TITLE: JC Virus Antibody Testing for Patients with MS: A Review of the Diagnostic Accuracy and Guidelines

DATE: 14 March 2013

CONTEXT AND POLICY ISSUES

Multiple sclerosis (MS) is an inflammatory disease in which the fatty myelin sheaths around the axons of the brain and spinal cord are damaged, resulting in demyelination and a broad spectrum of symptoms. There is no cure for MS, but therapies have been developed to restore function and prevent disability. Natalizumab is a humanized monoclonal antibody against the cell adhesion molecule α4-integrin that reduces the ability of inflammatory immune cells to attach and pass through the cell layers lining the intestines and blood-brain barrier. Natalizumab has been shown to be effective at decreasing the frequency of relapses and reducing disability progression in MS patients.

Despite its efficacy in treating MS patients, natalizumab has been associated with the risk of developing progressive multifocal leukoencephalopathy (PML), a rare and often fatal disease characterized by progressive damage or inflammation of the white matter of the brain at multiple locations. PML is caused by John Cunningham virus (JCV), named after the patient from which it was originally identified. Approximately 60% to 80% of humans produce antibodies against JCV, indicating previous exposure to the virus and a latent infection. However, JCV is normally controlled by a healthy immune system and will only produce effects a host that is severely immunocompromised.

Screening for JCV by detecting its DNA in blood and urine samples does not appear to have sufficient sensitivity or correlate with PML risk. Detecting JCV antibodies in the infected host is an alternative method. The availability of anti-JCV antibody tests may allow for the identification of MS patients who may be at increased risk of developing PML with natalizumab use. The StratifyJCV test (Biogen Idec) is a two-step, virus-like particle-based enzyme-linked immunosorbent assay (ELISA) designed to detect serum anti-JCV antibodies in MS patients. Quantitative anti-JCV antibody assays have also been developed.

The purpose of this review is to assess the diagnostic accuracy of tests for JCV antibodies in patients with MS in addition to guidelines and protocols for the administration of these tests.

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RESEARCH QUESTIONS

1. What is the diagnostic accuracy of tests for JC virus antibodies in patients with multiple sclerosis?

2. What are the evidence-based guidelines and testing algorithms for JC virus antibodies in patients with MS?

KEY FINDINGS

Anti-JCV antibody assays were able to identify anti-JCV antibodies in approximately 50-60% of natalizumab-treated MS patients or MS patients considering treatment with natalizumab. The StratifyJCV test was found to have a low false negative rate when urinary JCV-DNA was used as a positive reference. No evidence-based guidelines and testing algorithms for JCV antibodies in patients with MS were identified.

METHODS

Literature Search Strategy

A limited literature search was conducted on key resources including PubMed, Ovid EMBASE, The Cochrane Library (2013, Issue 1), University of York Centre for Reviews and Dissemination (CRD) databases, Canadian and major international health technology agencies, as well as a focused Internet search. No filters were applied to limit the retrieval by study type. Where possible, retrieval was limited to the human population. The search was also limited to English language documents published between January 1, 2003 and February 12, 2013.

Selection Criteria and Methods

One reviewer screened the titles and abstracts of the retrieved publications and evaluated the full-text publications for the final article selection, according to selection criteria presented in Table 1.

Table 1: Selection Criteria

<table>
<thead>
<tr>
<th>Population</th>
<th>Patients with multiple sclerosis (MS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intervention</td>
<td>Anti-JCV antibody testing</td>
</tr>
<tr>
<td>Comparator</td>
<td>N/A</td>
</tr>
<tr>
<td>Outcomes</td>
<td>Diagnostic accuracy, evidence-based testing protocols, best practice guidelines</td>
</tr>
<tr>
<td>Study Designs</td>
<td>Health technology assessments, systematic reviews, meta-analyses, randomized controlled trials (RCTs), non-randomized studies and evidence-based guidelines</td>
</tr>
</tbody>
</table>
Exclusion Criteria

Studies were excluded if they did not meet the selection criteria, were duplicate publications, were included in a selected systematic review or were published prior to 2003.

Critical Appraisal of Individual Studies

The quality of diagnostic accuracy studies were assessed using the revised version of the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2). A numeric score was not calculated for each study. Instead, strengths and weaknesses of each study were summarized and described.

