JC Virus Persistence Following Progressive Multifocal Leukoencephalopathy in Multiple Sclerosis Patients Treated with Natalizumab

Caroline F. Ryschkewitsch, MT, Peter N. Jensen, BS, Maria Chiara Monaco, PhD, and Eugene O. Major, PhD

JC virus (JCV) DNA in the cerebrospinal fluid (CSF) provides the laboratory confirmatory diagnosis of progressive multifocal leukoencephalopathy (PML) in patients whose clinical symptoms and magnetic resonance imaging findings are consistent with PML. The Laboratory of Molecular Medicine and Neuroscience (LMMN), National Institute of Neurological Disorders and Stroke (NINDS), National Institutes of Health (NIH), made the confirmatory laboratory diagnosis in 35 multiple sclerosis (MS) patients treated with natalizumab. Thirteen patients had 3 or more CSF samples taken from weeks to months following PML diagnosis. Seven of the 13 patients demonstrated persistence of JCV DNA in the CSF even though all patients experienced immune reconstitution inflammatory syndrome (IRIS), 11 patients had plasma exchange, and 2 had immunoabsorption. Specific anti-JCV antibody was measured in plasma/sera samples from 25 of the 35 patients. Most of the samples showed moderate to high or rising antibody levels from the time of PML diagnosis. However, plasma from 1 patient at or near the time of PML diagnosis had a titer considered seronegative and 2 other plasma samples from patients had titers considered at baseline for seropositivity. In several PML cases, viral persistence and neurological deficits have continued for several years, indicating that once initiated, JCV infection may not entirely clear, even with IRIS.

ANN NEUROL 2010;68:384–391

Immunomodulatory therapies such as natalizumab (Tysabri; Biogen Idec, Weston, MA) show an increased risk of progressive multifocal leukoencephalopathy (PML) compared with other immune-altering treatments.[1] As of June 7, 2010, Biogen Idec reports 55 cases of PML in MS patients on natalizumab with an approximate incidence of 1 per 1,000, and incidence increasing with dosing (https://medinfo.biogenidec.com). In a recent report, Clifford and colleagues[2] outlined the clinical status in 28 cases, describing immune reconstitution in most patients following plasma exchange (PLEX), and recommending increased clinical vigilance and monitoring of patients beyond 3 years from diagnosis of PML. It was anticipated that central nervous system inflammatory reactions (immune reconstitution inflammatory syndrome [IRIS]) in these patients would clear JC virus (JCV) infection because many of these patients had an "early" diagnosis of PML. However, analysis of cerebrospinal fluid (CSF) samples from 35 PML patients (1 in the Sentinel trial[3] and 34 postmarketing) showed that 7 of these patients sustained viral persistence in the CSF for weeks to months after PLEX and/or immunoabsorption (IA) and IRIS. One of these cases[4] has been followed, and periodic, detectable viral DNA has been found in the CSF 3 years after PML diagnosis. Six patients' CSF samples showed decreasing viral load, with 4 resulting in undetectable viral load.

Materials and Methods

Real-Time Quantitative Polymerase Chain Reaction Assay for Detection of Viral DNA in CSF

The Laboratory of Molecular Medicine and Neuroscience (LMMN) established and maintains Clinical Laboratory Improvement Amendment (CLIA)-validated and certified real-time quantitative polymerase chain reaction (qPCR) assays for the detection of the JCV genome in clinical samples.[5] The assay targets a highly-conserved region of the viral genome that codes for the amino-terminal region of the multifunctional T protein, which is required for successful viral infection. The limit of detection of the assay is 10 copies of the viral genome per milliliter based on standards of viral DNA and testing samples that are available from the National Institutes of Health (NIH).[6] CSF is sent to the LMMN frozen, thawed just prior to assay, in which 200μl is used to extract and concentrate template to 25μl.[7] Samples are sent to the LMMN from centers that treat patients on a voluntary basis. There is no protocol or study that requires testing at the LMMN. Consequently, not all samples from PML patients have been analyzed at the LMMN.

From the Laboratory of Molecular Medicine and Neuroscience (LMMN), National Institute of Neurological Disorders and Stroke (NINDS), National Institutes of Health (NIH), Bethesda, MD.

Address correspondence to Dr Major, Laboratory of Molecular Medicine and Neuroscience (LMMN), National Institute of Neurological Disorders and Stroke (NINDS), National Institutes of Health (NIH), Bethesda, MD 20892. E-mail: majorg@ninds.nih.gov

Received May 19, 2010, and in revised form Jun 18, 2010. Accepted for publication Jun 25, 2010.

View this article online at wileyonlinelibrary.com. DOI: 10.1002/ana.22137

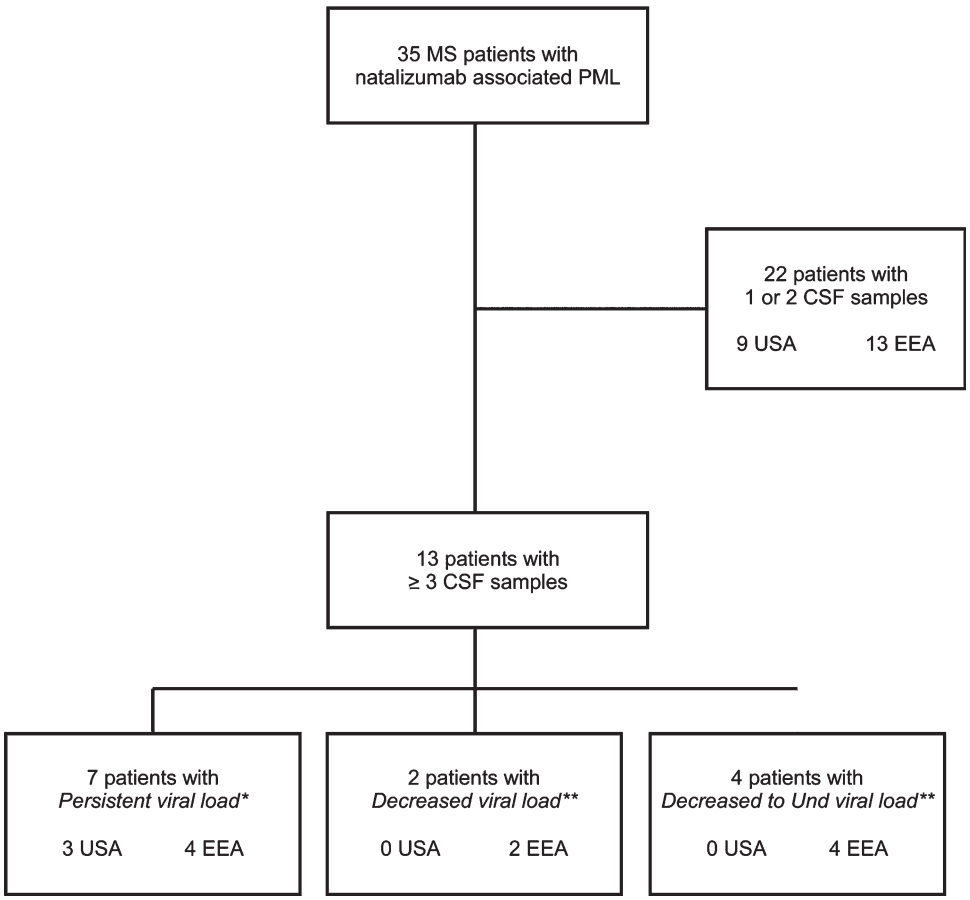


FIGURE 1: Flow diagram shows number of patients and categorization of CSF samples from natalizumab-treated MS patients who developed PML, and their associated geographic location. Persistent viral load is the detection of viral load in CSF at time of diagnosis of PML and in multiple samples that remained detectable over months. Decreased viral load is detection of viral load in CSF at time of diagnosis of PML and/or in multiple samples that decreased at least 10× or were undetectable in the last available sample. EEA = European Economic Area; und = undetected in qPCR assay; USA = United States.

Enzyme-Linked Immunosorbent Assay for the Determination of Specific Antibodies to JCV

The viral VP1 gene which codes for the capsid protein has been cloned into a baculovirus vector that is used to infect insect cells for large scale production of the major capsid protein that self-assembles into a virus-like particle and to which both antibody and T cell responses are directed.[8–10] Purified recombinant VP1 is used to coat wells in plastic plates as the target antigen in standard enzyme-linked immunosorbent assay (ELISA) format using 4-fold serial dilutions of serum or plasma.[11] The titer of the antibody is reported as the dilution of the sample that achieves 0.05 absorption at 450nm optical density reading above controls, and was also reported under the CLIA process. Only immunoglobulin G has been measured in the data reported here. The antibody titer was benchmarked against the hemagglutination inhibition assay previously used to determine serological status.[12] Antibody titers at ≥2,560 are considered a baseline level for seropositive and those below; ie, ≥160–640, are considered seronegative.

Results

Viral DNA in CSF of MS Patients with PML

Figure 1 shows the distribution of 35 MS patients on natalizumab whose CSFs were assayed for viral DNA and whose single or serial plasma (or sera) were also available for antiviral antibody testing. Clinical descriptions of 20 patients have been reported recently, as referenced in Tables 1 and 2, which also includes the treatment history that accompanied the initial sample.[2] The LMMN provided the initial laboratory diagnosis of PML in 21 cases and confirmed PML in 14 others. Over the course of weeks to months, 13 of these patients had 3 or more CSF samples tested for JCV DNA following their clinical progress after discontinuation of natalizumab, PLEX, or IA, to reduce the pharmacological effects of natalizumab and initiation of IRIS that all patients developed. Seven of these patients demonstrated viral persistence, which is defined as consistent detection of viral DNA in the CSF over months (see Table 1). One of the first cases of PML diagnosed from the Sentinel trial[4] has had CSFs tested since 2005, over 3 years to 2008,with detectable viral DNA 3 years after PML diagnosis (see Table 1, Patient 6). This patient has periodic neurological deficits that may be attributable to viral reactivation and persistent JCV infection (E. Frohman, personal

communication). In a retrospective review of longitudinal plasma samples on this patient from January 2003, July 2003, December 2003, and August 2004, there was an indication of viremia for years prior to PML occurrence in December 2004 based on the assay used at the time, in which only 1 of 2 duplicate samples was positive.[13] An acquired immune deficiency syndrome (AIDS)-associated case of PML not related to natalizumab therapy had JCV DNA detected in the CSF at the time of PML diagnosis and then 5 years later (2,300 copies/ml). This

TABLE 1: JC Virus DNA Copy Number in ≥3 CSF Samples and Plasma/Serum Including Antiviral Antibody

Patients[a]	Treatment History	Sample Collection Date[b]	CSF Viral Copies (copies/ml)	Plasma/Serum Viral Copies (copies/ml)	Antibody Titer[c]
Persistent viral load					
EEA					
1	PLEX, CDV	6/15/09	415	NA	
		7/3/09	745	NA	
		9/4/09	201	NA	
2 (#4)	IA, mefloquine	11/26/08	69		
		12/5/08	215	426 s	2,560
		1/19/09	117	19 s	10,240
		1/28/09	181	329 s	40,960
		3/12/09	48	61 s	163,840
		4/24/09	26	und p	163,840
				und s	
3 (#9)	IA, IVIG, mirtazipine	4/17/09	68		
		5/13/09	50		
		6/10/09	134		
		7/22/09	3,879	2,640 s	40,960
		10/14/09	89	79 s	2,621,440
4 (#18)	PLEX, mefloqine, mirtazipine	9/29/09	35		
		10/12/09	66	49 p	40,960
				30 s	40,960
		10/21/09	155	295 p	163,840
				396 s	163,840
		11/4/09	81	87 p	2,261,440
				111 s	2,261,440
		11/18/09	75	148 p	2,261,440
				95 s	2,261,440
		12/8/09	21	27 p	2,261,440
		3/8/10	12	und p	2,261,440
				und s	2,261,440
5 (#5)	PLEX, mefloquine	11/26/09	41	NA	
		12/7/09	126	NA	
		12/16/09	227	NA	

TABLE 1: Continued

Patients[a]	Treatment History	Sample Collection Date[b]	CSF Viral Copies (copies/ml)	Plasma/Serum Viral Copies (copies/ml)	Antibody Titer[c]
USA					
6[4]	PLEX, Ara C, CDV	3/2/05	6,050	2500 s	40,960
		3/29/05	2,275	und s	655,360
		9/20/05	42	und s	655,360
		6/11/08	37	14 p	
		9/12/08	26	108 p	
7 (#23)	PLEX	10/9/09	40,646	und s	10,240
		11/3/09	101,900		
		11/17/09	769,000		
Decreased viral load					
EEA					
8 (#6)	PLEX	4/7/09	96,436	463 p	10,240
				275 s	10,240
		4/21/09	601,825	12,487 p	40,960
				10,812 s	40,960
		5/5/09	2,241,062	10,900 p	40,960
				8,350 s	40,960
		5/29/09	3,482,575	18,012 p	40,960
				21,875 s	40,960
		7/16/09	13,825	4,063 p	10,485,760
		9/3/09	1,114	1,100 p	10,485,760
				1.374 s	10,485,760
USA					
9 (#7)	PLEX, mefloquine	5/18/09	1,220,175	NA	
		6/2/09	444,325	NA	
		7/7/09	9,937	NA	
Decreased to undetected viral load					
EEA					
10 (#1)	PLEX, IA, mefloquine	7/24/08	53	NA	
		8/15/08	10	NA	
		8/28/08	und	12 p	163,840
11 (#28)	PLEX, mefloquine	10/9/2009	11	NA	
		1/8/2010	26	6 s	655,360
		3/16/2010	und	und s	163,840
12	PLEX, IVIG, mefloquine, mirtazipine	12/22/09	51	NA	
		1/26/09	39	NA	
		2/25/09	70	NA	
		3/25/10	9		
		4/12/10	und		

