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Short report

Longitudinal analyses of anti-JCV antibody index for risk assessment of progressive multifocal leukoencephalopathy

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Abstract

Risk assessment for natalizumab-associated progressive multifocal leukoencephalopathy (Nat-PML) comprises the anti-JC virus (JCV) antibody index (AI). The anti-JCV AI was longitudinally determined in a natalizumab-treated MS cohort (Nat-MS, $n = 468$) and samples of Nat-PML patients ($n = 15$). In Nat-MS, the median AI was 0.8 (25th to 75th percentile, 0.2–2.8) with an intra-individual coefficient of variation (CV) of 9.8% (4.8–17.6). Patients with an AI ≤ 0.9 exhibited higher CV. The AI was higher (3.4 (3.1–3.6)) in samples before Nat-PML diagnosis than in seropositive Nat-MS (2.4 (1.0–3.4), $n = 298$, $p = 0.010$). AIs ≥ 3.0 were associated with a 14.5-fold (95% CI 2.3–90.4) increased PML risk ($p = 0.002$). Groups with an AI below 1.5 exhibit higher variability or even serostatus fluctuation. AI dynamics require further investigation.

***Keywords*:** Multiple sclerosis, natalizumab, PML, biomarker

Introduction

Treatment of Multiple sclerosis (MS) with natalizumab (Nat) is associated with progressive multifocal leukoencephalopathy (PML). The presence of antibodies against the causative JC virus (JCV) increases the risk to develop Nat-associated PML (Nat-PML).[1] The level of antibody reactivity is proposed to refine this risk in patients without prior immunosuppressive treatment.[2–4] Although one study suggested increasing antibody reactivity towards Nat-PML diagnosis,[5] persistently high anti-JCV antibody indices (AIs) prior to diagnosis have been reported.[2,6] We examine longitudinal variation of anti-JCV AIs in an independent large German cohort of natalizumab-treated MS patients and in the context of Nat-PML and PML of different etiology.

Methods

Ethics approval was obtained (Ruhr-University Bochum, registration-no. 3814-10). The anti-JCV AI was retrospectively determined by STRATIFY JCV™ DxSelect™[,7] in the following cohorts: a longitudinal cohort of Nat-treated MS patients in the post-marketing setting (Nat-MS, $n = 468$, Table 1); Nat-PML patients ($n = 15$, Table 2); patients with PML of other etiology (lymphoma: $n = 2$; HIV: $n = 1$; Fumaderm® treatment[8]: $n = 1$; Table 3, all PML diagnosed according to ref. 9).

Clinical data of 10/15 Nat-PML patients (Table 2, patients 1–10) were presented in our previous study;[3] 4/15 (patients 2, 6–8) were included in a previous study[2]. Samples were available prior to Nat-PML ($n = 9$ patients) and longitudinally (before/at/after PML-diagnosis, $n = 8$ patients, Table 2). Of the multiple samples taken before PML diagnosis, the earliest was included in the cross-sectional analyses.

Statistical analyses were performed using SPSS 22 with p-values < 0.05 considered significant. Data are presented as median (25th to 75th percentile) or (95% confidence interval (CI)). Odds ratios (ORs) of the CI were calculated with the Cox–Hinkley–Miettinen–Nurminen method.

Results

Nat-MS cohort

63.7% ($n = 298$) were anti-JCV seropositive at first sampling. Irrespective of serostatus, the anti-JCV AI

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Table 1. Demographic and clinical characteristics of longitudinal Nat-MS cohort.

