Journal of Medical Virology 89:528–534 (2017)

Polyomavirus Detection in Multiple Sclerosis Patients Under Natalizumab Therapy: Profile and Frequency of Urinary Shedding

Luiz Henrique Nali,[1] Maria Cristina Fink,[1] Guilherme S. do Olival,[1,2] Lenira Moraes,[3] Dagoberto Callegaro,[3] Charles Peter Tilbery,[2] Jose Ernesto Vidal,[4] Laura Masami Sumita,[1] Augusto C. Penalva de Oliveira,[2,4] and Camila M. Romano[1]*

[1]*Laboratório de Virologia, Instituto de Medicina Tropical de São Paulo, Universidade de São Paulo, São Paulo, Brazil*
[2]*Departamento de Neurologia, Santa Casa de Misericórdia de São Paulo, São Paulo, Brazil*
[3]*Hospital das Clínicas de São Paulo—HCFMUSP, São Paulo, Brazil*
[4]*Grupo de Neurociências, Instituto de Infectologia Emílio Ribas, São Paulo, Brazil*

Patients undergoing Natalizumab (NTZ) therapy are at risk of progressive multifocal leukoencephalopathy (PML). Besides John Cunningham virus (JCV), BK polyomavirus might represent an additional concern for such patients since it can also infect CNS cells. Currently, data regarding the presence of anti-JCV antibodies added to previous immunosuppressive therapy and prolonged NTZ therapy has been used to classify patients at risk of developing PML. Here, we investigated the profile shedding of JCV and BKV in multiple sclerosis (MS) patients during treatment with NTZ. Serial blood and urine samples from 97 MS patients receiving either NTZ or β-interferon were investigated for polyomavirus shedding. While all blood samples tested negative, 36% of the patients shed polyomavirus in the urine in at least one time point. From these, 21.7%, 9.3%, and 5.1% shed JCV, BKV, and both polyomavirus, respectively. No difference was observed between the rates of urinary shedding of patients treated with NTZ (38.9%) and patients treated with other drugs (34.5%), also no PML event was diagnosed during the follow-up. Therefore, urinary shedding might not be interfered by therapy condition. In our study, we also observed 14/27 (52%) of anti-JCV antibodies prevalence, and nearly half of them (42%) did not present any event of urinary shedding during the follow-up. ***J. Med. Virol. 89:528–534, 2017***.

KEY WORDS: JCV; BKV; natalizumab; multiple sclerosis; progressive multifocal leukoencephalopathy; risk stratification

BACKGROUND

MS and Natalizumab

Multiple sclerosis (MS) is an autoimmune disease with a crucial inflammatory stage and is usually characterized by a progressive and degenerative course [Owens and Sriram, 1995; Lassmann et al., 2007]. The disease affects around 1.3 million people worldwide [Milo and Kahana, 2010] and the traditional therapies present moderate effect, providing low efficiency on the reduction of relapse events and the progression of the disease [Etemadifar et al., 2007; Lanzillo et al., 2011].

Natalizumab (NTZ) is a humanized monoclonal antibody against α4β1 integrin, which acts blocking the interaction between lymphocytes and endothelial cells [Yednock et al., 1992]. In fact, NTZ has offered a great therapeutic gain for MS patients, being able to reduce significantly the relapses and the disease progression [Polman et al., 2006]. Today, NTZ is used with rigorous control due to patients who developed progressive multifocal leukoencephalopathy (PML), caused by the reactivation of the polyomavirus JC.

Grant sponsor: Fundação de Amparo a Pesquisa do Estado de São Paulo; Grant number: 2010/10619- 0; Grant sponsor: MsC CAPES scholarship

*Correspondence to: Camila M. Romano, Laboratório de Virologia, Instituto de Medicina Tropical de São Paulo, Universidade de São Paulo, Rua Dr. Enéas de Carvalho Aguiar, 470, 05403-000 São Paulo, Brazil. E-mail: cmromano@usp.br

Accepted 17 July 2016

DOI 10.1002/jmv.24646
Published online 9 August 2016 in Wiley Online Library (wileyonlinelibrary.com).