TABLE 1: Continued

Patients[a]	Treatment History	Sample Collection Date[b]	CSF Viral Copies (copies/ml)	Plasma/Serum Viral Copies (copies/ml)	Antibody Titer[c]
13 (#15)	PLEX, mefloquine, mirtazipine	9/9/09	1387	28 p	40,960
				18 s	655,360
		10/7/09	169	26 p	655,360
		1/29/10	und	und p/s	

[a]Information for Patients 6 and 10 is in Refs. 4 and 5, respectively. Persistent viral load is the detection of viral load in CSF at time of diagnosis of PML and in multiple samples that remained detectable over months. Decreased viral load is detection of viral load in CSF at time of diagnosis of PML and/or in multiple samples that decreased at least 10× or were undetectable in the last available sample.
[b]First date indicates sample at time of PML diagnosis.
[c]Antibody titers are greater than or equal to reported value.
(#) = patient number and information in Ref. 2; Ara C = cytosine arabinoside; CDV = cidofovir; CSF = cerebrospinal fluid; EEA = European Economic Area; IA = immune absorption; IVIG = intravenous immunoglobulin; NA = sample(s) not available; p = plasma; PLEX = plasma exchange; s = serum; und = undetected in qPCR assay; USA = United States.

patient had a history of seizures and headaches that were attributed to human immunodeficiency virus (HIV)-1 disease. CSF samples were not taken until recently (J. Rumbaugh, personal communication). Multiple CSF samples from 2 other patients (see Table 1, Patients 8 and 9) showed a decrease of viral load over weeks from an extraordinarily high viral load of 3×10^6 and 1×10^6, which decreased to 1×10^3 and 1×10^4 in 4 and 2 months, respectively. CSFs in 4 patients (see Table 1, Patients 10–13) resulted in undetectable viral load in the last sample that was taken. No other CSF samples are available to determine whether any of these 6 patients have had viral DNA reappear in the CSF.

Table 2 shows the data from 22 patients with only 1 or 2 CSF samples taken at the time of suspected PML and a few weeks later. No further CSF samples have been available to assess whether any of these PML patients have reactivated or persistent infection. Figure 2 shows a box-plot of the distribution of viral copy number from all 35 patients' initial CSF at time of PML diagnosis with a mean approximating 800 copies with an upper margin of 9,000 copies/ml and a lower margin of 90 copies/ml. Interestingly, 57% of samples fell below the mean while 43% were above. Thirteen samples (37%) had fewer than 90 copies/ml at the time of PML diagnosis, a low copy number that should be within the limits of a qPCR assay coupled with $\geq$40% DNA template extraction efficiency.

Viral DNA and Antibody Titers in Plasma/Serum

Table 1 also shows that 9 patients had plasma/serum samples available, 8 of whom were viremic at multiple time points. However, all patients had moderate to rising antibody titers to JCV, even in the viral persistent group that reached the highest titer measured, 10×10^6, indicating robust humoral immune responses in PML patients. One PML patient with a CSF viral load of 297 copies/ml (see Table 2, Patient 16), had a negative serum antibody titer at the time of diagnosis, 160–640, but was also viremic, 85 copies/ml, and had high copy number in the urine, 27,212 copies/ml. This patient was also reported as being HIV-1 seropositive. Two other PML patients (see Table 1, Patient 2, and Table 2, Patient 18) had antibody levels at baseline for seropositivity, 2,560, with CSF viral loads of 2,900 copies/ml and 69 copies/ml, respectively. Patient 2's antibody titer increased to $\geq$163,840 over 5 months, with decreasing viral copy number in the CSF and plasma (see Table 1, Patient 2).

Table 3 shows data from a series of CSF and plasma samples from MS patients from the AFFIRM and SENTINEL clinical studies that were assayed as controls from non-PML samples. None of the non-PML patients had viral genome copies in the CSF. Antibody titers determined by the LMMN ELISA certified assay showed seropositives in 65% of MS patients, 90% of Crohn's disease patients, and 80% of rheumatoid arthritis patients.

Discussion

The availability of clinical samples from PML patients at the time of laboratory diagnosis and follow-up for extended periods of time has revealed viral persistence that can occur for years.

TABLE 2: JC Virus DNA Copy Number in 1 or 2 CSF Samples and Plasma/Serum Including Antiviral Antibody

Patients[a]	Treatment History	Sample Collection Date[b]	CSF viral copies (copies/ml)	Plasma/serum viral copies (copies/ml)	Antibody Titer[c]
EEA					
14 (#8)	IA	1/15/09	92	54 s	40,960
		1/28/09	250	und p	10,240
				32 s	10,240
15	No history	5/29/09	18		
		6/4/09	762		
16 (#14)	PLEX, mefloquine	7/24/09	297		
		7/29/09		und p	640
		7/29/09		85 s	160
17 (#17)	PLEX, mefloquine, mirtazipine, CDV	9/24/09	122		
18 (#21)	PLEX	10/7/09	2,927	und p	2,560
				und s	
19	PLEX, IA	12/1/09	197	und p	10,240
				und s	
20	No history	11/19/09	57		
21	PLEX	1/15/10	1,424		
		1/19/10		4,448 p	163,840
22	PLEX	2/16/10	31	und p	163,840
				und s	
23	PLEX, mefloquine	2/25/10	38,005	239 s	10,240
24	PLEX	1/21/10	12		
		3/4/10		157 p	12,621,440
		3/2/10		58 s	
25	IA, mirtazipine	4/28/10	91	25 p	
26	PLEX	9/30/10	2374	9 s	≥163,840
		11/16/09	2574	131 p	≥40,960
USA					
27 (#3)	PLEX, mefloquine	10/28/08	34,500		
				2,900 p	
28 (#11)	PLEX, mefloquine, levetiracetam	7/23/09	322		
		9/2/09	2,988		
29 (#12)	PLEX, miretazipine	8/4/09	3,600,000		
30 (#19)	PLEX, mirtazipine	10/2/09	257		
31 (#24)	PLEX, mefloquine	10/22/09	39		
		1/8/10	1,081		
		2/23/10			163,840

TABLE 2: Continued

Patients[a]	Treatment History	Sample Collection Date[b]	CSF viral copies (copies/ml)	Plasma/serum viral copies (copies/ml)	Antibody Titer[c]
32 (#26)	PLEX, mirtazipine	10/26/09	90,125		
33	PLEX	3/1/10	8,925		
		4/19/10	613	628 p	2,621,440
				511 s	
34	No history	4/21/10	380	und s	40,960
35	PLEX	4/23/10	21,736		

[a]Information for Patients 6 and 10 is in Refs. 4 and 5, respectively. Persistent viral load is the detection of viral load in CSF at time of diagnosis of PML and in multiple samples that remained detectable over months. Decreased viral load is detection of viral load in CSF at time of diagnosis of PML and/or in multiple samples that decreased at least 10× or were undetectable in the last available sample.
[b]First date indicates sample at time of PML diagnosis.
[c]Antibody titers are greater than or equal to reported value.
(#) = patient number and information in Ref. 2; Ara C = cytosine arabinoside; CDV = cidofovir; CSF = cerebrospinal fluid; EEA = European Economic Area; IA = immune absorption; IVIG = intravenous immunoglobulin; NA = sample(s) not available; p = plasma; PLEX = plasma exchange; s = serum; und = undetected in qPCR assay; USA = United States.

This observation might be important, if not critical, when evaluating new clinical episodes of neurological deficits and may warrant periodic assessment for viral genome in the CSF as well as in the plasma. Perhaps surprising in some PML patients is that viral persistence occurs even after IRIS and in the presence of high antibody titers to JCV. The observations that 2 PML patients had antibody titers to JCV at the time of diagnosis which were baseline as seropositive and 1 PML patient's sample was seronegative in the LMMN ELISA assay recommend a closer look on the use of antibody levels for either PML risk assessment[14] or as a prognostic indicator. A cross-reference analysis of identical samples tested in several laboratories experienced with the ELISA assay might clarify the interpretation of antibody titers that define serological status. At this point, presence of and rise in antibody levels may be a valuable indication of virus exposure and active infection, respectively. It is noteworthy that of 8 PML patients with multiple CSF samples and plasma/sera in Table 1, antibody titers increased from the time of PML diagnosis to extremely high titers and in a few cases reached to levels of 1×10^6. This would indicate that PML patients have the ability to respond to JCV infection, evidenced also as viremia in these patients, with a robust humoral immune response. A clear assumption would be that JCV eludes antibody-dependent clearance of JCV because these patients not only responded to infection but also were seropositive before PML. Assessment of patients' cell-mediated immune responses to multiple viral antigens and their immune surveillance remains a key feature in determining the ability to prevent PML or limit infection after PML occurs.[15]

It is not known whether the origin of CSF viral DNA during persistence is derived from the brains of PML patients or if virus periodically enters the brain from the periphery. It is anticipated that PML will continue to occur in natalizumab-treated patients and in other patients on therapies that modulate the immune system. In many if not all of these PML patients,

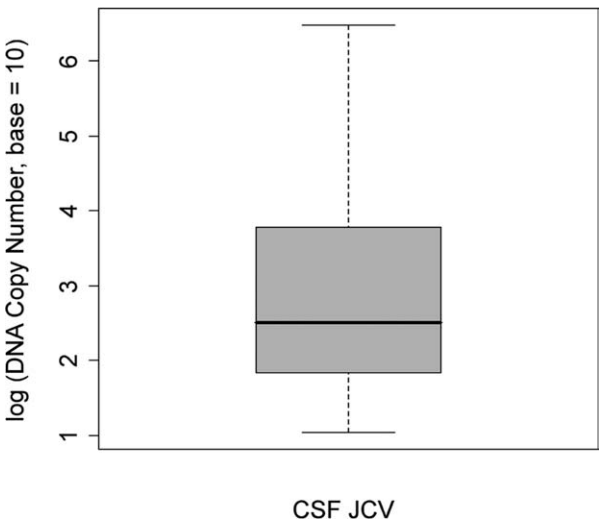


FIGURE 2: Box-plot of the distribution of viral copy number in 35 CSF samples taken at or near the time of PML diagnosis: median = 2.923; 25th percentile = 1.794; 75th percentile = 3.866; minimum value = 1.041; maximum value = 6.477. The data are normally distributed about the mean ($p = 0.3137$).

TABLE 3: JCV Status of Plasma or CSF Samples from Patients in the AFFIRM/SENTINEL Trial

	ELISA on Plasma or Serum for Anti-JCV Antibody Titers				
	MS	**Crohns**	**RA**	**NK**	**Total**
Samples, n	(214)	(40)	(5)	—	(259)
Percent positive	65%	90%	80%	—	69.9%
Percent negative	35%	10%	20%	—	30.1%
Antibody titer	2,560–10,240[a]	10,240–40,960[a]	10,240	—	
CSF qPCR	0/346	0/34	0/4	0/4	0/388

[a]Average antibody titer in ELISA assay using 4× serial dilutions.
— = not applicable; CSF = cerebrospinal fluid; MS = multiple sclerosis; NK = diagnosis unknown; RA = rheumatoid arthritis.

regardless of resolution of acute disease, clinical signs of neurological disability suggests long-term pathological consequences of PML.

Acknowledgments

We thank all Laboratory of Molecular Medicine and Neuroscience (LMMN) members; K. Johnson, PhD, Senior Statistician, National Institute of Neurological Disorders and Stroke (NINDS) for data analysis in Fig 2; the staff at the Centers that provided the samples; and the cooperation of Biogen/Idec in their acquisition. LMMN, NINDS, receives support from the Division of Intramural Research, NINDS. There is no cost for the assays nor does the LMMN receive any form of compensation. All reports on sample tests go directly to the Centers and physicians who send the samples.

Potential Conflict of Interest

None of the authors have any conflicts of interest.