Total cohort ($n = 468$)	n[1]	Median	25th to 75th percentile				
Age at MS diagnosis, y	415	28.6	21.6–35.3				
MS duration at Nat-initiation, y	413	6.6	3.0–10.8				
Nat-duration at 1st sampling, m	449	5.0	3.2–6.5				
Interval between 1st and 2nd sampling, m	463	5.1	3.0–7.6				
			anti-JCV positive (1st sample)		anti-JCV negative (1st sample)		
	n[1]	Median (IQR)	n	Median (IQR)	n	Median (IQR)	p-Value[2]
Age at 1st sampling, y	465	38.6 (30.0–44.3)	297	39.3 (31.1–45.2)	168	36.1 (28.9–42.9)	0.005
	n[1]	%[3]	n	%[3]	n	%[3]	p-Value[4]
Gender							
male	126	27.0	86	29.1	40	23.5	n.s.
female	340	73.0	210	70.9	130	76.5	
Pre-treatment of any kind (immunomodulators and immunosuppressants)							
yes	387	95.1	247	95.7	140	94.0	n.s.
no	20	4.9	11	4.3	9	6.0	
Immunomodulatory pre-treatment							
yes	364	89.4	229	88.8	135	90.6	n.s.
-no	43	10.6	29	11.2	14	9.4	
Immunosuppressive pre-treatment (incl. mitoxantrone)							
yes	60	14.7	42	16.3	18	12.1	n.s.
no	347	85.3	216	83.7	131	87.9	

(continued)

Table 1. Continued.

	n[1]	%[3]	anti-JCV positive (1st sample) n	%[3]	anti-JCV negative (1st sample) n	%[3]	p-Value[4]
Mitoxantrone pre-treatment							
yes	43	10.6	32	12.4	11	7.4	n.s.
no	364	89.4	226	87.6	138	92.6	
Anti-Nat antibody status							
positive	9	1.9	7	2.4	2	1.2	n.s.
negative	458	98.1	290	97.6	168	98.8	

Refer to table 2 for an explanation of the footnotes.

showed modest fluctuation over time: intra-individual coefficient of variation (CV) 9.8% (4.8–17.6); median AIs of 0.8 (0.2–2.8) at first, 0.9 (0.2–2.8) at second time point. Initially seropositive patients exhibited an AI of 2.4 (1.0–3.4) at first testing with a CV of 7.7% (3.7–15.3). Eight of 298 seropositive patients had no AI change, 113/298 an AI increase, and 177/298 an AI decrease.

Stratified by AI thresholds,[2] groups with lower AIs demonstrated higher CV ($AI \leq 0.4$ ($n = 193$): CV 12.9% (6.7–22.6); $AI > 0.4 - \leq 0.9$ ($n = 45$): CV 15.5% (8.1–26.2) versus $AI > 1.5 - \leq 3.0$ ($n = 95$): CV 9.4% (4.2–15.2); $AI > 3.0$ ($n = 103$): CV 4.6% (2.4–7.8)). A significant difference in CV was also observed between AI groups $> 0.9 - \leq 1.5$ ($n = 32$, CV 12.2% (4.3–27.1)) versus >3.0 and $>1.5 - \leq 3.0$ versus >3.0 (all $p < 0.001$, Kruskal–Wallis/Dunn's post hoc test, no differences in sampling intervals, Figure S1 (available online)).

Serostatus change from negative (36.3% at first time point) to positive was observed in 11% over 7.6 (4.6–12.2) months. Twelve of 19 exhibited an $AI \leq 0.9$ at second testing. The fastest seroconversion from negative to positive with an AI increase of 2.0 was observed over 1.8 months; a maximum AI increase from 0.1 to 3.5 was seen within 11.8 months.

Serostatus change from positive (63.7% at first time point) to negative occurred in 4% over 4.6 (2.8–7.1) months. The highest initial AI in this subgroup was 0.83. In 9/12 patients, confirmatory testing determined seronegative status at second testing.

Despite high CV of 33.3% (19.8–88.4) in patients with change in serostatus ($n = 31$), AIs remained low (first AI 0.3 (0.2–0.4); second AI 0.4 (0.3–0.7)).

Seropositive patients were older than seronegative patients (39.3 (31.1–45.2), $n = 297$, versus 36.1 (28.9–42.9), $n = 168$, $p = 0.005$, Mann–Whitney test, two-sided). Gender, pre-treatment groups (comprising any pre-treatment prior to initiation of Nat (i.e. immunomodulators and immunosuppressants), immunomodulators, immunosuppressants including mitoxantrone, mitoxantrone alone), anti-Nat antibody status were neither associated with serostatus (Table 1) nor anti-JCV AI in seropositive patients (Figure 1).