SUMMARY OF EVIDENCE

Quantity of Research Available

The literature search yielded 193 citations. Upon screening titles and abstracts, 188 citations were excluded and 5 potentially relevant articles were retrieved for full-text review. No potentially relevant reports were identified through grey literature searching. Of the 5 potentially relevant reports, one did not meet the inclusion criteria. Four publications were included in this review. The study selection process is outlined in a PRISMA flowchart (Appendix 1). Four prospective studies met inclusion criteria. No evidence-based testing protocols or guidelines were identified.

Summary of Study Characteristics

Details on study characteristics can be found in Appendix 2.

Study design and country of origin

Four prospective, observational studies were included. One study was from Germany, one study was from Italy, and two studies were from the USA.

Patient characteristics

All of the studies included MS patients who were treated or considering treatment with natalizumab. The mean age of patients ranged from 37.4 to 44.4 years in two studies, and 69 to 75.7% of the patients were female. The age and gender ratio in the other two studies were not specified. In the study by Warnke et al, 74% of the patients received 300 mg natalizumab for a median of 26.5 months. In the study by Laroni et al, all patients were treated with natalizumab for a median of 22 months. In the study by Bozic et al (STRATIFY-1), 85.3% of the patients received natalizumab for a mean of 21 infusions. In the study by Gorelik et al (STRATA), all patients received 300 mg natalizumab for 48 weeks.

Test characteristics

Three studies used the StratifyJCV test to identify anti-JCV antibodies in serum or plasma samples. StratifyJCV is a two-step assay that incorporates an initial screening and subsequent confirmation ELISA in which the presence or absence of anti-JCV antibodies in serum or plasma samples is determined spectrophotometrically at 450 nm.
In the screening step, anti-JCV antibodies are captured onto JCV-coated plates and visualized spectrophotometrically through a horseradish-peroxidase-linked secondary antibody. In the confirmation step, serum samples are pre-incubated with either serum or JCV virus-like particles (VLPs) before performing the ELISA and the amount of inhibition is calculated to determine decrease in ELISA reactivity after samples were pre-adsorbed with JCV VLPs. Predetermined cutoff values are used to distinguish whether a sample is positive or negative for anti-JCV antibodies.

One study evaluated a quantitative anti-JCV antibody assay in which sera samples were pre-adsorbed with BK virus (BKV) in order to remove anti-BKV antibodies before performing an ELISA. BKV is closely related to JCV and both viruses share cross-reactive epitopes and this may contaminate results of anti-JCV antibody tests. In the quantitative anti-JCV antibody assay, samples that fell within an “indeterminate zone” were tested using the same confirmation step as the StratifyJCV test where sera was pre-incubated with JCV VLPs. In this study, the quantitative anti-JCV antibody assay was compared with the Stratify JCV assay.

In two studies, JCV-DNA detection in urine was used as a positive reference. In one study, JCV-DNA was used as a comparator. The presence of JCV-DNA in urine was determined by a real-time quantitative polymerase chain reaction (qPCR). The limit of detection was 500 copies/mL in two studies and 166 copies/mL in one study.

**Outcomes measured**

The study by Warnke et al. evaluated the inter-assay agreement between a quantitative anti-JCV antibody assay and the StratifyJCV test. Warnke et al. also looked at the correlation in anti-JCV antibody reactivity between the quantitative anti-JCV antibody assay and the StratifyJCV test. The study by Laroni et al reported sensitivity and positivity values for the StratifyJCV test using urinary JCV-DNA as a comparator. The studies by Bozic et al. and Gorelik et al. both reported the prevalence of anti-JCV antibodies detected by the StratifyJCV test and false-negative rates using urinary JCV-DNA as a positive reference.

**Summary of Critical Appraisal**

Details on critical appraisal can be found in Appendix 3.

The assays that were used in all of the included studies were described in enough detail in the study or referenced elsewhere to be reproducible. Both serum and urine samples were collected at the same time in two studies, but collected up to 6 months apart in one study. In the study by Warnke et al., the quantitative anti-JCV antibody assay was performed on frozen sera collected from patients who had already been analyzed with the StratifyJCV test. Samples were drawn at the same time, but the length of time samples were stored before testing was not reported.

No positive reference standard was used in two studies. JCV-DNA from urine samples was used as a positive reference in two studies to calculate a false-negative rate, but the timeframe in which the reference standard was performed in relation to the StratifyJCV test was not stated.

It was unknown whether the investigators performing the tests were blinded to the result of the other tests in all of the included studies. However, the results of the anti-JCV antibody assays
was determined by spectrophotometric data and pre-set cut-off values and it is unlikely that results would be biased even if prior knowledge of the reference standard results was known.