Polyomavirus JC and PML

John Cunningham virus (JCV) is ubiquitous in the human population, and the seroprevalence may be higher than 50% in some populations [Egli et al., 2009; Kean et al., 2009; Antonsson et al., 2010; Bozic et al., 2011; Trampe et al., 2012]. JCV genome has around 5,130 base pairs in a circular double-stranded DNA genome [Frisque et al., 1984]. The virus establishes latency in the renal tissue and under particular situations variants may arise. Variants presenting mutations and insertions/deletions mainly in regulatory region (RR) improve the tropism for neurological tissues [Daniel et al., 2001; Pfister et al., 2001] and are associated to PML onset. Point mutations within the VP1 gene are also frequently found in viruses from the brain and cerebrospinal fluid (CSF) of PML patients [Sunyaev et al., 2009; Gorelik et al., 2011].

PML in MS Patients

Since the first introduction of NTZ on the market, several reports were made regarding the patients who developed PML after a period of treatment. It is important to remind that around 24% of patients who develop PML as a consequence of NTZ dies and survivors may present neurological sequel [Dong-Si et al., 2015]. Considering this scenario, it was established risk stratification parameters for PML in MS patients under NTZ treatment [Bloomgren et al., 2012; Laroni et al., 2012; Sorensen et al., 2012; Tur et al., 2012].

Since BK polyomavirus also has the ability to infect and cause disease in CNS [Vidal et al., 2007], this virus might represent an extra concern for patients undergoing NTZ therapy. In fact, BKV reactivation in patients with MS under NTZ therapy occurs in a frequency of 22.2% in plasma [Lonergan et al., 2009] and 5% in CSF [Sadiq et al., 2010].

Herein, we investigated for up to 1 year the profile of urinary shedding and monitored the putative reactivation of both viruses (JC and BK) in the blood of patients with MS under NTZ treatment.

METHODS

Patients

MS patients were diagnosed by clinical and imaging criteria at the outpatient care units of the centers of the study (Hospital das Clínicas in Sao Paulo and Irmandade Santa Casa de Misericordia de São Paulo). Patients were classified according to the therapy as follows: (i) NTZ Group (NG)—included patients undergoing NTZ therapy; (ii) Control Group (CG)—patients receiving β-interferon. This study was approved by the ethical committee of Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, #016611.

Blood and urine samples from NG were collected monthly and samples from CG were sampled every 3 months.

Viral DNA Detection

Viral DNA was obtained from urine and blood with QIAamp mini blood kit (Qiagen, Hilden, Germany). Samples were screened for the presence of both polyomaviruses through generic conventional PCR, which amplifies a 173 nucleotides fragment from the AgT region [Arthur et al., 1989; Fink et al., 2006]. Positive samples were submitted to specific real-time PCR (TaqMan® system) assays [Pal et al., 2006] that discriminates between BKV and JCV and also determines the viral load.

Regulatory Region (JCV-RR) and JCV VP1 Molecular Characterization

JCV-RR was also amplified [Pfister et al., 2001] and amplicons from patients presenting continuous shedding were cloned in order to detect putative variants that would be not detected through sequencing of bulk PCR. TOPO TA (Invitrogen, Thermo Fisher, Waltham, MA) was used for cloning and plasmids were inserted in DH5alpha *Escherichia coli* through electroporation. Plasmids were recovered using Gene Jet Plasmid Miniprep Kit (Fermentas, Thermo Fisher). Cloned fragments were sequenced by Sanger method (from 15 to 20 clones per sample). Chromatograms were inspected for quality according to phred index and also for the presence of subpopulations. JCV-RR sequences were aligned with reference sequences of each form (archetype and rearranged). Also, JCV-VP1 was amplified with protocol previously described [Nali et al., 2014], and then sequenced in order to investigate for any putative mutations related to PML. JCV-VP1 sequences were aligned to sequences with mutations on VP1 related to PML, previously described [Sunyaev et al., 2009; Gorelik et al., 2011]. All sequences were then visually inspected with Se-Al program (http://tree.bio.ed.ac.uk/software/seal/).

Anti-JCV ELISA Assay

Antibodies against JCV were investigated in 27 patients from NTZ group by using the second- generation Stratify (Biogen Idec, Cambridge, MA) JCV enzyme-linked immunosorbent assay (ELISA). Serum samples were shipped to Norway to perform the test.

RESULTS

Ninety-seven patients were included in the study. Sixty-one (62.9%) were under immune-modulatory treatment and were included into the control group (CG) and the remaining 36 (37.1%) were included in the NTZ group (NG). All patients enrolled in the study presented the relapsing-remitting form of MS.

