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Review

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Review

Molecular neuroimaging of inflammation in HIV

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Summary

People with HIV now have near-normal life expectancies due to the success of effective combination antiretroviral therapy (cART). Following cART initiation, immune recovery occurs, and opportunistic diseases become rare. Despite this, high rates of non-infectious comorbidities persist in treated people with HIV, hypothesized to be related to persistent immuno-activation. One such comorbidity is cognitive impairment, which may partly be driven by ongoing neuro-inflammation in otherwise effectively treated people with HIV. In order to develop therapeutic interventions to address neuro-inflammation in effectively treated people with HIV, a deeper understanding of the pathogenic mechanisms driving persistent neuro-inflammatory responses and the ability to better characterize and measure neuro-inflammation in the central nervous system is required. This review highlights recent advances in molecular neuroimaging techniques which have the potential to assess neuro-inflammatory responses within the central nervous system in HIV disease. Proton magnetic resonance spectroscopy (^{1}H-MRS) has been utilized to assess neuro-inflammatory responses since early in the HIV pandemic and shows promise in recent studies assessing different antiretroviral regimens. ^{1}H-MRS is widely available in both resource-rich and some resource-constrained settings and is relatively inexpensive. Brain positron emission tomography (PET) imaging using Translocator Protein (TSPO) radioligands is a rapidly evolving field; newer TSPO-radioligands have lower signal-to-noise ratio and have the potential to localize neuro-inflammation within the brain in people with HIV. As HIV therapeutics evolve, people with HIV continue to age and develop age-related comorbidities including cognitive disorders. The use of novel neuroimaging modalities in the field is likely to advance in order to rapidly assess novel therapeutic interventions and may play a role in future clinical assessments.

Keywords: human, immunodeficiency diseases, inflammation, viral

Introduction to HIV

Over three decades ago, human immunodeficiency virus (HIV) was identified as the causative agent of acquired immune deficiency syndrome (AIDS). In the absence of effective antiretroviral treatment, the prognosis for most people living with HIV is poor with severe immunodeficiency developing after several years. People living with untreated HIV present with characteristic opportunistic infections and/or cancers and typically progress to death over a period of 10–20 years [1]. Since the mid-1990s, the field of HIV therapeutics and clinical management progressed, with significant reductions in morbidity and mortality for people living with treated HIV [2]. However, an effective cure for HIV has still not yet been discovered, and the number of people living with HIV presently is the highest it has been since the beginning of the epidemic. In 2019, an estimated 38 million people were living with HIV worldwide, with around 1.7 million people newly acquiring HIV infection in 2019 alone [3].

Natural history of untreated HIV infection

In the absence of antiretroviral treatment, the natural history of HIV infection comprises a primary phase lasting up to 6 months following HIV acquisition, followed by a chronic phase which generally lasts 10–20 years and eventually progresses to death in most people living with HIV [4].

HIV rapidly spreads systemically within days to weeks of HIV acquisition, due to the absence of any memory adaptive immune responses, culminating in a massive rise in viraemia frequently reaching a zenith of 10^6–10^7 plasma HIV RNA copies/ml and a significant decrease in CD4+ T-cell counts. Some individuals may present symptomatically during this period known as acute seroconversion illness, with often transient symptoms that include fever, generalized lymphadenopathy, a non-specific rash, myalgia, and headache [5] and rarely, AIDS-defining illnesses if significant decline in CD4+ T-cell count has occurred.

After several months, the immune system gains some form of control; HIV replication falls and reaches a set-point while CD4+ T-cell count somewhat recovers. The individual then progresses into what has traditionally been termed the clinically latent phase known as chronic HIV infection, whereby the individuals experiences few symptoms, but viral replication remains ongoing. Persistent viral replication is associated with a state of chronic immune activation and inflammation. This results in pro-inflammatory cytokine production and thymus dysfunction, which is associated with high rates of non-infectious co-morbidities such as cardiovascular and cerebrovascular disease and progressively leads to a reduction in

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CD4+ T-cell counts and immunodeficiency [6, 7]. Once CD4+ T-cell counts fall below 200 cells/μl, most people living with untreated chronic HIV infection will develop AIDS-defining condition(s) and ultimately progress to death.

HIV and the central nervous system

HIV is neurotropic and neurologic manifestations are a common feature of HIV disease [8]. HIV crosses into the central nervous system early on in the course of acute HIV infection, when systemic infection is displaying significant viraemia, reductions in CD4+ T-cell counts and elevations of CD8+ T-cell counts. The 'Trojan horse' hypothesis postulates that HIV crosses the blood–brain barrier into the central nervous system via infected CD4+ T cells or monocytes performing routine surveillance, then either these cells go on to differentiate into macrophages or HIV goes on to infect resident brain immune cells like macrophages, microglial cells, and astrocytes [9] (Fig. 1). The 'free virus transfer' hypothesis theorizes that free HIV virions from the peripheral blood compartment traverse the blood–brain barrier due to blood–brain barrier impairment caused by uncontrolled HIV infection, which then go on to infect the resident brain immune cells, as above [10] (Fig. 1). Once infected, activated central nervous system perivascular macrophages, microglial cells, and astrocytes release pro-inflammatory cytokines, chemokines, and neurotoxins, which leads to further blood–brain barrier disruption, immune activation, and neuro-inflammation, culminating in neuronal injury and apoptosis (Fig. 1). Persistent central nervous system immune activation and neuro-inflammation is purported to continue throughout the course of untreated HIV infection, which leads to synaptic dysfunction and neuronal injury, and can result in neurological sequelae such as cognitive impairment.