References

1. Major EO. Progressive multifocal leukoencephalopathy in patients on immunomodulatory therapies. Ann Rev Med 2010;61:35–47.
2. Clifford D, De Luca A, Simpson DM, et al. Natalizumab associated progressive multifocal leukoencephalopathy in patients with multiple sclerosis: lessons learned from 28 cases. Lancet Neurol 2010; 9:438–446.
3. Yousry TA, Major EO, Ryschkewitsch C, et al. Evaluation of patients treated with natalizumab for progressive multifocal leukoencephalopathy. N Engl J Med 2006;354:924–933.
4. Langer-Gold A, Atlas SW, Green AJ, et al. Progressive multifocal leukoencephalopathy in a patient treated with natalizumab. N Engl J Med 2005;353:375–381.
5. Linda H, von Heijne A, Ryschkewitsch C, et al. Diagnosis and treatment of progressive multifocal leukoencephalopathy after natalizumab monotherapy in a person with multiple sclerosis. N Engl J Med 2009;361:1081–1087.
6. National Institutes of Health, Office of Technology Transfer Federal Register Announcement, HHS Reference No. E-152-2009/0, July 6, 2009. qPCR assay for detection of JC virus. Available at: http://www.ott.nih.gov/Technologies/abstractDetails.aspx?RefNo=1977. Accessed July 28, 2010.
7. Ryschkewitsch C, Jensen P, Hou J, et al. Comparison of PCR-southern hybridization and quantitative real-time PCR for the detection of JC and BK viral nucleotide sequences in urine and cerebrospinal fluid. J Virol Methods 2004;121:217–221.
8. Lenz P, Day PM, Pang YY, et al. Papillomavirus-like particles induce acute activation of dendritic cells. J Immunol 2001;166: 5346–5355.
9. Li J, Melenhorst J, Hensel N. et al. T cell responses to peptide fragments of the BKV T antigen-implications for cross reactivity of immune response to JC virus. J Gen Virol 2006;87:2951–2960.
10. Koralnik IJ, Du Pasquier RA, Kuroda MJ, et al. Association of prolonged survival in HLA-A2+ progressive multifocal leukoencephalopathy patients with a CTL response specific for a commonly recognized JC virus epitope. J Immunol 2002;168:499–504.
11. Hamilton R, Gravell M, Major EO. Comparison of antibody titers determined by hemagglutination inhibition (HI) and enzyme immunoassay (EIA) for JC virus and BK virus. J Clin Microbiol 2000;38: 105–109.
12. Knowles W, Pipkin P, Andrews N, et al. Population based study of antibody to the human polyomaviruses BKV and JCV and the simian polyomavirus SV40. J Med Virol 2003;71:115–123.
13. Rudick R, O'Connor P, Polman C, et al. Effects of natalizumab treatment on the presence of JC virus DNA in blood and urine in multiple sclerosis patients. Presented at the American Academy of Neurology (AAN) Annual Meeting, Toronto, April 10–17, 2010. St. Paul, MN: American Academy of Neurology, 2010.S31.002.
14. Gorelik L, Bixler S, Lerner M, et al. Evaluation of the incidence of anti-JCV antibodies in a cohort of natalizumab-treated MS patients. Presented at the American Academy of Neurology (AAN) Annual Meeting, Toronto, April 10–17, 2010. St. Paul, MN: American Academy of Neurology, 2010:S31.003.
15. Ransohoff RM. Natalizumab and PML. Nat Neurosci 2005;8:1275.

Natalizumab Drug Holiday in Multiple Sclerosis: Poorly Tolerated

Joep Killestein, MD, PhD,[1] Anke Vennegoor, MD,[1] Eva M. Strijbis, MD,[1] Alexandra Seewann, MD,[1] Bob W. van Oosten, MD, PhD,[1] Bernard M. J. Uitdehaag, MD, PhD,[1,2] and Chris H. Polman, MD, PhD[1]

It has been suggested that natalizumab-associated progressive multifocal leukoencephalopathy may be prevented by structured interruptions of treatment. Evidence supporting such a drug holiday is not yet available. Here we present initial observations in 10 multiple sclerosis patients who were stringently monitored up to 6 months after discontinuation of the infusions. Cumulatively, a combination of clinical relapse and new and/or enhanced lesions on magnetic resonance imaging had occurred in 7 of 10 patients. Although numbers are small, our data suggest that in patients who were switched to natalizumab because of disease activity despite first-line treatment, a natalizumab drug holiday without reinstatement of alternate disease-modifying therapy is poorly tolerated.

ANN NEUROL 2010;68:392–395

Natalizumab is a monoclonal antibody with specificity for alpha-4 integrin that inhibits the entry of mononuclear cells to sites of inflammation in the central nervous system. It has been approved worldwide for treatment of relapsing-remitting multiple sclerosis (MS) based on its efficacy in phase 3 trials.[1,2] Use of natalizumab is associated with a rare risk of development of progressive multifocal leukoencephalopathy (PML), a potentially lethal demyelinating disease of the central nervous system caused by lytic infection of oligodendrocytes by the Jamestown Canyon virus (JCV).

As of January 2010, natalizumab has been prescribed to >50,000 individuals with MS, and the number of confirmed PML cases has now risen to>30 worldwide.[3] Although details on many of these cases have not been published, the US Food and Drug Administration concluded in September 2009 that the risk for PML appears to increase with the number of natalizumab infusions received, the current rate ranging from 0.4 to 1.3 per 1,000 patients who have received at least 24 infusions.[4] In January 2010 the European Medicines Agency recommended additional measures to better manage risk of PML with natalizumab.[3] Already in 2007, accompanying the worldwide introduction of natalizumab, Kappos and colleagues provided recommendations for patient selection and monitoring, including diagnostic algorithms to apply in natalizumab-treated patients with clinical or magnetic resonance imaging (MRI) findings suggestive of PML.[5]

However, clinical vigilance is the most important indicator of suspected PML, because laboratory markers that predict the likelihood for PML are not yet available. Findings on whether JCV DNA is increased in plasma and peripheral blood mononuclear cells after natalizumab therapy are inconsistent, and there is no firm evidence that JCV DNA can predict which patients ultimately develop PML.[6–8]

In addition to focusing on early recognition of PML, some experts have questioned whether PML can be prevented by using natalizumab in treatment cycles with limited duration or whether structured interruptions of treatment should be instituted.[9,10] Evidence supporting such a drug holiday is not yet available, neither on its most appropriate timing nor on its most appropriate duration. It can, however, be assumed that such duration should be at least 3 months, because it has been shown that it takes about this amount of time for natalizumab serum concentrations to drop below 1 μg/ml, a level below which desaturation of alpha4-integrin is observed.[11]

Here we report initial observations in 10 patients with relapsing MS who after 12 months of treatment had a favorable response to the drug, both clinically and on MRI, and thereafter decided to discontinue treatment for a variety of reasons. Clinical and MRI assessments were prospectively planned to be performed 3 and 6 months after discontinuation of monthly infusions.

Patients and Methods

Since 2006, about 100 patients have started treatment with intravenous natalizumab (Tysabri, Biogen Idec, Cambridge, MA) 300mg every 4 weeks at the MS Center of the VU Academic Medical Center in Amsterdam, the Netherlands. These patients all had relapsing MS that was clinically active (2 relapses, or 1 relapse and new lesions on brain MRI during the past year)

From the [1]Department of Neurology, MS Center Amsterdam; [2]Department of Epidemiology and Biostatistics, VU University Medical Center, Amsterdam, the Netherlands.

Address correspondence to Dr Killestein, VU Medical Center, Dept of Neurology, PO Box 7057, 1007 MB Amsterdam, the Netherlands. E-mail: j.killestein@vumc.nl

Received Feb 5, 2010, and in revised form Apr 26, 2010. Accepted for publication Apr 30, 2010.

View this article online at wileyonlinelibrary.com. DOI: 10.1002/ana.22074

despite treatment with first-line disease-modifying agents (interferon beta or glatiramer acetate). Patients had monthly visits for relapse assessment, whereas Expanded Disability Status Scale (EDSS) and brain MRI were performed at baseline and every year thereafter.

From those patients who had a favorable response after 1 year of treatment (no relapses, not more than 1 new T2 lesion on 1 year MRI compared to baseline, no gadolinium-enhancing lesions at 1 year MRI), several have now discontinued treatment, for a variety of reasons. Patients who discontinued natalizumab agreed to have clinical visits and gadolinium-enhanced brain MRI at treatment discontinuation and thereafter at 3-month intervals (MRI was only performed if patients were not pregnant). Here we report on the first 10 patients who were monitored according to the aforementioned algorithm after their discontinuation of treatment.

Patient characteristics are given in the Table. All patients had been pretreated with interferon beta. Three had also been on glatiramer acetate. Their mean age at start of treatment with natalizumab was 39 years. They discontinued natalizumab after a mean treatment duration of 23 months (from 12 to 40 months). Reasons for discontinuation were desire to become pregnant in 2 patients, subjective disease worsening in 1 (in absence of relapse, or objective EDSS worsening, or new lesional activity on MRI), and fear for future risk of PML in 7.

Results

At discontinuation of treatment, all patients were clinically stable; none of them revealed new lesions on their MRI in comparison to the last MRI on treatment.

At 3 months, 2 patients had clinical activity, and 3 had new and/or enhancing lesions on brain MRI (see Table). The patient who discontinued treatment because of subjective worsening had a severe clinical relapse, with EDSS worsening of >1 full point 12 weeks after discontinuation; his MRI showed many new lesions that were enhanced after administration of gadolinium contrast (Fig). The other patient had a clinical relapse 2 months after her last natalizumab infusion; her MRI showed a big gadolinium-enhanced lesion. These patients both opted to restart treatment with natalizumab. At 6 months, of the remaining 8 patients, 5 had had a clinical relapse, and all of these patients also had new and/or enhanced lesions on brain MRI. Of the 3 patients who were clinically well, 2 had become pregnant (no MRI performed at month 6 because of pregnancy).

Cumulatively, at the end of 6 months, a combination of clinical relapse and new and/or enhanced lesions on MRI had occurred in 7 of 10 patients (see Table). The mean interval between treatment discontinuation and occurrence of relapse in these patients was 17 weeks (range, 8–22 weeks). All 7 active patients opted to restart with natalizumab.

Discussion

In 10 consecutive patients who responded well to natalizumab infusions, but decided to discontinue for a variety of reasons (mainly fear for PML risk and desire to become pregnant), we observed clinical relapses and accompanying active lesions in 7 at 6 months after discontinuation of natalizumab. Only 3 patients (2 of whom had become pregnant) were still clinically well at 6 months, although 1 of the patients who became pregnant had had new T2 lesions on MRI at month 3. Strikingly, and well in line with pharmacokinetic data, disease activity started to occur about 3 months after discontinuation of natalizumab.

In our view, although the patient number is quite limited, these data strongly suggest that in patients who were switched to natalizumab because of disease activity on first-line immune modulatory therapy and responded well, discontinuation of natalizumab is poorly tolerated. None of the patients received other disease-modifying drugs after discontinuation of natalizumab, because they all had failed on first-line immunomodulatory treatment, and starting immunosuppressive drugs was assumed to keep the risk of PML elevated. In this small observational study, a drug holiday of 6 months leads to undesired disease activity in the large majority of patients; by that time 7 of 10 patients had received treatment with intravenous steroids because of a relapse, and these patients all opted for immediate restart with natalizumab.

Although the extent of clinical and radiological disease activity after discontinuation of natalizumab is striking, the limited sample size and the amount of disease activity before natalizumab initiation do not allow interpretation of these data as evidence for an overshoot of disease activity compared to the prenatalizumab treatment period. None of the patients showed clinical or radiological signs and symptoms that were suspect for either active PML or an immune reconstitution inflammatory syndrome.

For these patients, who probably represent a subgroup with active disease because they all had documented clinical disease activity while on first-line therapy, we do not recommend discontinuation of natalizumab as a drug holiday without reinstatement of alternate disease-modifying therapy.

Potential Conflicts of Interest

J.K. has received consulting fees from Merck-Serono and Novartis. B.M.J.U. has received honoraria for consultancy from Merck Serono and Novartis in the past year. C.H.P. has accepted consulting fees or speaking fees from Actelion, Biogen Idec, Bayer Schering, Teva, Merck-Serono, Novartis, GlaxoSmithKline, UCB, Roche, and Antisense Ther-

TABLE: Clinical and Radiological Characteristics of 10 Relapsing-Remitting MS Patients Who Discontinued Natalizumab

Patients[a]	Months[b]	Reasons for Discontinuation	Relapses in Year Prenatalizumab	Baseline MRI Prenatalizumab	Clinical Month 3 after Discontinuation	MRI Month 3 after Discontinuation	Clinical Month 6 after Discontinuation	MRI Month 6 after Discontinuation
F, 53	27	Fear of PML	2 exacerbations	No active lesions	Exacerbation	1 large enhanced lesion + 1 new T2	Stable after IVMP and restart natalizumab	No active lesions
F, 29	28	Fear of PML	1 exacerbation	3 new T2, no enhanced lesions	Stable	No active lesions	Exacerbation treated with IVMP	25 enhanced lesions
F, 26	22	Fear of PML	4 exacerbations	26 enhanced lesions	Stable	No active lesions	Exacerbation treated with IVMP	19 enhanced lesions
F, 34	13	Desire to become pregnant	3 exacerbations	2 new T2, 1 enhanced lesion	Stable	No active lesions	Pregnant, stable	Not done
F, 41	22	Fear of PML	1 exacerbations	No active lesions	Stable	No active lesions	Exacerbation treated with IVMP	3 enhanced lesions
M, 48	40	Fear of PML	0 exacerbations	2 new T2 lesions	Stable	No active lesions	Stable	No active lesions
F, 49	23	Fear of PML	2 exacerbations	6 new T2, 2 enhanced lesions	Stable	No active lesions	Exacerbation treated with IVMP	4 enhanced lesions
M, 51	21	Subjective worsening	1 exacerbation	5 new T2, 3 enhanced lesions	Exacerbation	>50 enhanced lesions	Stable after IVMP and restart natalizumab	1 enhanced lesion, no new T2
F, 26	12	Desire to become pregnant	2 exacerbations	3 enhanced lesions	Stable	2 new T2 lesions, no enhanced lesions	Pregnant, stable	Not done
F, 41	24	Fear of PML	2 exacerbations	4 new T2, 3 enhanced lesions	Stable	No active lesions	Exacerbation treated with IVMP	4 enhanced lesions

Baseline MRI prenatalizumab: scan performed during first-line disease modifying-treatment (interferon-beta or glatiramer acetate) within 3 months before start natalizumab. New T2 lesions compared to baseline scan before initiation of first-line treatment. Active lesions: new T2 and/or gadolinium-enhanced lesions.

[b]Gender, age in years at the time of initiation of natalizumab therapy.