JCV and BKV Excretion in Blood and Urine Samples

Polyomavirus DNA were investigated in urine and blood of these patients and all blood samples were

TABLE I. Polyomavirus Urinary Shedding in MS Patients

Groups (#)	Polyomavirus (%)	JCV (%)	BKV (%)	JCV+BKV (%)
CG (61)	21 (34.5)	13 (21.3)	5 (8.2)	3 (4.9)
NG (36)	14 (38.9)	8 (22.2)	4 (11.1)	2 (5.6)
Total (97)	35 (36)	21 (21.7)	9 (9.3)	5 (5.1)
P^*	0.668	0.440	0.722	0.367

*Chi-square test.

negative for both JCV and BKV. However, 36% of the patients presented polyomavirus in the urine in at least one of the serial-samples analyzed, where 21 (21.6%) patients shed JCV; eight (8.2%) shed BKV, and six (6.2%) patients shed both polyomaviruses. The frequency of urinary shedding was evaluated in both groups (CG and NG), and the values were similar (Table I).

BKV and JCV Shedding Profile

Thirty-three patients from CG and 20 from NG were followed up for at least 3 months (maximum 12 months). Of those, seven patients from NG and 12 from CG had at least one event of urinary shedding during the study (Tables II and III).

In general, patients from both groups did not present any specific pattern regarding to the viral shedding. As demonstrated on Tables II and III, some patients from both groups presented continuous shedding (Patients 09, 29, 63, and 40). Others presented a single positive sample along the study (Patients 18, 68, 44, and 77); and five patients presented intermittent shedding (Patients 24, 38, 65, 75, and 91).

It was also noticed that some patients started to shed polyomaviruses during the follow-up. Three patients from the CG started to shed BKV; three patients from NG started to shed BKV and two JCV.

TABLE II. Polyomaviruses Urinary Shedding in Serial Samples From Control Group

	Follow-up months				
ID	1	3	6	9	12
PMS05	n	n	○	n	n
PMS17	○	n	n	–	–
PMS18	n	n	n	○	–
PMS23	⊙	⊙	⊙	–	–
PMS24	•	•	n	•	–
PMS38	•	n	n	•	–
PMS40	•	•	•	–	–
PMS41	○	○	n	–	–
PMS44	–	•	n	n	–
PMS53	•	•	•	–	–
PMS63	•	•	•	•	–
PMS68	n	n	n	○	–

(•): JC-positive samples (○): BK-positive samples, (⊙): JC- and BK-positive samples, n: negative samples, –: follow-up not performed.

JCV-RR and JCV-VP1 Sequencing in Urine Samples

We amplified and sequenced the JCV-RR from all positive patients. In addition, the JCV-RR from patients with continual shedding was cloned to investigate the presence of minor variants. All sequences including the cloned ones did not present variation and were similar to the archetype form of RR. We also amplified and sequenced the JCV-VP1 from all positives samples. No mutations related to the PML were detected, except for one patient who presented the emergence of a variant, with a single polymorphism not related to PML. Such variant was detected during few months of the follow-up and was related to the increased viral load. These data were reported in detail by Nali et al. [2014].

Viral Load

JCV median viral load from CG ranged from 6.00×10^2 to 2.21×10^9 copies/ml, and from 6.00×10^3 to 4.03×10^8 copies/ml for NG. Although the NG average viral load was slightly higher, it did not reach significance (Fig. 1). Nevertheless, substantial increase in JC viral load was observed in two patients from NG during the follow-up. Patient 09, included in the study 1 month before NTZ introduction experienced a large increase in the JC load from 7.00×10^3 to 1.00×10^9 copies/ml after 12 months [Nali et al., 2014]; and Patient 29, included in the study 1 year after beginning NTZ treatment experienced an increase from 7.00×10^3 copies/ml at the first positive sample to 6.78×10^5 at the last time-point (after 10 months). The remaining patients did not present significant fluctuation of JCV viral load during the follow-up.

BK viral load ranged from 4.00×10^3 to 3.75×10^7 copies/ml in the CG and from 5.00×10^3 to 3.75×10^6 copies/ml in the NG (Fig. 2). As well as for JCV, no difference was observed in the average viral load between the groups.