Microglial cells are the main resident immunocytes in the brain and are thought to be highly susceptible to HIV infection [11]. They are the main starting point for most immune and inflammatory cascades within the brain and play a major role in immune surveillance, synaptic pruning, and the elimination of microbes, dead cells, and aggregates such as amyloid plaques [12–14]. Both resting and activated microglial cells demonstrate ramifications which closely link in with neurons, astrocytes, and blood vessels. Microglial cells are continuously scanning the microenvironment and can rapidly migrate to the area of brain insult and become phagocytic [14]. Traditionally, activated microglial cells were thought to evolve into a M1 pro-inflammatory phenotype, characterized by the release of pro-inflammatory cytokines or a M2 anti-inflammatory phenotype, characterized by healing and debris clearance. However, it is becoming increasingly accepted that the binary classification of microglial activation may be an oversimplification and multiple combinations with varying degrees of both pro- and anti-inflammatory phenotypes of activated microglia may co-exist. Microglial dysfunction may result in excessive cytokine, chemokine, and neurotoxin production, which can then lead to neuroinflammation and neuronal injury [15]. Microglial cells have a low turnover and may harbour integrated HIV for prolonged periods of time [16, 17]. In contrast, macrophages are not thought to undergo cell division within the central nervous system and are continuously replenished from peripheral monocytes traversing the blood–brain barrier.

Astrocytes also play a crucial role in synaptic pruning and maintaining central nervous system function and homeostasis [18]. Similar to microglial cells, astrocytes may behave in a pro- and/or anti-inflammatory manner in response to the microenvironment or insult and persistent astrocyte activation can also cause neuro-inflammation which can lead to neuronal injury. However, it is estimated that only 5–10% of the total astrocyte population are infected with HIV and astrocytes are not thought to be capable of supporting productive viral replication.

More recently, there is evidence to suggest that blood–brain barrier pericytes can also be productively infected with HIV and may lead to the development of HIV-associated central nervous system injury [19, 20].

HIV reservoirs

For the vast majority of people with HIV on virologically suppressive antiretroviral treatment, upon treatment cessation, plasma viral load rebounds within 8 weeks [21]. The source of the rebounding virus is cells latently infected with HIV, known as the HIV reservoir, formed when HIV DNA integrates into the genome of the host cell [22, 23]. HIV reservoirs are formed soon after acquiring HIV, are stable despite plasma viral suppression on antiretroviral treatment, and can persist for the lifetime of the infected host cell [24]. The infected host cells do not display viral antigens on their cell surface, thus can evade immune system surveillance and removal [25].

Data suggest that the main cellular HIV reservoir are resting memory CD4+ T cells in lymph nodes and blood, but other proposed anatomical HIV reservoir sites include the central nervous system, adipose tissue, and genital tract [26, 27].

HIV in the antiretroviral treatment era

The mainstay of HIV treatment at present is combination antiretroviral treatment, with the aim being to suppress plasma HIV RNA to <50 copies/ml, accompanied by a recovery in CD4+ T-cell count and immune function [28]. Subsequently, people living with HIV on virologically suppressive antiretroviral treatment rarely present with AIDS and have near-normal life expectancies. Thus, effectively treated HIV infection is now considered a chronic manageable condition and the emphasis is shifting from the management of opportunistic infections and immunosuppression to the consequences of a chronic viral infection, its treatment and ageing.

However, it is becoming increasingly apparent that individuals on virologically suppressive antiretroviral treatment continue to be at higher risk of developing non-AIDS related conditions including neurological, cardiovascular, and kidney disease [29–31]. This is further corroborated by the evidence demonstrating that despite an improvement in immune function in people living with treated HIV, these individuals continue to demonstrate persistently elevated systemic and intrathecal biomarkers of immune activation and inflammation, compared to HIV-negative control populations [32–35]. The reason for persistent immune activation and inflammation in people living with treated HIV remains unclear but important contributors include non-infectious co-morbidities (cardiovascular disease and diabetes mellitus), co-infections (Cytomegalovirus, Epstein-Barr virus, hepatitis B, and hepatitis