PML cohorts

Nat-PML and PML patients of other etiology tested seropositive at all time points (Tables 2 and 3). In samples 26.3 (19.2–34.5) months before Nat-PML

Table 2. Demographic and clinical characteristics of Nat-PML patients.

Case no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Sex	F	F	F	F	M	F	F	F	F	F	M	F	M	F	F
Age at PML-diagnosis, y	35	40	35	45	42	45	58	30	34	41	33	46	43	46	52
Sample collection in relation to PML–diagnosis (- before), m	−2.9	a) −29.5 b) −25.8	−20.6	−17.7	a) −36.7 b) 0	a) −33.6 b) −24.5	a) −26.3 b) 0	a) 6.8 b) 15.1	0	a) −35.3 b) −27.8 c) 0.2[5]	0.9[5]	a) 0 b) 0.1[5] c) 12.5	3.2	0.6[5]	a) −23.2 b) −8.0 c) 0.4[5]
No. of Nat infusions at PML–diagnosis	31	29	n/a	24	n/a	39	31	30	27	37	n/a	30	38	40	>70
Immunosuppressive pre-treatment	yes	no	no	no	no	no	no	yes	no	no	no	no	no	no	no
AI	3.6	a) 3.6 b) 3.8	1.8	3.4	a) 3.4 b) 3.8	a) 3.2 b) 3.6	a) 3.0 b) 3.9	a) 3.1 b) 3.5	3.9	a) 3.5 b) 3.6 c) 3.9	3.3	a) 3.9 b) 3.9 c) 3.9	2.9	3.9	a) 3.95 b) 4.43 c) 3.32
JCV DNA, copies per ml (CSF)[6]	72	120	24	29,750	9	n/a	660	255	37	8	22[7]	544	neg[7]	6,930	120
JCV DNA, copies per ml (s)[6]	n/a	n/a	30	neg	66	n/a	neg	533[8]	neg[9]	neg	2	1435	neg[9]	270	neg

[1]Indicates data available for number of patients, due to the restrospective character, diverging numbers are explained by missing data. [2]Mann–Whitney test, two-sided; [3]Refer to the respective subgroup. [4]Fisher's exact test, two-sided, [5]For samples <1 month after diagnosis, effects of plasma exchange (PLEX) may be considered, PLEX dates not known. [6]Collected at the time point of diagnosis, if not indicated otherwise. [7]3 months after diagnosis. [8]4 months after diagnosis. [9]6 months after diagnosis. AI: antibody index; CSF: cerebrospinal fluid; DNA: deoxyribonucleic acid; F: female; IQR: interquartile range; JCV: John Cunningham virus; m: months; M: male; n/a: not available; n.s.: not significant; Nat: natalizumab; neg: negative; no.: number; PML: progressive multifocal leukoencephalopathy; s: serum; y: years

Table 3. Demographic and clinical characteristics of patients with PML of different etiology.

Case no.	1	2	3	4
PML etiology	Fumaderm®	HIV	lymphoma	lymphoma
Sex	M	M	M	M
Age at PML-diagnosis, y	69	45	64	56
Sample collection in relation to PML-diagnosis, m	0.5	0	3.8	0
Immunosuppressive pre-treatment	no	no	yes	yes
AI	3.6	3.9	1.2	3.8
JCV DNA, copies per ml (CSF)[1]	16	2400	neg[2]	4125
JCV DNA, copies per ml (s)[1]	neg	n/a	n/a	n/a

[1]Collected at the time point of diagnosis, if not indicated otherwise. [2]4 months after diagnosis. AI: antibody index; CSF: cerebrospinal fluid; DNA: deoxyribonucleic acid; HIV: human immunodeficiency virus; JCV: John Cunningham virus; m: months; M: male; n/a: not available; neg: negative; PML: progressive multifocal leukoencephalopathy; s: serum; y: years

