

## Short Communication

## CD8 encephalitis with CSF EBV viraemia and HIV drug resistance, a case series



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## ABSTRACT

**Introduction:** CD8 encephalitis is a relatively recently described condition in the setting of HIV infection. It is becoming increasingly recognised in recent years though is still likely underdiagnosed.

**Methods:** We present three cases of encephalitis in HIV-positive black African females initially presenting with neurological pathology. Two cases concern recent presentations of patients attending HIV services at a large tertiary referral hospital and the third case involves a retrospective analysis of an archived case.

**Results and discussion:** MRI brain demonstrated periventricular white matter changes in 2 cases and a cerebellar lesion in the third case. CSF examination revealed lymphocytosis and elevated protein levels. CSF HIV viral load analysis showed viral escape along with new antiretroviral drug resistance mutations. CSF flow cytometry studies demonstrated a reversed CD4:CD8 ratio with a high CD8<sup>+</sup> cells percentage. All patients had EBV DNA detected in their CSF. Brain biopsy in two patients confirmed CD8 encephalitis and also revealed isolated cells demonstrating EBV positivity by in-situ hybridization using EBER (Epstein-Barr virus-encoded small RNAs). Treatment with steroids and ART optimisation led to significant clinical and radiological improvements in all cases.

**Discussion:** CD8 encephalitis should be considered as a cause of neurological symptoms and confusion in the HIV-positive patient, particularly if poor ART adherence or viral resistance are suspected. Brain biopsy should be considered in HIV-positive patients with encephalopathy of uncertain cause. Early treatment with high-dose corticosteroids when suspecting this diagnosis is essential for a favourable outcome. The prognosis is variable but can be favourable even following severe encephalopathy. The presence of new INSTI mutations in the CSF but absent peripherally in two INSTI-era patients is a novel finding for this case series in the context of CD8 encephalitis. The role played by EBV in this disease remains unclear and warrants further investigation.

## 1. Introduction

CD8 encephalitis is an under-recognised condition that forms part of the spectrum of HIV neurocognitive disorders (Le and Spudich, 2016). It appears to be more prevalent among HIV-positive black African individuals (Lescure et al., 2013). Several triggers have been identified, including HIV viral escape in the CSF. Patients typically present with

headaches, confusion and/or seizures. The condition is characterised by a high CD8:CD4 cell ratio on CSF flow cytometry and the presence of inflammation and CD8 lymphocyte infiltration on brain biopsy (Gray et al., 2013). Clinical suspicion for the disorder, the prompt administration of corticosteroids and ensuring HIV viral suppression in CSF and serum is essential to a favourable outcome (Zarkali et al., 2017).

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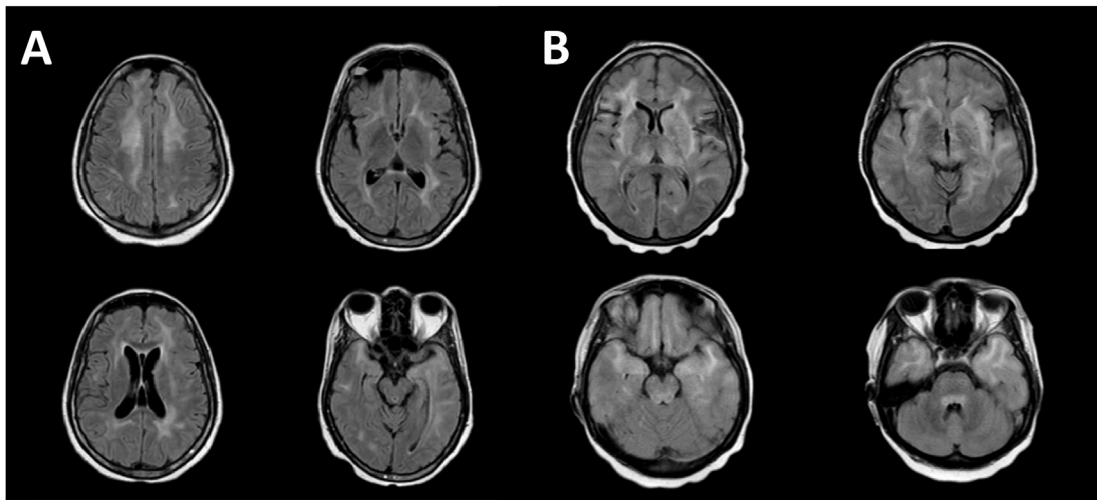
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**Fig. 1.** (A&B)A: MRI brain of patient 1 demonstrating bilateral periventricular deep white matter changes with high signal involving both temporal lobes on axial T2 FLAIR sequences. B: MRI brain axial T2 FLAIR sequences of patient 2 revealing diffuse non-restricting T2 hyperintensities throughout the white matter involving the pons, temporal poles, insulae and periventricular white matter.