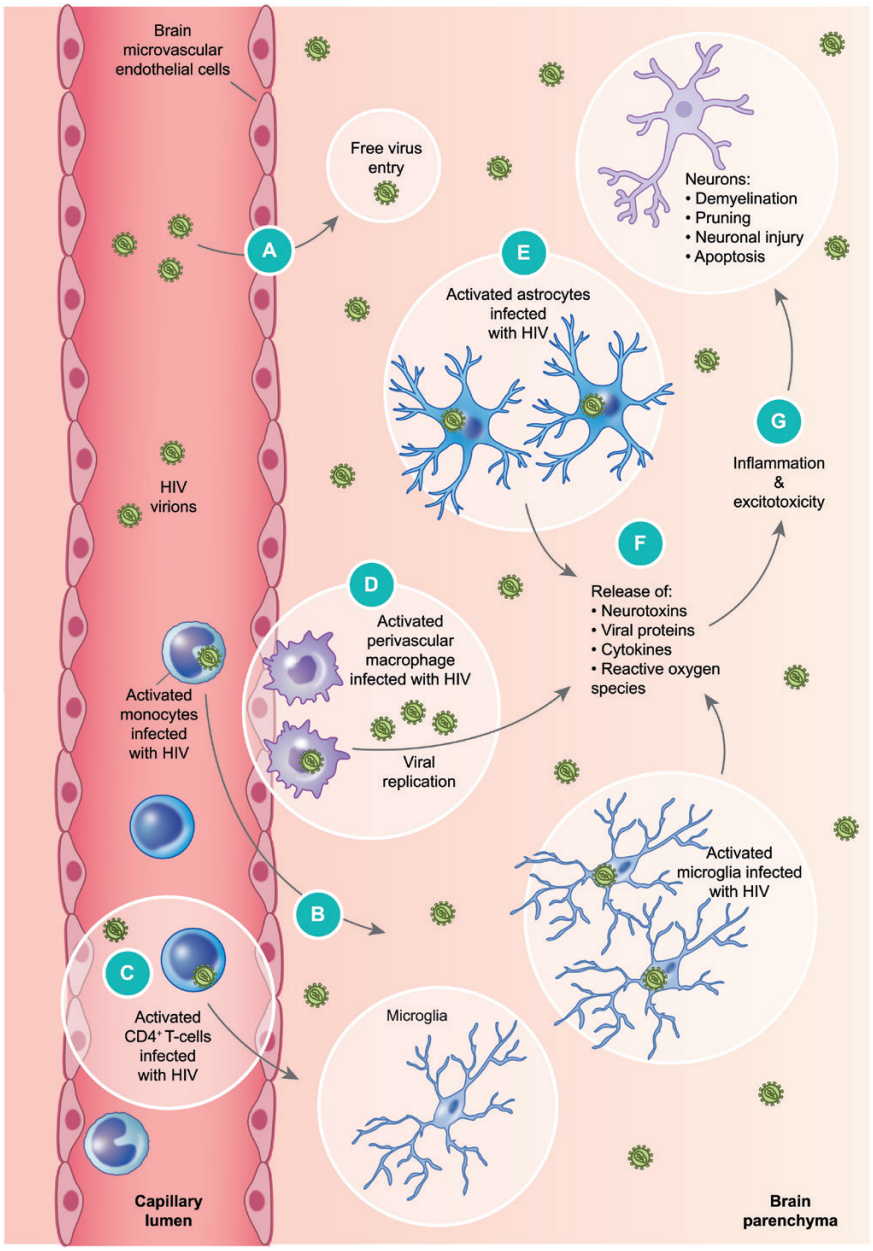

Figure 1: Schematic representation of the potential routes for HIV entry and pathogenesis in the central nervous system. (A) Direct entry of virus into the brain parenchyma ('free virus hypothesis') is possible when blood–brain barrier permeability is enhanced due to dysfunction. (B) Activated monocytes infected with HIV may migrate across the blood–brain barrier, trafficking HIV into the central nervous system ('Trojan horse hypothesis'). Once in the brain parenchyma, this virus can then infect microglial cells, astrocytes, and macrophages. (C) Activated CD4+ T cells infected with HIV may also traffic HIV across the blood–brain barrier, into the central nervous system. (D) Infected monocytes that have traversed the blood–brain barrier from the systemic circulation into the brain parenchyma may differentiate into perivascular macrophages, which allows HIV replication and the production of free virions that may infect neighbouring microglial cells. (E) Astrocytes may harbour HIV infection but are not thought to be capable of supporting HIV replication within the central nervous system. (F) Activated and infected cells may release viral proteins, neurotoxins, cytokines, and chemokines which can aid the influx of more monocytes by perpetuating increased blood–brain barrier breakdown. (G) The release of chemotactic, inflammatory factors, and neurotoxic factors maintain the inflammatory cycle within the central nervous system and ultimately results in neuronal injury and apoptosis.

C), and lifestyle factors (tobacco, alcohol, and recreational drug use) which have been shown to be more prevalent in people living with HIV [36]. Certain HIV-specific factors such as antiretroviral drug toxicity, lower nadir CD4+ T-cell count, lower CD4:CD8 ratio, and longer duration of untreated HIV may also affect immune activation and neuroinflammation. Evidence is emerging to suggest that a complex interaction exists between HIV reservoirs and low-level viral transcription with immune activation and inflammation, which can cause end-organ disease [37]. It remains unclear whether the elevated T-cell activation in people living with HIV increases HIV reservoir size, or whether higher viral reservoirs lead to elevated immune activation.

HIV and cognitive dysfunction

In the absence of antiretroviral treatment, a spectrum of HIV-associated cognitive dysfunction was observed in people

living with HIV. The most severe form is HIV-associated dementia, an AIDS-defining condition, that occurred more frequently when CD4+ T-cell count was <200 cells/μl and with a prevalence rate of 15–20% in untreated cohorts of people with HIV. HIV-associated dementia characteristically causes severe cognitive impairment (concentration and memory), behavioural changes (apathy and withdrawal), and motor symptoms (weakness and slowness of hand movements) [38].