**Summary of Findings**

Details on study findings can be found in Appendix 4.

What is the diagnostic accuracy of tests for JC virus antibodies in patients with multiple sclerosis?

Three studies evaluated the ability of the StratifyJCV test to identify anti-JCV antibodies in MS patients that were considering treatment or had undergone treatment with natalizumab. The study by Gorelik et al. detailed the development of the StratifyJCV test for the identification of patients at higher or lower risk of developing PML.9 This study used plasma and serum samples from patients in the STRATA study (Safety of TYSABRI Redosing and Treatment) and found that 53.6% of natalizumab-treated patients tested positive for anti-JCV antibodies with a false negative rate of 2.5%.9 All patients that developed PML tested positive for anti-JCV antibodies prior to diagnosis.9 Bozic et al. analyzed baseline anti-JCV antibodies in MS patients prior to any natalizumab treatment in the STRATIFY-1 study.13 Similar to the analysis conducted by Gorelik et al., 56.3% of patients tested positive for anti-JCV antibodies with a false negative rate of 2.7%, and all patients tested positive prior to the diagnosis of PML.13 Laroni et al. found that the StratifyJCV test had a higher positive test rate than urinary JCV-DNA qPCR.12

Warnke et al. compared a quantitative anti-JCV antibody assay to the StratifyJCV test and found good inter-assay agreement for anti-JCV antibody status and a strong correlation for antibody reactivity between the two assays in seropositive individuals.10 There was a slightly lower positivity in the quantitative anti-JCV antibody assay compared to the StratifyJCV test (53% vs. 62%) and a higher proportion of patients tested positive in the StratifyJCV test only. The study found that 90% of discordant samples tested positive for anti-BKV antibodies, suggesting that cross-reactivity with BKV may contribute to differences between StratifyJCV and this quantitative assay.

What are the evidence-based guidelines and testing algorithms for JC virus antibodies in patients with MS?

No evidence-based guidelines and testing algorithms for JCV antibodies in patients with MS were identified.

**Limitations**

In all but one study, the timeframe during which both the anti-JCV antibody assay and JCV-DNA urine analyses were performed was not specified. The time intervals between comparative tests may have affected the results due to deterioration of samples over time despite being frozen.

Urinary JCV-DNA was used as a positive reference standard in two studies.9,13 The use of JCV-DNA as a reference standard is concerning as only 20-30% of JCV-infected individuals shed urinary JCV-DNA.6,7 True sensitivities and false-positive rates were not calculated in any included study as there is no universally accepted method to identify all JCV-infected patients.13 Therefore, it is not possible to determine the diagnostic accuracy of anti-JCV antibody tests at the moment.
Only one included study (STRATIFY-1) analyzed JCV serostatus in patients who had not yet received natalizumab treatment, so evidence is limited to support the use of this test as a screening tool for PML risk prior to treatment.\(^{13}\) However, patients would likely be tested after natalizumab treatment begins to monitor any seroconversions.

BK virus was found to have similar epitopes to JCV, which may be responsible for cross-reactivity in anti-JCV antibody assays. However, this issue was addressed in only one study.\(^{10}\)

**CONCLUSIONS AND IMPLICATIONS FOR DECISION OR POLICY MAKING**

According to the included studies, anti-JCV antibody assays were able to detect anti-JCV antibodies in patients who went on to develop PML and exhibited low false negative rates when JCV-DNA was used as a positive reference. The StratifyJCV test was found to identify more JCV-positive patients than urinary JCV-DNA. A quantitative anti-JCV antibody assay that removed cross-reactive anti-BKV antibodies was found to have slightly lower positivity than the StratifyJCV test. Since these assays vary in definitions of lower and higher cut-off points and there is no true positive reference, it is difficult to determine whether one assay is more sensitive than the other.

In the studies that used the StratifyJCV test, the number of patients who had detectable JCV-DNA in the urine and undetectable serum anti-JCV antibodies, demonstrated a low false negative rate.\(^{9,12,13}\) The authors in Gorelik et al. suggest that the false negative results in the StratifyJCV test are unlikely to be due to varying amino acid sequences in different JCV strains or very recent infection with JCV as the test is able to detect several JCV strains equivalently and the patients with false negative results had detectable JCV-DNA up to 6 months prior to serological testing.\(^{9}\) Therefore, the false negative results may be explained by inter-individual differences in mounting an immune response to JCV infection.