## 2. Methods

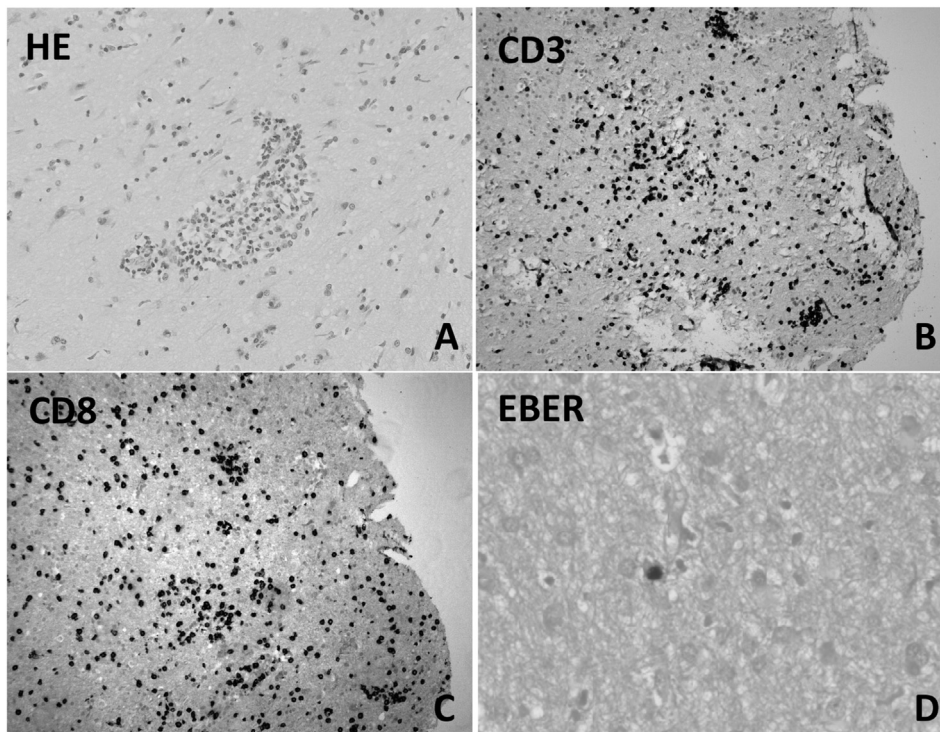
### 2.1. Case 1

A 39-year-old HIV-positive black African female presented to hospital following a seizure. She had a history of poor ART adherence with acquired NNRTI resistance mutations (V106M and F227L) since diagnosis but had good viraemic control in recent years. Her medical history was significant for multiple episodes of aseptic meningitis presenting as headaches and episodes of confusion over the preceding years, one of which was treated empirically as TB meningitis. At an outpatient clinic 3 months prior to her admission she reported good adherence to her ART regimen of tenofovir alafenamide/emtricitabine and dolutegravir. Her

HIV viral load was undetectable.

The patient became progressively more obtunded post-admission and required intubation. CT brain imaging revealed extensive low-density changes throughout the white matter of both cerebral hemispheres. A lumbar puncture revealed clear CSF with WBC of 42 per  $\text{cm}^2$  (100% lymphocytes), elevated protein 121 mg/dL (normal range 15–45 mg/dL) and glucose 2.5 mmol/L (normal range 2.22–3.89 mmol/L). No acid-fast bacilli were identified and no organisms were identified on Gram staining. GeneXpert TB and cryptococcal antigen testing were negative. The patient was treated empirically with intravenous antimicrobials to cover a possible meningoencephalitis and was commenced on anti-epileptics while further test results were awaited.