While overt HIV-associated dementia is rarely seen in people living with treated HIV, the global prevalence of minor cognitive disorders remains high with reported prevalence rates ranging from 15 to 69%, depending on the clinical and geographical setting and the definition of cognitive impairment used [39–43]. In the antiretroviral treatment era, cognitive dysfunction in people with HIV frequently impairs the domains of attention, fine movement, executive function, processing speed, and learning [44–46]. Symptomatic cognitive impairment can reduce quality of life for people with HIV and can affect ability to maintain employment and/or education, and medication adherence, which can have serious consequences on long-term effectiveness of antiretroviral treatment for the individual and onward HIV transmission [47, 48].

To date, it remains unclear whether the underlying pathogenesis of cognitive impairment in people living with treated HIV is caused by HIV- or non-HIV-related factors. One hypothesis is that the HIV reservoir and low-level viral transcription in the central nervous system may lead to immune activation and neuroinflammation, causing neuronal injury and culminating in neuronal injury. Data in support of this hypothesis demonstrate an association between the detection of cells latently infected with HIV in the cerebrospinal fluid of people living with treated HIV and cognitive impairment [49].

Neuropsychometric testing and structural neuroimaging have traditionally been the mainstay for the diagnosis of cognitive impairment. However, these tools are generally descriptive, lack sensitivity, and often cannot discriminate between active and historical brain injury. Routine brain biopsies are not ethical, hence there has been a strong push to identify other methods of identifying the neuro-HIV processes associated with neuroinflammation and neuronal injury in order to further elucidate the underlying pathogenesis of cognitive impairment in people living with HIV. Cerebrospinal fluid and blood biomarkers of immune activation, neuroinflammation, and neuronal injury have been pursued but neuroimaging has the advantage of being able to non-invasively define anatomical regions of the brain displaying changes in signal, whereas cerebrospinal fluid and blood biomarkers produce a more diluted signal from the brain, as a whole. Over the past 30 years, molecular imaging studies have increasingly been utilized to investigate neuroinflammation in a variety of diseases, and more recently, in the investigation of people with HIV. In this review, we will be focussing on two molecular neuroimaging techniques for the identification of neuroinflammation in people with HIV, specifically concentrating on proton magnetic resonance spectroscopy (^{1}H-MRS) and Translocator Protein 18 kDa (TSPO) positron emission tomography (PET).

Molecular imaging of inflammation

Molecular imaging utilizes imaging techniques to display molecular and cellular events in living organisms. In contrast, traditional structural imaging techniques such as computed tomography (CT) rely on physical characteristics to produce images. Molecular imaging enables examination into the more basic science of disease and has revealed important insights into the pathogenesis of a wide variety of diseases.

The basic framework of molecular neuroimaging relies on: (i) a ligand or probe, that (ii) interacts with the molecular or cellular marker being investigated, and (iii) displays a signal that can be amplified, that can (iv) be utilized to produce an image.

Magnetic resonance spectroscopy (MRS)

Proton MRS (^{1}H-MRS)

Cerebral proton MRS (^{1}H-MRS) allows measurement of central nervous system metabolites in different areas of the brain. Proton MRS is based on the principle that protons in different chemical environments, or in different molecules, have different resonance properties. During imaging, the brain can be divided up into very small areas, known as voxels, and the distribution of resonance in protons in such voxels can be assessed and displayed in a spectrum.

Each spectral peak can then be measured with the area under the peak representing a quantitative measurement of the relative abundance of specific compounds. With proton MRS, by far the largest peak will be the water (H_2O) peak. Other peaks include choline (Cho, a marker of cell turnover), creatine (Cr, a marker of cell metabolism), N-acetylaspartate (NAA, a marker of neuronal viability), and myoinositol (mI, a marker of gliosis), as illustrated in Fig. 2. It is therefore possible to characterize and quantify the chemical profile of areas of the brain with this non-invasive technique [50]. Of specific interest to this manuscript, elevations in cerebral mI can be considered a direct marker of glial cell activation and an indirect marker of neuroinflammation.

Proton MRS in people living with HIV

Proton MRS has been studied quite extensively in HIV-positive cohorts. Prior to the advent of effective cART, not unsurprisingly, cerebral MRS studies reported an increase in frontal white matter mI [51] and a decrease in NAA [52] in the white matter and basal ganglia when compared with HIV-negative individuals. These abnormal metabolite findings are likely to represent neuroinflammatory processes and loss of neuronal tissue.

Although these reports were not unsurprising, these initial studies paved the way to utilize proton MRS in the effective antiretroviral treatment era. Studies during the initial antiretroviral treatment era described differences in cerebral proton MRS findings in people living with HIV on different antiretroviral treatment combinations. One study reported lower NAA concentrations in frontal lobe voxels in people living with HIV on antiretroviral treatment regimens containing didanosine or stavudine compared to those on regimens containing zidovudine and lamivudine [53]. Didanosine and stavudine are two of the earliest antiretroviral agents and have much greater toxicity profiles compared to more modern agents, and it was hypothesized that the reduction in the NAA measurement could be related to neuronal damage or loss due to toxicities from these drugs.

