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ABBREVIATIONS

Aβ – amyloid-β

ACh – acetylcholine

AchEI – cholinesterase inhibitor

AD – Alzheimer's disease

AIDS – acquired immune deficiency syndrome

ANI – asymptomatic neurocognitive impairment

APOE – apolipoprotein E

APP – amyloid precursor protein

AP1 – activator protein 1

ART – antiretroviral therapy

BACE1 – beta-site APP Converting Enzyme 1

BBB – blood- brain barrier

BOLD – blood oxygen levels dependent

cART – combination antiretroviral therapy

CDC – US Centers for Disease Control and Prevention

ChAT – choline acetyltransferase

CNS – central nervous system

COMT – catechol- O-methyl transferase

CPE – CNS Penetration Effectiveness

CSF – cerebrospinal fluid

CYP – cytochrome P

CYP2E1 – cytochrome P 450 2E1

DC-SIGN – dendritic cell-specific intercellular adhesion molecule-3- grabbing non-integrin

fMRI – functional magnetic resonance imaging

GFAP – glial fibrillary acidic protein

gp – glycoprotein

HAART – highly active antiretroviral therapy

HAD – HIV-associated dementia

HAND – HIV-associated neurocognitive disorders

HCV – hepatitis C virus

HIV – human immunodeficiency virus

HIVE – HIV encephalitis

HLA – human leukocyte antigen

IDE – insulin degrading enzyme

IFN-d – interferon-delta

IL – interleukin

IN – integrase

IRIS – immune reconstitution inflammatory syndrome

LAMP2A – lysosomal-associated membrane protein 2A

LC3II – microtubule-associated protein-1 light chain β II

LPS – lipopolysaccharide

LRP – low density lipoprotein receptor

LRRK2 – leucine-rich repeat kinase 2 gene

LTR – long terminal repeat

M – Major

MEF2C – myocyte enhancer factor 2C

meth – methamphetamine

miRNA – microRNA

MND – mild neurocognitive disorder

mPFC – medial prefrontal cortex

NAc – nucleus accumbens

NF-κB – nuclear factor-κB

NMDAR – N-methyl-D-aspartate glutamate receptor

NNRTI – non-nucleoside reverse transcriptase inhibitor

NO – nitric oxide

NRTI – nucleoside reverse transcriptase inhibitor

NtRTI – nucleotide reverse transcriptase inhibitor

O – Outlier

PBMC – peripheral blood mononuclear cell

PD – Parkinson's disease

PFC – prefrontal cortex

PI – protease inhibitor

PLHIV – people living with HIV

PR – protease

PSEN1 – presenilin 1

PSEN2 – presenilin 2

p-Tau – phosphorylated Tau

ROS – reactive oxygen species

RT – reverse transcriptase

SIVE – simian immunodeficiency virus encephalitis

SNCA – alpha-synuclein gene

SP1 – specificity protein 1

SQSTM1 – sequestosome 1

TNF-α – tumor necrosis factor-alpha

t-Tau – total Tau

UNAIDS – United Nations Declarations and Goals

WHO – World Health Organization

RESUMO

O síndrome da imunodeficiência adquirida (SIDA) é uma doença incurável que afeta milhões de pessoas em todo o mundo e é causada pelo vírus da imunodeficiência humana (VIH). 50% dos pacientes com VIH podem desenvolver uma patologia em paralelo, designada doenças neurocognitivas associadas ao VIH (do inglês HAND). O objetivo deste trabalho é destacar os mecanismos associados ao VIH que podem levar a défices neurocognitivos e também, como comportamentos lesivos, especialmente o abuso de drogas podem desencadear essas mudanças. Além disso, é fornecido aqui uma visão geral de como o tratamento crónico anti-retrovírico pode contribuir para este resultado. A população idosa infetada pelo VIH é particularmente suscetível ao desenvolvimento destas doenças neurológicas ou neurodegenerativas relacionadas com a idade, como é o caso da doença de Alzheimer (DA) e da doença de Parkinson (PD). Esta evidência possivelmente ocorre devido ao efeito sinérgico entre o processo de envelhecimento natural e o vírus. Portanto, as mudanças fisiológicas naturais do envelhecimento, juntamente com a polifarmácia, os efeitos colaterais cumulativos, a baixa penetração no sistema nervoso central (SNC) pelos agentes anti-retrovirais e a inibição da P-glicoproteina, por exemplo, podem potenciar os efeitos do VIH em alterações neurocognitivas. Assim, as partículas virais e os vários processos desencadeados pela infeção que são exacerbados pelas drogas de abuso, podem também contribuir para a neurotoxicidade. O aumento da severidade da disfunção cognitiva está intimamente relacionado com o aumento da morbidade e mortalidade na população infetada pelo VIH.