An MRI brain demonstrated bilateral periventricular deep white



**Fig. 2.** (A-D) Brain biopsy showing inflammation (A) characterised by lymphocytes, predominantly T cells (B), with CD8 cells predominant (C). ISH using EBER displaying positive nuclei (D).

matter changes involving both temporal lobes (left > right) (Fig. 1A). The patient's CD4 count was 125 (19%) and serum HIV viral load was <40 copies/ml. A serum vasculitic screen was non-contributory. Syphilis and toxoplasma serology were negative. CSF PCR testing for JC virus, CMV, VZV, enterovirus, HSV 1&2, *Listeria monocytogenes*, *Streptococcus pneumoniae*, *Neisseria meningitidis* and *Haemophilus influenzae* were negative and cultures were sterile. EBV DNA was detected at a low level in the CSF but was not detected in serum. Autoimmune encephalitis antibodies were negative in serum and CSF. Further testing of the CSF sample revealed an elevated HIV viral load of 13,085 copies/ml and new resistance mutations: M184V and K219N and, on a further CSF sample, an integrase strand transfer inhibitor mutation, R263RK. This latter mutation confers intermediate resistance to dolutegravir. No malignant cells were seen in the CSF, but flow cytometry studies demonstrated a reversed CD4:CD8 ratio with 81% of the lymphocytes comprising of CD8<sup>+</sup> cells.

The patient deteriorated further, developing diminished lower limb reflexes and mute plantars. An EEG showed diffuse generalised slowing without evidence of epileptiform activity. As the CSF flow cytometry results raised the possibility of CD8 encephalitis, a brain biopsy was performed. This demonstrated meningeal, grey and white matter inflammation characterised by lymphocytes, predominantly T-cells, with CD8 cells substantially outnumbering CD4 cells at a ratio of 5:1 (Fig. 2A–C). This confirmed a diagnosis of CD8 encephalitis. Interestingly, in-situ hybridization (ISH) of the brain biopsy using EBER (Eps-tein-Barr virus-encoded small RNAs) also showed 3 positive nuclei (Fig. 2D).

Intravenous methyl prednisolone was commenced. Within 48 h, the patient's level of consciousness returned to normal allowing for successful extubation. Her ART regimen was optimised to take account of her new CSF resistance mutations (tenofovir alafenamide/emtricitabine/darunavir/cobicistat, dolutegravir 50 mg BD and rilpivirine). Repeat MRI brain imaging prior to discharge demonstrated a stable appearance of the extensive signal abnormality in the subcortical white matter. A repeat lumbar puncture prior to discharge revealed 1 WBC/cm<sup>2</sup> and a protein of 59 mg/dL. HIV viral load was now undetectable in the CSF. EBV DNA was again detected but at a low level (1815 copies/ml). The patient was discharged after 3 weeks on suppressive valaciclovir therapy, a decreasing dose of steroids and optimised ART. Subsequent serum and convalescent CSF revealed HIV viral suppression. The patient remains well at 18 months of follow up.

### 3. Case 2

Our second case involves a 42-year-old black African female with a history of HIV infection who presented with collapse. She had a history of ART adherence issues with archived M184V mutation. Her ART regimen consisted of raltegravir BD, darunavir and ritonavir. She had been last seen as an outpatient 2 months prior where her CD4 count was 234 cells/mm<sup>3</sup> (19%) and her HIV viral load was undetectable.

On admission she was disorientated and exhibited slurred speech. MRI brain imaging revealed diffuse periventricular and subcortical non-restricting T2 hyperintensities (Fig. 1B). CSF showed a WBC count of 33 per cm<sup>2</sup> (100% lymphocytes) and an elevated protein of 219 mg/dL. Anti-epileptics and meningoencephalitis treatment were commenced.

