Title: Progressive hemispheric atrophy in HIV: A Rasmussen's-like variant of CD8 encephalitis?

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Key points

- We report two cases of progressive lateralising encephalopathy in adult patients with treated HIV in the absence of opportunistic infection or vasculitis.
- One case was characterised by CD8 cortical infiltrates and was steroid responsive and may represent a variant of CD8 encephalitis.

- The other case presented with focal seizures and episodes of status epilepticus and pathology showed severe cortical atrophy with features reminiscent of the chronic phase of Rasmussen's encephalitis.

CD8 encephalitis (CD8E) was first recognised in 2004 in HIV-positive patients receiving anti-retroviral treatment (ARV) [1] and further delineated in a larger series [2]; it is characterised by diffuse bilateral involvement of the cerebral white and grey matter on MRI with CD8 infiltrates on histology in the absence of opportunistic infection or HIV encephalitis. We report two HIV-positive patients receiving ARV who developed progressive lateralising encephalopathies and discuss the possible autoimmune pathomechanisms and similarities to Rasmussen's encephalitis (RE).

Case 1: A 50 year old Nigerian female, HIV-positive since age 35 was receiving anti-retroviral (ARV) treatment but with poor compliance. She was found collapsed and hypernatraemic at home. On admission to intensive care, an MRI showed a hyperintense FLAIR/T2 signal in the right insular region, hippocampus, head of caudate, cingulate, frontal and parietal cortex (Figure 1Bi), HIV viral load (VL) was 308 copies/ml (CSF VL < 60 /ml), CD4 count 172/ µL and CSF was negative for opportunistic infection (including JCV, HSV, VZV, CMV, mycobacteria, syphilis, CrAg). An EEG did not detect any seizure activity. MRI at one month showed progression, with right sided cortical laminar necrosis, subacute cortical changes (Figure 1Bi), and an interval pontine infarct, but angiography was normal. She was treated with methyl-prednisolone with some neurological recovery but re-presented four months later with hypoactive delirium, CSF VL 2415/ml (paired plasma VL 442/ml). In addition, an elevated plasma CD8 count 2154/ µL and low CD4:8 ratio of 0.3 was noted. HIV encephalopathy due to viral escape was diagnosed and ARV treatments intensified. She had several similar episodes over the next months, with some clinical response to steroids. After a negative autoimmune screen, including NMDA antibodies, she was commenced mycophenolate mofetil as a steroid-sparing agent. A right frontal brain biopsy was carried out which showed superficial cortical laminar necrosis with neuronal loss (Figure 1Ai), numerous $CD8^+$ and $TiA1^+$ T cell infiltrates, some of which were "typical cytotoxic" lymphocytes expressing Granzyme-B and perforin (Figure 1Aii,v,vi,vii); TCR-δ^+, $CD4^+$ and $CD20^+$ lymphocytes (Figure Aviii, iii and iv) were much less frequent by comparison. HLADR labelled extensive macrophages and microglial infiltrates (Figure 1Aiv, inset). No vasculitis or opportunistic infections were found. MRI at one year showed further significant volume loss of the right hemisphere and new T2 signal changes in the subcortical white matter of the frontal and parietal lobes (Figure 1Biii). Eighteen months after her initial presentation, she was admitted with sepsis and deteriorated suddenly (See supplemental file for summary).

The brain at post-mortem weighed 942g with atrophy of the right hemisphere (Figure 1Biv), patchy thinning and discolouration of the cortical ribbon and blurring of the grey-white boundaries, particularly involving right frontal, insular, parietal cortex with softening and reduced volume of the underlying white matter and compensatory ipsilateral ventricular dilation. Mild atrophy of ipsilateral deep grey nuclei and hippocampus was noted and midline cystic cavitation in the pons. Histology of right frontal, parietal, insular and occipital cortex showed laminar (layer II-IV) to pan-cortical subacute to chronic necrosis (Figure 1Bv) with 'skip-like' foci of atrophy in the occipital lobe. There was evidence of some neuronal preservation, even in the most damaged cortical regions and the pontine cavity, against ischaemic necrosis. Focal cortical clusters of $CD8^+$ cells in layer II and hippocampal CA1 region were noted (Figure Bvii) with some $CD3^+$/$CD8^+$ T lymphocytes in close proximity to neurones (Figure 1Bix,x) and $TiA1^+$ T cells, some of which still expressed Granzyme-B were seen (Figure 1Bxi,xiii), but TCR-δ^+ lymphocytes were much rarer. GFAP stain highlighted cellular gliosis but regions with reduced GFAP and aquaporin 4 labelling also noted (Figure 1Bvi,vii). The white matter of the right hemisphere was

