

# Next-generation Sequencing with cerebrospinal fluid for the diagnosis of progressive multifocal leukoencephalopathy in HIV-infected patients

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## Research article

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## Abstract

**Background** To investigate the clinical manifestations, imaging findings, characteristics of cerebrospinal fluid, treatment and prognosis in patients with AIDS-related progressive white matter lesions diagnosed by second-generation sequencing with cerebrospinal fluid.

**Methods** Patients with clinically suspected PML were diagnosed by second-generation sequencing with cerebrospinal fluid, and the clinical data of these patients were **retrospectively** reviewed.

**Results** Ten patients with AIDS-related PML were diagnosed, including eight males (80%). The average age was 38.7±8.2 years, and the median CD4+ T cell count was 46×10<sup>6</sup>/L. The median **HIV** RNA was 5.99 × 10<sup>4</sup> copies/ml. Main clinical manifestations included: dyskinesia in 4 patients (40%) and dizziness in 3(30%), cognitive decline in 2(20%), and speech disorder in 1(10%). Three patients (30%) developed convulsions throughout the course of the disease. Image findings involved lesions cerebellum in 3 cases (30%), and lesions above the cerebellum in 7 cases (70%). Mild increase of total protein was observed in the cerebrospinal fluid of 4 cases (40%), while white blood cell count, sugar and chloride were normal in all of the cases. The copies of JCV sequence in the cerebrospinal fluid ranged from 3-12531 reads (median=67 reads). All the patients received antiviral therapy including integrase inhibitors. Among the 10 patients, 2 died in hospital and 8 survived, 2 of them have survived for more than 1 year. Symptoms were improved in 5 patients and unchanged in 3 patients.

**Conclusions** PML is common in patients with **HIV**/AIDS. It is reliable to diagnose the disease through clinical manifestations, routine detection of cerebrospinal fluid, imaging and cerebrospinal fluid NGS sequencing. Strong and effective antiretroviral treatment may improve the prognosis of patients.

## Background

Progressive multifocal leukoencephalopathy (PML) is a rare demyelinating disease of the central nervous system (CNS) caused by reactivation of the JC polyomavirus (JCV) which occurs almost exclusively in immunocompromised patients. In the pre-antiretroviral therapy (ART) era, 3-7% of human immunodeficiency virus (HIV)-infected patients developed PML[1-3]. With the widespread use of ART, the incidence and mortality of PML have declined significantly[4-8]. PML typically manifested as altered mental status, motor deficits, ataxia, and visual symptoms and is progressive and usually fatal. Currently, no specific treatment is available, but restoration of immune function with ART may lead to disease remission or improved survival.

More importantly, diagnosis of PML in HIV-infected patient is still challenging due to the complicated clinical characteristics in late stage of diseases, various imaging manifestations and the sensitivity of JCV DNA detection in cerebrospinal fluid (CSF)[9, 10]. Next-generation sequencing technology has been successfully used in detecting pathogens in CSF[11-13]. Here, we reported a series of cases diagnosis of PML through detecting the JC virus in CSF using NGS in clinically suspected patients.

## Methods

HIV-infected patients was suspected of PML by clinical manifestations, typical cranial imaging features and examination of CSF which rule out common CNS infections (e.g., cryptococcal meningitis, tuberculosis meningitis and toxoplasmic encephalitis). CSF of suspected patients was sent for NGS.

### *CD4<sup>+</sup> T cell count and HIV viral load*

CD4 T cell count and HIV viral load were quantified using flow cytometry with FACSCalibur (BD Company, USA) and COBAS AmpliPrep/COBAS TaqMan HIV-1 Test (Roche, Switzerland) at the clinical laboratory of SPHCC, respectively.