Anti-JCV Antibodies Detection

The second-generation Stratify (Biogen Idec) JCV ELISA was performed in 27 patients from NG, and 14 had positive result (52%). Among them, six patients (42%) did not shed the virus in urine during the follow-up. Interestingly, one patient presented JCV DNA in the urine but did not present anti-JCV antibodies.

TABLE III. Polyomavirus Urinary Shedding in Serial Samples From NTZ Group

		Follow-up months												
ID	# of NTZ infusions[a]	1	2	3	4	5	6	7	8	9	10	11	12	13
PMS09	12	•	•	•	•	•	⊙	⊙	⊙	⊙	⊙	⊙	⊙	⊙
PMS29	37	•	–	•	•	⊙	⊙	⊙	⊙	–	⊙	–	–	–
PMS54	15	n	n	n	n	•	n	n	n	–	–	–	–	–
PMS65	19	n	•	•	•	•	n	n	⊙	⊙	–	–	–	–
PMS75	6	n	n	n	n	•	•	n	n	n	–	–	–	–
PMS77	6	n	n	n	n	O	–	–	–	–	–	–	–	–
PMS91	6	⊙	n	⊙	⊙	–	–	–	–	–	–	–	–	–

(•): JC-positive sample, (O): BK-positive sample, (⊙): JC- and BK-positive samples, n: negative sample, –: follow-up not performed.
[a]The total number of infusions was based on the number of infusion until the last tested sample was collected.

DISCUSSION

Today, NTZ represents a real gain for MS patients who had failed other therapies. However, risks to develop PML prevent several patients to be treated. For that reason, it is really necessary to understand and to evaluate the real risk that a MS patient might be at while treating with NTZ.

In this work, the overall proportion of MS patients who shed JCV (21.7% considering at least one time-point) was similar to that observed for healthy individuals, which is around 18.9–27% [Markowitz et al., 1993; Egli et al., 2009; McClure et al., 2012; Urbano et al., 2016]. Our data were also similar to immunocompromised groups, such as renal and liver recipients [Drachenberg et al., 2007; Kusne et al., 2012] and HIV-infected population, which range from 25% to 28% [Markowitz et al., 1993; Behzad-Behbahani et al., 2004]. We did not observe any particular profile of viral shedding among our patients, which is in touch with previous studies [Polo et al., 2004; Urbano et al., 2016].

Laroni et al. [2012] followed a MS patient under NTZ therapy who developed PML and observed continuous viral shedding with graduall increase in the viral load before PML onset. Two of our patients from NG also presented an increase in the viral load throughout the follow-up, and one of them with the emergence of variants (evaluated by sequencing on the VP1 gene) during the period of highest viral load [Nali et al., 2014]. Nevertheless, none of them developed PML during the follow-up.

BKV Urinary Shedding and NTZ

Polyomavirus BK is an opportunistic virus that behaves very similarly to JCV in terms of genome organization, sites of latency, and reactivation and as JCV, BK can also be neurotropic [Vidal et al., 2007].

The overall shedding observed in this study (9.3%) contrasts to the 21% of BKV urinary shedding in MS patients who use NTZ reported elsewhere [Chen et al., 2009; Lonergan et al., 2009]. Also, Lonergan et al. [2009] described that 22.2% of the patients started to shed BKV after NTZ therapy introduction. Despite this, no patient developed BKV- related diseases [Lonergan et al., 2009]. Although there is no evidence that BKV is associated with diseases in patients receiving NTZ, we observed that nearly all patients treating longer than 1 year started to shed BK continuously with slightly higher viral load when compared to CG, suggesting that BK viral replication might be interfered by prolonged use of NTZ. However, little information about the influence of NTZ in BKV shedding and reactivation is available to confirm this hypothesis [Delbue et al., 2014].

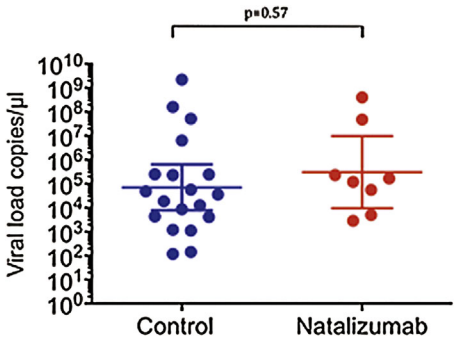

Fig. 1. Average JCV viral load in urine of MS patients.