gliotic with reduction in axons and myelin with no active demyelination but scattered CD8 cells around blood vessels, including periventricular regions was present. No giant cells, granulomas, vasculitis or other vasculopathy was identified. Immunohistochemistry for HIV p24 and opportunistic infections, including AFB, fungi, toxoplasma, SV40, VZV and *in situ* hybridisation for VZV and EBER were negative. Samples from the left hemisphere showed minimal pathology, and T cell infiltrates were not identified in other organs to suggest diffuse infiltrative lymphocytosis syndrome (DILS).

Case 2: A 53 year old Caucasian male, HIV since age 48, which he contracted from a blood transfusion while working in Africa. He presented with onset of focal seizures with loss of consciousness following an influenza vaccination one month earlier. He was receiving ARV with stable CD4 counts and undetectable VL. An EEG showed frequent short delta waves in the right central-parietal regions, and anti-seizure medication (ASM) was started. CSF for infection screen was negative and MRI showed a presumed vascular-ischaemic lesion in the right parietal region (Figure 2A). A month later, he presented in generalised convulsive status epilepticus and ASM were modified. An MRI at six months showed more extensive right frontal T2 signal abnormality (Figure 2B). A biopsy from the right frontal gyrus showed no vasculitis or opportunistic infection. Seizures and serial right hemisphere MRI signal changes progressed (Figure 2C, D) with a small drop in CD4 from 437 to 311/ µL documented a year later, but VL remained undetectable in serum and CSF with no CSF lymphocytosis or infection and negative anti-neuronal antibody screens. Further episodes of refractory focal and generalised status epilepticus continued; he was treated with immunotherapy but died at age 58 (See supplemental file for summary).

The brain at post-mortem weighed 1215g with extensive atrophy and collapse of the right hemisphere (Figure 2E), brown discolouration of the cortex, loss of white matter, including the corpus callosum and ipsilateral ventricular dilatation. Occipital, medial temporal, fronto-basal, cingulate cortex, basal ganglia and thalamus were somewhat better preserved (Figure 2E, 2I). Histology confirmed pan-cortical atrophy and gliosis but no vasculitis or small vessel pathology (Figure 2F, H). Despite the degree of cortical collapse, preserved neurones in damaged regions remained identified (Figure 2G, 2I inset). Phagocytic macrophages (Perls positive) (Figure 1T,1U), occasional CD8 T cells (Figure 2J) and iron-encrusted neurones were prominent (Figure 2K) but no neuronophagia or microglial nodules. Immunohistochemistry for p24, SV40, toxoplasma, stains for micro-organisms and viral PCR were negative. The left hemisphere showed mild gliosis but no significant pathology (Figure 2L), and both hippocampi showed preserved subfields. No other organs were examined at post-mortem.

These cases share the common finding of progressive lateralising brain atrophy in the setting of ARV treated HIV. In both, vascular-ischaemic causes were clinically suspected, but not supported by pathology and with neuronal preservation shown in regions of cortical damage. Underlying opportunistic infections including VZV vasculitis or HIV-related encephalitis were not identified. In the first case, CD8 infiltrates were present, suggestive of CD8E.