### *DNA extraction and next-generation sequencing*

Sterile cerebrospinal fluid (CSF) of patients was retained for high-throughput sequencing. The glass bead grinding process was carried out firstly. A glass bead with a diameter of 0.5mm was added to the broken tube, and then 0.6ml of the sample was added in. The sample was vibrated at 2800-3200 rounds/minute for 30 minutes. Then 300µl was used for nucleic acid extraction. The genome extraction kit (DP316) of Tiangen microsample was operated according to the instructions. Five hundred nanogram of extracted DNA was interrupted, repaired, jointed, amplified, repositored and sequenced according to the standard process. A CSF sample produced 20M sequence data. Low-quality and short sequence (less than 35bp) was removed from the sequence data, so as to obtain high-quality sequence. The sequence was then compared with the human reference genome (H19), with the software of Burrows-Wheeler Alignment. After removing the human sequence, the sequence was compared with 4 databases of bacteria (2473 species), fungi (199 species), viruses (4061 species) and parasites (135 species), and the numbers of sequences matched to any a certain pathogen were obtained. The possible pathogens were judged according to the number of sequences and other clinical judgement.

### *Treatment and follow-up*

Patients with positive JCV in CSF were diagnosis of PML. ART were continued during hospitalization or initiated immediately after exclusion of the previous-mentioned common CNS infection. Patients were followed-up on the outpatient clinic or by phone call. Patients with aggravation of clinical symptoms and CD4 count increase following ART were diagnosis of immune reconstruction syndrome (IRIS) after excluded other causes.

### *Statistical methods*

Descriptive analysis to describe demographic data. The baseline characteristics of patients were described by median values with continuous variables. The classified variables were counted and proportioned. All the data were processed using STATA 13 software (StataCorp, Texas, USA).

## **Results**

### *Basic information of patients:*

From Oct 2017 to Jan 2019, a total of 10 cases were diagnosed of PML, including eight males (80%) and two females (20%). These patients were at their middle age (38.7±8.2 years) with advanced immunodeficiency (CD4 T cell count 46 (2-241) cells/ml). HIV RNA was detectable in the majority patients (80%). The value of HIV RNA was ranged from less than 40 to 2.81 \*10<sup>5</sup> copies/ml, with a median of 5.99 \*10<sup>4</sup> copies/ml.

HIV infection was not diagnosed until onset of PML symptoms in four patients. Two patients never received ART although HIV infection has been diagnosed for 6 and 8 years, respectively. Two patients who had resistance to current ART regimens developed symptoms after ART for 6 months and 12 years, with HIV viral load of 1.3 \*10<sup>5</sup> copies/ml and 2.3 \*10<sup>4</sup> copies/ml, respectively. One patient developed symptoms after 1 month of ART. Two cases were complicated with pulmonary non-tuberculosis Mycobacterium infection, and no other co-infection was found in all the patients.

### Symptom, imaging and CSF examination

The onset of the disease is occult, gradually aggravated. The time from symptoms onset to admission was 0.5-3 months. Dyskinesia (40%), convulsions (30%), dizziness (30%), cognitive impairment (20%) and speech impairment (10%) were the most common symptom. Symptoms in case 9 were related to immune reconstruction syndrome after antiviral therapy. (**See Table 1**)

Lesions were found in various parts of the brain in MRI examination. The lesions were located under the tentorium cerebellum in 3 cases (30%) and above the tentorium cerebellum in the rest (70%) (**see Figure 1**). For CSF test, levels of white blood cell, glucose and chloride were normal while slight increase of protein was observed in four patients (40%).

JCV sequence in the cerebrospinal fluid varied from 3-12531 reads, median 67 reads (**see Table 2**).

### *Treatment*

All patients received ART. Four of them started antiretroviral treatment immediately, 4 had been already treated for 1-3 months, 2 adjusted regimen according to drug resistance reports. Either raltegravir or dolutegravir was included in the regimen for every patient. Three patients received quadruple regimen to strengthen antiviral therapy. Supportive treatment were also given to alleviate symptoms.

### *Therapeutic Response*

Two patients died at 2 and 5 months after onset of symptoms respectively. The remaining 8 cases survived and were followed-up for 2-18 months. Two patients had survived for more than one year. Symptoms in five of the patients were relieved, but did not improve in the other three patients.

