Case Report

Cytology of progressive multifocal leukoencephalopathy revisited: A case report with special reference to JC polyomavirus-infected oligodendrocytes and astrocytes

Mieko Doi1 | Keisuke Ishizawa1,2 | Kei Ikeda2 | Kazuo Nakamichi3 | Yoshihiko Nakazato2 | Toshimasa Yamamoto2 | Atsushi Sasaki1

1Division of Diagnostic Pathology, Saitama Medical University Hospital, Saitama, Japan
2Department of Neurology, Saitama Medical University, Saitama, Japan
3Department of Virology 1, National Institute of Infectious Diseases, Shinjuku-ku, Japan

Correspondence
Keisuke Ishizawa, Division of Diagnostic Pathology & Department of Neurology, Saitama Medical University Hospital, Morohongo 38, Moroyama-town, Irumagun, Saitama 350-0495, Japan.
Email: ishizawa@saitama-med.ac.jp

1 | INTRODUCTION

Progressive multifocal leukoencephalopathy (PML) is a central nervous system demyelinating disease which primarily affects immunocompromised individuals, including those with human immunodeficiency virus (HIV) infection, rheumatological diseases, and hematological malignancies.1,2 The disease is caused by JC polyomavirus (JCV), and oligodendrogial intranuclear inclusions – which are identified by the presence of enlarged, hyperchromatic, “ground-glass” nuclei – are the pathological hallmark of the disease.1,3-8 Recently, due to the advent of immunosuppressants and molecularly targeted drugs, it is not uncommon to encounter PML in routine diagnostic practice.2

In addition to histology, cytology was also of great help in reaching the diagnosis of PML in this patient. The cytological profiles are described here, with special reference to JCV-infected oligodendrocytes and astrocytes, and the role of cytology in the diagnosis of PML is discussed.

2 | CASE PRESENTATION

The patient was a female in her seventies. She was diagnosed as having autoimmune hemolytic anemia, and oral administration of prednisolone was initiated. She had been taking it for about 1 year, when, at the dose of 9 mg day−1, she noticed muscle weakness in her left upper limb. This symptom became worse, and she was admitted to our institution. On neurological examination, she showed left hemiparesis (4/5 on manual muscle testing). Deep tendon reflexes were not exaggerated, and pathological reflexes were not present. A series of blood investigations, including blood cell count, blood chemistry, and serum autoantibodies, were unremarkable, and the serum anti-HIV antibodies were negative. Brain magnetic resonance imaging (MRI) disclosed irregularly shaped, patchy areas confined to the deep white matter of the right frontoparietal lobe without mass effect, which showed high intensity on T2-weighted and fluid attenuated inversion recovery images and low intensity on T1-weighted images. These areas showed high intensity on both diffusion weighted images and an apparent diffusion coefficient map. Gadolinium (Gd)-enhancement was negative. These MRI findings suggested that leukoencephalopathy was the most likely diagnosis, although differentiation from infiltrating gliomas and multiple sclerosis (MS) was required. A stereotactic brain biopsy targeting the right parietal white matter was performed.

The intra-operative touch imprint and squash smear materials were stained with hematoxylin & eosin (H&E) and Papanicolaou (Pap) for cytological evaluation. The remaining materials were embedded in paraffin, and the sections were stained with H&E and...
Klüver-Barrera (KB), and immunostained for neurofilament protein (NFP) (mouse monoclonal, 2F11; 1:100; Dako), Iba-1 (rabbit polyclonal; 1:1000; FUJIFILM Wako Pure Chemical Corporation), p53 (mouse monoclonal, DO-7; 1:100; Dako), and SV401,7 (mouse monoclonal, PAb416; 1:100; Calbiochem) using heat-treated antigen retrieval pretreatment. The immunostained sections were visualised with diaminobenzidine.

On squash smear specimens, many cells were sampled. On H&E, many cellular aggregates composed of variable cells were noted (Figure 1A). In these, admixed with macrophages and reactive/gemistocytic astrocytes, another type of cell with enlarged, hyperchromatic, and ground-glass nuclei was observed. Most of these cells harbored round/oval, almost naked nuclei and scant cytoplasm (Figure 1B,C), morphologically suggesting oligodendrocytes; however, a fraction of them harbored irregular, peripherally located nuclei and plump, eosinophilic cytoplasm (Figure 1D,E), morphologically suggesting astrocytes (Figure 1D,E). The precise identity of large and multinucleated astrocytic cells, which were thus considered to be “Creutzfeldt cells,” was not confirmed. Many cellular aggregates were also noted on Pap. Although the nuclei and cytoplasm were less clearly visible than on H&E, many cells with enlarged, hyperchromatic, and ground-glass nuclei, which were reminiscent of those on H&E, were noted (Figure 1F). On touch imprint specimens only a few cells were present, and these specimens were therefore of little use for cytological evaluation.