In a recent meta-analysis of cerebral proton MRS in people living with HIV, the characteristics of metabolite alterations during different stages of HIV are described [54]. During early HIV infection, the predominant signal alterations

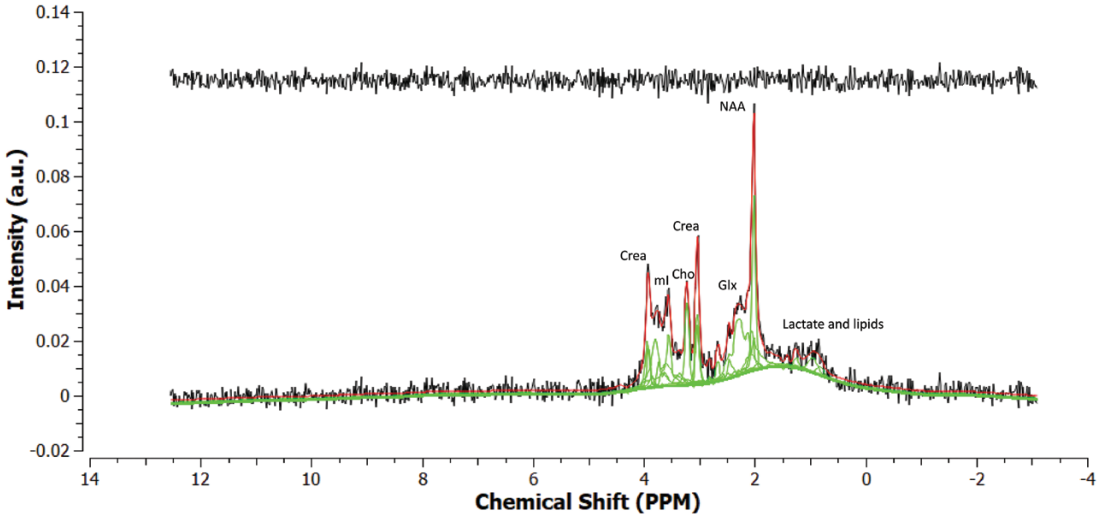

Figure 2: [1]H-magnetic resonance spectra obtained from an individual living with HIV, illustrating peaks relating to creatine at 3.9 and 3.0 ppm, myo-inositol at 3.6 ppm, choline at 3.2 ppm, glutamate/glutamine at 2.1 ppm, N-acetyl aspartate at 2.0 ppm, and lactate and lipids at 0.9 to 1.3 ppm. Crea, creatine; mI, myo-inositol; Cho, choline; Glx, glutamate/glutamine; NAA, N-acetyl aspartate; a.u., arbitrary unit; ppm, parts per million. Figure obtained from authors' own work, unpublished.

observed are in the basal ganglia and include elevations in Cho. Whereas in chronic treated HIV, consistent reductions in NAA have been observed across several brain areas which is associated with cognitive dysfunction.

More recent studies from our group have assessed changes in cerebral metabolites and cerebral metabolite ratios in people living with HIV commencing or modifying antiretroviral therapy [55, 56]. Maraviroc, a licensed antiretroviral drug, works via blockade of the C-C chemokine receptor type 5 (CCR5), thereby preventing HIV entry into cells. Blockade of the CCR5 chemokine receptor has also been postulated to have anti-inflammatory properties. In a cohort of people living with HIV intensifying antiretroviral therapy with maraviroc, increases in NAA concentration in the basal ganglia were observed associated with maraviroc plasma concentration, which may have been related to changes in the inflammatory milieu within the central nervous system [55].

As HIV treatments continue to be developed and novel strategies assessed, cerebral proton MRS may continue to play a role as a non-invasive modality to assess changes in neuroinflammation and central nervous system responses to treatment.

TSPO PET imaging

Microglial activation is accompanied by dramatic changes in molecular expression, one of which is the TSPO, previously referred to as the peripheral benzodiazepine receptor (PBR). TSPO is located on the outer mitochondrial membrane and is thought to play a role in steroidogenesis [57] (Fig. 3). TSPO expression is amplified during immune activation mainly in microglia, but also in astrocytes. The high TSPO density on activated microglia (confirmed on histochemistry), combined with its associations with neuroinflammation makes it a suitable ligand for molecular imaging [58] and has been used in a variety of neurological disorders [59, 60].

TSPO PET imaging methods

The first-generation TSPO ligand, [11C] PK11195, demonstrated neuroinflammation in a variety of conditions including Alzheimer's disease and multiple sclerosis, however [11C] PK11195 expresses high non-specific binding and poor central nervous system penetration through the blood–brain barrier, resulting in a low signal-to-noise ratio [61].