In descriptions of CD8E, microglial activation, gliosis, diffuse parenchymal and perivascular infiltrates of polyclonal CD8 > CD4 T cells involve primarily the white matter [1-3] but with bilateral involvement on histology and MRI [3, 4]. Although cortical involvement is recognised [2, 5, 6] this does not seem to be preferentially targeted and the extensive, progressive and lateralised cortical destructive lesions observed in the current cases are not typical features. Recognised triggers for CD8E include CNS IRIS, virological 'escape' (with increased CSF VL), trivial systemic infections (including in well-controlled patients), or interruptions to ARV treatment [2, 3, 7]. In case 1, there was poor ARV compliance, systemic infection and viral escape and CD8 infiltrates in the brain. In the second case, the pathology

was 'burnt out' with minimal inflammation and the history revealed only mild drops in CD4 counts, low/undetectable VL, with a history of recent flu-vaccination as the only possible trigger.

CD8E is considered to arise through an imbalance of CNS parenchymal cytotoxic CD8 to CD4 cells, driving paradoxical and autonomous immunopathological cascades with resulting inflammatory neuropathology [2]; steroid responsiveness supports an inflammatory pathogenesis [5]. RE is regarded as a CD8 cytotoxic autoimmune condition against an as yet unidentified neuronal/glial antigen [10]. Although cytotoxic T cell receptors in CD8E have not been characterized, the emergence of autoimmune targets remains highly plausible. Indeed, CD8E in HIV has drawn comparisons with ADEM [2] and more recently with autoimmune GFAP astrocytopathy where similar vascular radial enhancement is seen on MRI (including in one HIV-positive patient) [8]. In case 1, regions with reduced GFAP and aquaporin labelling in damaged cortex was observed but anti-GFAP auto-antibodies were not investigated during life. Refractory epilepsy was the primary symptom in case 2, with a clinical course similar to RE, which can present in adulthood [9]. Different pathological stages of RE are recognised, from active/inflammatory to chronic/'burnt-out' phases with progressive cortical destruction [10]. Cytotoxic $CD8^+$, (Granzyme B/perforin-positive) T cells in proximity to neurones and astroglia predominate over clonal γδ-T and CD4 populations in the autoimmune cellular responses in active RE with fewer $CD20^+$ cells [11]. Similar lymphocyte phenotypes were seen in case 1 with active disease but T cell subsets remain relatively unexplored in CD8E [4].

Paradoxically, a wide spectrum of auto-immune disorders, including DILS, and involving several organs is recognized in HIV; these occur during the early stages of the infection where CD4 counts are high, in the context of effective ARV treatment or during IRIS [12, 13]. Interestingly, a recent report, based on MRI only, described a possible *N*-methyl-*D*-aspartate receptor antibody-mediated encephalitis in the setting of HIV [14]. These current cases may also represent autoimmune pathology, reminiscent of Rasmussen's encephalitis and broadening the phenotype of encephalitides encountered in the setting of HIV.

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Conflicts of interest

All authors have no conflicts of interest to declare.

Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

FIGURE LEGEND

Figure 1. Neuroimaging and pathology of CASE 1

Case 1.

(A) From the cortical biopsy: (Ai) regions of inflammatory cell infiltration of the cortex and reduction of NeuN-positive neurones. The lymphocytes were mainly CD8-positive (Aii) with rarer CD4 (Aiii) and CD20-positive cells (Aiv). Inset in (Aiv) shows marked macrophage and microglial infiltrates, positive for HLA-DR. (Av) More numerous lymphocytes were TiA1- expressing, compatible with cytotoxic cells and fewer cells expressed Granzyme B (Avi), perforin (Avii) or TCRδ (Aviii) (positive cells arrowed in all).

(B) MRI imaging and post-mortem findings: (Bi) T2 MRI weighted images at initial presentation, (Bii) one month and (Biii) one year following presentation, confirming the progressive right sided cortical and subcortical atrophy with signal change over this period. (Biv) Coronal section of the fixed brain at the level of the lateral geniculate nucleus shows loss of volume on the right side, ventricular dilatation, regions of cortical thinning and hippocampal volume loss. (Bv) Right frontal cortex showing laminar spongiosis and gliosis in the superficial cortex (layer I-III) with patchy small lymphocytic infiltrates. (Bvi) The same region with GFAP showing reactive astrocytes in the subpial region and loss of labelling in layer II, as also reflected by reduced aquaporin 4 (AQP) immunolabelling (Bvii). (Bviii) The right hippocampus with regions of skip like atrophy in CA1; predominant infiltrates of CD3 (Bix) and CD8 cells (Bx) were seen in proximity to surviving neurones (arrows). In regions with lymphoid infiltrates TiA1 positive (Bxi) and Granzyme B positive (Bxiii) were present, with much fewer cells noted to be TCRδ (Bxii).