[b]Number of natalizumab infusions.

MS = multiple sclerosis; MRI = magnetic resonance imaging; F = female; PML = progressive multifocal leukoencephalopathy; IVMP = intravenous methylprednisolone 1,000mg for 3 days; M = male.

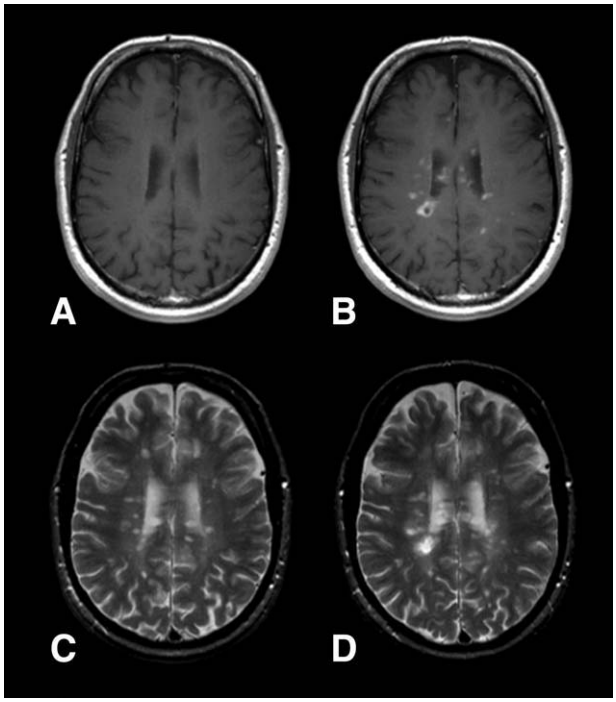


FIGURE: T1-weighted postgadolinium contrast and T2-weighted images just before (A, C) and 12 weeks after (B, D) discontinuation of natalizumab in a patient (male, aged 51 years) who discontinued treatment after 21 infusions (completely stable Expanded Disability Status Scale [EDSS] and magnetic resonance imaging [MRI] without new lesions during natalizumab treatment). He had a severe clinical relapse with EDSS worsening of >1 full point. His MRI showed >50 new enhanced lesions (B) and new T2 lesions (D) 12 weeks after discontinuation of natalizumab, whereas no enhancement (A) was observed just before discontinuation.

apeutics, and has received grant support from Biogen Idec, Bayer Schering, GlaxoSmithKline, Novartis, UCB, Merck-Serono, and Teva. VU Medical Center has received financial support for research activities from Bayer Schering Pharma, Biogen-Idec, GlaxoSmithKline, Merck Serono, Novartis, and Teva.

References

1. Polman CH, O'Connor PW, Havrdova E, et al. A randomized, placebo-controlled trial of natalizumab for relapsing MS. N Engl J Med 2006;354:899–910.
2. Rudick RA, Stuart WH, Calabresi PA, et al. Natalizumab plus interferon beta-1a for relapsing MS. N Engl J Med 2006;354:911–923.
3. European Medicines Agency. Questions and answers on the review of Tysabri (natalizumab). Available at: http://www.ema.europa.eu/humandocs/PDFs/EPAR/tysabri/Tysabri_A20-29_Q&A.pdf Accessed January 21, 2010.
4. US Food and Drug Administration. Information on natalizumab (marketed as Tysabri). Available at: http://www.fda.gov/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/ucm107198.htm. Accessed February 1, 2010.
5. Kappos L, Bates D, Hartung HP, et al. Natalizumab treatment for multiple sclerosis: recommendations for patient selecting and monitoring. Lancet Neurol 2007;6:431–441.
6. Chen Y, Bord E, Tompkins T, et al. Asymptomatic reactivation of JC virus in patients treated with natalizumab. N Engl J Med 2009;361:1067–1074.
7. Rudick RA, Polman CH, O'Connor PW, et al. Evaluation of natalizumab treatment on the presence of JC virus in blood and urine from MS patients. Mult Scler 2009;15(suppl 9):S274–S275.
8. Varnier OE, McDermott JL, Giacomazzi CG, et al. Non invasive surveillance of JCV reactivation in blood and urine from natalizumab treated MS patients, blood donors and immunocompromised patients identifies five PML cases in HIV and congenital immunodeficiency patients. Mult Scler 2009;15(suppl 9):S276–S277.
9. Hartung HP. New cases of progressive multifocal leukoencephalopathy after treatment with natalizumab. Lancet Neurol 2009;8:28–31.
10. Hauser SL, Johnston SC. Balancing risk and reward: the question of natalizumab. Ann Neurol 2009;66:A7–A8.
11. Khatri BO, Man S, Giovannoni G, et al. Effect of plasma exchange in accelerating natalizumab clearance and restoring leukocyte function. Neurology 2009;72:402–409.

Natalizumab Dosage Suspension: Are We Helping or Hurting?

Timothy W. West, MD,
and Bruce A. C. Cree, MD, PhD, MCR

The risk of developing progressive multifocal leukoencephalopathy increases with the duration of treatment with natalizumab. Planned dosage interruptions have been proposed as a means of decreasing cumulative risk. The clinical consequences of dosage interruption were evaluated in a single center cohort of natalizumab-treated patients. Medical records were reviewed for 84 patients identified with multiple sclerosis who received 12 or more infusions of natalizumab at an academic multiple sclerosis center. Eighty-one percent (68/84) underwent a dosage interruption, and 19% (16/84) had no interruption in natalizumab treatment. Of those with a treatment interruption, 27.9% (19/68) experienced a clinical

From the Multiple Sclerosis Center, University of California at San Francisco, San Francisco, CA.

Address correspondence to Dr West, 350 Parnassus Ave, Suite 908, San Francisco, CA 94117. E-mail: Timothy.West@ucsf.edu

Additional Supporting Information can be found in the online version of this article.

Received Jun 18, 2010, and in revised form Jul 06, 2010. Accepted for publication Jul 17, 2010.

View this article online at wileyonlinelibrary.com. DOI: 10.1002/ana.22163

relapse within 6 months of the suspension, whereas none of the patients with ongoing treatment experienced a flare during months 12 to 18 of treatment ($p = 0.017$, Fisher exact test). Survival analysis showed that Kaplan-Meier curves comparing dosage interruption to ongoing treatment diverged ($p = 0.025$). Median time from treatment interruption to relapse onset was 3 months. No clinical predictors associated with an increased risk of developing flares during dosage interruption were identified. Among the 19 patients who had a flare, 7 had severe flares, with a mean number of 16 Gad+ lesions on brain magnetic resonance imaging (range, 6–40). Their median Expanded Disability Status Scale at natalizumab interruption was 3.0 and increased to 6.0 during the flare ($p = 0.0008$). Natalizumab dosage interruption is associated with clinical flares and return of radiographic inflammatory disease activity. Some of these flares can be clinically severe, with a high number of contrast-enhanced lesions, suggesting a possible rebound of disease activity.

ANN NEUROL 2010;68:395–399

Natalizumab is a humanized anti–α-4 integrin monoclonal antibody that decreases lymphocyte trafficking across the blood-brain barrier. It has been approved by the US Food and Drug Administration for treatment of relapsing multiple sclerosis (MS) and is proven to reduce the relapse rate, progression of disability, and lesions on brain magnetic resonance imaging (MRI).[1,2] Exposure to natalizumab treatment is associated with risk of developing progressive multifocal leukoencephalopathy (PML). As of April 6, 2010, 46 cases of PML associated with natalizumab treatment have been reported. The risk of PML increases with duration of exposure.[3] Estimates of the risk of PML are 1:50,000 during the first year, 1:3,000 during the second year, and 1:750 in the third year of treatment (personal communication, Biogen Idec Medical Affairs).

In clinical practice, natalizumab is sometimes discontinued because of the dosage-dependent risk of PML. Planned dosage interruptions are hypothesized to decrease the cumulative risk of developing PML.[4] Those who undergo a planned interruption usually restart natalizumab after a period of 3 to 6 months. The rationale behind this duration of interruption is based on the phase II natalizumab trial that showed no difference in gadolinium-enhancing lesions between the active treatment and placebo groups 3 months after discontinuation.[5,6] Furthermore, in a cohort of patients who discontinued natalizumab, intrathecal gamma-globulin synthesis increased 6 months after natalizumab cessation.[7] It is not known whether planned dosage interruptions restore immunological function sufficiently to reduce the risk of PML. Nevertheless, dosage interruption is commonly used in clinical practice despite its unproven benefit.

The safety and tolerability of dosage interruption has not been assessed in clinical trials, and observational cohort studies have shown conflicting results. Two studies showed evidence of a return of MRI activity after cessation of natalizumab,[8,9] but another group found no worsening of disease activity after stopping natalizumab therapy.[7] The aim of the present study was to evaluate the clinical consequences of natalizumab dosage interruption.

Patients and Methods

Patients at the University of California, San Francisco (UCSF) MS Center who had received at least 12 natalizumab infusions were identified using the Tysabri Outreach Unified Commitment to Health program (TOUCH program). Only clinically definite MS patients with at least 13 months of follow-up were included. Treatment of a minimum of 12 months was selected to exclude patients who discontinued natalizumab after only a few infusions. At UCSF, dosage suspension is often planned following at least 1 year of treatment because the risk of PML during the first treatment year is low.

The time to relapse was defined in months from the date of the last infusion to the onset of clinical symptoms. A reference group of patients without dosage interruption was defined as those patients who received ongoing treatments for >12 infusions without interruption. In this group, the time to relapse was defined from the date of the 12th infusion to the onset of clinical symptoms. Both dosage interruption and ongoing treatment groups were censored at 6 months, because dosage interruption patients typically either restart natalizumab or undergo another disease-modifying treatment.

Brain MRI studies were performed for surveillance of subclinical MS disease activity, for monitoring for possible PML, or for evaluation of worsening neurological symptoms. Because these MRI scans were obtained for clinical reasons, the pulse sequences and timing of MRI with respect to treatment interruption were not standardized.

Statistical analysis was performed using STATA (College Station, TX). Chi-square, rank sum, sign rank, Student *t*, and log-rank tests were used to compare the 2 groups. This study received approval from the UCSF Committee on Human Research.

Results

Eighty-four relapsing MS patients (29 male, 55 female) received at least 12 infusions of natalizumab. Of those, 81% (n = 68) underwent a dosage interruption, and 19% (n = 16) had uninterrupted treatment with natalizumab. Those with treatment interruption were similar to those without interruption (Table 1).

Of the 68 who underwent a dosage interruption, 53 were taking part in a planned dosage suspension, 9

TABLE 1: Cohort Characteristics

Characteristic	Treatment Interruption	No Treatment Interruption	*p*
Number of subjects	68	16	
Women:men	44:24	11:5	0.760
Number of flares in year prior to natalizumab start, mean ± SD (range)	1.85 ± 1.06 (0–6)	1.29 ± 0.90 (1–4)	0.082
Number of flares during first year of natalizumab treatment	0.15 ± 0.36	0.36 ± 0.50	0.075
Prior immunomodulatory treatment, No. of patients	Yes: 67 No: 1	15 1	0.259
Prior immune suppression treatment, No. of patients	Yes: 18 No: 50	2 14	0.238
Reason for starting natalizumab	Tx failure: 62 Tx intolerance: 5 Not specified: 1	14 1 1	0.083
EDSS at natalizumab start, median (range)	3.5 (0–8)	3.5 (1.5–6.5)	0.477
Race/ethnicity	White: 55 African American: 5 Asian: 1 Latino: 6 Other: 0 Not specified: 1	11 2 1 1 1 0	0.262
Disease course	RRMS: 56 SPMS: 6 PRMS: 4	16 0 0	0.263

SD = standard deviation; Tx = treatment; EDSS = Expanded Disability Status Scale; RRMS = relapsing-remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis; PRMS = progressive relapsing multiple sclerosis.

were discontinued due to treatment failure, 3 did not tolerate natalizumab, 2 self-discontinued the therapy for personal concerns, and 1 developed recurrent melanoma. During the treatment interruption, only 4 of the 68 patients were treated with another disease-modifying therapy. None of these 4 patients had a clinical flare or an increase in MRI activity during the first 6 months off of natalizumab.

Of the patients who did not undergo a dosage suspension, none (0/16) experienced a clinical flare, whereas for those who underwent a dosage interruption, 28% (19/68) experienced a clinical relapse within the first 6 months off of therapy ($p = 0.017$, Fisher exact test). Because the duration of follow-up was not precisely matched for the 2 groups, survival functions were calculated to provide better estimates of the risk and timing of relapses. The survival functions for time to first relapse after dosage interruption or following the 12th natalizumab infusion for the ongoing treatment group were significantly different using the log-rank test ($p = 0.025$, Supporting Information Fig). The median time from interruption to clinical flare onset was of 3 months (range, 1–6).

Three additional treatment interruption patients experienced radiographic disease activity without clinical relapse, as evidenced by new gadolinium-diethylenetriamine pentaacetic acid (DPTA)-enhancing lesions on brain MRIs performed after 6 months of treatment interruption. MRI scans were not performed for the ongoing treatment group during the 6 months of follow-up; however, brain MRIs for these patients were performed near the first and second year of natalizumab treatment. Only 1 of the 16 patients had MRI evidence of subclinical disease activity, with 5 new T2 lesions identified on the second annual brain MRI.

