HHS Public Access

Author manuscript

*Ann Neurol*. Author manuscript; available in PMC 2015 December 01.

Published in final edited form as:

*Ann Neurol*. 2014 December ; 76(6): 789–791. doi:10.1002/ana.24302.

# Finger pointing to JC virus: a tale of two indexes

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Pointing to an object is a natural behavior in humans. This is usually performed using the second finger of the dominant hand, named *index* –from the Latin *indicare*, to indicate. By extension, the term “index” has been used to describe an indirect shortcut derived from and pointing into, a greater volume of values, data, information or knowledge.

This issue of the Annals of Neurology contains two articles describing a JC virus (JCV) antibody index (1, 2). However, despite their similar name, they are inherently quite different. Why try to detect and quantify antibody production to JCV? Indeed, JCV is a ubiquitous polyomavirus, which infects a majority of people without causing any disease. It remains quiescent in the kidneys, and can be found in the urine at any given time in approximately a third of healthy individuals. In the setting of immunosuppression, JCV can reactivate, spill into the bloodstream and reach the central nervous system. There, it can productively infect and destroy oligodendrocytes and astrocytes, leading to a demyelinating disease called progressive multifocal leukoencephalopathy (PML). In addition, JCV variants have been shown to infect cerebellar granule cells or cortical pyramidal neurons, causing JCV granule cell neuronopathy (JCV GCN) or JCV encephalopathy (JCVE) respectively (3). Finally, JCV can also infect meningeal and choroid plexus cells, causing meningitis and hydrocephalus (4).

In immunosuppressed patients with suggestive clinical and neuroradiological findings, the diagnosis of JCV-associated brain disease is usually established by brain biopsy or by detection of JCV DNA in the CSF by polymerase chain reaction (PCR) (5). Unlike other common human viruses for which serological tests have been used clinically for a long time, JCV has lagged behind. After its discovery in 1971, it was found that JC virions had the capability to agglutinate type O erythrocytes, leading to the first generation serological test, called hemagglutination inhibition test (HAI)(6). In this test, serial dilutions of plasma samples are mixed in microtiter wells together with live JC virions and type O erythrocytes, and the presence of JCV antibodies in the plasma is surmised by blockade of hemagglutination, which can be visually detected. However, JC virions needed for the assay are tedious to grow in culture, and it is therefore not astonishing that this imprecise and somewhat cumbersome assay was only used in few virology research laboratories.

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Nevertheless, sero-epidemiologic studies demonstrated that anti-JCV antibodies could be detected in teenagers, and that JCV seroprevalence increased with age, with a range of 30–90% of healthy adults, depending on the population tested (7).

It was also shown that all patients had JCV antibodies in their blood prior to the development of PML, indicating that the disease was caused by a reactivation rather than primary infection, and that the humoral immune response was unable to prevent disease onset and subsequent progression. Since most people are infected by JCV, and since the presence of anti-JCV antibodies was neither diagnostic nor prognostic of PML, development of more modern ELISA tests for JCV was restricted to few research laboratories (8–12), and there was little financial incentive for any company to license or commercialize those assays.

This situation changed, however, when natalizumab, an immunomodulatory medication for multiple sclerosis (MS) and Crohn's disease (CD), was associated with the development of PML in 2005. Natalizumab is a humanized monoclonal antibody against $\alpha 4\beta 1$ and $\alpha 4\beta 7$ integrin receptors located on white blood cells, which blocks the egress of leukocytes from the bloodstream into the CNS and gut. In doing so, this medication prevent lymphocytes bent on attacking normal components on the CNS and gut to reach their target organs. Conversely, natalizumab also prevents JCV-specific $CD4^+$ and $CD8^+$ lymphocytes from patroling the CNS, which leads to viral reactivation and resulted in the development of PML in 484 MS and 2 CD patients as of August 6, 2014(13).