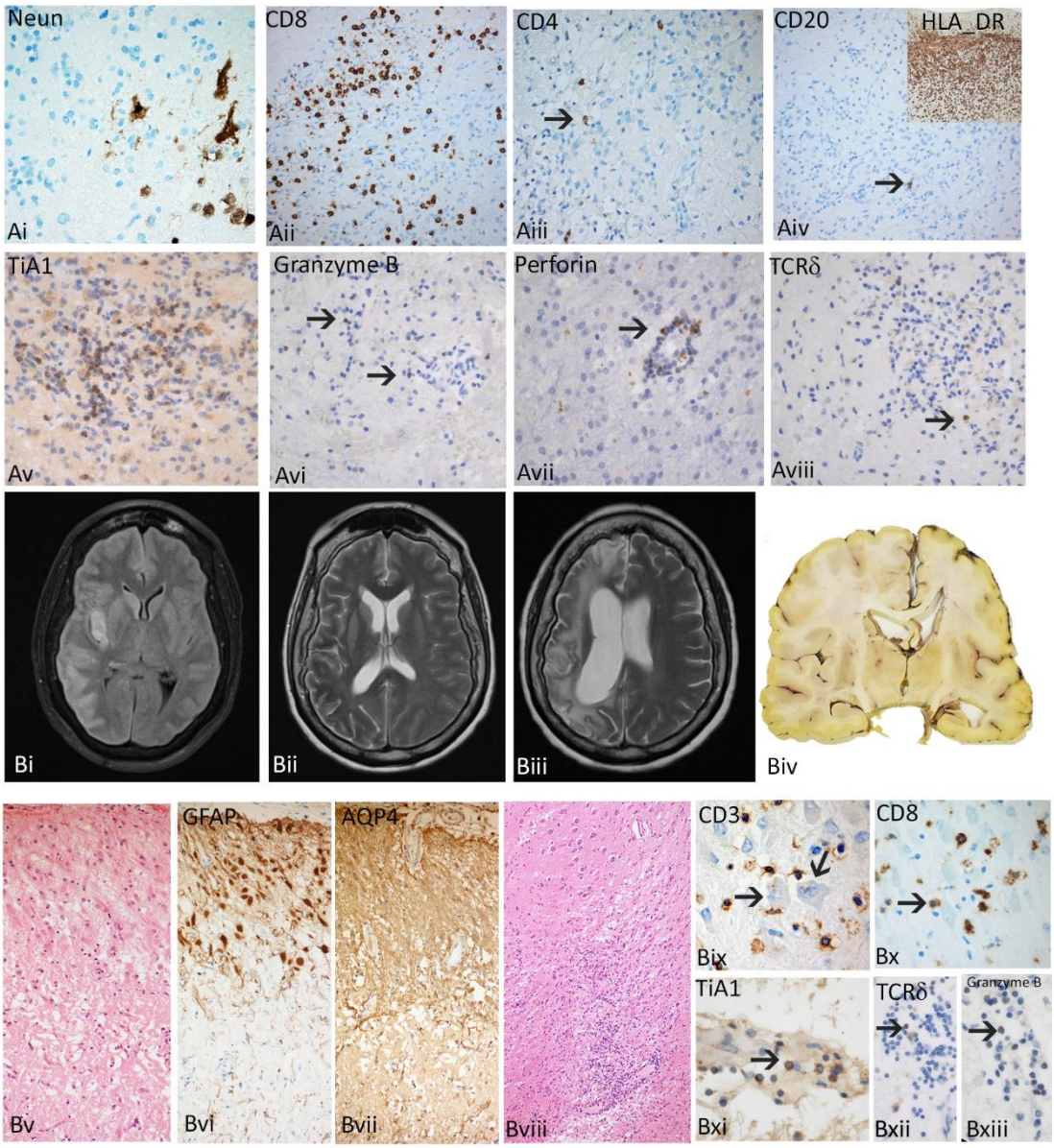
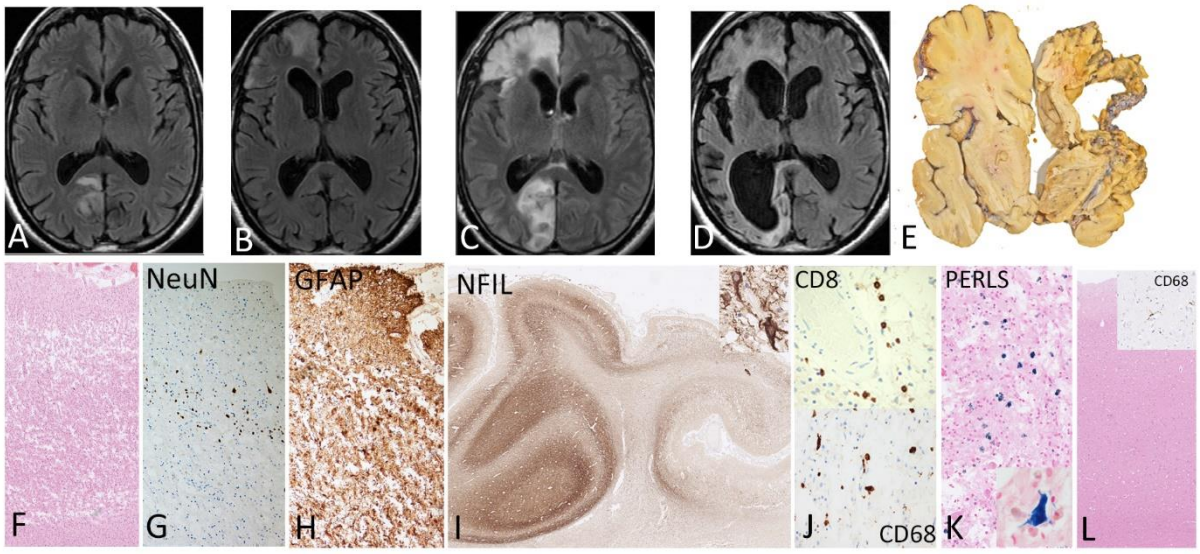