($n = 9$), the AI was higher (3.4 (3.1–3.6)) than in seropositive Nat-MS (2.4 (1.0–3.4), $n = 298$, $p = 0.010$, Mann–Whitney test, two-sided). The AI before occurrence of Nat-PML was ≥3.0 in eight out of nine patients resulting in a 14.5 higher (95% CI 2.3–90.4) PML risk with an AI threshold dichotomized as ≥3.0 versus <3.0 ($p = 0.002$, Fisher's exact test, two-sided). The lowest AI threshold with increased PML risk was ≥2.2 (OR 6.9 (95% CI 1.1–42.0); $p = 0.044$).

Longitudinal Nat-PML samples demonstrated persistently high AIs ≥3.0. AIs remained ≥3.0 in all but one sample at/after Nat-PML diagnosis (Table 2). PML of other etiology exhibited an AI of 3.7 (1.2–3.9) around time point of diagnosis (Table 3).

Discussion

Seroprevalence in this Nat-MS cohort was higher (63.7%) than in other studies[3,5,6,10] but proportions of patients with serostatus change were similar.[3,5] Seropositivity was consistently associated with age.[3,10] Significant associations of AI levels with age, gender, pre-treatment, and anti-Nat antibodies within the seropositive cohort were absent.

AI levels in our seropositive Nat-MS patients are higher than in the initial report[2], but similar to a more recent study.[6] The reasons are not clear; our cohort and the latter with open-label treatment in different clinical settings may reflect an unbiased, mixed collective.

Of note, variation of anti-JCV AI is common, with only eight out of 298 seropositive patients exhibiting no AI change at all. Yet, there are differences in the magnitude of variability. Stratified by anti-JCV AI, groups with low AI (≤0.4; >0.4–≤0.9) exhibited higher variability partially leading to change in serostatus despite advances of the second generation assay[6,7]. Especially in these subgroups, determination of serostatus may be less predictive than absolute AI level to determine low PML-risk.

The lowest AI variability was seen in AI-groups >1.5–≤3.0 and >3.0; however, for AIs above 2.5 the assay signal is saturated, partially explaining lower CV in high AI groups.

As an AI threshold of ≤1.5 versus >1.5 is proposed to provide a conservative risk estimate[2], evaluation of AI fluctuation in the group of >1.5–≤3.0 may help to refine PML risk assessment: individual dynamics of anti-JCV AI exceeding the projected inter-test variability defined as more than twofold median CV in this group, i.e. 18.8%, may point to an underlying biological process.

The lowest anti-JCV AI in samples prior to PML diagnosis was 1.8; high anti-JCV AIs were thus consistently detected in Nat-PML including samples up to 3 years prior to Nat-PML diagnosis in line with other studies[2,6] without an increase of antibody reactivity towards PML diagnosis[5].

Owing to the retrospective character, we were only able to include JCV DNA findings in serum/CSF of some PML patients impairing conclusions on potential relationships between AI and viral load.

In our cohorts, an AI ≥3.0 was associated with a 14.5-fold PML risk. However, an exact threshold

