Molecular and Cellular Probes 29 (2015) 54–62

Contents lists available at ScienceDirect

Molecular and Cellular Probes

journal homepage: www.elsevier.com/locate/ymcpr

Molecular diagnostic tests to predict the risk of progressive multifocal leukoencephalopathy in natalizumab-treated multiple sclerosis patients

Francesca Rossi [a, *], Scott D. Newsome [b], Raphael Viscidi [a]

[a] Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA
[b] Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

ARTICLE INFO

Article history:
Received 27 September 2014
Accepted 26 November 2014
Available online 5 December 2014

Keywords:
PML
JCV
Natalizumab
Multiple sclerosis

ABSTRACT

Natalizumab is a humanized monoclonal antibody against the alpha$_4$ chain of the alpha$_4$beta$_1$ and alpha$_4$beta$_7$ integrin heterodimers used with high effectiveness in the treatment of multiple sclerosis. The use of this drug can unfortunately be associated with the onset of progressive multifocal leukoencephalopathy, a possibly fatal infection of the central nervous system, caused by polyomavirus JC. To understand and quantify the risk of developing PML is important for patients who are about to start therapy with natalizumab and for patients who already are under treatment with this drug. In this review we describe and critique molecular diagnostic tests proposed in the last years to assess the risk of PML.

1. Introduction

Progressive multifocal leukoencephalopathy (PML) is a rare and often fatal neurological infection. The disease is characterized by demyelination which is typically multifocal, and can involve any location in the central nervous system. Prominent histopathological features include hyperchromatic, enlarged oligodendrocytes and enlarged bizarre astrocytes. Clinical manifestations are highly varied given the widespread distribution of the disease in the brain. Behavioral and cognitive abnormalities, visual field deficits and motor abnormalities are common, while sensory loss, seizures and headaches are less common.

PML was first described in 1958 in two patients with chronic lymphatic leukemia and one patient with Hodgkin's disease [1]. Up until the early 1980s, this infection was rare and occurred mostly in patients with hematological or solid organ malignancies, autoimmune disorders or immunodeficiency states. The incidence of PML increased dramatically during the AIDS pandemic and by the 1990s the majority of PML cases occurred in AIDS patients [2] [3]. Since the introduction of combined antiretroviral therapy (cART), the incidence of AIDS-related PML has declined. Since their introduction, monoclonal antibody biologics used for treatment of autoimmune diseases and cancers have emerged as a new cause of PML [4–6].

2. JC virus and PML

PML is caused by JC polyomavirus (JCPyV). JCPyV is a non-enveloped, small, double stranded DNA virus. Primary infection is believed to be acquired through the respiratory or gastrointestinal tract and is clinically silent or non-specific. The virus then establishes a latent or low level persistent infection. The best documented site of persistent infection is uroepithelial cells. Asymptomatic viral shedding in the urine is common in healthy individuals [7]. Other sites where the virus may persist include bone marrow, tonsils and spleen. Whether the virus is latent or persistent in the brain is controversial. Because primary infections are generally asymptomatic or associated with non-specific signs and symptoms [8], exposure to JCPyV has been defined serologically. Serological studies indicate that infections occur in childhood and early adolescence and may continue to occur throughout adult life. Seroprevalence is approximately 10% in children less than 10 years of age, rises rapidly during late childhood and adolescence to an average of 50–60% and then continues to rise slowly to a peak of 70–80% in persons over 70 years of age [9,10]. The rise in

* Corresponding author. Present address: Department of Public Health Science and Infectious Diseases "Sapienza" University of Rome, Piazzale Aldo Moro 5, 00185, Rome, Italy. Tel.: +39 3891017492.
E-mail address: francesca12.rossi@uniroma1.it (F. Rossi).

http://dx.doi.org/10.1016/j.mcp.2014.11.007
0890-8508/

seroprevalence with age may be due to incident infections or an increasing titer of antibody resulting in more individuals being classified as seropositive. The pathogenesis of PML is not well understood. Since JCPyV infection is common and PML is rare, multiple factors must conspire to lead to the disease. Chief among these are: loss of immune surveillance leading to reactivation, entry of the virus into the brain, and the evolution of neurotropic variants [11].

3. PML and monoclonal antibody therapeutics

The drug most commonly associated with an increased risk of PML is natalizumab (TYSABRI®). Natalizumab is a humanized monoclonal antibody directed against the $alpha_4$ chain of the $alpha_4beta_1$ and $alpha_4beta_7$ integrin heterodimers [12].

The antibody blocks the binding of the $alpha_4$ chain to vascular cell adhesion protein 1 (VCAM1), thus inhibiting the adhesion and penetration of lymphocytes into tissues. In multiple sclerosis (MS) patients, the drug prevents lymphocytes from entering the brain. Natalizumab is highly effective in the treatment of MS, resulting in a 68% decrease in relapses and significantly reducing MRI measures of disease activity [13]. The drug was approved by the US Food and Drug Administration (FDA) in November 2004 for treatment of relapsing forms of MS. However, in 2005 the drug was withdrawn from the market when reports appeared of two natalizumab treated MS patients who developed PML. In the summer of 2006, the drug was reintroduced into the market and a global risk management program was established [5]. As of August 5 2014, there have been 486 confirmed cases of PML worldwide among approximately 129,100 patients treated with natalizumab. Based on these numbers, the overall risk of PML is estimated to be 3.69 per 1000 patient (95% confidence interval: 3.37 to 4.0 per 1000 patients (TYSABRI® (natalizumab): PML Incidence in Patients Receiving TYSABRI® (natalizumab) BIOGEN [14].

