Accurate Risk Assessment for the Development of PML in Natalizumab Treated MS Patients Requires CSF Analysis

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INTRODUCTION

Progressive multifocal leukoencephalopathy (PML) is a disease of the central nervous system (CNS) characterized by multiple lesions in brain white matter. The disease is caused by JC Virus (JCV) activation due to immunosuppression or immune system altering therapies such as Natalizumab. In our laboratory we are hoping to establish assays that are able to detect JCV activation at a stage before it causes PML.

OBJECTIVE

To determine if it is possible to detect patients who are at higher risk for getting PML as a result of Natalizumab treatment by establishing methods of detecting JCV activation in the CNS using the following:

1. JCV DNA detection using highly sensitive quantitative PCR (qPCR) with a low limit of detection (LOD) to maximize the sensitivity of viral detection
2. JCV specific antibody detection in the cerebral spinal fluid (CSF) using an enzyme-linked immunosorbant assay (ELISA). This will help determine if viral activation is taking place as the antibody levels rise..

METHODS

Real Time Polymerase Chain Reaction (RT-PCR) Detection of JC Virus (JCV) DNA in CSF

Cell-free CSF samples (140 µl) were used for viral DNA extraction using the QIAamp Viral RNA Mini Kit (Qiagen Inc., Cat. 52904). The final elution is then used as the template for detection of JCV DNA described below.

RT-PCR detection of JCV DNA was established on the Applied Biosystems 7900HT Fast Real-Time PCR System. Primers and probes specific for the VP2 capsid protein of JCV and TaqMan® Gene Expression Master Mix were used. Reaction volumes were 20 µl and cycling parameters were an initial hot start hold of 10 min at 95 C. This was followed by 50 cycles of 15 seconds at 95 C and 1 minute at 60 C.

JCV MAD1 Strain Quantitated Viral DNA (Advanced Biotechnologies Inc) was used to establish a low copy range standard curve as well as a positive control. Serial dilutions were made to obtain 12, 5, 3, 2, 1, and <1 copies of JCV DNA per well. A positive control of 1 JCV DNA copy per well was prepared using serial dilutions of the stock JCV MAD1 Strain Quantitated Viral DNA in artificial CSF (aCSF) and then subjected to the same extraction kit, QIAamp Viral RNA Mini, used for the CSF samples.

ELISA detection of JCV Specific Antibodies in the CSF

High Binding microplates were coated overnight at 4 C with 0.1 µg per well of JCV major capsid VP1 protein in Phosphate Buffered Saline (PBS). The plates were then blocked with 3% non-fat dry milk in PBS-Tween (0.1% Tween-20) for one hour at Room Temperature (RT). Each plate contained a standard curve using a mouse anti-JCV VP1 antibody serially diluted to 312.5-20,000 pg/mL and CSF samples in serial dilutions of 1:2-1:32. All standards and samples were assayed in duplicates and incubated for 2hr at RT. Mouse monoclonal anti-human IgG detection antibody (1:5000) was added followed by HRP-conjugated goat anti-mouse IgG antibody (1:4000) to all wells. The plates were then developed in the dark with 0.3mg of ABTS substrate per well for 30 min at RT. The reaction was stopped with 1% SDS solution, and absorbance at 405nm was measured in a microplate reader. In all incubations a volume of 100 µl was used unless specified, and all antibodies were diluted in blocking solution. In addition, all antibody incubation steps were followed by five washes of 250 µl blocking solution.

RESULTS

JCV DNA Detection in CSF

Our JCV qPCR assay shows a consistent LOD of around 7-20 copies of JCV DNA per ml of CSF. This translates into 1-3 copies of DNA per well on the assay plate. Our positive control of known quantitated DNA amount, purified from the Qiagen kits used to extract potential DNA from CSF samples, was consistently detected from plate to plate. Out of the 182 patients tested for JCV viral DNA, not one had a detectable result.

However, a patient with a possible PML diagnosis was sent to our lab to be analyzed. Although commercial assays determined this sample to be negative, our center detected <30 copies/mL of JCV DNA in the CSF of this patient. In addition, this patient had a highly positive CSF anti-JCV antibody concentration.

JCV Antibody Detection in CSF

We have established an assay to reliably detect JCV antibody levels in the CSF. A summary of the patients tested are delineated in Table I.

Table I.

Total CSF Samples Tested	819
Total Number of Patients Tested	320
Total Number of Patients With post-treatment data (Either A Single Determinant or Multiple Determinants post-treatment)	280
Total Number of Multiple Determinant Patients	194
Total Number of Multiple Determinant Patients with Pre-Treatment samples	137
Total Number of Patients with Serum and CSF Determinants	83