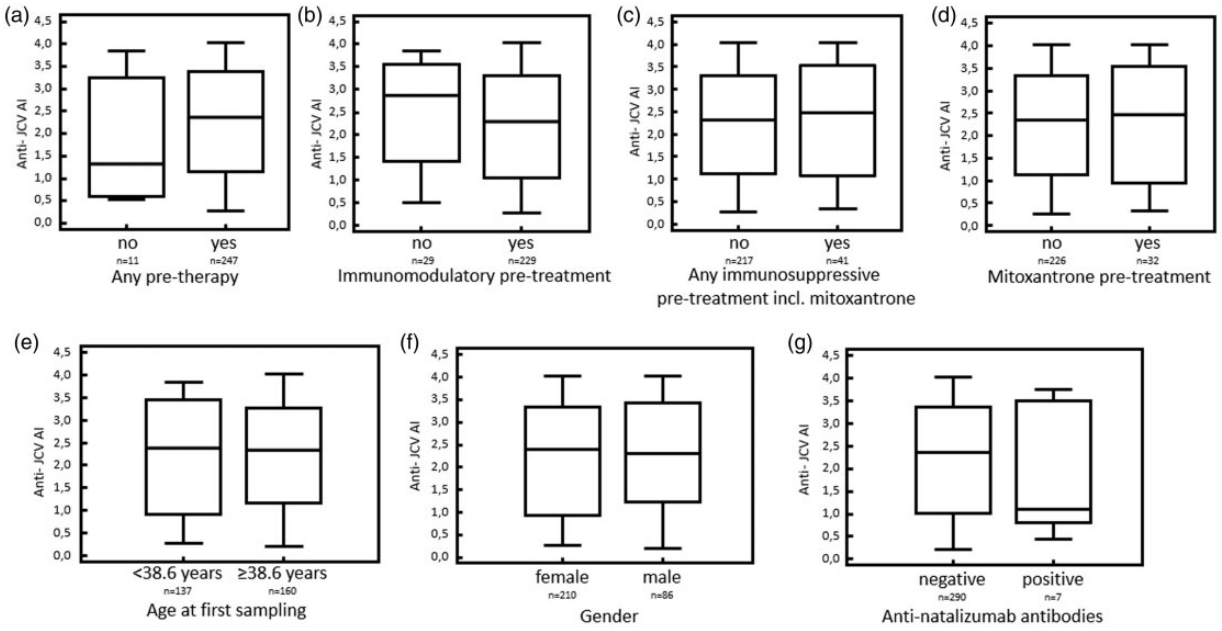

Figure 1. (a–d) Anti-JCV-AI of initially seropositive cohort ($n = 298$) stratified by previous treatments. (a) Any pre-treatment; (b) immunomodulatory pre-treatment; (c) immunosuppressive pre-treatment; and (d) mitoxantrone pre-treatment; (e) stratified by median age of the whole cohort; (f) stratified by gender; (g) stratified by presence of anti-natalizumab antibodies. Tukey box-and-whisker plots. $p =$ not significant for all subgroups, Mann–Whitney test.

remains unclear taking into account higher AIs in comparison with previous observations[2] and the small number of samples before PML diagnosis ($n = 9$) in our study. Still, even a more conservative threshold, e.g., at an AI level of 1.5,[2] can only reflect statistical risk estimations that need to be combined with individual risk-assessment.

Whether determination of AI dynamics, especially in AIs > 1.5, may be additionally helpful in PML risk assessment, deserves further investigation in a prospective setting. Our study supports repeated determination of anti-JCV-AI in addition to serostatus in Nat-PML risk stratification.

Conflict of interest

A.S. received personal compensation for activities with Novartis, Sanofi and Almirall Hermal GmbH. N.v.A. and A.K.T. report no disclosures. R.H. received research and travel grants from Biogen and Novartis. T.P. and M.S. are employees of Biogen and hold stocks. G.K. was a Biogen employee at the time work was completed and holds stocks at Biogen. R.G. received personal compensation for activities with Bayer Healthcare, Biogen and Teva Neuroscience and in an editorial capacity from Therapeutic Advances in Neurological Disorders, and also received patent payments from Biogen and research support from Bayer Healthcare, Biogen, Merck Serono, Teva Neuroscience, Novartis and from the German Ministry for Education and Research (BMBF, ''German Competence Network Multiple Sclerosis'' (KKNMS), CONTROL MS, 01GI0914). A.C. received consulting fees, speaker honoraria (Almirall, Bayer Schering, Biogen, Genzyme, Merck Serono, Novartis, Sanofi, Teva); research support (Biogen, Genzyme, Novartis); and research grants from the German Ministry for Education and Research (BMBF, ''German Competence Network Multiple Sclerosis'' (KKNMS), CONTROL MS, 01GI0914).

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