In order to overcome these challenges, second-generation TSPO radiotracers such as [11C] PBR28 and [11C] DPA713 were developed, which display greater TSPO affinity binding, higher penetration through the blood–brain barrier and higher signal-to-noise ratio [62]. Binding affinities for second-generation TSPO radiotracers have been observed to be highly dependent on the single-nucleotide (SNP) rs6971 polymorphism, with an alanine (Ala) to threonine (Thr) substitution in the TSPO gene [63]. Individuals homozygous for Ala/Ala display high specific TSPO radiotracer binding and are considered high-affinity binders (HABs), individuals homozygous for Thr/Thr demonstrate minimal specific TSPO radiotracer uptake and are considered low-affinity binders (LABs) while individuals heterozygous for Ala/Thr have reduced TSPO radiotracer uptake and are deemed medium-affinity binders [64]. Considering [11C] PBR28, HABs (Ala/Ala) demonstrate 50-fold higher TSPO radiotracer binding compared to LABs (Thr/Thr) [65]. In view of this, TSPO genotypic testing is performed prior to PET imaging and individuals who are LABs are generally excluded from second-generation TSPO radiotracer imaging studies.

Intra-individual differences in TSPO radiotracer uptake have been observed and one way of correcting for this variability is to normalize the concentrations of TSPO radiotracer within the brain to the concentration in the blood using a metabolite-corrected plasma input function. To do this, arterial line insertion and frequent blood sampling is required, which is invasive in nature, time consuming, and expensive. An alternative method is to normalize radiotracer binding to a pseudo-reference region; however, this is challenging as TSPO receptors are expressed throughout the human brain and a true TSPO-free region of the brain in people living with HIV has yet to be determined. To date, areas of the brain such as the cerebellum and cortical grey matter have been used as pseudo-reference regions [66–68].

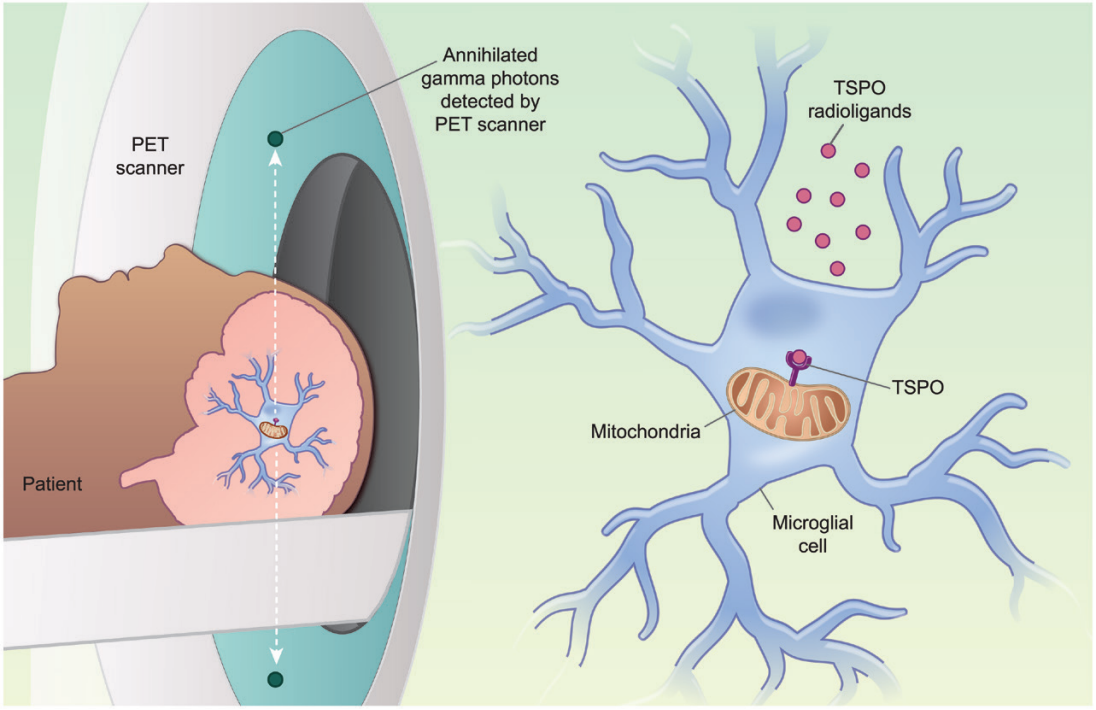

Figure 3: Schematic illustration of PET imaging of activated microglia with radioligands that bind 18 kDa TSPO. TSPO is localized to the outer mitochondrial membrane. TSPO radioligands bind to TSPO in activated microglial cells. The radioligand decays by emitting a positron, which upon combining with an electron, culminates in the annihilation of both particles. Two gamma ray photons are concurrently released at an angle of 180° from each other. The PET scanner detects both the gamma rays and generates a three-dimensional PET image.