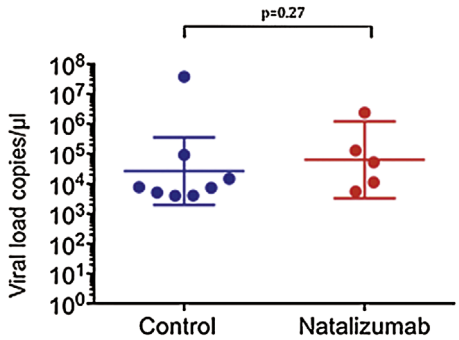

Fig. 2. Average BKV viral load in urine of MS patients.

JCV Antibodies and Urinary Shedding

JCV antibodies prevalence observed here (52%) was similar to those found by others [Egli et al., 2009; Kean et al., 2009; Antonsson et al., 2010; Ribeiro et al., 2010; Bozic et al., 2011; Trampe et al., 2012]. But, anti-JCV was not related to viral replication or shedding since 42% of NG patients with anti-JCV antibodies did not present a single event of JCV urinary shedding during the follow-up. It is important to remind that patients who developed NTZ-related PML presented urinary shedding prior PML onset [Reid et al., 2011; Laroni et al., 2012], suggesting that those who do not shed JCV might be at low risk to develop PML, at least in a short to medium term.

Intriguingly, one patient from NG (Patient 54, see Table III for details) shed JCV in the urine in one time point but did not present anti-JCV antibodies. Although we do not have the precise date of the antibody testing, this finding may be explained by two possibilities: (i) this single shedding event could represent a primary infection, or (ii) it is a false negative result. It was reported previously that 37% of patients who has no detectable antibodies can present viruria, suggesting that the current anti-JCV ELISA may underestimate the real number of JCV-infected patients [Berger et al., 2013].

The key for a safer treatment is to gather enough information that could predict PML onset. Based on this, it has been proposed that anti-JCV antibodies titer could be used as additional tool to stratify patients at a higher risk, once PML patients may present higher titers than non-PML patients [Vennegoor et al., 2015]. Also Plavina et al. [2014] proposed a cut-off for anti-JCV antibodies, where indexes higher than 1.5 absorbance OD units could indicate higher risk for PML. Although these data may be used to stratify patients at risk of PML, it might not be a very useful tool to actually predict PML, since not always the anti-JCV antibodies titers is related with duration of NTZ exposure and PML onset [Antoniol and Stankoff 2014; Plavina et al., 2014; Gagne Brosseau et al., 2016]. Most strikingly, a patient without previous immunosuppressive therapy who developed PML presented no anti-JCV antibodies until 2 weeks before PML onset [Gagne Brosseau et al., 2016].

Perspectives for MS Patients Under NTZ Treatment

JCV is the main reason that prevents neurologists from prescribing NTZ to a MS patient. PML is a very complex disease and unfortunately, the knowledge regarding JCV pathogeny and mechanisms of reactivation is not fully understood [Reid et al., 2011; Laroni et al., 2012; Delbue et al., 2014]. Therefore, it is not possible yet to precisely predict whether and when patients will develop PML. Although many questions remain unanswered, researchers had established criteria for PML risk based on three conditions: anti-JCV antibodies status; prolonged NTZ therapy—for more than 2 years; and previous immunosuppressive therapy. JCV is ubiquitous in human population, but we found that 42% of the patients presenting anti-JCV antibodies did not shed JCV in the urine during the NTZ therapy and all patients who presented anti-JCV antibodies but no viruria were treating with NTZ for at least 6 months. Crucially, nearly all reported NTZ- treated patients who developed PML presented JCV viruria before PML onset. Is it possible to suggest that patients with JCV antibodies but without urinary shedding (or sporadic shedding) present lower risk to develop PML than those who shed the virus in the urine continuously? It is certainly a difficult question to answer.

Additionally, the fact that both the control and NTZ groups had similar profile of JCV shedding is in line with the idea that treatment itself does not induce viral replication, at least in short to medium term. Unfortunately, thousands of patients who could benefit themselves from NTZ therapy are considered not suitable for the treatment. Although it is important to classify patients as high- or low-risk patients, to predict which patients are in fact under imminent risk to PML is pivotal to assure the best assistance to MS patients.