TABLE 2: Severe Flare Group Characteristics

	Patient						
Characteristic	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Gender	Male	Male	Female	Female	Female	Female	Female
Disease course	SPMS	RRMS	RRMS	RRMS	RRMS	RRMS	RRMS
Race	White	White	Asian	White	White	White	White
Age of onset	18	35	16	20	18	32	25
Length of disease in years	30	15	14	12	14	5	25
EDSS at initiation	3.5	4.0	2.5	6.0	2.0	2.0	5.0
EDSS at interruption	3.5	4.0	2.5	5.5	2.0	2.5	N/A
EDSS at flare (nadir)	8.5	6.0	6.0	5.5	4.0	2.5	N/A
EDSS at convalescence	6.5	6.0	6.0	6.0	4.0	2.5	5.0
Number of Gad+ lesions at flare	40	6	10	8	16	8	10

SPMS = secondary progressive multiple sclerosis; RRMS = relapsing-remitting multiple sclerosis; EDSS = Expanded Disability Status Scale; N/A = not available; Gad = gadolinium.

Baseline clinical characteristics were similar between those with a flare and those without flares during dosage suspension (Supporting Information Table). Thus, clinical predictors of an increased risk of flares with dosage interruption were not identified.

To compare the clinical severity of post-natalizumab dosage interruption relapses, the Expanded Disability Status Scale (EDSS) scores at the time of dosage interruption were compared to the nadir EDSS scores at the time of relapse by the sign rank test. The median (range) natalizumab EDSS score at time of dosage interruption was 3 (0–8), and the median EDSS score during the relapse was 6 (1–8.5), $p = 0.0008$. The EDSS score at time of dosage interruption was not significantly different from the EDSS score at time of first natalizumab dose ($p = 0.253$).

Among the 19 treatment interruption patients who manifested a return of disease activity (clinical and/or MRI), 7 experienced unusually severe flares (Table 2). PML was ruled out by either MRI or lumbar puncture. All of these patients had failed other disease-modifying therapies. All experienced a clinical flare within the year prior to starting natalizumab, and 1 experienced 2 clinical flares. Six were on physician-advised dosage suspension, and 1 self-discontinued treatment due to concern of inefficacy. The median time to flare was 3 months. The median number of infusions prior to interruption was 18 (range, 8–24). On brain MRI at the time of the flare, there was a mean of 16 (range, 6–40) gadolinium-DPTA–enhancing lesions in this group (Fig).

Discussion

This study found that natalizumab treatment interruption is associated with recurrence of clinically significant MS disease activity. There are several important limitations inherent in this study's design that can potentially bias the conclusions. First, data were acquired retrospectively through nonblinded medical record review. Second, all assessments of disease activity were made during routine neurological care without standardized follow-up. Third,

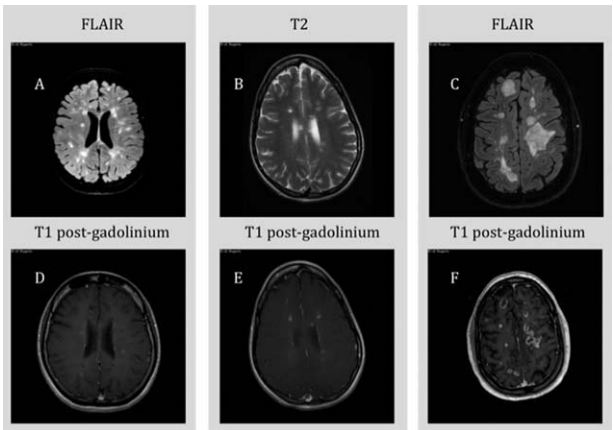


FIGURE: Refer to subjects from Table 2. (A) Fast fluid-attenuated inversion recovery (FLAIR) image and (D) T1-weighted magnetic resonance imaging (MRI) with gadolinium in Patient #5 with 16 gadolinium-enhancing lesions. (B) T2-weighted MRI and (E) T1-weighted MRI with gadolinium in Patient #3 with 10 gadolinium-enhancing lesions. (C) FLAIR image and (F) T1-weighted MRI with gadolinium in Patient #1 with 40 gadolinium-enhancing lesions.

subjects were not randomized to ongoing treatment versus dosage interruption. Despite being rigorous with data abstraction, we cannot exclude the possibility of unintentional bias; however, that radiographic disease activity was present at the time of all clinical relapses provides some reassurance, because these studies were evaluated independently. We attempted to control for the nonrandomized design by comparing the characteristics of the treatment interruption and ongoing-treatment groups and found no statistically significant differences. However, there were trends for those in the treatment interruption group to have more flares during the year prior to starting natalizumab ($p = 0.082$), to be more likely to begin natalizumab due to intolerance of other therapies ($p = 0.083$), and to have fewer clinical relapses during the first year of natalizumab treatment ($p = 0.075$).

That most natalizumab-treated patients have had ongoing disease activity despite treatment with other immunotherapies raises the concern for the risk of a return of clinical activity following dosage interruption.[4] Our study supports this concern; 28% of patients who underwent an interruption experienced a clinical flare (32% if isolated MRI activity was included). This is similar to the previously reported rate of 36 to 41% of patients developing new brain lesions following 6 months of drug suspension.[5]

Perhaps what is more alarming in this cohort is the subgroup of 7 patients (37%) who had a severe flare, with a nearly 3-point increase in median EDSS score accompanied by a large number of gadolinium-enhancing lesions and associated with limited recovery of neurological function (see Table 2). This level of disease activity was unusual for these patients, given their prior disease course, and raises concern of an overshoot effect or possibly an immune reconstitution inflammatory syndrome. When natalizumab is removed by plasmapheresis or immunoabsorption for PML treatment, the inflammatory syndrome associated with immune reconstitution is robust.[3]

Two case series describe an increase in disease activity following natalizumab dosage interruption. The first study found that the median annualized number of active T2 lesions on brain MRI increased; however, this observation seemed to be driven by patients with brief exposures to natalizumab (range of 1–8 infusions).[8] The second study found that 7 of 10 patients had clinical and/or MRI activity following dosage suspension.[9] In contrast, no evidence of rebound following natalizumab discontinuation was found in a study in which 19 of 23 patients were treated with another disease-modifying therapy during the 14-month period of follow-up.[7] Within our cohort, only 4 patients received a disease-modifying therapy following natalizumab interruption, and none experienced increased disease activity. If alternate treatment could minimize the risk of clinical flares, then planned dosage interruption might be a rational, albeit theoretical, option for PML prevention.

Acknowledgments

We thank C. Rodriguez, for her help in identifying natalizumab-treated patients, and the patients at the UCSF MS Center who are described in this study.

Potential Conflicts of Interest

T.W.W. has nothing to disclose. B.A.C.C. has received personal compensation for consulting for Biogen-Idec and Elan.

References

1. Polman CH, O'Connor PW, Havrdova E, et al; AFFIRM Investigators. A randomized, placebo-controlled trial of natalizumab for relapsing multiple sclerosis. N Engl J Med 2006;354: 899–910.
2. Rudick RA, Stuart WH, Calabresi PA, et al; SENTINEL Investigators. Natalizumab plus interferon beta-1a for relapsing multiple sclerosis. N Engl J Med 2006;354:911–923.
3. Clifford D, Luca A, Simpson DM, et al. Natalizumab associated progressive multifocal leukoencephalopathy in patients with multiple sclerosis: lessons from 28 cases. Lancet Neurol 2010;9: 438–446.
4. Hartung HP. New cases of progressive multifocal leukoencephalopathy after treatment with natalizumab. Lancet Neurol 2009;8: 28–31.
5. Miller DH, Khan OA, Sheremata WA, et al. A controlled trial of natalizumab for relapsing multiple sclerosis. N Engl J Med 2003; 348:15–23.
6. Dalton CM, Miszkiel KA, Barker GJ, et al. Effect of natalizumab on conversion of gadolinium enhancing lesions to T1 hypointense lesions in relapsing multiple sclerosis. J Neurol 2004;251:407–413.
7. Stüve O, Cravens PD, Frohman EM, et al. Immunologic and clinical status 14 months after cessation of natalizumab therapy. Neurology 2009;72:396–401.
8. Vellinga MM, Castelijns JA, Barkhof F, et al. Postwithdrawal rebound increase T2 lesional activity in natalizumab-treated MS patients. Neurology 2008;70(13 pt 2);1150–1151.
9. Killestein J, Vennegoor A, Strijbis E, et al. Natalizumab drug holiday in MS: poorly tolerated. Ann Neurol 2010;68:392–395.

Pathological Gambling in Parkinson Disease Is Reduced by Amantadine

Astrid Thomas, MD, PhD,[1,2]
Laura Bonanni, MD, PhD,[1,2]
Francesco Gambi, MD, PhD,[3]
Angelo Di Iorio, MD, PhD,[4]
and Marco Onofrj, MD[1,2]

To investigate the possible efficacy of amantadine in the control of pathological gambling (PG) associated with Parkinson disease (PD), 17 PD patients with PG were randomly selected for a double-blind crossover study with amantadine 200mg/day versus placebo and an open follow-up. Assessments included PG-specific scales (Yale-Brown Obsessive-Compulsive Scale for PG, Gambling-Symptom Assessment Scale, South Oaks Gambling Screen) and assessment of expenditures and time spent gambling. Amantadine abolished or reduced PG in all treated patients, as confirmed by scale score and daily expenditure reduction. Amantadine might be useful to treat PG. The effect of amantadine, acting as an antiglutamatergic agent, also opens new insights into the pathogenesis of PG.

ANN NEUROL 2010;68:400–404

Impulse control disorder (ICD) is defined as failure to resist an impulse, or as the drive or temptation to carry out a harmful act. It is considered, along with dopamine dysregulation syndrome (DDS) and repetitive non–goal-oriented behaviors (punding), to be a frequent disorder in Parkinson disease (PD).[1,2]

In PD, typical ICD includes hypersexuality, pathological gambling (PG), compulsive shopping, or compulsive eating; 6 to 7% of PD patients meet criteria for 1 of these disorders.[3] Lifetime prevalence of ICD is 6.1% in all PD patients, and 13.7 to 17.1% in PD patients on dopamine agonists (DA).[4]

Recent studies hypothesize that L-dopa–induced dyskinesias and behavioral alterations observed in DDS and ICD[5,6] depend on common mechanisms involving alterations of glutamate homeostasis with combined activation of sensitized dopamine and N-methyl-d-aspartate (NMDA) glutamatergic receptors.[5,6] Imbalance between synaptic and nonsynaptic glutamate might result in failure of prefrontal cortex control.[5,6]

No treatments are at present validated for ICD or DDS; reduction and withdrawal of DA are considered possible options, but DA withdrawal may induce severe worsening of motor control.[7] Clozapine has been shown in anecdotal reports to reduce hypersexual behaviors,[8] but no evidence supports its use in other ICDs. The antiglutamatergic acamprosate (Ca acetyl-homotaurine) is undergoing phase 2 studies.[9]

Amantadine, an antiglutamatergic drug with NMDA receptor antagonist properties,[10] introduced for the treatment of early PD motor symptoms based on original serendipitous findings and also able to reduce dyskinesias,[11] reduced punding behavior in a PD patient. [12]

In the present study, we aimed to test the ability of amantadine to possibly reduce ICD in PD. We selected PD patients recently affected by PG, as this ICD is frequent, economically disruptive, and quantifiable and has been a source of complex legal actions.

Patients and Methods

Seventeen patients with PD according to UK Brain Bank Criteria,[13] with severe PG identified in the last 10 months that was not decreased by DA reduction or withdrawal or behavioral strategies,[7] were selected from a cohort of 1,096 PD patients regularly followed at our Movement Disorder Clinic.

PG was identified according to Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) (rule of 5 of 9 items) and South Oaks Gambling Scale (SOGS) criteria.[14] Patients affected by manic episodes or bipolar disorder and patients receiving antipsychotics or anticholinergics or previously exposed to amantadine were excluded from the study.

PD symptoms were evaluated with the Unified Parkinson's Disease Rating Scale,[15] PD stage with the Hoehn/Yahr (H/Y) scale, cognition with the Mini-Mental State Examination, and behavioral and mental functions with the Neuropsychiatry Inventory.[16]

The study received approval by our local ethical committee, according to the Declaration of Helsinki and subsequent revisions.[17] After complete description of the study, all subjects signed written informed consent.

From the [1]Department of Neurology, University G. d'Annunzio; [2]Aging Research Center (CeSI), Gabriele d'Annunzio University Foundation; [3]Department of Oncology and Neuroscience, Institute of Clinical Psychiatry, University G. d'Annunzio; [4]Laboratory of Clinical Epidemiology, Geriatric Unit, Department of Medicine and Aging, University G. d'Annunzio, Chieti and Pescara, Italy.

Address correspondence to Prof Onofrj, Department of Neurology, Aging Research Center, Ce.S.I., Gabriele d'Annunzio University Foundation, Chieti-Pescara, Via dei Vestini, 66013 Chieti, Italy. E-mail: onofrj@unich.it

Additional Supporting Information can be found in the online version of this article.

Received Jan 25, 2010, and in revised form Mar 8, 2010. Accepted for publication Mar 12, 2010.

View this article online at wileyonlinelibrary.com. DOI: 10.1002/ana.22029

Study Design

To assess the effect of amantadine on PG, a 17-week, double-blind, placebo-controlled, crossover open extension study on amantadine treatment was designed, consisting of 4 weeks baseline and 8 weeks amantadine/placebo crossover with 1 week washout and 4 weeks follow-up.