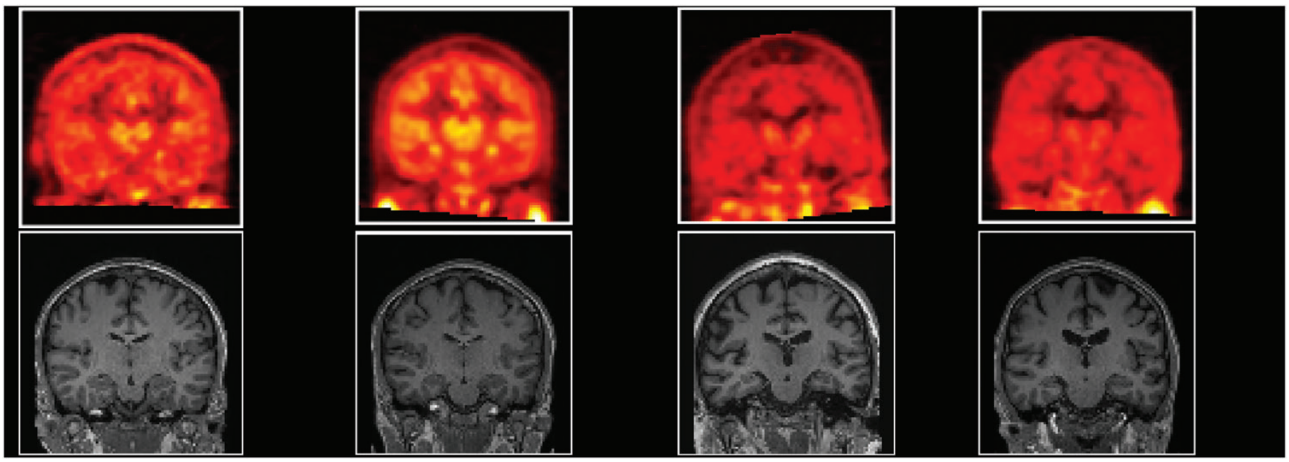

Figure 4: Representative images of volume of distribution parametric maps (0–90 minutes) of [^{11}C] PBR28 PET scans registered to magnetic resonance imaging. The images illustrate the brain distribution of [^{11}C] PBR28 binding in people living with HIV on virologically suppressive antiretroviral treatment. All four individuals are high affinity binders. Images obtained from authors' own work, unpublished.

TSPO PET imaging in HIV

Since 2005, molecular imaging using TSPO PET has increasingly been used to investigate neuroinflammation in the development of HIV-associated cognitive impairment in people living with HIV (Fig. 4), as given in Table 1. To date, the results have been conflicting, likely related to the different methodologies used and small study sample sizes. Some studies investigated people living with HIV with and without cognitive impairment, while others only investigated people living with treated HIV without symptomatic cognitive impairment. Furthermore, the diagnostic criteria and tests used to identify cognitive impairment were different throughout the studies, with some studies using the Frascati criteria (the currently recommended standardized research definition of acquired cognitive impairment in people living with HIV), while others used the increasingly popular Cogstate™ computerized cognitive testing battery or the now less-commonly used Memorial Sloan-Kettering dementia scale, thus making direct comparisons of levels of cognitive impairment between the studies difficult. The different studies also utilized different methodologies of measuring TSPO radiotracer uptake, firstly with some studies using a regions-of-interest (ROI)-based approach while others used a voxel-based analysis and secondly, different pseudo-reference regions were selected for normalization in the various studies.

Table 1: Summary of TSPO PET imaging studies in people living with HIV

	TSPO radiotracer	Participants (mean age in years, % male)	ART status	Plasma HIV RNA in PWH	Cognitive status of PWH	TSPO radiotracer uptake measurement methodology	Reference region	Significant findings of cognitively unimpaired PWH versus CP ($P < 0.05$)	Cognitive domains significantly correlating with TSPO radiotracer uptake in PWH ($P < 0.05$)
Hammoud *et al.*, 2005	[^{11}C] PK11195	10 PWH (45, 100%) 5 CP (41, 80%)	7 on ART, 3 ART-naive	NA	5 unimpaired, 5 impaired	Standardized uptake value ratio	White matter	No differences noted	NA
Wiley *et al.*, 2006	[^{11}C] PK11195	12 PWH (49, NA) 5 CP (44, NA)	All on ART	9: <50 HIV RNA cp/ml 3: >50 HIV RNA cp/ml	6 unimpaired, 6 impaired	Binding potential	Cluster with normal ligand kinetics	No differences noted	NA
Garvey *et al.*, 2014	[^{11}C] PK11195	7 PWH (48, NA) 9 CP (31, NA)	All on ART	All <50 HIV RNA cp/ml	All no symptomatic cognitive impairment	Binding potential	Normal behaving cortical grey matter	ROI analysis: No differences noted Voxel-wise analysis: corpus callosum, anterior and posterior cingulate, temporal and frontal lobes	Executive function
Coughlin *et al.*, 2014	[^{11}C] DPA-713	23 PWH (46, NA): 12 HABs 12 CP (40, NA) HABs	All on ART	All <50 HIV RNA cp/ml	8 unimpaired 12 impaired	Volume of distribution	Total grey matter	No differences noted	NA
Vera *et al.*, 2016	[^{11}C] PBR28	12 PWH (42, 100%): 8 HABs, 4 MABs 10 CP (41, 100%) HABs and MABs	All on ART	All <50 HIV RNA cp/ml	All no symptomatic cognitive impairment	Distribution volume ratio	Cortical grey matter	ROI analysis: whole brain, parietal lobe, occipital lobe, globus pallidus	Global cognition, speed, accuracy/visual learning, memory/visual learning
Rubin *et al.*, 2018	[^{11}C] DPA-713	21 PWH (48, 81%) 13 HABs No CP	All on ART	All <50 HIV RNA cp/ml	7 unimpaired 14 impaired	Volume of distribution	Total grey matter	NA	Verbal memory, processing speed/attention/concentration, executive function, working memory, motor function, visual memory, visual construction
Boerwinkle *et al.*, 2020	[^{11}C] PBR28	24 PWH (57, 75%) 13 HABs 13 CP (59, 69%) HABs	All on ART	All <50 HIV RNA cp/ml	All no symptomatic cognitive impairment	Binding potential	Cerebellum cortex, total grey matter, unsegmented white matter	No differences noted	Global cognition, executive function