ACKNOWLEDGMENTS

The authors wish to thank all the members of Group of Neurosciences of the Instituto de Infectologia Emílio Ribas for the scientific and administrative support for this study.

REFERENCES

Antoniol C, Stankoff B. 2014. Immunological markers for PML prediction in MS patients treated with natalizumab. Front Immunol 5:668.

Antonsson A, Green AC, Mallitt KA, O'Rourke PK, Pawlita M, Waterboer T, Neale RE. 2010. Prevalence and stability of antibodies to the BK and JC polyomaviruses: A long-term longitudinal study of Australians. J Gen Virol 91:1849–1853.

Arthur RR, Dagostin S, Shah KV. 1989. Detection of BK virus and JC virus in urine and brain tissue by the polymerase chain reaction. J Clin Microbiol 27:1174–1179.

Behzad-Behbahani A, Klapper PE, Vallely PJ, Cleator GM, Khoo SH. 2004. Detection of BK virus and JC virus DNA in urine samples from immunocompromised (HIV-infected) and immunocompetent (HIV- non-infected) patients using polymerase chain reaction and microplate hybridisation. J Clin Virol 29:224–229.

Berger JR, Houff SA, Gurwell J, Vega N, Miller CS, Danaher RJ. 2013. JC virus antibody status underestimates infection rates. Ann Neurol 74:84–90.

Bloomgren G, Richman S, Hotermans C, Subramanyam M, Goelz S, Natarajan A, Lee S, Plavina T, Scanlon JV, Sandrock A, Bozic C. 2012. Risk of natalizumab-associated progressive multifocal leukoencephalopathy. N Engl J Med 366:1870- 1880.

Bozic C, Richman S, Plavina T, Natarajan A, Scanlon J, Subramanyam M, Sandrock A, Bloomgren G. 2011. Anti-John Cunnigham virus antibody prevalence in multiple sclerosis patients: Baseline results of STRATIFY-1. Ann Neurol 70:742–750.

Chen Y, Bord E, Tompkins T, Miller J, Tan CS, Kinkel RP, Stein MC, Viscidi RP, Ngo LH, Koralnik IJ. 2009. Asymptomatic reactivation of JC virus in patients treated with natalizumab. N Engl J Med 361:1067–1074.

Daniel DC, Wortman MJ, Schiller RJ, Liu H, Gan L, Mellen JS, Chang CF, Gallia GL, Rappaport J, Khalili K, Johnson EM. 2001. Coordinate effects of human immunodeficiency virus type 1 protein Tat and cellular protein Puralpha on DNA replication initiated at the JC virus origin. J Gen Virol 82: 1543–1553.

Delbue S, Elia F, Carloni C, Pecchenini V, Franciotta D, Gastaldi M, Colombo E, Signorini L, Carluccio S, Bellizzi A, Bergamaschi R, Ferrante P. 2014. JC virus urinary excretion and seroprevalence in natalizumab-treated multiple sclerosis patients. J Neurovirol 21:645–652.

Dong-Si T, Gheuens S, Gangadharan A, Wenten M, Philip J, McIninch J, Datta S, Richert N, Bozic C, Bloomgren G, Richman S, Weber T, Clifford DB. 2015. Predictors of survival and functional outcomes in natalizumab-associated progressive multifocal leukoencephalopathy. J Neurovirol 21:637–644.

Drachenberg CB, Hirsch HH, Papadimitriou JC, Gosert R, Wali RK, Munivenkatappa R, Nogueira J, Cangro C, Haririan A, Mendley S, Ramos E. 2007. Polyomavirus BK versus JC replication and nephropathy in renal transplant recipients: A prospective evaluation. Transplantation 84:323–330.

Egli A, Infanti L, Dumoulin A, Buser A, Samaridis J, Stebler C, Gosert R, Hirsch HH. 2009. Prevalence of polyomavirus BK and JC infection and replication in 400 healthy blood donors. J Infect Dis 199:837–846.

Etemadifar M, Janghorbani M, Shaygannejad V. 2007. Comparison of interferon beta products and azathioprine in the treatment of relapsing-remitting multiple sclerosis. J Neurol 254:1723–1728.