A study published in 2010 found that measuring JCV DNA in blood or urine using methods available at that time was not successful at predicting PML risk in natalizumab-treated MS patients.\(^{7}\) A study published in 2012 found that screening for anti-JCV antibodies in a large German natalizumab-treated MS cohort had potential clinical utility in stratifying patients for PML risk as all patients who developed PML tested positive for anti-JCV antibodies.\(^{14}\) These studies, along with this review, suggest that anti-JCV antibody assays may be a useful tool to identify patients at risk for developing PML and who should not receive natalizumab treatment. Despite promising results, additional research is required to evaluate the clinical utility of anti-JCV antibody assays and optimize test protocols.

No evidence-based guidelines and testing algorithms for JCV antibodies in patients with MS were identified. Gorelik et al. collected and analyzed sera samples from patients over 5 years and found an annual seroconversion rate of 2%, suggesting that even seronegative patients should be tested periodically in clinical practice.\(^{9}\)
REFERENCES


APPENDIX 1: Selection of Included Studies

193 citations identified from electronic literature search and screened

188 citations excluded

5 potentially relevant articles retrieved for scrutiny (full text, if available)

0 potentially relevant reports retrieved from other sources (grey literature, hand search)

5 potentially relevant reports

1 report excluded: irrelevant intervention (1)

4 reports included in review
### APPENDIX 2: Summary of Study Characteristics

<table>
<thead>
<tr>
<th>First Author, Publication Year, Country</th>
<th>Study Design</th>
<th>Patient Characteristics</th>
<th>JCV Tests</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warnke* 2013 Germany</td>
<td>Prospective observational study</td>
<td>175 MS patients who were tested for anti-JCV antibodies using the 1st generation StratifyJCV test (129 patients received 300 mg natalizumab for median of 26.5 months, range 1-56 months) – mean age 38.7 (95% CI 37.2-40.2), 69% female</td>
<td>Quantitative anti-JCV antibody assay</td>
<td>Inter-assay agreement, antibody reactivity</td>
</tr>
<tr>
<td>Laroni* 2012 Italy</td>
<td>Prospective observational study</td>
<td>73 MS patients treated with natalizumab (median exposure 22 infusions)</td>
<td>StratifyJCV test vs. JCV-DNA urine test (qPCR) – limit of detection 166 copies/mL</td>
<td>Sensitivity, positivity</td>
</tr>
<tr>
<td>Bozic* 2011 USA</td>
<td>Prospective observational study (STRATIFY-1)</td>
<td>1096 relapsing MS patients being treated or considering treatment with natalizumab (85.3% patients received mean 21 infusions) – mean age 44.4±10.87, 75.7% female</td>
<td>StratifyJCV test</td>
<td>Prevalence of anti-JCV antibodies, false-negative rate</td>
</tr>
<tr>
<td>Gorelik* 2010 USA</td>
<td>Prospective observational study</td>
<td>831 MS patients from the STRATA study who received 300 mg natalizumab for 48 weeks</td>
<td>StratifyJCV test</td>
<td>Prevalence of anti-JCV antibodies, false-negative rate</td>
</tr>
</tbody>
</table>

CI=confidence interval; DNA=deoxyribonucleic acid; JCV=John Cunningham virus; MS=multiple sclerosis; qPCR=quantitative polymerase chain reaction
## APPENDIX 3: Summary of Critical Appraisal

<table>
<thead>
<tr>
<th>First Author, Publication Year</th>
<th>Strengths</th>
<th>Limitations</th>
</tr>
</thead>
</table>
| Warnke et al. 2013            | • The protocol used for the qJCV assay was detailed enough to reproduce | • It was unclear whether assessors were blinded to results of other test  
• qJCV test was performed on frozen sera and the length of time after the StratifyJCV test was used was not specified  
• No reference standard was used |
| Laroni et al. 2012             | • Serum and urine samples were analyzed fresh and not from frozen stores  
• The protocol for the StratifyJCV test was reproducible | • It was unclear whether assessors were blinded to results of other test  
• Both tests were performed on samples collected up to 6 months apart, which may have affected the results  
• No reference standard was used |
| Bozic et al. 2011              | • Analyzed serum and urine samples were collected at the same time  
• The protocol for the StratifyJCV test was reproducible | • It was unclear whether assessors were blinded to results of other test  
• The time between when the test and reference standard was performed was not stated  
• JCV DNA from urine was used as a reference standard, which does not guarantee 100% positivity |
| Gorelik et al. 2010            | • Analyzed serum and urine samples were collected at the same time  
• The protocol for the StratifyJCV test was reproducible  
• Assessors of the samples of PML patients were blinded to patient status | • It was unclear whether assessors were blinded to results of other test  
• The time between when the test and reference standard was performed was not stated  
• JCV DNA from urine was used as a reference standard, which does not guarantee 100% positivity |
# APPENDIX 4: Summary of Findings