An EEG demonstrated abnormal bilateral slowing without epileptiform activity. CSF PCR testing for JC virus, CMV, VZV, enterovirus, HSV 1&2, *Listeria monocytogenes*, *Streptococcus pneumoniae*, *Neisseria meningitidis* and *Haemophilus influenzae* was negative. Further CSF investigations showed HIV viral load of 1240 copies/ml. A new INSTI mutation, N155H, was found in this sample which conferred high-level resistance to raltegravir. EBV DNA was also detected in the CSF at a low level. CSF flow cytometry revealed a reversed CD8:CD4 ratio with CD8<sup>+</sup> cells at 70% and CD4<sup>+</sup> at 30%.

These clinical, radiological and laboratory findings suggested a diagnosis of CD8 encephalitis. The patient started on high-dose intravenous steroids and completed 21 days of intravenous acyclovir. Her ART

was broadened to tenofovir alafenamide/emtricitabine/darunavir/cobicistat, zidovudine and BD dolutegravir, reflecting the new HIV mutations. She responded excellently to this treatment, returning to her premorbid baseline after 2 weeks with complete HIV suppression in her CSF. Her steroids were weaned to zero and the patient had no further episodes of confusion or collapse after 9 months of follow up.

## 4. Results and discussion

CD8 encephalitis is a relatively recently described condition in the setting of HIV infection. It is becoming increasingly recognised in recent years though is still likely underdiagnosed (Langford and Letendre, 2013).

Presenting signs and symptoms of CD8 encephalitis can include headache, confusion and seizures (Lescure et al., 2013), though ataxia and slurred speech have also been described (Salam et al., 2016). Triggers for the condition have been postulated to include infection, immune reconstitution inflammatory syndrome (IRIS) and HIV viral escape in the CSF as a result of poor ART adherence, viral mutation or other causes (Lescure et al., 2013; Morioka et al., 2016). A genetic component is believed to play a role as the condition seems to predominantly affect black Africans (Lescure et al., 2013; Miller et al., 2004).

Imaging features associated with the condition include high-intensity signal in the white and grey matter on T2 MRI brain imaging as well as multiple gadolinium-enhancing perivascular lesions that exhibit restriction on diffusion-weighted imaging (Lescure et al., 2013). Flow cytometry results of Lescure's 14 patient CD8 encephalitis case series revealed a high percentage of CD8<sup>+</sup> lymphocytes in the CSF with a high CD8:CD4 ratio (Lescure et al., 2013). Histopathological hallmarks of the condition include diffuse infiltration of the perivascular space by CD8<sup>+</sup> lymphocytes, with a much lower ratio of CD4<sup>+</sup> T cells (Lescure et al., 2013; Gray et al., 2013) in the context of perivascular contrast enhancement on T1 weighted imaging and in the absence of alternate, particularly infectious, cause.