The study design and Consolidated Standards of Reporting Trials (CONSORT) statement checklist are reported as supplementary material (Supplementary Study Design and Supplementary CONSORT Statement, respectively).

PG was quantified by blinded raters with the Gambling-Symptom Assessment Scale (G-SAS)[18] and the Yale-Brown Obsessive-Compulsive Scale (Y-BOCS) for PG.[19] Daily diaries assessed the time spent gambling and gambling cost in each day of the week. Patients' reports were double-checked with caregivers.

Assessments were performed twice during the baseline (run-in) period of 4 weeks (T1 and T2) and twice during the follow-up period of 4 weeks, where only 12 patients received amantadine (T6, T7). Randomization at the end of the baseline period (T2) assigned amantadine/placebo with a ratio 1:1. During the crossover period, assessments were done at T3 after 2 weeks of treatment (first crossover branch), at the end of the 1-week washout period (T4), and at T5 after 2 weeks of treatment (second crossover branch). Amantadine was administered as an add-on to the current antiparkinsonian medications, consisting of DA in monotherapy (4 patients), L-dopa in monotherapy (4 patients), L-dopa and DA therapy (9 patients), entacapone (7 patients), and rasagiline (all), unmodified throughout the study.

Amantadine tablets were triturated and inserted into polyamide capsules; identical capsules containing agar gel were used as placebo.

Amantadine or placebo were administered by a nurse unaware of patients assignments, with a titration schedule of 50mg twice daily (bid) for 2 days and 100mg bid in the following 2 weeks, and was withdrawn in 2 days (50mg bid) during period T4.

All patients had 24-hour access to clinicians to inform about effects of treatments or of withdrawals.

Statistics

Baseline characteristics were compared between treatments using analysis of variance for continuous variables and chi-square test analyses for dichotomous or categorical variables.

Differences in the G-SAS and Y-BOCS scores between treatments, at baseline and at follow-up, were tested by mixed models analysis of covariance.[20] Analyses were adjusted for age, sex, H/Y stage, and disease duration.

For dropped-out patients, the worst score reached at the G-SAS and Y-BOCS during the run-in phase was considered as the score at the end of the study (intention to treat analysis).

Order of drug administration was the variable added in the mixed model to evaluate potential carryover effects.

TABLE: Demographics and Clinical Characteristics

Characteristic	Value
Sex, M/F	13/4
Age, yr (range)	61.0 ± 1.6 (53–74)
PD DD, mo (range)	52.4 ± 7.8 (8–106)
H/Y stage (range)	1.9 ± 0.2 (1–3)
	6 patients H/Y stage 1–1.5; PD DD, 3–14 mo
	5 patients H/Y stage 2–2.5; PD DD, 39–52 mo
	6 patients H/Y stage 3; PD DD, 48–106 mo
L-dopa dose, mg (range)	223.5 ± 49.2 (0–500)
DA Eq dose, mg (range)	1.2 ± 0.4 (0–3)
Duration of L-dopa treatment, mo (range)	18.7 ± 5.7 (22—81)
Duration of DA treatment, mo (range)	47.4 ± 7.3 (8—92)
Duration of PG, mo (range)	7.1 ± 0.4 (4–9)
Daily expenditures, % of salary	B 2.0 ± 0.2
	A-d4 0.01 ± 0.1
	A-d14 0.01 ± 0.1
SOGS	15.1 ± 2.3
SAS	B 30.9 ± 0.7
	P 31.2 ± 0.2
	A 21.6 ± 0.9
Y-BOCS	B 28.0 ± 0.6
	P 28.0 ± 0.1
	A 17.3 ± 0.7
UPDRS-IV items 32–33 (complications of therapy)[a]	B 4.2 ± 1.5
	P 4.1 ± 1.6
	A 2.2 ± 0.4

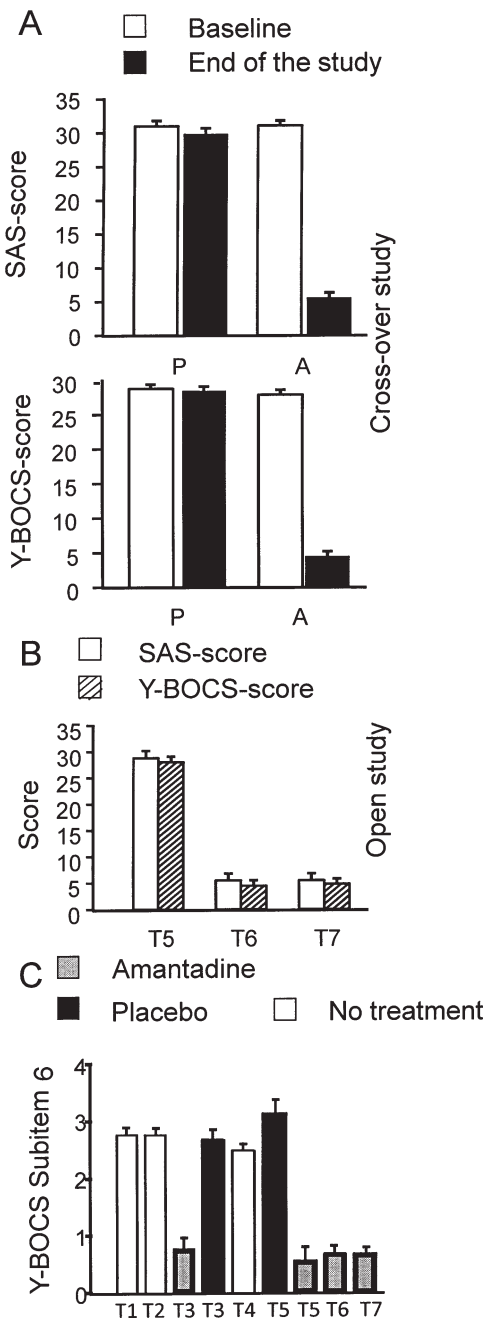
SAS and Y-BOCS are calculated on the 12 patients taking amantadine during the study.
[a]UPDRS-IV items 32–33 report dyskinesias, as amantadine is known to reduce dyskinesias. Only 4 of 6 patients in H/Y stage 3 had dyskinesias.
M = male; F = female; PD = Parkinson disease; DD = disease duration; H/Y = Hoehn/Yahr; DA = dopamine agonist; Eq = equivalent (1mg pramipexole equals 4mg ropinirole); PG= pathological gambling; B = before treatment; A-d4 = 4 days after amantadine treatment introduction; A-d14 = 14 days after amantadine treatment introduction; SOGS = South Oaks Gambling Scale; SAS = Symptom Assessment Scale; P = placebo; A = amantadine; Y-BOCS = Yale-Brown Obsessive-Compulsive Scale; UPDRS-IV = Unified Parkinson's Disease Rating Scale IV.

The power of the study was >0.90 (post hoc for possible non-normal distribution, alpha error ≤0.05).

Results

The Table reports demographics and clinical characteristics of patients entering the study.

PG consisted of instant lottery scratch games in all patients. Six patients also gambled on slot machines. Average daily expenditures (as percentage of daily salary) are reported in the Table.



PG had appeared in all patients in the past year and was a constant problem, as evidenced by DSM-IV and SOGS. In all patients, initial attempts to reduce/switch DA to L-dopa or change DA, gambling counseling, and behavioral strategies had been unsuccessful. PG had appeared in patients with different PD characteristics, as reported in the Table. Five patients dropped out from the study because of side effects consisting of confusion, orthostatic hypotension, insomnia (2 patients), and visual hallucinations, all on amantadine branches (Supplementary Patient Data show further details).

Amantadine abolished daily expenditures, resolving PG in 7 patients; in 5 patients, amantadine reduced G-SAS and Y-BOCS scores, daily expenditures (by 75–90%), and time spent gambling.

Amantadine effect was evidenced on day 2 to 4 of treatment (see Table); as assessed by diaries and caregiver interviews, it was equally effective in the run-in and in the second period of the crossover. Supplementary Figures 1 to 4 show reduction of daily expenditure as percentage of monthly salary. Placebo had no effect in run-in and induced reoccurrence of PG in 2 to 3 days during switch-over. In patients receiving placebo during the T3 and T5 crossover period, G-SAS and Y-BOCS scores and daily expenditures were at the same level as in baseline.

Comparison of G-SAS, Y-BOCS, and total gambling expenditures between amantadine and placebo revealed a probability of $p < 0.01$ (see Table, Fig, and Supplementary Figs 1–4).

G-SAS and Y-BOCS scores after 2 weeks of amantadine treatment were reduced by 80% compared to baseline, whereas no changes occurred during the placebo treatment (see Fig 1A). Differences between treatments

FIGURE 1: (A) Symptom Assessment Scale (SAS) and Yale-Brown Obsessive-Compulsive Scale (Y-BOCS) score changes during the crossover study. Both scores are reduced by amantadine ($p < 0.001$ compared to baseline). Baseline indicates scores (averaged for each patient) in T1, T2; placebo (P) and amantadine (A) scores indicate averaged scores in T3 and T5 of the crossover. (B) SAS and Y-BOCS score changes during the open study in the 7 patients treated with placebo in T5 (second crossover branch). Notice that reductions of SAS and Y-BOCS scores persisted from T6 to the end of study, T7 ($p < 0.01$). (C) Y-BOCS subitem 6 (time spent gambling) reported throughout the crossover and open extension study (periods T1–T7). White bars at T1, T2, and T4 indicate periods without pathological gambling (PG) treatments. Notice that PG scores are reduced by amantadine during the crossover study (T3 and T5), whereas placebo (T3, T5) has no effect. T6 and T7 show a preserved effect of amantadine administration during the open follow-up. Notice that during the T4 washout period, PG scores increase in 1 week, evidencing the short-lived effect of amantadine (Supplementary Figs 1–4 detail the finding).

in the crossover study were statistically significant (G-SAS: $F = 522.9$; $p < 0.001$; Y-BOCS: $F = 698.2$; $p < 0.001$), regardless of whether dropped-out patients were included. No carryover effect was observed (G-SAS: $F = 0.17$; $p = 0.69$: Y-BOCS: $F = 1.59$; $p = 0.17$). Supplementary Statistical Data provide a detailed analysis.

Figure 1B shows scale score changes during open follow-up branches. A reduction of the G-SAS ($p < 0.001$) and Y-BOCS scores ($p < 0.001$) was observed in both assessments of open study (T6 and T7) in the 7 patients treated with placebo in the second phase of the crossover study.

Timing of amantadine effect is evidenced in Supplementary Figures 1–4 and in Figure 1C, showing incremental increase of time spent gambling during the 1-week washout T4 period in comparison with study effects.

No patient had side effects because of amantadine withdrawal.

Discussion

The effect of amantadine was beyond expectations, as a financially devastating compulsive behavior was completely abolished or markedly reduced in all patients. Evidence of this effect might help neurologists who are forced to deal with PD patients affected by PG. Compulsive gambling is a major problem only in a restricted percentage of PD patients, yet it has led worldwide to litigation, including accusations of inadequate management of antiparkinsonian treatments, on the assumption of its relationship with DA.[4]

Amantadine is an old drug showing surprising new qualities; its effect on dyskinesias was discovered only a few years ago,[11,21] and its effect on behavioral disorders in PD has not been addressed, except for anecdotic reports describing occurrence of confusion, psychosis, or hallucinations during its use.[22,23] Hallucinations and confusion appeared also during our study, leading to premature withdrawal of the drug in 5 patients, providing evidence that amantadine might be poorly tolerated in PD patients with PG (29.4%), as in the general PD population. Theoretical issues suggest that amantadine might induce psychosis because of its antiglutamatergic activity[24] and thus might be contraindicated in patients at risk of, or already affected by, behavior disorders. Contrasting with this assumption, the present report showed that PG could be suppressed in 2 to 3 days by amantadine and that amantadine withdrawal induced, in a few days, reappearance of the disorder. This short time effect of amantadine on PG suggests that synaptic remodulation of glutamate/dopamine imbalance is the most likely mechanism in agreement with the introductory hypotheses, whereas plastic reorganization would require longer exposition time. The effect of amantadine gives new perspectives for research on understanding and treatment of PG.

Acknowledgments

We thank Drs G. Sepede and N. Cera for reading and discussing the manuscript.

Potential Conflicts of Interest

Nothing to report.