## **Discussion**

The diagnosis of PML can be challenging in HIV-infected patients as variable CNS infections and tumors can presents in the late stage of immunodeficiency. The clinical manifestations of PML can be varying, depending on the size, location and number of lesions. The most common symptoms in the current study included limb dyskinesia, dizziness and convulsions, which are neither sensitive nor specific. Cranial MRI can be helpful to identify the white matter lesions of PML. Frontal lobes and parieto-occipital regions are the regions that most affected. However, in HIV-infected patients, many other diseases including tuberculosis meningitis, cytomegalovirus infection, varicella-zoster leukoencephalitis, CNS vasculitis, as well as AIDS dementia may affect the white matter in a similar manner.

Routine CSF analysis is helpful to diagnose CNS infections. It has been reported that CSF white blood cells counts generally below 20/ul while protein level can be elevated in HIV-infected patients with PML[14, 15]. However, other infections including viral infection, tuberculosis infection, as well as fungi infection can also lead to this change of CSF. Detection of JCV in cerebrospinal fluid by PCR is essential for confirming PML as autopsy of brain maybe difficult. In patients without ART, the sensitivity and specificity of this technique were 72-92% and 92-100%, respectively[9, 10, 16]. The sensitivity may be restricted by the concentration of DNA in the CSF and the volume of CSF obtained. Frequent lumbar puncture for testing is not always convenient, since the volume of CSF is usually small and the number of suspected pathogens is high. More importantly, not all the clinicians would suspect PML in symptomatic HIV-infected patients. Therefore, JCV PCR may not be performed and patients maybe misdiagnosed and treated.

NGS can identify pathogenic microorganisms by high throughput screening, which can overcome the shortage of CSF volume. Taking full advantage of unbiased sequencing, NGS could also detect JCV in CSF “unexpectedly” in clinic where physicians are not aware of PML. In addition, NGS could provide result in a short time which could facilitate clinical management as delays in CNS infection and misdiagnosis can be fatal. In the current study, we reported for the first time a series of suspected PML cases confirmed diagnosis by NGS with CSF. Our results implicated that NGS could be a useful to help diagnose PML in HIV-infected patients.

## Conclusions

PML is prevalent in AIDS patients. NGS sequencing of the CSF may be a resolution for confirmation the diagnosis, combine with clinical and radiological findings, as well as routine examination of the CSF.

## Abbreviations

PML: Progressive multifocal leukoencephalopathy; CNS: central nervous system; JCV: JC polyomavirus; ART: antiretroviral therapy ; HIV: human immunodeficiency virus; CSF: cerebrospinal fluid; IRIS:immune reconstruction syndrome.

## Declarations

-Ethics approval and consent to participate

This study was approved by the Ethic Committee of Shanghai Public Health Clinical Center. Written informed consent were obtained from all the study patients.

-Consent for publication

Written informed consent to publish this information was obtained from study participants.

-Availability of data and material

Not applicable.

-Competing interests

There is no competing interests to be declared.

-Funding

Not applicable.

-Authors' contributions

Conceived and designed the research: HZL,RFZ. Analyzed the data: RFZ,TKQ, JC,JJS,LL,WS,ZYW,YT. Wrote the paper:RFZ, TKQ, JC,JJS. All authors drafted the article, revised it critically for important intellectual content, and approved the final article.