On histology, the lesion showed marked infiltration of macrophages and astrocytes, and perivascular lymphoid cuffing (Figure 2A). A severe loss of myelin was observed (Figure 2B), while axons were well preserved (Figure 2C, NFP (2F11) immunostain), suggesting a demyelinating disease. On Iba-1-immunostained sections, the infiltration of macrophages was remarkable (Figure 2D). A closer look at the specimens disclosed enlarged, hyperchromatic, and ground-glass nuclei that were similar to those observed on cytology (Figure 2E,G). Most of these nuclei were round/oval and almost naked, with scant cytoplasm (Figure 2E), and likely correspond to the cells pictured in Figure 1B,C. Some of these cells were immunoreactive for SV40 (Figure 2F, SV40 (PAb416) immunostain); however, others were observed that were morphologically irregular and peripherally located, with plump, eosinophilic cytoplasm (Figure 2G), likely corresponding to the cells pictured in Figure 1D,E. A number of these cells were also immunoreactive for SV40 (Figure 2H, SV40 (PAb416) immunostain). Interestingly, not only the enlarged nuclei but also the small ones were immunoreactive for SV40 (Figure 2F), as has previously been reported elsewhere. On p53-immunostained sections, a number of p53-immunoreactive nuclei, which were either enlarged or small, were noted (Figure 2I, p53 (DO-7) immunostain). In general, the morphological details of nuclei were less clearly discernible on histology than on cytology.

The lumbar puncture, which had been performed some time before the brain biopsy, was found to be positive for variant JCV (3959 copies ml\(^{-1}\), prototype) in the cerebrospinal fluid (CSF) of the patient. After the introduction of combined mefloquine and mirtazapine therapy, the progression of the left hemiparesis stopped. About 1 year after admission, the titer of variant JCV in the CSF decreased to 978 copies ml\(^{-1}\), and the patient remained symptomatically unchanged.

![Figure 1](image-url)  
**Figure 1** Cytological findings on squash smear specimens. (A) A cellular aggregate composed of variable cells (hematoxylin & eosin (H&E)). (B, C) Cells with enlarged, round/oval, and ground-glass nuclei, appearing almost naked with scant cytoplasm. (arrows, H&E). (D, E) Cells with peripherally located, irregular nuclei and relatively plump, eosinophilic cytoplasm. (arrows, H&E). (F) An enlarged, ground-glass nucleus (in the center; Papanicolaou). Magnification: (A) ×100, (B, D) ×200, (C, E) ×400, (F) ×600.
DISCUSSION

Thus far, there have been several reports on the cytology of PML. A paper by Yu et al.\textsuperscript{5} reported the cytomorphology of PML in a series of 16 patients infected with HIV. This paper analyzed cytological parameters such as JCV-infected oligodendrocytes, nuclear atypia of reactive astrocytes, and others (cellularity, etc). The JCV-infected oligodendrocytes were characterised by enlarged nuclei with homogenous, smudged chromatin, imparting a "glassy" appearance, and little or no cytoplasm. By contrast, the cells with eccentrically placed nuclei, abundant fibrillary cytoplasm, and mild to moderate nuclear atypia, but without observations of chromatin smudging or intranuclear inclusions, were classified as reactive astrocytes. However, in a previous paper detailing the cytology from two cases of PML,\textsuperscript{3} a population of atypical cells with abundant dense eosinophilic cytoplasm forming multiple cytoplasmic processes was described; the nuclei of these were enlarged, hyperchromatic, multinuclear, and smudged, lacking discrete chromatin structures. This population of atypical cells could be JCV-infected astrocytes, but the authors did not mention whether or not they were. In our investigations of the literature relating to atypical astrocytes, those that have been documented on cytology of PML have been placed in the category of "reactive astrocytes."\textsuperscript{5,6,10} However, as was seen in the present case, JCV-infected astrocytes, whose nuclei are reminiscent of...
those of JCV-infected oligodendrocytes, do exist in PML. It has been previously reported elsewhere that astrocytes can be infected with JCV.11,12 Although JCV-infected astrocytes may account for a small proportion of JCV-infected cells in PML, relative to JCV-infected oligodendrocytes, the recognition of JCV-infected astrocytes likely enhances the diagnostic accuracy of PML. Moreover, although both JCV-infected astrocytes and JCV-infected oligodendrocytes are recognizable on cytology and histology alike, the cytology could provide better samples for the recognition of the characteristic nuclei.