PALAVRAS-CHAVE: SIDA, VIH, envelhecimento, tratamento anti-retrovírico, doenças neurocognitivas associadas ao VIH, drogas de abuso, doença de Alzheimer, doença de Parkinson.

ABSTRACT

Acquired immune deficiency syndrome (AIDS) is an incurable disease that affects millions of people worldwide and is caused by the human immunodeficiency virus (HIV). 50% of HIV patients may develop a parallel pathology, called HIV-associated neurocognitive disorders (HAND). The aim of this work is to highlight the mechanisms associated with HIV that may lead to neurocognitive deficits and also, how injurious behaviours, particularly drug abuse, may trigger these changes. Furthermore, it is provided here a broad overview of how chronic antiretroviral therapy (ART) may contribute to this outcome. The elderly HIV-infected population is particularly susceptible to the development of these neurological or even neurodegenerative age-related diseases, as is the case of Alzheimer's disease (AD) and Parkinson's disease (PD). This evidence possibly occurs due to the synergistic effect between natural ageing process and the virus. Therefore, natural physiological changes of aging, together with polypharmacy, cumulative side effects, low penetration in the central nervous system (CNS) of antiretroviral agents and inhibition of P-glycoprotein, for example, can potentiate the effects of HIV in neurocognitive abnormalities. Thus, viral particles and several processes triggered by the infection that may be exacerbated by drugs of abuse, also can contribute to neurotoxicity. The increased severity of cognitive dysfunction is closely related to increased morbidity and mortality in HIV-infected population.

KEYWORDS: AIDS, HIV, ageing, antiretroviral therapy, HIV-associated neurocognitive disorders, drugs of abuse, Alzheimer's disease, Parkinson's disease.

INTRODUCTION

Acquired immune deficiency syndrome (AIDS) is a severe and incurable disease caused by a specific agent, the human immunodeficiency virus (HIV), which dramatically damages the organism immune defences, exposing it to opportunistic complications [1]. According to most recent data from UNAIDS in 2014, 36.9 million people around the world were infected with HIV [2]. Since the discovery of the HIV in 1983, more than 30 million people died from the infection itself, leading AIDS to be considered one of worst global pandemics [3].

After introduction of the antiretroviral therapy (ART), the natural correlation between HIV-related diseases with accelerated and inevitable death has been changing. Most people living with HIV (PLHIV) or at risk of contracting the infection, inhabit at low or middle-income countries, and their access to prevention, care and treatment are underrepresented relative to their burden of disease; however, in the last decade, the number of people with HIV receiving treatment in resource-poor countries has significantly increased. With the worldwide expansion of ART, 15.8 million people were receiving treatment in June 2015, which contributed to a healthier life during longer periods. This correlates with an increased prevalence of HIV infection among PLHIV, from 31 million in 2002 to 36.9 million in 2014. On the other hand, the incidence of newly infected people with HIV had declined, from 3.3 million in 2002 to 2 million in 2014 (Figure 1), due not only to the lower transmission of the virus and to ART, but also to a better knowledge about HIV infection and preventive strategies [1, 2, 4]. With the change in HIV global epidemiology by the application of ART, millions of infected people are now ageing with the disease, even though they are being affected by other diseases associated with ageing. Among them, neurocognitive-associated impairments are probable to arise. Clinical manifestations of HIV-associated neurologic

debilities can range from subtle changes in asymptomatic HIV-positive patients to a substantial neurocognitive impairment in patients treated chronically. Neurodegenerative diseases, such as Alzheimer's or Parkinson's diseases (AD, PD respectively) are debilitating and incurable conditions that result in the progressive degeneration and/or death of neurons and whose prevalence has increased, also due to the longer life expectancy. In the past few years, there has been an interest in the effects of HIV in the central nervous system (CNS) and an increasing, despite limited, research to understand how HIV infection can trigger a neurocognitive disease. Indeed, HIV-associated neurocognitive disorders (HAND) emerge in infected individuals treated effectively by ART. These comorbidities change cognitive, motor and behavioural domains in people's lives, increasing substantially morbidity and mortality rates. However, at present, antiretroviral agents are the only resource available for treatment of patients affected by HAND [5].