Fink MC, Penalva de Oliveira AC, Milagres FA, Vidal JE, Picerno-Pouza AF, Duarte Neto A, Pannuti CS. 2006. JC virus DNA in cerebrospinal fluid samples from Brazilian AIDS patients with focal brain lesions without mass effect. J Infect 52:30–36.

Frisque RJ, Bream GL, Cannella MT. 1984. Human polyomavirus JC virus genome. J Virol 51:458–469.

Gagne Brosseau M-S, Stobbe G, Wundes A. 2016. Natalizumab-related PML 2 weeks after negative anti-JCV antibody assay. Neurology 86:484–486.

Gorelik L, Reid C, Testa M, Brickelmaier M, Bossolasco S, Pazzi A, Bestetti A, Carmillo P, Wilson E, McAuliffe M, Tonkin C, Carulli JP, Lugovskoy A, Lazzarin A, Sunyaev S, Simon K, Cinque P. 2011. Progressive multifocal leukoencephalopathy (PML) development is associated with mutations in JC virus capsid protein VP1 that change its receptor specificity. J Infect Dis 204:103–114.

Kean JM, Rao S, Wang M, Garcea RL. 2009. Seroepidemiology of human polyomaviruses. PLoS Pathog 5:e1000363.

Kusne S, Vilchez RA, Zanwar P, Quiroz J, Mazur MJ, Heilman RL, Mulligan D, Butel JS. 2012. Polyomavirus JC urinary shedding in kidney and liver transplant recipients associated with reduced creatinine clearance. J Infect Dis 206:875–880.

Lanzillo R, Quarantelli M, Bonavita S, Ventrella G, Lus G, Vacca G, Prinster A, Orefice G, Tedeschi G, Brescia Morra V. 2011. Natalizumab vs interferon beta 1a in relapsing- remitting multiple sclerosis: A head-to-head retrospective study. Acta Neurol Scand 126:306–314.

Laroni A, Giacomazzi CG, Grimaldi L, Gallo P, Sormani MP, Bertolotto A, McDermott JL, Gandoglia I, Martini I, Vitello G, Rinaldi F, Barzon L, Militello V, Pizzorno M, Bandini F, Capello E, Palu G, Uccelli A, Mancardi GL, Varnier OE. 2012. Urinary JCV-DNA testing during natalizumab treatment may increase accuracy of PML risk stratification. J Neuroimmune Pharmacol 7:665–672.

Lassmann H, Brück W, Lucchinetti CF. 2007. The immunopathology of multiple sclerosis: An overview. Brain Pathol 17:210–218.

Lonergan RM, Carr MJ, De Gascun CF, Costelloe LF, Waters A, Coughlan S, Duggan M, Doyle K, Jordan S, Hutchinson MW, Hall WW, Tubridy NJ. 2009. Reactivation of BK polyomavirus in patients with multiple sclerosis receiving natalizumab therapy. J Neurovirol 15:351–359.

Markowitz RB, Thompson HC, Mueller JF, Cohen JA, Dynan WS. 1993. Incidence of BK virus and JC virus viruria in human immunodeficiency virus-infected and -uninfected subjects. J Infect Dis 167:13–20.

McClure GB, Gardner JS, Williams JT, Copeland CM, Sylvester SK, Garcea RL, Meinerz NM, Groome LJ, Vanchiere JA. 2012. Dynamics of pregnancy-associated polyomavirus urinary excretion: A prospective longitudinal study. J Med Virol 84:1312–1322.

Milo R, Kahana E. Multiple sclerosis: Geoepidemiology, genetics and the environment. Autoimmun Rev 9:A387–A394.

Nali LH, Moraes L, Fink MC, Callegaro D, Romano CM, Oliveira ACP 2014. Natalizumab treatment for multiple sclerosis: Updates and considerations for safer treatment in JCV positive patients. Arq Neuropsiquiatr 72:960–965.

Owens T, Sriram S. 1995. The immunology of multiple sclerosis and its animal model, experimental allergic encephalomyelitis. Neurol Clin 13:51–73.

Pal A, Sirota L, Maudru T, Peden K, Lewis AM Jr. 2006. Real-time, quantitative PCR assays for the detection of virus-specific DNA in samples with mixed populations of polyomaviruses. J Virol Methods 135:32–42.