CP, control participants; cp/ml, copies per 1 ml plasma; MABs, medium-affinity binders; NA, not available; PWH, people with HIV.

Overall, varying results have been reported when comparing TSPO radiotracer uptake amongst people with HIV compared to control participants (Table 1). Hammoud *et al.* [69], Wiley *et al.* [70], Garvey *et al.* [71] (using the ROI approach), Coughlin *et al.* [72], and Boerwinkle *et al.* [73] observed no significant difference in TSPO radiotracer uptake amongst cognitively unimpaired people living with HIV compared to control participants. However, in studies comparing cognitively unimpaired people living with HIV compared with control participants, Garvey *et al.* [71] demonstrated increased [^{11}C] PK11195 uptake using the voxel-based analysis within the corpus callosum, anterior and posterior cingulate, temporal cortex, and frontal cortex and Vera *et al.* reported increased [^{11}C] PBR28 uptake in the parietal lobe, occipital lobe, and globus pallidus using the ROI approach [74].

When comparing people living with HIV with and without cognitive impairment, Wiley *et al.* [70] and Hammoud *et al.* [69] observed no significant difference in [^{11}C] PK11195 radiotracer uptake; however, Coughlin *et al.* [72] demonstrated increased [^{11}C] DPA713 radiotracer uptake in the frontal cortex of cognitively impaired people with HIV. In addition, when comparing cognitively impaired people with HIV with control participants, Wiley *et al.* [70] observed no significant differences in TSPO radiotracer uptake while Hammoud *et al.* [69] noted increased [^{11}C] PK11195 radiotracer uptake in the thalamus, putamen, frontal cortex, temporal cortex, and occipital cortex in the people living with HIV.

With regards to the associations between cognitive function domains and TSPO radiotracer uptake, inconsistent associations between the different brain regions and certain cognitive domains have been noted. Garvey *et al.* [71] observed a negative correlation between executive function and increased [^{11}C] PK11195 uptake in the anterior cingulate, posterior cingulated, and corpus callosum, whereas Rubin *et al.* demonstrated an association between poorer cognitive performance in the verbal memory, processing speed, attention, concentration, executive function, working memory, and motor function domains and higher [^{11}C] DPA713 uptake at the frontal, temporal, and occipital cortices and hippocampus [75]. Conversely, Vera *et al.* noted a negative correlation between global cognitive performance score and [^{11}C] PBR28 uptake in the hippocampus, amygdale, and thalamus [74]. Overall, the TSPO PET neuroimaging studies in people with HIV to date suggest that an association exists between TSPO radiotracer uptake and cognitive impairment, and the inconsistencies in the exact domains and brain regions implicated likely reflects the global, rather than focal nature of neuroinflammation in people with HIV.

TSPO PET imaging interpretation remains challenging because TSPO radiotracer uptake will depend on the inflammatory component of the disease being investigated and the phase of the disease process when imaging occurs. Until a gold-standard method of measuring TSPO radiotracer uptake is developed and accepted, comparing results from TSPO PET neuroimaging studies using different radioligands and methodologies will remain challenging. TSPO PET imaging studies are costly, which limits the study sample sizes and may explain the paucity of significant results. Fluorine-18 [^{18}F]--based agents are in development which have a longer half-life (110 minutes) and would allow wider use, compared to Carbon-11 [^{11}C]-based agents which have a 20-minute half-life and thus, require on-site synthesis and a cyclotron. TSPO radiotracers with more specificity for microglia versus astrocytes and pro- versus anti-inflammatory phenotypes are currently in development, which will develop our understanding of the immunocytes and types of responses involved in neuroinflammation.

Conclusion

Effective combination antiretroviral treatment has undoubtedly improved the prognosis for people living with HIV. However, treated people living with HIV continue to present with symptomatic cognitive disturbances that can affect quality of life. Molecular neuroimaging is advantageous over its structural neuroimaging counterparts due to its ability to detect subtle dynamic changes. However, methodological approaches to analysis need to be standardized to ensure that differences in signals observed is due to true pathology, rather than differences in the analytical methods. Further studies investigating longitudinal molecular neuroimaging in people living with HIV and novel PET radioligands highly specific for pro-inflammatory processes in activated microglia are underway, which will provide further insight into the development of cognitive impairment in people living with treated HIV.

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J.A. wrote the first draft of the manuscript. A.W. undertook substantial review of the first draft of the manuscript. Both authors approved the final version of the manuscript.

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Data availability

Not applicable.

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