Figure 2. Neuroimaging and pathology of CASE 2

Case 2. MRI (T1 with contrast) (A) at time of presentation of seizures and at (B) 6 months, (C) 11 months, (D) at 19 months following seizures onset showing progressive atrophy and signal change in the right hemisphere. (E) Coronal slice through the fixed brain at the level of the anterior basal ganglia with collapse and discolouration of the cortex and white matter of the right hemisphere with some sparing of the mesial temporal cortex and cingulate. (F) Right frontal (F1) gyrus with pan cortical collapse and spongiosis; inset top left showing that even in the regions of maximal cortical destruction surviving neurones were easily seen. (G) Incomplete loss of neurones was supported by residual labelling with NeuN. (H) GFAP shown in the same region as in F showed pan cortical reactive cellular gliosis. (I) The occipital cortex on the right showed regions of cortical sparing adjacent to atrophic and spongiotic cortex (arrow) stained with neurofilament (SMI32); inset confirming frequent neurones within the region of cortical collapse. (J) CD68 showing numerous cortical macrophages in the atrophic cortex but

no microglial nodules or neuronophagia (bottom); top shows rare CD8 T cells near a cortical vessel. (K) Pigment laden macrophages in the cortex were Perls positive and inset, ferruginated neurones were frequent. (L) The left frontal cortex in contrast shows relative preservation and no inflammation with minimal gliosis and normal microglial density on CD68 (inset).



Supplemental Figure

Diagrammatic summary of the time line for the clinical course for Case 1 and Case 2.

Time zero equates to the point of HIV diagnosis and the plots are not shown to scale.