<table>
<thead>
<tr>
<th>First Author, Publication Year</th>
<th>Main Study Findings</th>
<th>Authors’ Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warnke 2013</td>
<td>The quantitative anti-JCV antibody assay (qJCV) was compared to the StratifyJCV test to determine positivity for anti-JCV antibodies. Inter-assay agreement: 83%</td>
<td>“When comparing the results for the anti-JCV antibodies from the StratifyJCV® and the anti-VP1 GST capture ELISA, we noted a good agreement for the anti-JCV antibody status, and a strong correlation for antibody reactivity in positive individuals...However, a slightly lower positivity was observed with the anti-VP1 GST capture ELISA (53% vs. 62%). A higher proportion of patients tested positive in the StratifyJCV® test only (13% vs. 4%), which indicates an effect of different cut-off definitions or a systematic difference in specificity. The patient samples with discordant anti-JCV antibody status exhibited low anti-JCV antibody reactivity. As 90% of the discordant samples were positive for anti-BKV antibodies, cross-reactivity with BKV might contribute to these findings” (p. 7)</td>
</tr>
<tr>
<td>Laroni 2012</td>
<td>Positivity StratifyJCV: 52% JCV-DNA (urine): 42.5% Positive both tests: 37% Positive StratifyJCV, Negative JCV-DNA: 15% Positive JCV-DNA, Negative StratifyJCV: 5.5% Negative both tests: 42.5%</td>
<td>“The anti-JCV antibodies test confirmed its higher sensitivity (52% positive patients) compared to urinary JCV-DNA (42.5%), and, as expected due to the possibility of intermittent excretion of JCV-DNA, a number of urinary JCV-DNA negative patients had anti-JCV antibodies. However, 4/73 (5.5%) of patients had detectable JCV-DNA in urine and undetectable serum anti-JCV antibodies.” (p. 671)</td>
</tr>
<tr>
<td>Bozic 2011</td>
<td>Anti-JCV antibody detection by StratifyJCV at baseline (no natalizumab treatment) Positivity: 56.0% (95% CI 53.0-59.0) False negative rate: 2.7% (95% CI 0.9-6.2)</td>
<td>“Baseline results from STRATIFY-1 and TYGRIS-US confirm that the anti-JC virus antibody prevalence using the 2-step anti-JC virus antibody assay is approximately 50 to 60%. Furthermore, these data indicate that the prevalence of being anti-JC virus antibody positive is not affected by the presence of established risk factors for PML, prior immunosuppressant use and natalizumab treatment duration. These results, together with data at the time of this writing showing that 100% of patients with PML, in whom serum samples were available, were anti-JC virus antibody positive prior to the diagnosis of PML, suggest that anti-JC virus antibody positive status combined with established PML risk factors may serve as a useful tool for PML risk stratification.” (p. 749)</td>
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</table>
| Gorelik 2010                  | Anti-JCV antibody detection by StratifyJCV® Positivity: 53.6% (95% CI 49.9-57.3) False negative rate: 2.5% (upper 95% CI 4.4) | “Using a large, geographically diverse population of natalizumab-treated MS patients, we have found an approximate 54% seropositivity rate for anti-JCV antibodies. Pre-
<table>
<thead>
<tr>
<th>First Author, Publication Year</th>
<th>Main Study Findings</th>
<th>Authors’ Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>17/17 (100%) MS patients tested positive for anti-JCV antibodies prior to the diagnosis of PML – samples were collected 16 to 180 months prior to PML diagnosis and analyzed in a blinded fashion</td>
<td>PML samples available from 17 natalizumab-treated patients who were eventually diagnosed with PML tested seropositive in the ELISA. This rate is significantly different from the theoretically expected 9 of 17 based on the 54% seropositivity observed in our general MS population (p&lt;0.0001)&quot; (p. 300)</td>
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</tbody>
</table>

BKV=BK virus; CI=confidence interval; JCV=John Cunningham virus;