References

1. Fenu S, Wardas J, Morelli M.Impulse control disorders and dopamine dysregulation syndrome associated with dopamine agonist therapy in Parkinson's disease. Behav Pharmacol 2009;20:363–379.
2. Evans AH, Lees AJ. Dopamine dysregulation syndrome in Parkinson's disease. Curr Opin Neurol 2004;17:393–398.
3. Szarfman A, Doraiswamy PM, Tonning JM, et al. Association between pathologic gambling and parkinsonian therapy as detected in the Food and Drug Administration Adverse Event database. Arch Neurol 2006;63:299–300.
4. Antonini A, Tolosa E, Mizuno Y, et al. A reassessment of risks and benefits of dopamine agonists in Parkinson's disease. Lancet Neurol 2009;8:929–937.
5. Kalivas PW. The glutamate homeostasis hypothesis of addiction. Nat Rev Neurosci 2009;10:561–572.
6. Linazasoro G. Dopamine dysregulation syndrome and levodopa-induced dyskinesias in Parkinson disease: common consequences of anomalous forms of neural plasticity. Clin Neuropharmacol 2009;32:22–27.
7. Galpern WR, Stacy M. Management of impulse control disorders in Parkinson's disease. Curr Treat Options Neurol 2007;9:189–197.
8. Ruggieri S, De Pandis MF, Bonamartini A, et al. Low dose of clozapine in the treatment of dopaminergic psychosis in Parkinson's disease. Clin Neuropharmacol 1997;20:204–209.
9. Fox SH, Brotchie JM, Lang AE. Non-dopaminergic treatments in development for Parkinson's disease. Lancet Neurol 2008;7:927–938.
10. Movement Disorders Society.Management of Parkinson's disease: an evidence-based review. Amantadine and other antiglutamate agents. Mov Disord 2002;17(suppl 4):S13–S22.
11. Thomas A, Iacono D, Luciano AL, et al. Duration of amantadine benefit on dyskinesia of severe Parkinson's disease. J Neurol Neurosurg Psychiatry 2004;75:141–143.
12. Kashihara K, Imamura T. Amantadine may reverse punding in Parkinson's disease—observation in a patient. Mov Disord 2008;23: 129–130.
13. Hughes AJ, Daniel SE, Kilford L, et al. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. J Neurol Neurosurg Psychiatry 1992;55:181–184.
14. Lesieur HR, Blume SB. The South Oaks Gambling Screen (SOGS): a new instrument for the identification of pathological gamblers. Am J Psychiatry 1987;144:1184–1188.
15. Fahn S, Elton RL, Members of the Unified Parkinson's Disease Rating Scale Development Committee. Unified Parkinson's disease rating scale. In: Fahn S, Marsden CD, Calne DB, et al, eds. Recent development in Parkinson's disease. Florham Park, NJ: Macmillan Healthcare Information, 1987:153–164.
16. Cummings JL, Mega M, Gray K, et al. The Neuropsychiatric Inventory: comprehensive assessment of psychopathology in dementia. Neurology 1994;44:2308–2314.

17. World Medical Association Declaration of Helsinki.Recommendations guiding physicians in biomedical research involving human subjects. JAMA 1997;277:925–926.

18. Kim SW, Grant JE, Potenza MN, et al. The Gambling Symptom Assessment Scale (G-SAS): a reliability and validity study. Psychiatry Res 2009;166:76–84.

19. Pallanti S, DeCaria CM, Grant JE, et al. Reliability and validity of the pathological gambling adaptation of the Yale-Brown Obsessive-Compulsive Scale (PG-YBOCS). J Gambl Stud 2005;21:431–443.

20. Littell RC, Pendergast J, Natarajan R. Modelling covariance structure in the analysis of repeated measures data. Stat Med 2000;19:1793–1819.

21. Rajput AH, Rajput A, Lang AE, et al. New use for an old drug: amantadine benefits levodopa-induced dyskinesia. Mov Disord 1998;13:851.

22. Poewe W. When a Parkinson's disease patient starts to hallucinate. Pract Neurol 2008;8:238–241.

23. Smith EJ. Amantadine-induced psychosis in a young healthy patient. Am J Psychiatry 2008;165:1613.

24. Riederer P, Kornhuber J, Gerlach M, et al. Glutamatergic-dopaminergic imbalance in Parkinson's disease and paranoid hallucinatory psychosis. In: Rinne UK, Nagatsu T, Horowski R, eds. International Workshop Berlin, Parkinson's disease. Bussum, the Netherlands: Medicom, 1991:10–23.

Central Facial Palsy Revisited: A Clinical-Radiological Study

Luigi Cattaneo, MD, PhD,[1] Elena Saccani, MD,[2] Piero De Giampaulis, MD,[2] Girolamo Crisi, MD,[2] and Giovanni Pavesi, MD, PhD[2]

We investigated the pattern of volitional facial motor deficits in acute stroke patients. We assessed the strength of single facial movements and correlated it to the site of infarct classified on computed tomography scans. Exclusion criteria were previous stroke, cerebral hemorrhage, and subcortical stroke. Results showed that weakness in eyelid closure was associated with anterior cerebral artery (ACA) stroke. Weakness in lip opening was associated with middle cerebral artery (MCA) stroke. We suggest that sparing of upper facial movements in MCA stroke is due to the presence of an upper face motor representation in both the MCA and ACA territories.

ANN NEUROL 2010;68:404–408

Facial palsy caused by supranuclear lesions affects usually the voluntary movements of the lower face contralateral to the affected hemisphere, sparing the movements of the upper hemiface. The classical model interprets this pattern with bilateral corticonuclear projections from each primary motor area to the motoneurons innervating the upper facial muscles and uniquely contralateral projections to the motoneurons innervating the lower facial muscles.[1] This model, however, has been recently challenged in a study in nonhuman primates based on the existence of multiple (up to 5) distinct cortical motor representations of the face.[2] Accordingly, movements of the lower face would be represented mainly in the lateral Rolandic region, namely in primary motor cortex and in the ventral premotor cortex, and movements of the upper face would be represented mainly in the anterior cingulate and mesial frontal cortex. According to this model, stroke in the territory of the middle cerebral artery (MCA) affects only lower face movements because of sparing of corticonuclear projections from facial representations in the territory of the anterior cerebral artery (ACA).

In this work, we assessed systematically in consecutive acute ischemic stroke patients the strength of a series of facial movements. Then we correlated in single subjects the weakness in distinct facial movements with the involvement of different vascular territories.

Patients and Methods

Patients

We assessed consecutive stroke patients from our clinic over a 2-years period. All of them were evaluated between the 2nd and the 5th day from the stroke. Exclusion criteria were: (1) hemorrhage; (2) previous ischemic stroke; (3) previous peripheral facial palsy; (4) lack of compliance to the examination; and (5) presence on radiological examination of ischemia in the territories other than A, M1, M4, and M5 of the Alberta Stroke Program Early CT Score (ASPECTS) (see below).[3] It should be noted, therefore, that all patients with lesions in the deep subcortical segments of the ASPECTS system, such as the internal capsule (I segment) or the basal ganglia (C and L segments), were excluded from the study.

Clinical and Radiological Assessment and Evaluation of Voluntary Facial Movements

We collected the data of age, gender, and handedness. Computed tomography (CT) scans obtained 2 or 3 days from stroke were evaluated by an expert neuroradiologist for ASPECTS scoring. This is a topographic scoring system applied in predicting outcome of hyperacute stroke, which considers 3 cerebral

From the [1]Center for Mind/Brain Sciences, University of Trento, Trento, Italy; and [2]Department of Neuroscience, Azienda Ospedaliero-Universitaria, University of Parma, Parma, Italy.

Address correspondence to Dr Cattaneo, Center for Mind/Brain Sciences (CIMeC), University of Trento, Via delle Regole, 101, I - 38100 Mattarello (TN), Italy. E-mail: luigi.cattaneo@unitn.it

Additional Supporting Information can be found in the online version of this article.

Received Nov 20, 2009, and in revised form Apr 7, 2010. Accepted for publication Apr 26, 2010.

View this article online at wileyonlinelibrary.com. DOI: 10.1002/ana.22069

TABLE 1: Mean Scores and Distribution in the Population of Weakness in Facial Movements

Facial Movement	Subjects, %[a]	Mean Score[b]
Forehead elevation	32.1	0.36
Eyelid closure	53.6	0.57
Lip elevation	64.3	1.11
Lip abduction	75.0	1.32
Lip depression	71.4	1.21
Lip closure	60.7	0.61

[a]N = 28.
[b]0 = normal; 4 = no movement.

vascular regions: ACA (A), posterior cerebral artery (P), and MCA, the last divided into 10 territories, only 6 of which (M1–6) are corticosubcortical regions. We considered lesions in 4 ASPECTS regions: A, which encompasses mesial motor areas (ie, supplementary motor area and cingulate gyrus); M1, which encompasses the inferior frontal gyrus and the utmost caudal portion of the medial frontal gyrus and precentral gyrus; M4, which encompasses the rostral portion of the medial frontal gyrus; and M5, which encompasses the rostral portion of the precentral and postcentral gyrus and therefore the main portion of the primary motor cortex. Conversely, the M1 and M4 regions encompass the premotor areas.

Voluntary facial movements were assessed by an expert neurologist who was blinded as to the radiological picture of the patient apart from the side of the lesion. Six distinct movements were assessed: (1) forehead elevation, (2) eyelid closure, (3) lip elevation, (4) lip abduction, (5) lip depression, and (6) lip closure. The first 2 were referred to as *upper facial movements* and the latter 4 as *lower facial movements*. Force was evaluated against resistance by comparing the side contralateral to the lesion with the ipsilateral side. Patients were asked to oppose as hard as they could the movements of the examiner's finger, which was positioned as follows: (1) pushing the middle of the eyebrow downward for testing forehead elevation; (2) pushing the lower and the upper eyelid downward and upward, respectively, in 2 distinct operations for testing eyelid closure; (3) pushing downward the skin of the cheek laterally to the nasal pinna for testing upper lip elevation; (4) pushing medially the skin overlying the modiolus for lip abduction; (5) pushing upward the lower lip for lower lip depression; and (6) pulling laterally the corner of the mouth by inserting the finger in the oral cavity for lip closure. Force was graded on a 5-point scale with 0 indicating the same force as the contralateral side, 1 = 3/4 of the contralateral side, 2 = 1/2 of the contralateral side, 3 = 1/4 of the contralateral side, and 4 = absence of movement. In this way, the measure was by no means an absolute measurement of force but rather a side-to-side comparison, which made the scoring easy to perform. The same neurologist (G.P.), experienced in evaluation of cranial muscles, performed the clinical examination in all patients, so no interexperimenter variability needed to be taken into account. Additionally, all patients were assessed for the presence of impairment of emotional facial movements by testing spontaneous smile and frowning.

Data Analysis

Data analysis was performed using the SPSS 15 software. To characterize the pattern of facial weakness, the force scores of each of the 6 facial movements were analyzed as within-subjects dependent variables with the nonparametric Friedman analysis of variance (ANOVA) in the subpool of subjects with facial palsy.[4] Post hoc comparisons were made with Wilcoxon test for paired samples.

The correlation with radiological data was made on the whole population, including patients without facial weakness, by means of ordinal logistic regression, using as ordinal dependent variable the single scores of facial force and as categorical predictors the involvement of the 4 arterial territories: A, M5, M4, and M1.[5] The maximum iterations were set at 100, and a complementary log-log link function was used. The pseudo R^2 coefficient was calculated using the Nagelkerke method.[6]

Results

A total of 211 consecutive patients were recruited. Of these, 164 were excluded from the study. Thirty-six had hemorrhage; 87 had previously existent strokes; 2 had previous facial palsy; 30 were not compliant; and 41 had strokes in territories other than the 4 that we considered (A, M5, M4, and M1). Exclusion criteria overlapped in a few patients.

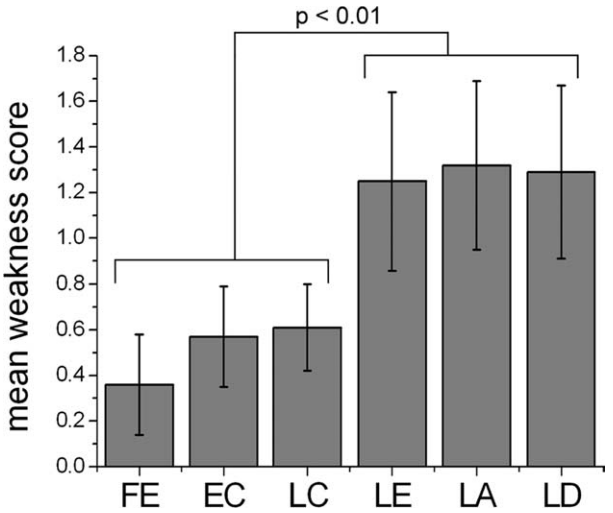


FIGURE 1: Plot of the involvement of different facial movements. Error bars indicate 95% confidence intervals, and *p* values indicate results of Wilcoxon test for paired samples. FE = forehead elevation; EC = eyelid closure; LC = lip closure; LE = lip elevation; LA = lip abduction; LD = lip depression.

TABLE 2: Results of Ordinal Logistic Regression

Force Score	Predictive Value of the Model (X^2; *df*; *p*)	Pseudo R^2	Significant Predictor	*p*	Estimate
Forehead elevation	20.378; 4; <0.0001	0.525	—	—	—
Eyelid closure	9.724; 4; <0.045	0.240	A	0.035	2.397
Lip elevation	40,033; 4; <0.0001	0.664	M5	0.013	2.863
Lip abduction	39.272; 4; <0.0001	0.638	M5	0.019	1.431
Lip depression	41.665; 4; <0.0001	0.667	M5	0.028	1.410
Lip closure	2.963; 4; 0.564	0.084	—	—	—

Of the remaining 47 patients (aged 43–90 years; mean age, 73 years; 18 women), 28 had facial weakness, and 19 did not. The distribution and entity of weakness in the different facial movements are shown in Table 1 and Figure 1. The clinical and radiological features of the patients are shown in Supplementary Table.

Of the 28 patients with facial palsy, 3 had isolated weakness of the upper facial movements (all 3 involving the eyelid closure only); 7 had facial weakness isolated to the lower facial movements. The remaining 18 had facial weakness in both quadrants; however, weakness was prominent in some lower facial movements (see Supplementary Table). The results of Friedman ANOVA showed a clear main effect (chi-square ANOVA, n = 47, $df = 5$: 30.82905, $p < 0.00001$). Post hoc analysis using multiple Wilcoxon tests showed that lip elevation, abduction, and depression were all significantly weaker than forehead elevation, eyelid closure, and lip closure (all *p* values < 0.01; see Fig 1).