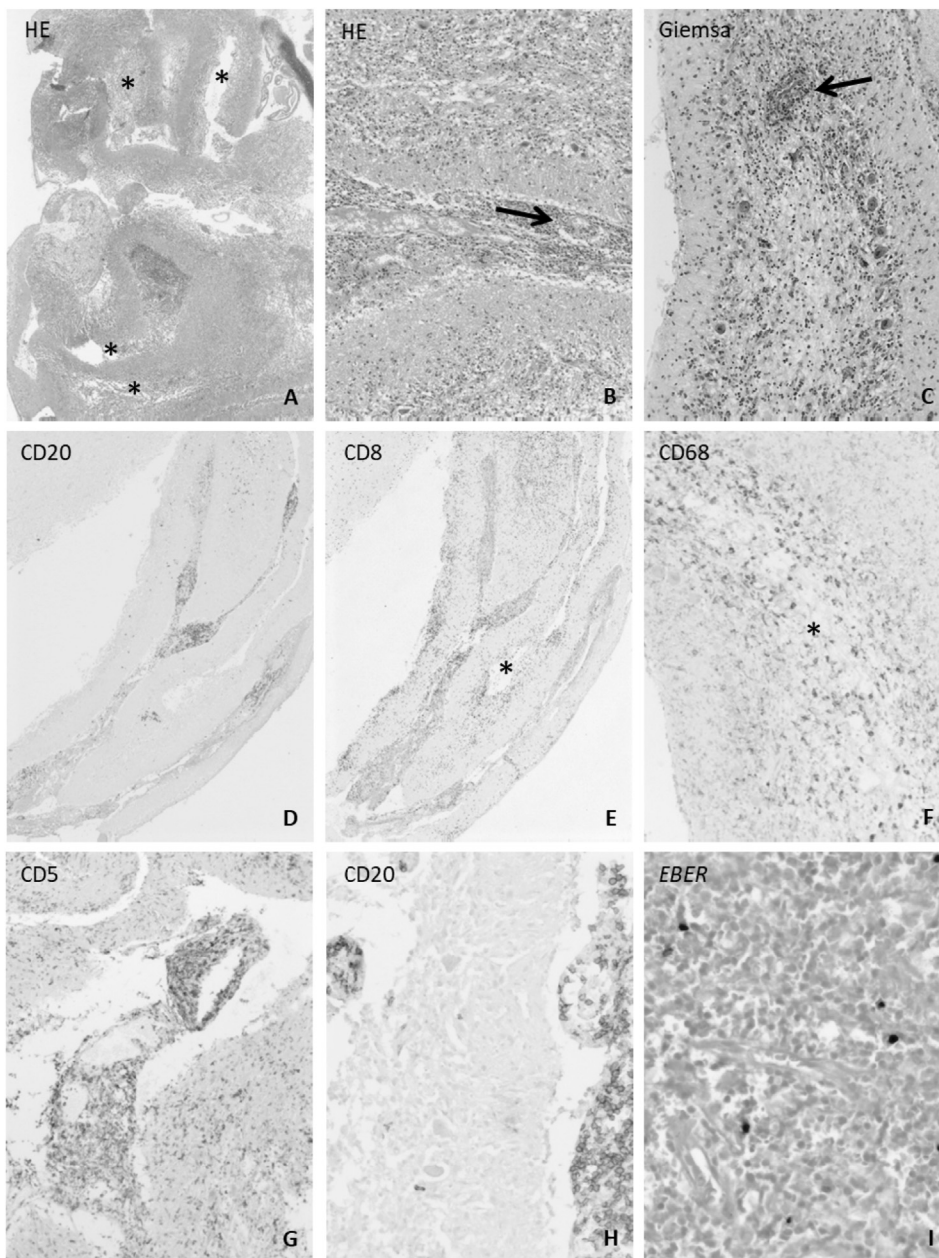
Another feature of CD8 encephalitis is its responsiveness to steroids (Lescure et al., 2013; Zarkali et al., 2017; Moulignier et al., 2013). Several case reports and series have demonstrated that patients respond well to early treatment with steroids and can relapse if steroids are withdrawn or tapered too quickly (Salam et al., 2016). Long term immunosuppression with steroid sparing agents has been used in other cases (Salam et al., 2016) but a steroid taper and ART optimisation was sufficient in our patients to lead to clinical remission. If the condition is unrecognised and steroids are not administered promptly, the prognosis can be very poor with a high mortality rate (Lescure et al., 2013).

Our patients initially presented with some of the classic phenotypic, symptomatic and diagnostic hallmarks of CD8 encephalitis. Both patients had histories of poor ART adherence and resistance issues. CSF findings revealed CSF HIV viral escape with new drug-resistant HIV mutations, not present in their serum, requiring ART regimen optimisation. CSF flow cytometry established clinical suspicion for CD8 encephalitis, and the subsequent initiation of high dose corticosteroids led to a dramatic clinical improvement. The presence of new INSTI mutations in the CSF that was not detected peripherally is a novel finding for this case series in the context of CD8 encephalitis.

The presence of EBV at low levels in the CSF of these patients, as well as the presence of positive nuclei on brain biopsy, were interesting findings. This prompted us to consult international colleagues, a collaboration which led to the revisiting of an archived case that had similar histological findings.