How can we predict which natalizumab-treated MS patient will develop PML? Based on the premise that people who have not been exposed to JCV should not be at risk of PML, Gorelik et al. established a JCV ELISA which was used to screen all natalizumab-treated MS patients (14). Risk stratification analyses indicated that the risk of PML ranged from 1/10,000 in seronegative patients, to 1/90 in seropositive patients after 24 months of natalizumab monotherapy, if they had received prior immunosuppressive medications (15).

Building upon those findings, Plavina et al investigated whether anti-JCV serum antibody level could further define the risk of PML.

They used a second generation ELISA test, now commercially available, which includes a cutoff calibrator, consisting of pooled sera from JCV-seropositive healthy volunteers, as well as a positive and negative control. The index value for the patient sample is calculated by dividing the mean optical density (OD) value of the sample by the OD of the cutoff calibrator, to normalize results across plates. An index >0.40 denotes JCV seropositivity, and higher values indicate higher amount of antibodies in the sample. Using serum or plasma samples from 71 natalizumab-associated PML patients and 2,522 JCV-antibody positive patients without PML, including a test dataset and verification dataset, the authors showed that there was a wide overlap in index values between natalizumab-associated PML cases and non-PML JCV seropositive patients. However, they showed an increased JCV-index in PML patients with no prior immunosuppressant medication before natalizumab compared with non-PML JCV seropositive patients, while there was no difference in the

index if the PML patients had been treated with immunosuppressant drugs before natalizumab.

More specifically, of 51 PML cases with no prior immunosuppression, only one (2%) had a JCV ab index < 0.9, while 45/51 (88%) had an index >1.5. PML risk estimates were approximately 10 times higher with an index >1.5 compared to an index < 1.5.

Interestingly, patients with natalizumab-associated PML often have JC viral load in CSF below 100 copies/ml, which may be below the limit of detection of clinical laboratories. Warnke et al assessed whether concomitant measurement of JCV antibody in CSF and serum could have a complementary value in the diagnosis of PML. Using a different ELISA than the one of Plavina et al, they devised another JCV antibody index ($AI_{JCV}$), taking into account CSF and serum concentration of JCV antibody, IgG and albumin. An $AI_{JCV} > 1.5$ was considered as evidence for intra-thecal antibody synthesis. Tested on 37 patients with natalizumab-associated PML and 89 natalizumab-treated MS patients without PML, $AI_{JCV}$ >1.5 had a modest sensitivity at the time of PML diagnosis of 55%, and a specificity of 100%. In a few cases, retrospective analysis showed that $AI_{JCV}$ was elevated before JCV DNA was detectable in CSF by PCR.

What is the take home message of those two studies for natalizumab-treated MS patients and physicians caring for them?

First, these two studies illustrate that although the JCV ELISA methods are conceptually similar, using viral-like particles (VLPs) made of self-assembled JCV VP1 capsid proteins, they are technically different, and so side by side testing in serum samples may occasionally lead to discordant results (16).

Second, all JCV ELISA tests suffer from the shortcoming that JCV primary infection is clinically asymptomatic, and therefore a population of truly seronegative individuals is difficult to identify. This population is required to adequately determine the background “noise” of the assay.

Third, the JCV ELISA tests used in these two studies focus on detection of anti-JCV IgG, and therefore, acute JCV infection, that triggers an IgM response, could be missed.

Fourth, negative serological results do not necessarily mean absence of exposure to JCV. Several studies have found JCV DNA by PCR in urine or blood of JCV seronegative patients using different assays (17, 18).

Fifth, JCV primary infection may occur at any time and a negative serological result does not signify that the person is not at risk of becoming infected with this ubiquitous virus.

In practical terms, the commercially available JCV antibody index has already been widely used, even prior to the publication of the related paper, as an additional tool in PML risk stratification strategy. MS patients and physicians caring for them should remember that it is only valid in natalizumab-treated patients with no prior IS, and should balance the risks of PML with those of MS relapse when switching to different medications.

Finally, we can only hope that these two different indexes, each with an operational threshold of 1.5, will not be confused with each other. For this, we will just have to keep our fingers crossed.

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