The recognition of JCV-infected astrocytes is also important for differentiating PML from infiltrating astrocytomas. In the present study, many atypical cells, some of which were conspicuously astrocytic, were noted on both cytology and histology, potentially masquerading as infiltrating astrocytomas. This feature was all the more confusing due to the presence of p53-immunoreactive nuclei in both JCV-infected oligodendrocytes harboring ground-glass nuclei as well as marked infiltration of macrophages,1,5-7 can lead to a possible/probable intraoperative diagnosis of PML. On the other hand, nuclear pleomorphism, mitotic figures, glomeruloid vascular proliferation (endothelial proliferation), necrotic debris, and a relative paucity of macrophages compared to PML, are cytological features that favour the intraoperative diagnosis of infiltrating astrocytomas.6,9 In an immunohistochemical context, it should be borne in mind that PML, despite its non-neoplastic nature, is associated with nuclear p53-immunoreactivity7,14, as are infiltrating astrocytomas.13 This knowledge of p53-immunoreactivity on immunohistochemistry in PML could prevent the misdiagnosis of infiltrating astrocytomas and strengthen the diagnosis of PML. Recently, the identification of a number of immunohistochemical markers, including not only p53 but also mutated isocitrate dehydrogenase 1 (IDH1R132H) and alpha-thalassemia X-linked intellectual disability (ATRX), and molecular markers including epidermal growth factor receptor (EGFR), have been shown to be an indispensable tool for the diagnosis of infiltrating astrocytomas15; this immunohistochemical and molecular testing will be of great help in the differential diagnosis of PML vs infiltrating astrocytomas.

The differential diagnosis of PML vs other demyelinating diseases, most notably MS, is also worthy of discussion here. Clinically, MS usually presents in young (up to middle-aged) immunocompetent persons, about two thirds of whom are women.15 The variable neurological symptoms and signs of MS are typically disseminated in time and space.15 Neuroradiologically, the MS lesions, which are ovoid-shaped and spatially multiple, are often located periventricularly or, to a lesser extent, subcortically,8,15 and the “open ring sign” on Gd-enhanced MRI is considered to be fairly specific to demyelinating diseases, including MS.16 These clinical and neuroradiological features of MS are considerably different from those of PML. Moreover, in a cytological context, many macrophages and reactive astrocytes, the latter of which are sometimes large and multinucleated (“Creutzfeldt cells”), are noted in PML and MS alike.8,9 potentially misleading the differential diagnosis of PML vs MS; however, the ground-glass nuclei seen on cytology in PML are altogether absent in MS. These cytological features, along with the differing clinical and neuroradiological features, are helpful for the differential diagnosis of PML vs MS. It should be borne in mind, however, that because of the introduction of molecularly targeted drugs for the treatment of MS, the number of cases of PML overlying preexistent MS is on the rise.2

The clinical presentation in immunocompromised individuals,1,2 white matter lesions with little or no enhancement/mass effect on neuroimaging studies,7,8,17 detection of variant JCV in the CSF, cytological and histological recognition of ground-glass nuclei in both JCV-infected oligodendrocytes and JCV-infected astrocytes, and immunohistochemical detection, in particular, of SV40-immunoreactivity or even p53-immunoreactivity, strongly suggest the diagnosis of PML. The present study, in conjunction with previous reports,5-6 confirms the usefulness of cytology for the diagnosis of PML. It also suggests the importance of cytological and histological recognition not only of JCV-infected oligodendrocytes but also of JCV-infected astrocytes in the diagnosis of PML.

ACKNOWLEDGMENTS
The authors thank Mr. Toshinori Nagai for his excellent technical assistance.

CONFLICT OF INTEREST
The authors have no conflict of interest to declare.

AUTHOR CONTRIBUTIONS
M.D., K.I. (the second author), and A.S. evaluated the cytological and histological profiles of the specimens, made the pathological diagnosis of PML, and drafted the manuscript. K.I. (the third author), Y.N., and T.Y. took care of the patient in the clinical setting and made a great contribution to the diagnosis and treatment of the patient. K.N. analyzed the cerebrospinal fluid of the patient and disclosed the presence of variant JCV, contributing greatly to the definite diagnosis of PML.