Currently, HIV infection has no cure and has a great impact on a global scale, as well as the various pathologies affecting the CNS. Therefore, there is a great interest in exploring the association between these two types of diseases. Our attention will be focused in data concerning aged long-term HIV infected patients with growing cognitive impairments and in the interactions between the progression of HIV infection or the ART and neurological disorders including neurodegenerative diseases.

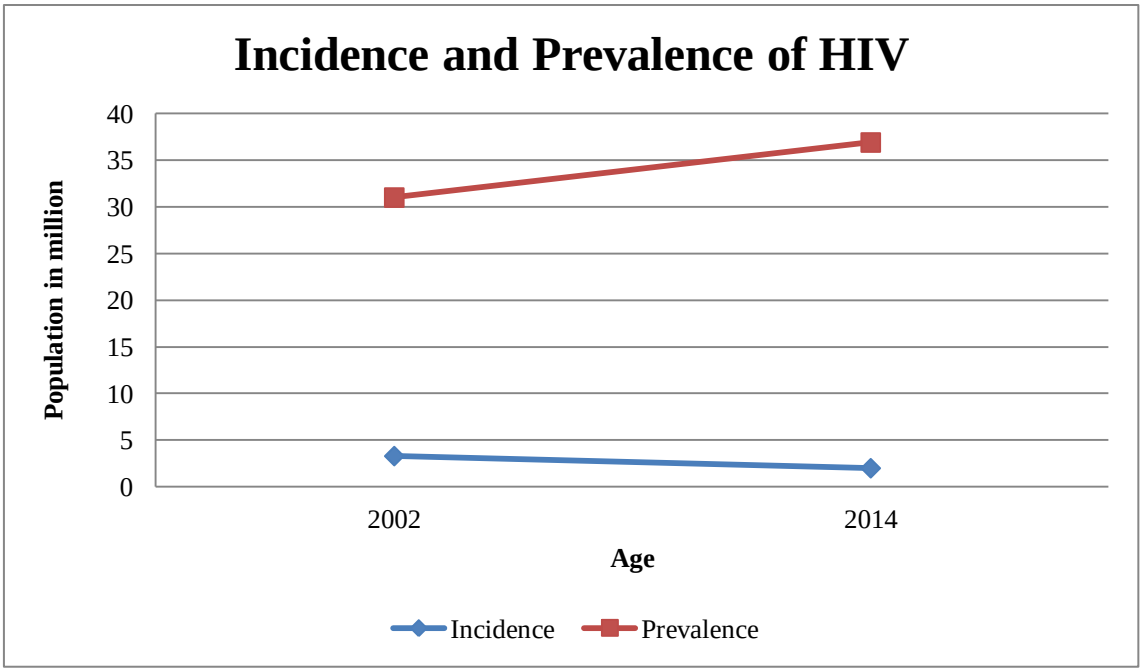

Figure 1 – Comparison of the increasing prevalence and decreasing incidence of HIV infection between 2002 and 2014.

MATERIAL AND METHODS

This literature review was based on scientific articles (reviews and original) published between 2005 and 2016. The search was conducted in the scientific and electronic database Medline (PubMed); using the keywords: AIDS, HIV, ageing, antiretroviral therapy, HIV-associated neurocognitive disorders, drugs of abuse, Alzheimer's disease, Parkinson's disease, separately and combined together, at first, to generally describe HIV and AIDS and then to disclose the link between HIV and neurocognitive-related impairments. The filter "Published in the last 10 years" was also used to gather information to write this manuscript. Despite having given more importance to articles from the last five years, information that revealed relevant from older articles was also taken into account. Other scientific databases as B-On and ClinicalKey were also consulted.

HIV DIVERSITY

There are two types of HIV known to cause AIDS, HIV-1 and HIV-2. The original viruses, the so-called simian immunodeficiency viruses, were transmitted from African primates to human species. Consequently, over several years, the viruses adapted to humans and began to disperse from individual to individual and then, from country to country [3].

HIV-1 is divided in major groups, M (Major), O (Outlier) and N (non-M, non-O) and P. Groups O, N and P are restricted to Central Africa. In contrast, group M exhibits a global widespread dispersion and is the principal responsible for HIV pandemic [4, 6]. HIV-2 is largely confined to West Africa and some societies in Europe, like Portugal,

with socioeconomic links to this African region. The opposite happens with HIV-1, which has a worldwide spread and causes almost all HIV infections [7].

