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Department of Pathology

To: Editor, World J. Virology

October 5, 2015

Dear Editor for World J. Virology;

Thank you for the invitation and rapid review of our MS #22039, "Neuropathology of JC virus in Remission".

We have made all the changes recommended by the reviewers, including a slight change to the title, which is now "Neuropathology of JC Virus in Progressive Multifocal Leukoencephalopathy in Remission".

We have added a new Figure 1 in response to Reviewer 1. We have included more detail about our methods, added citations, improved the description of the staining patterns, and revised the conclusions in response to Reviewer 2.

Please note that we have inserted .jpg images into the Word.doc version for ease of review. We also uploaded the two image files for each figure as higher resolution .TIFF format for publication per the instructions.

Our specific changes are detailed below:

Reply to Reviewers

Reviewer 1:

"excellent article, informative and to the point, could you include a radiograph (or two? —at diagnosis and prior to death, if possible)"

REPLY: Thank you for the suggestion to include imaging studies. We have retrieved all that were in our archives and now include two images (**new Figure 1**).

The initial imaging study from 1999 was no longer available to us, and no imaging was done at the time of death. However, the two studies that we were able to retrieve demonstrate no progression. We also added text to Methods and Results.

Reviewer 2:

1. Authors stated in the discussion (the first paragraph-lane 8-10) that “polyomaviruses integrate into the host DNA”. Polyomaviruses mostly stays episomal and do not commonly integrate with host chromosomes. Unlike retroviruses, polyomaviruses do not carry or encode integrase.

REPLY: The reviewer is correct, polyomaviruses integrate only rarely, although this has not been studied thoroughly. Our point was that, whether integrated or not, viral antigens may be produced without the production of infective particles. The absence of inflammation or spread of viral-induced lesions argues that staining for antigens does not necessarily indicate viral replication.

See first paragraph of Discussion for text changes and additional references regarding the integration of viral DNA into the host genome. It is not necessary to encode an integrase for a virus to integrate, and polyoma viruses, including JC, have been found to be integrated into host genome.

2. Unlike the conclusion made by authors, the postmortem histological evaluation of cerebellar lesions points demyelination, viral antigen (T-antigen) expression, enlarged bizarre astrocytes, and p53 expression that all indicates JCV reactivation and PML lesions.

REPLY: In the MS, we stated that the cerebellar lesion at post-mortem demonstrates demyelination. Evidence for active infection is absent. There are very few macrophages or other signs of inflammation, T-antigen staining is weak or absent, and only a few cells stain for p53. This is very different from the original biopsy. These are signs of a burnt-out inactive lesion. Scattered rare cells suggest a low level of viral protein presence, whether residual or newly synthesized cannot be determined. Lack of progression clinically and absence of new lesions pathologically rules out active infection. There are no “PML lesions” only the one lesion which is a scar from a previous infection.

3. Authors indicate that the T-antigen staining in Fig. 2E and p53 staining in Fig. 2F are nonspecific. It is important to have a non-PML cerebellar section as a control in parallel to make this conclusion. Additionally if examined carefully, the panel E in Fig.2 also shows nuclear staining of some cells for T-antigen.

REPLY: Of course we routinely perform both positive and negative controls for all antibody staining. We now state this in the Methods and also refer to this in the Results. See pg 5 para 2 of Methods, pg 8 para 2 of Results, and pg 10 para 2 of the discussion.

Additionally, the SV40 T-antigen antibody used in the study can recognize all the early gene products of polyomaviruses including small t antigen and T' proteins. Some of these proteins including small t antigen exclusively localizes to the cytoplasm. Therefore, the observed cytoplasmic staining does not exclude that these cells are not infected

REPLY: The reviewer is incorrect about the antibody—perhaps we were not clear in our methods section. We now describe the antibody in more detail (see Methods section pg 5 para 2). This antibody does not recognize small T antigen.

We purposefully showed cells exemplifying the weak staining for T antigen and for P53 in the histological images of the post-mortem in **Figure 3** (previous Figure 2). There may indeed be some low level of antigen expression and residual astrocyte activation. However, the lesion is not progressive and differs markedly from the biopsy with respect to the number of cells stained, and the intensity of the stain. The low level of background staining, which we termed “non-specific,” is present in control uninfected tissue and also in un-involved areas of the cerebellum in this patient to a similar degree as in the involved area. Hence this speckled cytoplasmic staining is not specific for virus and may not indicate presence of viral antigens (see new text pg 8 paras 2 &3)

It is important to distinguish expression of viral antigens, and productive active infection, which produces viral particles that may propagate the infection. In this case, the equivocal evidence for viral antigens p in the post-mortem specimens may indicate latent virus. The absence of new lesions argues that infective particles are not being produced in sufficient numbers to be termed an active infection, or viral reactivation. Reactivation of latent virus is a fascinating topic beyond the purview of this case report. We added a paragraph to the discussion to clarify this, pg 11 para 2.

4. In order to make the case and conclusions based on JCV remission, the postmortem CSF viral loads with strain sequencing (if possible) should be analyzed.

REPLY: The reviewer points out another interesting issue, and we have changed the title of the MS in response, and made other text changes throughout. This is a case of **PML** in remission. Our use of the term remission is incorrect for latent JC virus.

As to the viral loads and sequencing: Unfortunately due to the quiescence of the neurological symptoms, no CSF immediately prior to or at autopsy was obtained. The biopsy specimen is too small to permit harvest and sequencing of DNA, even if there were funding to perform those expensive analyses.

Minor comments: The antibody “middle T antigen” in several places (including figure legends) in manuscript must be corrected with T-antigen and stayed consisted. The antibody used recognizes all the early products of JCV T antigens.

REPLY: We have corrected the terminology of the T antibody throughout.

The reviewer is incorrect about the specificity of this antibody. We now describe it in more detail in the Methods section.

Thank you for the very helpful comments. We are please that our MS has been accepted for publication at WJV.

Sincerely,



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