CONSENT
Brain biopsy was performed as a routine diagnostic test with the written informed consent of the patient. This study was conducted according to the regulations of the Institutional Review Board (IRB) of Saitama Medical University Hospital.

DATA AVAILABILITY STATEMENT
No additional data are available since this is a case report of a single case.

ORCID
Keisuke Ishizawa https://orcid.org/0000-0003-4751-7691
REFERENCES

1. Gyure KA. Chapter 7 infections. In: Prayson RA, ed. Neuropathology. Elsevier Churchill Livingstone; 2005:287-338.
2. Cortese I, Reich DS, Nath A. Multifocal leukoencephalopathy and the spectrum of JC virus-related disease. Nat Rev Neurol. 2021;17:37-51.
3. Suhrland MJ, Koslow M, Perchick A, et al. Cytologic findings in progressive multifocal leukoencephalopathy. Report of two cases. Acta Cytol. 1997;41:481-486.
4. Cajulis RS, Hayden R, Frias-Hidvegi D, Brody BA, Yu GH, Levy R. Role of cytology in the intraoperative diagnosis of HIV-positive patients undergoing stereotactic brain biopsy. Acta Cytol. 1998;42:907-912.
5. Yu GH, Hidvegi DF, Cajulis RS, Brody BA, Levy RM. Cytomorphology of progressive multifocal leukoencephalopathy (PML): review of sixteen cases occurring in HIV-positive patients. Diagn Cytopathol. 1996;14:4-9.
6. Raisanen J, Goodman HS, Ghougassian DF, Harper CG. Role of cytology in the intraoperative diagnosis of central demyelinating disease. Acta Cytol. 1998;42:907-912.
7. Keith J, Pirouzmand F, Diamandis P, Ghorab Z. Intraoperative cytdiagnosis of progressive multifocal leucoencephalopathy. Cytopathology. 2014;25:59-61.
8. Burger PC. Section 19: Reactive and inflammatory lesions. In: Burger PC, ed. Smears and Frozen Sections in Surgical Neuropathology. PB Medical Publishing; 2009:599-612.
9. Burger PC. Section 4: Infiltrating gliomas. In: Burger PC, ed. Smears and Frozen Sections in Surgical Neuropathology. PB Medical Publishing; 2009:161-232.
10. Amberson JB, DiCarlo EF, Metroka CE, Koizumi JH, Mouradian JA. Diagnostic pathology in the acquired immunodeficiency syndrome. Surgical pathology and cytology experience with 67 patients. South Med J. 1993;86:1381-1384.
11. Wüthrich C, Batson S, Anderson MP, White LR, Koralink J. JC virus infects neurons and glial cells in the hippocampus. J Neuropath Exp Neurol. 2016;75:712-717.
12. Mázló M, Tariska I. Are astrocytes infected in progressive multifocal leukoencephalopathy (PML)? Acta Neuropathol. 1982;64:45-51.
13. Chapter 1 Diffuse astrocytic and oligodendrocytic tumours. In: Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, eds, WHO Classification of Tumours of the Central Nervous System. IARC; 2016:15-78.
14. Lammie GA, Beckett A, Courtney R, Scaravilli F. An immunohistochemical study of p53 and proliferating cell nuclear antigen expression in progressive multifocal leukoencephalopathy. Acta Neuropathol. 1994;88:465-471.
15. Kleinschmidt-DeMasters BK, Simon JH. Chapter 5 Dysmyelinating and demyelinating disorders. In: Prayson RA, ed. Neuropathology. Elsevier Churchill Livingstone; 2005:181-222.
16. Nappe TM, Niehaus MT, Goyke TE. Open ring sign diagnostic of multiple sclerosis in the emergency department. West J Emerg Med. 2015;16:579-580.
17. Thurnher MM, Thurnher SA, Mühlbauer B, et al. Progressive multifocal leucoencephalopathy in AIDS: initial and follow-up CT and MRI. Neuroradiology. 1997;39:611-618.

How to cite this article: Doi M, Ishizawa K, Ikeda K, et al. Cytology of progressive multifocal leukoencephalopathy revisited: A case report with special reference to JC polyomavirus-infected oligodendrocytes and astrocytes. Cytopathology. 2021;32:831–835. https://doi.org/10.1111/cyt.13042