Overall, both viruses share similarities including their basic genetic intracellular mechanisms of replication, modes of transmission (sexual contact, needle-sharing and transfusion, mother-to-child) and clinical outcomes, but, indeed, among them there are crucial differences.

HIV-2 causes a more slow progression of the disease and is less virulent, and therefore, frequently long-term non-progressors are noticed in HIV-2 infection. Also, HIV-2 has lower transmission rates, while HIV-1 has a general greater infectious component. Once progression occurred, the clinical manifestations, the range of opportunistic infections and the severity of AIDS are quite similar [4, 7, 8]. However, the evolution of HIV-2- related illness occurs with higher CD4+ counts and lower viral load than in HIV-1 infection, with these patients living longer. Furthermore, the transmission of HIV infection has been shown to be dependent on plasma viral load and CD4+ T-cell counts, which explains why HIV-2 is less transmissible from person to person. Thus, infected people with HIV-2 have a better prognosis due to a lesser virulence and to a better immune response from the host. Survival and mortality in HIV infection seem to be dependent on CD4+ count and plasma viral load [7]. In general, regarding the response of the virus to ART, HIV-1 and HIV-2 react similarly [6, 7].

HIV – THE VIRUS

HIV is a member of the viral family *Retroviridae* (retroviruses) that belongs to the sub-family *Retrovirinae*, genus *Lentivirus* [9, 10, 11, 12]. The term lentivirus means “slow virus” because since the beginning of the infection until the appearance of the

first symptoms it takes a long period [11]. These viruses are characterized by their use of viral reverse transcriptase (RT) and integrase (IN) for stable insertion of viral genomic information into the host genome. Lentiviruses can also replicate in non-dividing cells in their specific hosts, leading to slowly progressive diseases, including immunodeficiency, anaemia, pneumonitis and encephalitis [12].

HIV is structurally characterized by an envelope and an associated matrix wrapping a capsid, which itself involves the genome constituted by two single RNA chains and several enzymes [10]. The HIV envelope constitutes the outer coat and is composed by two lipid layers. This viral envelope is formed by different proteins; the glycoprotein (gp) 120 in the outside of the envelope that is essential to the virus to attach to the host cell and gp41, which is critical for the cell fusion process. The viral matrix protein, consisting of the p17 protein, extends between the envelope and the core [12]. HIV core involves the viral capsule protein p24, which in turn, surrounds two single strands of HIV RNA and the viral enzymes [10] (Figure 2).