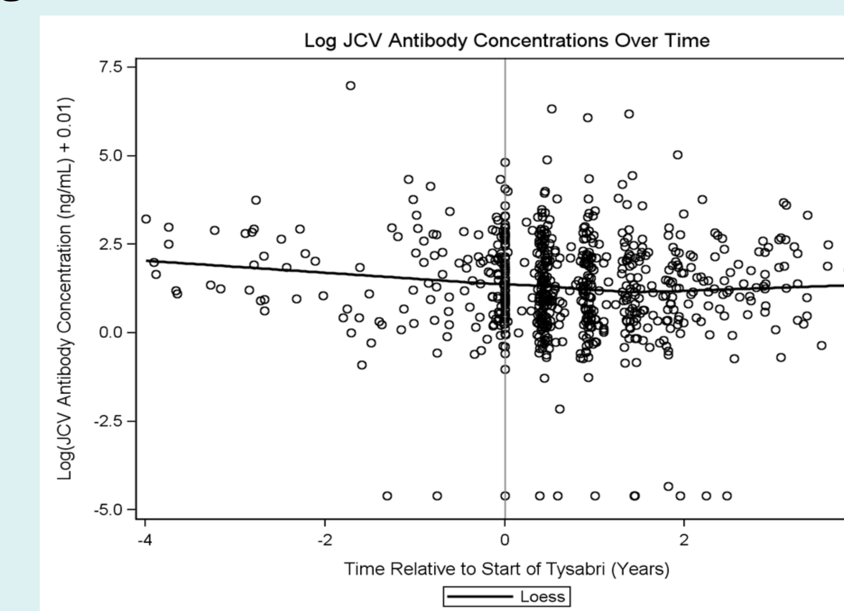
To establish CSF anti-JCV antibody parameters, we analyzed CSF anti-JCV antibody concentrations in 156 patients. All of these patients had not received natalizumab and their CSF antibody concentrations were separated into quintiles. Using these quintiles we categorized antibody concentrations as either undetectable, low, medium low, medium high, or high (Table II).

Table II.

Centile	Estimated Cut-off (ng/mL)	Boostrap 95% CI (ng/mL)	Categories
0-20th	≤1.64	1.45-2.04	Undetectable
20th-40th	≤2.85	2.50-4.09	Low
40th-60th	≤6.61	4.70-7.99	Medium Low
60th-80th	≤15.32	9.29-17.80	Medium High
80th-100th	>15.32	9.29-17.80	High

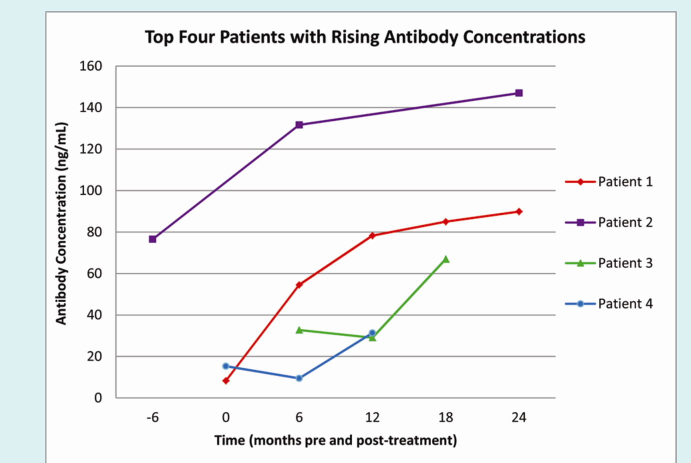
Statistical Analysis of 255 patients' JCV antibody concentrations pre and post-treatment demonstrated that the trend in antibody concentrations remained relatively stable for the general treatment population over several months. This is delineated by the LOESS model in Figure I.

Figure I.



Of the 194 patients in whom serial CSF antibody levels were obtained at approximately 6 month intervals, only 4 patients showed noticeable increases in anti-JCV levels indicating possibly early viral activation. These four patients antibody values are delineated in Figure II.

Figure II.



Serum versus CSF anti-JCV antibody values

A statistical comparison of 83 patient serum and CSF values indicated merely a moderate correlation (Spearman Rank Correlation Coefficient=0.38). In this group of 83 patients, none of the patients with detectable serum anti-JCV antibodies had absent CSF antibodies whereas 4 patients with undetectable serum anti-JCV antibodies had high baseline CSF antibodies. This is demonstrated in Table III.

Table III.

Categories	Log(Serum Normalized OD + 0.01) Quintiles	Log (CSF JCV + 0.01) Quintile Categories					
		Undetectable	Low	Medium Low	Medium High	High	
		0-20th	20th-40th	40th-60th	60th-80th	80th-100th	Total Patients
Undetectable	0-20th	9	1	1	2	4	17
Low	20th-40th	4	4	8	1	1	18
Medium Low	40th-60th	3	5	2	3	2	15
Medium High	60th-80th	1	5	4	4	3	17
High	80th-100th	0	2	1	7	6	16
	Total Patients	17	17	16	17	16	83

Furthermore, 55 of these 83 patients' serum samples were re-tested after approximately one year of therapy. It was found that two patients converted from a seronegative to a seropositive result, but most notably 3 of these patients converted from a seropositive to a seronegative result.

CONCLUSIONS

- Rising CSF antibody concentrations are only rarely associated with Natalizumab treatment, but likely suggest impending clinical disease
- Serum antibody values do not always correlate with CSF antibody values
- It is probable that the least likely patient to develop PML is a serum and CSF antibody negative patient