The ordinal logistic regressions indicated the infarction in the A vascular segment (ACA territory) as a significant predictor of eyelid closure weakness. Infarction in the M5 division of the MCA territory was a significant predictor of lip elevation, abduction, and depression, whereas no significant predictors were found for lip

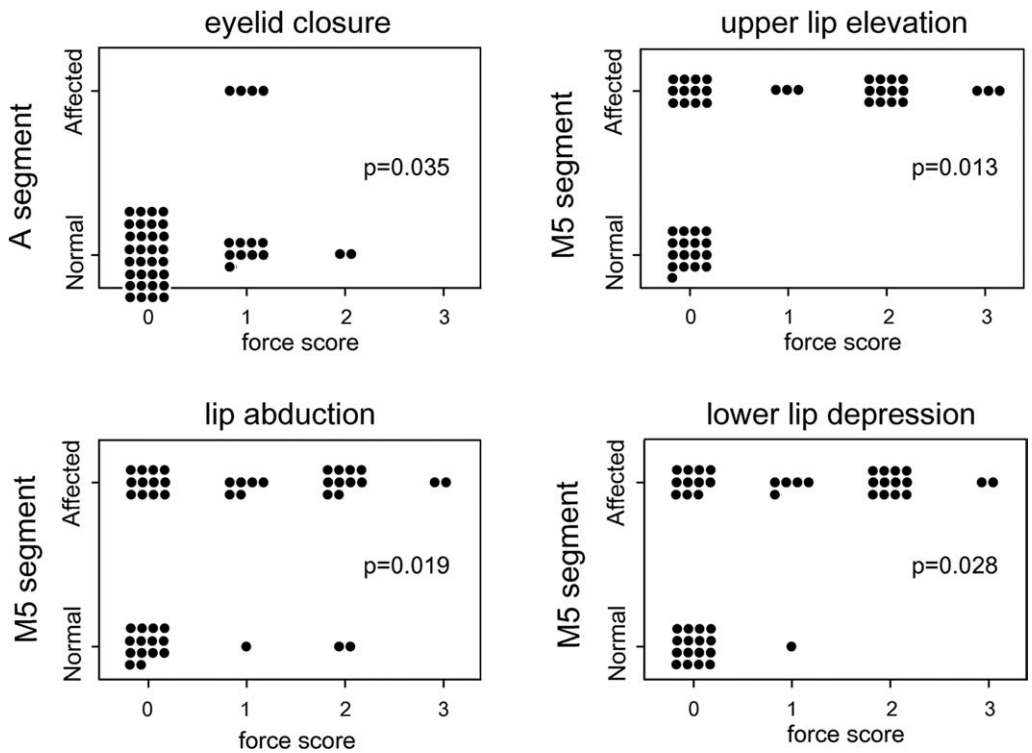


FIGURE 2: Graphical representation of the significant correlations found by ordinal regression between the force scores (0 = normal force, 4 = absence of force) in individual facial movements and the presence or absence of ischemia in definite segments of the ASPECTS (Alberta Stroke Program Early CT Score) system. Each dot represents a single patient.

closure and forehead. The results are shown in Table 2 and graphically presented in Figure 2.

Four cases with emotional facial palsy were found. All of them showed a concurrent impairment also in volitional movements. Three of them were in the group of patients excluded from the analysis. The reason for exclusion was a subcortical site of lesion that was found in the mesencephalon in 2 of them and in the internal capsule and basal ganglia in 1. The fourth patient with emotional palsy was among the included patients and had a lesion within the M5 territory (Patient 34 in Supplementary Table).

Discussion

The aim of this work was to investigate in acute stroke patients whether the phenotypical pattern of central volitional facial palsy is related to the presence of distinct facial cortical representations by means of a clinical-radiological correlation between the weakness of distinct facial movements and the arterial territories affected. We excluded therefore all lesions in the deep subcortical segments (namely the internal capsule and adjacent segments) of the ASPECTS system where descending pathways from vast cortical areas are in close contiguity. Among the segments that do comprise parts of the cerebral cortex, we limited our search to lesions in the arterial territories that may contain the facial representations postulated in the nonhuman primate model, namely in the frontal lobe and in the mesial surface of the hemispheres.[2,7] The selection therefore included the ASPECTS segments A, M1, M4, and M5. For this reason, our population as such is not representative of volitional central facial palsy of all stroke patients, but rather aims at a fine localization of facial representations within these cortical regions, to verify in humans the alternative model of central facial palsy.[2]

Our results show, first of all, that central volitional facial palsy is not a uniform entity. There is considerable variability in the involvement of different voluntary facial movements, and rather than a clear-cut distinction in upper and lower movements we found a more complex pattern (see Table 1 and Fig 1). The most affected movements were those that produce lip opening or centrifugal mouth movements, whereas lip closure or centripetal lip movements were less affected. We found only 7 of 28 patients with classical isolated lower quadrant palsy; in all the others, some form of upper face involvement was also present. Emotional facial palsy was found to be a rare entity, being present in 4 of 211 cases. It was never found dissociated from volitional palsy. The lesion sites associated with emotional palsy are consistent with previous reports in the literature, namely in the brainstem,[8] internal capsule,[9] and basal ganglia in 1 and in the white matter of the frontal region.[10]

Although to our knowledge no systematic phenomenological description of central facial palsy has been done, the idea of relative rather than absolute sparing of upper facial movements is not new.[11,12] Our results confirm this, by indicating a novel pattern of facial weakness in volitional central facial palsy; instead of lower face movements, what we find to be more severely affected are lip opening movements, whereas lip closing movements are only mildly affected, together with upper facial movements.

One limitation of the study is the use of CT scans instead of magnetic resonance imaging. However, our aim was not that of a fine-grained definition of the anatomy of the localization of facial weakness within the cortical mantle, but rather a correlation of vascular territories to facial weakness patterns. To this purpose, the spatial resolution of CT scans is entirely suitable, because the units of measurement of the ischemic area (the ASPECTS territories) are calibrated on CT scans.

Up to this point, the classical model of unilateral versus bilateral corticonuclear projection is still capable of explaining the phenotypical picture described. However, the correlation with the radiological features shows that eyelid closure movements are supported by a motor representation within the ACA territory, although present also in the Rolandic regions, because infarction in the ACA territory significantly predicts eyelid weakness, but eyelid weakness is present at times also in MCA infarction (see Fig 2). This result supports the idea of a duplex representation of voluntary eyelid movements, 1 in the mesial frontal cortex and the other in the Rolandic region. This datum reconciles the previous findings of a mesial representation of eyelid movements in humans[13,14] and in nonhuman primates[2] with the classical findings of projections from the primary motor cortex to the part of the facial nucleus innervating the upper facial muscles, as shown in humans and nonhuman primates.[2,15–17]

Conversely, centrifugal lip movements are clearly supported by a motor representation in the MCA territory, because their weakness is clearly predicted by MCA infarction and none of them was affected in ACA strokes. A similar effect on upper facial movement in patients with ACA stroke was also anecdotally reported.[18]

The movements of forehead elevation and mouth closure were observed in both ACA and MCA infarction. They had no significant predictor in the different vascular territories, and were always mildly affected. We can therefore presume that their representation is at least

duplex, in the ACA and in the MCA territory. In the literature, a forehead motor representation has been found in primary motor cortex. In accordance with previous data,[19,20] we show that voluntary forehead movements are present in the Rolandic region but also in the ACA territory. What is more puzzling is the presence of mouth closure weakness in ACA infarction. This could be partly explained by the existence of a vocalization center in the anterior cingulate cortex in both humans and monkeys, albeit devoted to emotional rather than volitional utterances.[21]

Our study has the limitation of not being able to account for the presence of bilateral corticonuclear projections. We suppose that the variability of the facial involvement is at least in part due to spared ipsilateral projections. For example, within the lower facial movements, the greater involvement of centrifugal movements compared to centripetal movements is probably in part due, as previously shown, to a more bilateral representation of mouth closure,[19,22] despite a mainly contralateral representation of centrifugal movements such as lip depression.[23]

Our study results indicate that the distinction of facial muscles in upper and lower quadrants is not entirely adequate. A clear distinction in the central representation of upper facial movements is found between eye closure and forehead elevation, and 2 distinct patterns of cortical representation characterize mouth closure and mouth opening muscles. The idea that the lower facial muscles are made of 2 clearly distinct subgroups, that is, those located toward the midline producing centrifugal movements and those located laterally and producing centripetal movements, is supported by several lines of evidence arising from different histological properties, reflex properties,[24] embryogenetic origin,[25] phylogenetic history,[26,27] and anatomical properties.[28]

Potential Conflicts of Interest

Nothing to report.

References

1. Strumpell A. Lehrbuch der speciellen pathologie und therapie der inneren krenkheiten. Leipzig, Germany: F.C.W. Vogel, 1884.
2. Morecraft RJ, Louie JL, Herrick JL, Stilwell-Morecraft KS. Cortical innervation of the facial nucleus in the non-human primate: a new interpretation of the effects of stroke and related subtotal brain trauma on the muscles of facial expression. Brain 2001;124:176–208.
3. Barber PA, Demchuk AM, Zhang J, Buchan AM. Validity and reliability of a quantitative computed tomography score in predicting outcome of hyperacute stroke before thrombolytic therapy. ASPECTS Study Group. Alberta Stroke Programme Early CT Score. Lancet 2000;355:1670–1674.
4. Sheldon MR, Fillyaw MJ, Thompson WD. The use and interpretation of the Friedman test in the analysis of ordinal-scale data in repeated measures designs. Physiother Res Int 1996;1:221–228.
5. Bender R, Grouven U. Ordinal logistic regression in medical research. J R Coll Physicians Lond 1997;31:546–551.
6. Nagelkerke NJD. A note on a general definition of the coefficient of determination. Biometrika 1991;78:691–692.
7. Morecraft RJ, Stilwell-Morecraft KS, Rossing WR. The motor cortex and facial expression: new insights from neuroscience. Neurologist 2004;10:235–249.
8. Khurana D, Sreekanth VR, Prabhakar S. A case of emotional facial palsy with ipsilateral anterior inferior cerebellar artery territory infarction. Neurol India 2002;50:102–104.
9. Trosch RM, Sze G, Brass LM, Waxman SG. Emotional facial paresis with striatocapsular infarction. J Neurol Sci 1990;98:195–201.
10. Hopf HC, Muller-Forell W, Hopf NJ. Localization of emotional and volitional facial paresis. Neurology 1992;42:1918–1923.
11. Bradley WG, Daroff RB, Fenichel GM, Marsden CD. Neurology in clinical practice. Oxford, UK: Butterworth-Heinemann, 2000.
12. Campbell WW. DeJong's The Neurologic Examination. Philadelphia, PA: Lippincott Williams & Wilkins, 2005.
13. Hanakawa T, Dimyan MA, Hallett M. The representation of blinking movement in cingulate motor areas: a functional magnetic resonance imaging study. Cereb Cortex 2008;18:930–937.
14. Sohn YH, Voller B, Dimyan M, et al. Cortical control of voluntary blinking: a transcranial magnetic stimulation study. Clin Neurophysiol 2004;115:341–347.
15. Jenny AB, Saper CB. Organization of the facial nucleus and corticofacial projection in the monkey: a reconsideration of the upper motor neuron facial palsy. Neurology 1987;37:930–939.
16. Kuypers HG. Corticobular connexions to the pons and lower brain-stem in man: an anatomical study. Brain 1958;81:364–388.
17. Kuypers HG. Some projections from the peri-central cortex to the pons and lower brain stem in monkey and chimpanzee. J Comp Neurol 1958;110:221–255.
18. Damasio AR. Descartes' error. New York, NY: G.P Putman's Sons, 1994.
19. Fischer U, Hess CW, Rosler KM. Uncrossed cortico-muscular projections in humans are abundant to facial muscles of the upper and lower face, but may differ between sexes. J Neurol 2005;252: 21–26.
20. Rodel R, Laskawi R, Markus H, Wenke K. Cortical transcranial magnetic stimulation of the frontalis muscle: characteristic features and details on motor-evoked potentials of the forehead. ORL J Otorhinolaryngol Relat Spec 2001;63:66–71.
21. von Cramon D, Jurgens U. The anterior cingulate cortex and the phonatory control in monkey and man. Neurosci Biobehav Rev 1983;7:423–425.
22. Costa J, Valls-Sole J, Valldeoriola F, et al. Motor responses of muscles supplied by cranial nerves to subthalamic nucleus deep brain stimuli. Brain 2007;130:245–255.
23. Paradiso GO, Cunic DI, Gunraj CA, Chen R. Representation of facial muscles in human motor cortex. J Physiol 2005;567:323–336.
24. Cattaneo L, Macaluso GM, Pavesi G. Inhibitory reflexes in human perioral facial muscles: a single-motor unit study. Clin Neurophysiol 2007;118:794–801.
25. Gasser RF. The development of the facial muscles in man. Am J Anat 1965;120:357–376.
26. Ross ED, Prodan CI, Monnot M. Human facial expressions are organized functionally across the upper-lower facial axis. Neuroscientist 2007;13:433–446.
27. Sherwood CC, Hof PR, Holloway RL, et al. Evolution of the brainstem orofacial motor system in primates: a comparative study of trigeminal, facial, and hypoglossal nuclei. J Hum Evol 2005;48: 45–84.
28. Happak W, Burggasser G, Liu J, et al. Anatomy and histology of the mimic muscles and the supplying facial nerve. Eur Arch Otorhinolaryngol 1994:S85–S86.