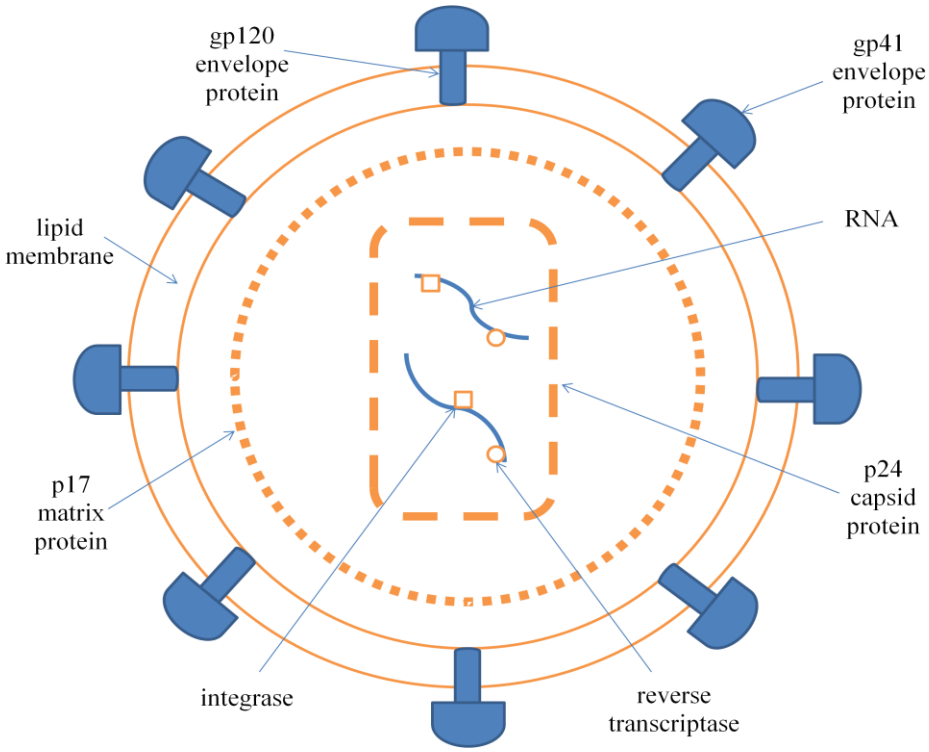

Figure 2 – Schematic representation of the HIV virion structure

The HIV virus is constituted by two single-stranded viral RNAs, RT, IN and capsid protein p24. Inner viral membrane and outer viral membranes are coated with matrix protein p17 and lipid membrane together with gp41 and gp120, respectively.

The viral genome contains three major genes encoding the three major structural proteins: Gag, Pol and Env, as well as essential enzymes that are found in all retroviruses [9, 12]. The gag gene provides the basic physical infrastructure of the virus, codes for the precursor Gag polyprotein which is processed by HIV protease (PR), originating the matrix protein p17, the capsid protein p24, among other core proteins [12]. Pol encodes for viral enzymes, namely RT, IN and HIV PR. HIV PR cleaves the precursor Gag polyprotein to produce structural proteins [12]; RT is required to transform RNA in viral DNA and IN is necessary to incorporate the double-stranded viral genome into the host DNA [9, 12]. The env gene encodes for gp160, a viral glycoprotein responsible for the synthesis of the viral envelope. Gp160 is cleaved by a host protease, resulting in a surface glycoprotein, gp120 or SU and a transmembrane glycoprotein or gp41, located in the viral envelope [9, 12]. Both gp120 and gp41 have a major role in the attachment and fusion of the virus with target cells [12]. These major proteins produce the structure of the viral core, called the virion [12]. In addition, HIV has several unique non-structural genes that encode proteins which have regulatory and auxiliary functions. HIV has two important regulatory elements: Tat and Rev and essential accessory proteins such as Nef, Vpr, Vif and Vpu [9, 12] (Figure 3).

Tat, or HIV transactivator of transcription, binds near the 5' LTR (long terminal repeat) region, activating the reverse transcription of the viral genome composed of RNA, ensures synthesis of viral mRNAs and high levels of viral protein production [9]. Furthermore, it regulates the release of virions from infected cells. Rev protein is important for the transport of viral mRNA from the nucleus to the cytoplasm and for the synthesis of major viral proteins and is hence essential for viral replication [9].

Regarding the accessory regulatory proteins, Vpr plays an important role in the replication of the virus through nuclear import of the preintegration complex [10]; Vif

improves the infectivity of HIV virions in lymphocytes and macrophages and Nef is involved in multiple functions during the replication cycle of the virus. It is believed to have a role in cell apoptosis and in the increase of virus infectivity [12]. Finally, Vpu is implicated in the successful release of virions from infected cells, as well as in CD4+ degradation involving the ubiquitin-proteasome pathway [12].