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## Tables

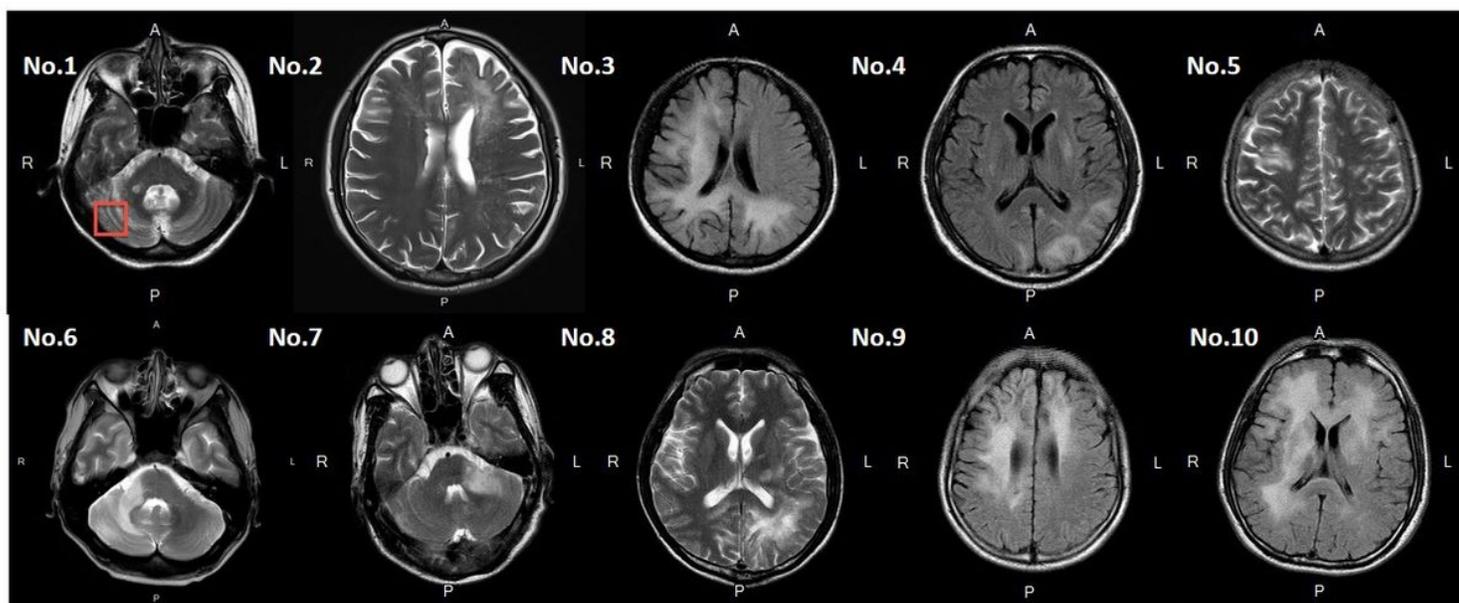
Table 1 Basic characteristic of patients with PML

Case no.	Age(year)	Gender	Initial symptom	Time from HIV diagnosis to PML onset (months)	Time from ART initiation to PML onset(months)	CD4 (cell/ $\mu$ l)	HIVRNA (copy/ml)	Site of lesions on MRI	Outcome	Survival time
1	24	Female	Ataxia	72	0	55	Undo	Right annular protuberance	Survived, not relieved	>9months
2	38	Male	Slow response	0	0	2	$2.81 \times 10^5$	Frontal lobe, parietal lobe	Survived, relieved	>4months
3	31	Male	Decreased muscle strength of both lower extremities	3	0	46	140	Fronto, temporal, parietal, pillow lobes	Survived, relieved	>17months
4	48	Male	Right Upper Limb Dyskinesia	0	0	63	$1.83 \times 10^4$	multifocal	Survived, relieved	>18months
5	42	Female	Dysphonia	144	144	16	$2.30 \times 10^4$	Paraventricular and fronto-parietal lobes	Died	3months
6	35	Male	Dizzy	0	0	4	$1.35 \times 10^5$	Cerebellar, hemisphere and brainstem	Died	2months
7	52	Male	Dizzy	96	0	46	209	Left brainstem, cerebellopontine angle	Survived, relieved	>2 months
8	37	Male	Right Limb Dyskinesia	6	6	241	$1.3 \times 10^5$	Left cerebral hemisphere	Survived, not relieved	>9 months
9	44	Male	Left Limb Dyskinesia	1	1	117	Undo	Bilateral frontal, temporal, parietal lobes	Survived, not relieved	>7 months
10	36	Male	Slow response	0	0	32	$9.68 \times 10^4$	Frontal lobe, right parietal lobe	Survived, relieved	>3 months

Table 2 CSF routine tests

Case no.	White blood cell counts $\times 10^6/L$	glucose (mmol/L)	chloride	protein (mg/L)	JCV sequence (reads)
1	1	4.48	122	400	12531
2	0	2.35	123	554	667
3	2	2.77	122	228	17
4	5	2.85	123	581	59
5	3	2.99	122	342	75
6	4	2.6	118	697	193
7	2	2.5	122	268	9
8	4	3.91	119	494	81
9	1	4.06	131	260	3
10	10	2.79	133	398	12

## Figures



**Figure 1**

Lesions locations in the brains of all the cases