## 5. Archived case

In 2001, a 33-year-old African woman presented with a several month-long history of ataxia. The patient tested positive for HIV, with a CD4 count of 71 cells/mm<sup>3</sup>, and a HIV viral load of 609,000 copies/ml. An MRI displayed a non-contrast enhancing focal cerebellar lesion with



**Fig. 3.** (A-I). These figures demonstrate cerebellitis characterised by meningoencephalitis with severe ischemic parenchymal loss (A). Dense meningeal and parenchymal lymphocytic infiltrates with vasculitis (arrows) and loss of Purkinje cells (B, C) are shown. The infiltrates were composed of B (D, H), CD8<sup>+</sup> T cells (E, G) and macrophages (F). Note the parenchymal loss (asterisk in A, D, E, F). ISH using EBER displayed numerous positive nuclei (I). (A, D, E x 25; B HE x 100; C, F, G x 200; H, I x 400).

low T1 and T2 high signal intensities, predominantly in the cortex, accompanied by severe cerebellar atrophy involving the hemispheres and the vermis. CSF PCR for HSV1&2, VZV, CMV, JCV and BK virus were negative. The patient was commenced on didanosine, lamivudine and nelfinavir.

Despite improvement in CD4 and viral load, repeat MRI imaging displayed T2 high signal intensities extending to mid cerebellar peduncles and the posterior pons. CSF analysis revealed raised WBC 39 per cm<sup>2</sup> (80% lymphocytes) and elevated protein (114 mg/dL). EBV was detected in the CSF. A brain biopsy was performed (Fig. 3). This demonstrated lymphocytic infiltrate composed of CD8<sup>+</sup> T-cells. ISH using EBER displayed numerous positive nuclei. A diagnosis of EBV cerebellitis was made. A HIV resistance profile demonstrated new ART resistance. The patient commenced treatment with ganciclovir, steroids (1 mg/kg) and her ART regimen was optimised, leading to a dramatic clinical improvement.

With hindsight, we believe that this patient presented with all the symptomatic, radiological and histological features of EBV-related CD8<sup>+</sup>

encephalitis. The brain biopsy was characterized by massive CD8<sup>+</sup> infiltrates and vasculitis. We hypothesise that this patient too had a diagnosis of CD8<sup>+</sup> encephalitis that responded to high dose steroids and ART optimisation. EBV was detected in the CSF of this patient just as it was in the two previously described cases, and positive nuclei were seen on brain biopsy.

In the literature, Raman and Nelson (2014) describe a case of EBV-associated cerebral vasculitis complicating HIV infection where CD8 infiltration and EBV positivity is seen on brain biopsy of a patient with an insidious course of neurological symptomatology. This patient responded to valganciclovir and steroids. Trevillyan et al. (2013) discuss a case of EBV encephalitis in a patient presenting with HIV associated neurocognitive disorder, a different presentation to that of our patients. This patient had known archived HIV ART resistance and responded to valganciclovir and ART optimisation. However, it remains unclear to us in the case of our patients if EBV plays a role in triggering this pathology or if it is merely an innocent bystander in the disease process of CD8<sup>+</sup> encephalitis.

## 6. Conclusion

CD8 encephalitis should be considered as a cause of confusion or neurological deficit in HIV-positive patients. MR imaging and CSF parameters may be helpful in the diagnostic work-up but brain biopsy should be pursued for a definitive diagnosis in patients exhibiting an encephalopathy of uncertain cause. Early treatment with high-dose corticosteroids is essential for a favourable outcome. The prognosis is variable but can be favourable even following severe encephalopathy. The presence of new INSTI mutations in the CSF but not detected peripherally in two INSTI-era patients is a novel finding for this case series in the context of CD8 encephalitis. A further novel finding includes the presence of EBV DNA in the CSF of all patients, and the presence of EBER positive nuclei on brain biopsy, in the context of HIV viral escape. Whether EBV reactivation is a trigger for, or a consequence of, the patients' CD8 encephalitis is unknown and warrants further investigation.

## 7. Authors contributions

CK, HAB, ED, SC, and FM contributed to the writing and the conceptualisation of the manuscript. JCB, PBM and SH contributed to the editing of the manuscript and the provision of figures included in the manuscript.

## Patient consent

Our case series contains details of three patients. Two cases concern patients who have provided written consent for the use of their clinical details and imaging in this case series. One case refers to archived samples from a patient who has since been lost to follow up. Brain biopsy images of this patient are used in this case series. The patient provided verbal consent at the time (2001) for her images to be used for future publication.

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## Declaration of competing interest

None of the authors have any competing interests to declare.

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