Efalizumab is an anti-CD11a antibody used for treatment of psoriasis. The drug inhibits T-cell activation and blocks trafficking of T cells into skin. It was removed from the market in 2009 following diagnosis of PML in four patients [15]. Rituximab is an anti-CD20 antibody directed toward B lymphocytes and is primarily used to treat lymphoproliferative disorders. Fifty-seven cases of PML associated with rituximab therapy have been reported, 52 with hematological malignancies and 5 with autoimmune diseases. Because the diseases which are treated with rituximab are also associated with development of PML, the causative role of the drug in inducing PML is not clearly established [16]. At least four cases of PML have been described in patients treated with alemtuzumab, a humanized monoclonal antibody against CD52 used in hematological malignancies and post-transplant rejection therapy [17,18]. Alemtuzumab has recently been approved in Europe for the treatment of MS. Sporadic PML cases have been described during therapy with infliximab for rheumatoid arthritis [19]. Dimethyl fumarate has also been rarely associated to PML in psoriasis patients [20]. [21,22]. Dimethyl fumarate hase been approved for the treatment of MS in USA (2013) and Europe (2014). The FDA has also issued warnings for mycophenolate mofetil and brentuximab vedotin, and the risk of PML must be considered for any therapy that alters immune function [15,23,24].

4. Clinical risk factors for PML in natalizumab treated patients

Two clinical factors have been associated with an increased risk of developing PML in patients receiving natalizumab: prior exposure to immunosuppressive medications, and duration of natalizumab treatment. Among patients receiving 1–24 infusions of natalizumab the risk of developing PML is low (0.7 per 1000 patients and 1.8 per 1000 with prior use of immunosuppressive drugs). The risk increases to 5.3 per 1000 (11.2 with prior use of immunosuppressive drugs) for those patients receiving 25–48 infusions and the peak incidence of PML is among patients receiving 49–72 infusions (6.1 per 1000). The use of immunosuppressive drugs is more common among patients who subsequently develop PML. A total of 34.5% of natalizumab-treated patients with PML, as compared to 20.3% of all natalizumab-treated patients in the Tysabri Global Observational Program in Safety trial (TYGRIS) had received one or more immunosuppressive drugs [5,14,25–28].

The third necessary factor for developing PML is infection with JCPyV.

In the next paragraphs we will analyze the molecular diagnostic tests developed to test for JCPyV infection in patients receiving natalizumab and to predict the risk of developing PML.

5. Molecular diagnostic tests to assess the risk of PML in natalizumab-treated patients

5.1. JC virus serology

5.1.1. 1st generation STRATIFY JC virus™ assay

The first commercial assay to detect JCPyV antibodies in natalizumab-treated patients became available in 2010 [29]. Scientists at Biogen Idec developed an ELISA test for JCPyV capsid antibodies (STRATIFY JC virus™ assay). The assay utilizes baculovirus-expressed JCPyV virus-like particles (VLPs) that contain the VP1 capsid protein from a reference neurotropic strain of JCPyV (the MAD-1 strain). The test is configured as a 2-step assay: an ELISA and a supplemental confirmation test. In the screening ELISA, JC VLPs are immobilized onto a microtiter plate. Serum samples are added to the plate and after incubation and washing, donkey anti-human IgG conjugated with horseradish peroxidase is added. The conjugate reacts not only with IgG, but also with IgA, IgM, IgD and IgE. Results of the test are reported as a normalized OD, obtained by dividing the mean OD of the sample by the mean OD of a positive control. Normalized OD values, also referred to as index values, values lower than 0.10 are scored as negative and values greater than 0.25 are scored as positive. Samples yielding index values between these two numbers are tested in the confirmatory step. In this step of the assay, samples are pre-incubated with or without soluble JCPyV VLPs to pre-adsorb antibodies prior to re-testing samples in the ELISA. Results are calculated as percent inhibition of signal by samples pre-incubated with JC VLP compared with the signal from samples pre-incubated with assay buffer. Inhibition of greater than 40% is considered specific.

In validation studies of the STRATIFY JC virus™ assay, the seropositivity rate was 97.5% among subjects secreting JC virus in the urine, with 5 of 204 virus secretors seronegative, yielding a false negative rate of 2.5% [29]. The intra-assay precision of the assay is in the range of ~3–6% and the inter-assay precision falls between ~7 and 10%. Analytical sensitivity of the assay is between 1.6 ug/ml and 3.1 ug/ml of purified polyclonal anti-JCPyV antisera. Analytical specificity of the assay was determined by evaluating its ability to discriminate between antibodies directed to JC virus and antibodies directed to the closely related human polyomavirus, BK virus (BKPyV). In the confirmatory test, only a small proportion of samples (2.8%, 5 out of 176) demonstrated inhibition by soluble BKPyV VLPs of greater than 40%. The robustness of the assay has been demonstrated by a high concordance in serostatus (88–98%) and significant correlation between normalized OD values (r values 0.91–0.97) in inter-laboratory comparisons.