ANA - anti-neuronal antibodies ; ARV - antiretroviral treatment ; ASM – anti-seizure medication; CLO – clobazam, EPC- epilepsy partialis continua; GS – generalised convulsions ; GCS – Glasgow coma scale ; HIVE – HIV encephalitis ; LEV – levetiracteam ; MCA – middle cerebral artery ; NMDA - -methyl D-aspartate receptor, PHB – phenobarbitone ; PTN – phenytoin ; RIP – time of death ; SE – status epilepticus ; VAL – sodium valproate ; VL – viral load ; UTI – urinary tract infection.

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Presentation : Collapse, hypernatraemic

Dx: HIV
ARV: Atazanavir 300mg, Cobicistat 150mg, Truvada) ((Tenofovir Disoproxil,Emtricitabine) once daily

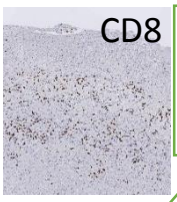
Poor ARV compliance

Re-presentation : Delirium
Dx : Viral escape

Methyl-prednisolone ARV restarted

Steroid dependent/Cushing's
Episodes of aspirations, UTIs, sepsis, low GCS with recovery

ARV intensified + Dolutegravir 50mg od
Some clinical response to steroids



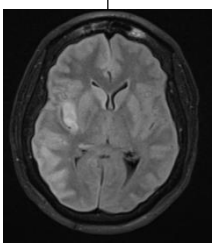
Brain biopsy : Cortical CD8>4
No HIVE/opp. infection

Further episodes sepsis, sudden death. RIP

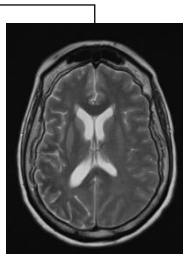
Autoantibody screen (including NMDA antibodies) negative

0 → 14yr → $14_{1/12}$ → $14_{4/12}$ → 15yr → $15_{6/12}$

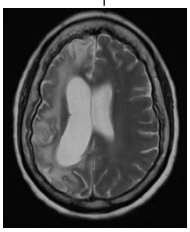
VL Serum	308	442	
VL CSF	<60	2415	
CD4/8	0.7	0.3	>1

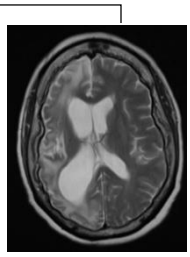


Hyperintense FLAIR/T2 signal in right MCA region



Progressive subacute cortical changes





Volume loss of right hemisphere
New T2 signal changes in the subcortical white matter of the frontal and parietal lobes

CASE 1

Dx HIV
ARV (Efavirenz, Emtricitabine, Tenofovir)

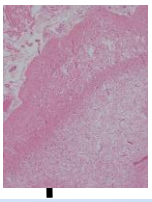
Viral load low
CD4 stable

Focal seizure onset

ASM : PTN, VAL CLO

Episodes of SE

Brain biopsy : 'chronic infarction' no vasculitis or opportunistic infection



CD4 583 cells/mm3
VL undetectable serum and CSF
ASM : LEV, PHB

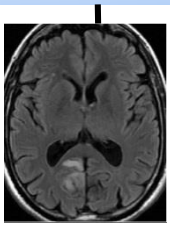
Focal motor seizures

Continued episodes of EPC, SE and GS

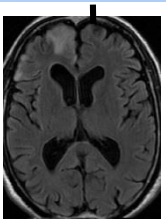
ANA screen negative (including NMDA antibodies) negative
Trials of immunotherapy (azathioprine, steroids and IV-Immunoglobulin)

RIP

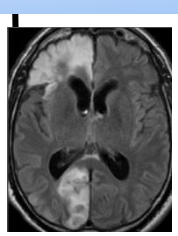
0 → 5 → $5_{1/12}$ → $5_{6/12}$ → 6 → 9yrs



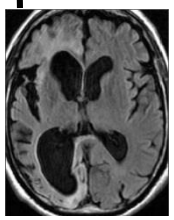
MRI : lesion in parietal region ? vascular-Ischaemic



More extensive right frontal T2 signal abnormality



Progression : right hemisphere signal change and subcortical white matter



19 Months since onset of seizures : Progressive hemispheric atrophy

CASE 2