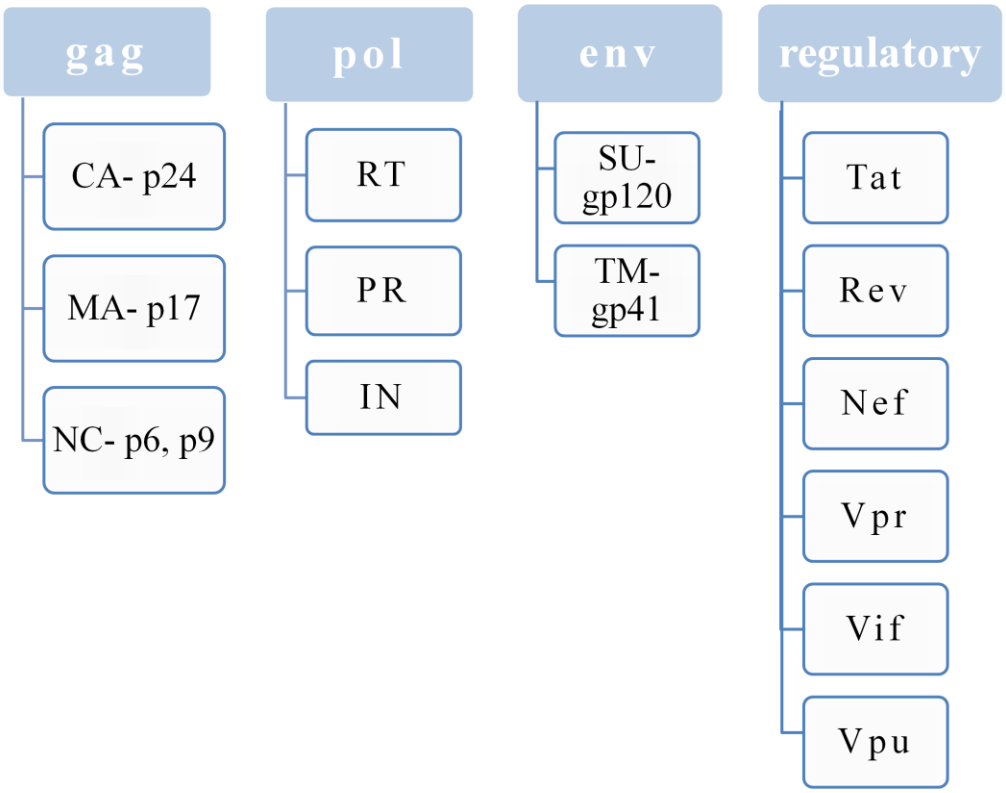

Figure 3 Schematic representation of the HIV viral genome

The viral genome encodes three structural (Gag, Pol and Env), regulatory (Rev and Tat) and accessory (Vif, Vpr, Vpu and Nef) proteins. The Gag precursor consists of the matrix, capsid protein and nucleocapsid proteins. The Pol precursor protein encodes three essential replication enzymes: RT, IN and PR. The Env precursor of HIV encodes the polyprotein envelope precursor (gp160), which is eventually cleaved by PR to generate viral SU and TM domains.

HIV reproduction involves essential steps, such as binding, fusion, viral entry, transcription, integration, replication, processing, budding, and maturation of the virus [4]. HIV life cycle begins when gp120 binds to a CD4+ receptor and to a co-receptor CXCR4 or CCR5 [9]. Thus, HIV infects mainly CD4+ cells [9], particularly T

lymphocytes, which have CXCR4 as co-receptors; monocytes, macrophages, dendritic cells and microglia which have the CCR5 co-receptor [12]. After this linkage, gp41 helps in the process of viral fusion with the host cell. HIV enters into the cell and releases its RNA genome into the cytoplasm [10]. Once infected, the cell turns into an HIV-replicating cell and loses its functions in the human immune system. HIV RT produces double-stranded DNA from viral RNA. Then, viral IN introduces pro-viral DNA into the host nucleus and incorporates the genetic material of the virus into the host genomic material [10], which structure is named "provirus" [11]. Once the viral genome is included into the host DNA, HIV can be transcribed using the cell machinery into new RNA molecules and viral proteins, which are managed into infectious particles and released from host cells to infect other cells and start a new life cycle [10]. The promoter site for transcription is positioned at the LTR at the 5'-end of the genome. A single infected cell can produce thousands of toxic viral particles [10, 12].

AGEING WITH HIV

The World Health Organization (WHO) and nearly all healthcare professionals defines elderly people as those aged 60 years or older. However, when infected with HIV, the US Centers for Disease Control and Prevention (CDC) classify elderly people as those aged 50 years or older. By 2025, about 2 billion old-aged people are expected globally, the majority of them, living in developing countries [5]. The ageing of the world's population is one of the most significant demographic trends of this era. This phenomenon is mainly occurring because in general, there are better conditions in everyday´s life, including superior health services complemented by improved care, treatment and follow-up systems and new treatment drugs with less or any side effects.

Despite all these upgrades, the rising of world's elderly populations are carrying new age-related diseases, such as AD and PD, whose incidence and prevalence are increasing [13, 14].

