as lesion size of >3cm), which were therefore not eligible for the multivariable model. Another limitation is that since the cases were collected retrospectively from a multicenter database, not all MRI scans were performed according to a standardized MRI protocol. However, this is hard to avoid as MRI data of a substantial number of asymptomatic PML patients from a single center lack. In addition we cannot exclude that when a new lesion was suspected to be either PML or MS, the lesion characteristics were scored in accordance with the suspected cause of the lesion. This may have led to an overestimation of the discriminatory power of the lesion characteristics. The ROC curve of the model was based on the same data as on which the model was designed, therefore potentially leading to overfitting of the data. In order to validate the model, it should be tested in an independent dataset. We were unable to include DWI in this study, which is unfortunate as a subset of asymptomatic PML lesions can be visible on high B-value DWI without showing a low signal on the apparent diffusion coefficient (ADC), meaning no real diffusion restriction is present.[21] Although new MS lesions are also frequently hyperintense,[39] there might still be an added value of DWI in differentiating asymptomatic PML from new MS lesions in a pharmacovigilance setting. However, this needs to be further investigated.

In conclusion, this study identifies MRI lesion characteristics which aid in differentiating asymptomatic PML lesions from new MS lesions in natalizumab-treated MS patients who do not

show new neurological symptoms. Most importantly, we present a prediction model of four lesion characteristics that shows a very high discriminating power in making this distinction. In our model the presence of punctuate T2 lesions, and cortical grey matter involvement strongly predict for the lesion being asymptomatic PML, while the presence of a focal lesion appearance, and a periventricular location strongly predict for the lesion to be a new MS lesion. Knowledge on these lesion characteristics can improve an early and specific diagnosis of natalizumab-associated asymptomatic PML and thereby lead to a better chance of survival and an improved functional outcome in these patients.

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Competing interest

MPW has received consultancy fees from Biogen and Roche. FB serves as a consultant for Bayer-Schering Pharma, Sanofi-Aventis, Biogen, Teva, Novartis, Roche, Synthron BV, Genzyme, Jansen Research. JK has accepted consulting fees from Merck-Serono, TEVA, Biogen, Genzyme, and Novartis. BMJU has received consultancy fees from Biogen Idec, Genzyme, Merck Serono, Novartis, Roche en TEVA. AV has received speaking fees from Teva. MTW, ES, COM, YL, SDR and BIW do not report any competing interest. The VUmc MS Center Amsterdam has received financial support for research activities from Bayer Schering Pharma, Biogen, Glaxo Smith Kline, Merck Serono, Novartis, and Teva.

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Legend to the figures:

Figure 1: Reference (A and B) and follow-up (C and D) axial T2-weighted images of a natalizumab-treated MS patient from our study. The follow-up MRI clearly shows the main PML lesion in the juxtacortical and deep white matter of the left frontal lobe and a second, smaller, PML lesion in the right frontal lobe (C and D, closed arrowheads). Punctuate T2 hyperintense lesions are clearly visible throughout the left hemisphere, both in the vicinity of the main PML lesion and further away with a rather perivascular spread (C and D, open arrowheads). The main PML lesion and the punctuate T2 hyperintense lesions did not show any enhancement after gadolinium administration on T1-weighted images (images not shown).

Figure 2: Reference axial T2-weighted images (A and B) and follow-up axial T2-weighted (C and D), and contrast enhanced T1-weighted (E and F) images of a natalizumab-treated MS patient from our study. The follow-up MRI shows a new PML lesion in the juxtacortical white matter and cortical grey matter of the right frontal lobe, which enhances after gadolinium administration (C and E, closed arrowheads). Punctuate T2 hyperintense lesions are visible with a rather perivascular spread (D, open arrowheads), and show a punctuate enhancement on T1-weighted images after gadolinium enhancement (F, open arrowheads).

Figure 3: Reference (A) and follow-up (C) axial T2-weighted images, and reference (B) and follow-up (D) sagittal fluid attenuated inversion recovery (FLAIR) images of a natalizumab-treated MS patient from our study. The follow-up MRI shows a new focal periventricular MS lesions in the corpus callosum (C and D, closed arrowheads).

Figure 4: Receiver operating characteristic (ROC) curve for the differentiation between asymptomatic PML and new MS lesions based on the multivariable model based on all patient ratings of the four raters combined.

SUPPLEMENTARY MATERIAL

Supplementary material 1

Figure S – 1

Algebraic expression of the multivariable model:

$$\text{Log(odds)} = -11.3 - (4.7 \times \text{focal appearance [absent = 0, present = 1]}) - (7.4 \times \text{periventricular localization [absent = 0, present = 1]}) + (4.1 \times \text{cortical grey matter involvement [absent = 0, present = 1]}) + (5.2 \times \text{punctuate T2 lesions [absent = 0, present = 1]}) + (5.0 \times \text{treatment duration [>12 months = 1, } \leq 12 \text{ months = 0]}) + (0.3 \times \text{age [years]})$$

Supplementary material 2

Legend to the figures:

Figure S – 2

Receiver operating characteristic (ROC) curve for the differentiation between asymptomatic PML and new MS lesions based on the multivariable model for all four raters separately.

Figure S – 2 (a)

ROC curve for rater 1. AUC: 0.997 (95% CI: 0.988 – 1.0) based on the rating of 18 asymptomatic PML patients and 20 patients with new MS lesions.

Figure S – 2 (b)

ROC curve for rater 2. AUC: 0.971 (95% CI: 0.922 – 1.0) based on the rating of 16 asymptomatic PML patients and 16 patients with new MS lesions.

Figure S – 2 (c)

ROC curve for rater 3. AUC: 0.994 (95% CI: 0.979 – 1.0) based on the rating of 16 asymptomatic PML patients and 17 patients with new MS lesions.

Figure S – 2 (d) ROC curve for rater 4. AUC: 1.0 (95% CI: 1.0 – 1.0) based on the rating of 15 asymptomatic PML patients and 9 patients with new MS lesions.