Pfister LA, Letvin NL, Koralnik IJ. 2001. JC virus regulatory region tandem repeats in plasma and central nervous system isolates correlate with poor clinical outcome in patients with progressive multifocal leukoencephalopathy. J Virol 75: 5672–5676.

Plavina T, Subramanyam M, Bloomgren G, Richman S, Pace A, Lee S, Schlain B, Campagnolo D, Belachew S, Ticho B. 2014. Anti-JC virus antibody levels in serum or plasma further define risk of natalizumab- associated progressive multifocal leukoencephalopathy. Ann Neurol 76:802–812.

Polman CH, O'Connor PW, Havrdova E, Hutchinson M, Kappos L, Miller DH, Phillips JT, Lublin FD, Giovannoni G, Wajgt A, Toal M, Lynn F, Panzara MA, Sandrock AW. 2006. A randomized, placebo-controlled trial of natalizumab for relapsing multiple sclerosis. N Engl J Med 354:899–910.

Polo C, Pérez JL, Mielnichuck A, Fedele CG, Niubò J, Tenorio A. 2004. Prevalence and patterns of polyomavirus urinary excretion in immunocompetent adults and children. Clin Microbiol Infect 10:640–644.

Reid CE, Li H, Sur G, Carmillo P, Bushnell S, Tizard R, McAuliffe M, Tonkin C, Simon K, Goelz S, Cinque P, Gorelik L, Carulli JP. 2011. Sequencing and analysis of JC virus DNA from natalizumab-treated PML patients. J Infect Dis 204: 237–244.

Ribeiro T, Fleury MJ, Granieri E, Castellazzi M, Martini F, Mazzoni E, Coursaget P, Tognon M. 2010. Investigation of the prevalence of antibodies against neurotropic polyomaviruses BK, JC and SV40 in sera from patients affected by multiple sclerosis. Neurol Sci 31:517–521.

Sadiq SA, Puccio LM, Brydon EW. 2010. JCV detection in multiple sclerosis patients treated with natalizumab. J Neurol 257:954–958.

Sorensen PS, Bertolotto A, Edan G, Giovannoni G, Gold R, Havrdova E, Kappos L, Kieseier BC, Montalban X, Olsson T. 2012. Risk stratification for progressive multifocal leukoencephalopathy in patients treated with natalizumab. Mult Scler 18:143–152.

Sunyaev SR, Lugovskoy A, Simon K, Gorelik L. 2009. Adaptive mutations in the JC virus protein capsid are associated with progressive multifocal leukoencephalopathy (PML). PLoS Genet 5:e1000368.

Trampe AK, Hemmelmann C, Stroet A, Haghikia A, Hellwig K, Wiendl H, Goelz S, Ziegler A, Gold R, Chan A. 2012. Anti-JC virus antibodies in a large German natalizumab-treated multiple sclerosis cohort. Neurology 78:1736–1742.

Tur C, Tintore M, Vidal-Jordana A, Castillo J, Galan I, Rio J, Arrambide G, Comabella M, Arevalo MJ, Horno R, Vicente MJ, Caminero A, Nos C, Sastre-Garriga J, Montalban X. 2012. Natalizumab discontinuation after PML risk stratification: Outcome from a shared and informed decision. Mult Scler 18:1193–1196.

Urbano PRP, Oliveira RR, Romano CM, Pannuti CS, Fink MC. 2016. Occurrence, genotypic characterization, and patterns of shedding of human polyomavirus JCPyV and BKPyV in urine samples of healthy individuals in São Paulo, Brazil. J Med Virol 88:153–158.

Vennegoor A, van Rossum JA, Polman C, Wattjes M, Killestein J. 2015. Longitudinal JCV serology in multiple

sclerosis patients preceding natalizumab-associated progressive multifocal leukoencephalopathy. Mult Scler 21: 1600–1603.

Vidal JE, Fink MC, Cedeno-Laurent F, Delbue S, Ferrante P, Dauar RF, Filho FB, Nogueira RS, Calore EE, Pannuti CS, Trujillo JR, de Oliveira AC. 2007. BK virus associated meningoencephalitis in an AIDS patient treated with HAART. AIDS Res Ther 4:13.

Yednock TA, Cannon C, Fritz LC, Sanchez-Madrid F, Steinman L, Karin N. 1992. Prevention of experimental autoimmune encephalomyelitis by antibodies against alpha 4 beta 1 integrin. Nature 356:63–66.