This demographic shift is also observed in HIV-positive patients, where the number of people aged 50 and older is increasing. In general, this can be largely, attributed to ART, which has been quite successful in prolonging and improving the quality of life of these patients. Currently, the life expectancy of an HIV-infected patient is considered similar to a healthy person whether achieved and maintained normal CD4+ counts [5]. Therefore, the prognosis of HIV-infected individuals suffered great advances. Additionally, older patients have good immunologic (CD4+ counts) and viral (viral loads) responses to highly active antiretroviral therapy (HAART) [15] although not so efficient as the younger counterparts [16]. Moreover, the immunologic response was shown to be inferior in patients over the age of 50, occurring a faster clinical progression, and still the viral response in these subjects is superior due to better adherence to antiretroviral treatment [17, 18]. Patients who positively respond to antiretroviral treatment are dying not due to AIDS, but due to non-HIV-related illnesses, such as non-AIDS malignancies including liver, anal and lung cancer and Hodgkin's lymphoma (23.5%), cardiovascular diseases (15.7%) and liver diseases (14.1%) [4, 5].

Interestingly, age-related neurodegenerative disorders, such as AD and PD, and HAND have some resemblance in their clinical complaints and neuropsychological patterns. In fact, several studies have demonstrated that there is an overlap in the brain tissue damage between ageing and the virus [19]. Additionally, both seem to be correlated with deterioration of the blood–brain barrier (BBB) and dopamine deficiency. Ageing can dysregulate the cellular homeostasis, thereby spoiling the cellular elimination of toxins as well as it is associated to an inflammatory status and elevated

plasma lipopolysaccharide (LPS) levels, similar to what happens in HIV infection [19]. Therefore, one can infer that age-related events combined with direct or indirect consequence of the HIV infection itself in the brain may act synergistically resulting in neurological impairments [20].

It is common to find a cognitively impaired population in an aged group and likewise, older individuals living with HIV, can present a scenario in which degeneration may occur, making them vulnerable to impairment of neurocognitive capacities. The effects of increased age linked to longer periods of HIV infection, HIV-related pathogenic factors, behaviours and morbidities and ART secondary effects, may probably be associated to a negative impact in cognitive domains. Antivirals toxicity is frequently observed in older patients, on one hand due to the fragility and weakness of the human organism and on the other hand due to the high quantity of medication that these patients take to control their chronic conditions. Therefore, the probability of occurrence of pharmacological interactions increases [18]. Indeed, older HIV-infected adults have worse cognitive abilities compared to the same-aged peers without HIV and younger adults with HIV [21].

Furthermore, HAND might be recognized as an accelerator of the ageing process due to the early onset of the decline of cognitive abilities. In HIV infected patients is commonly observed chronic inflammation, increased oxidative stress levels, hyper coagulation, multimorbidity and there are also other factors, such as substance abuse, contributing to the development of early ageing [22]. This is evidenced by recent studies on age-related markers such as phosphorylated Tau (p-Tau) protein, Amyloid and α-synuclein, showing that the levels of these latter in the cerebrospinal fluid (CSF) of some PLHIV are comparable to non-infected subjects 15 to 20 years older [23, 24].

Since the discovery of HIV pandemics, most of the available information about HIV infection is directed for younger groups, however, with the change of the proportion of disease burden to patients over the age of 50, there is a need to review the knowledge and to consider the intersection between age-related processes with HIV infection that may contribute to neurodegenerative impairment.

HIV IN THE CNS

HIV is a neurotropic virus, meaning it has the capacity to infect the brain and impair CNS functions [25]. Several studies have shown that HIV has been frequently detected in CSF and brain tissue from the beginning of the infection and throughout its evolution, regardless of the neurological symptoms. Particularly, in the first two weeks of HIV infection, the virus is able to penetrate CNS by the "Trojan horse" mechanism, where it is able to cross the BBB through HIV infected monocytes and lymphocytes [23]. In the brain, HIV infected monocytes differentiate into macrophages. These latter will then infect other cells in the CNS, such as microglia and perivascular macrophages. Neuronal cells are not directly infected by HIV itself, but neuronal damage and death are the result of secondary events following HIV infected macrophages and glial cells, the most commonly infected cells in the brain [5, 23, 24]. Furthermore, these cells become the preferred cellular deposits of viral replication and the chronic containers of HIV in the CNS [3, 5, 26]. As a result of HIV replication, activated macrophages and microglia express neurotoxic molecules, which among other purposes, activate astrocytes leading to increased BBB permeability and further monocyte and lymphocyte migration into the brain [23] (Figure 4). These astrocytes, other important cells affected by HIV, represent 85% of all the constituents of the brain and execute indispensable

functions, such as maintenance of BBB and support of neuronal communication. As a consequence, they directly interfere with the structure and function of the synapse and glutamate re-uptake [23, 26], important processes involved in normal neurologic function.

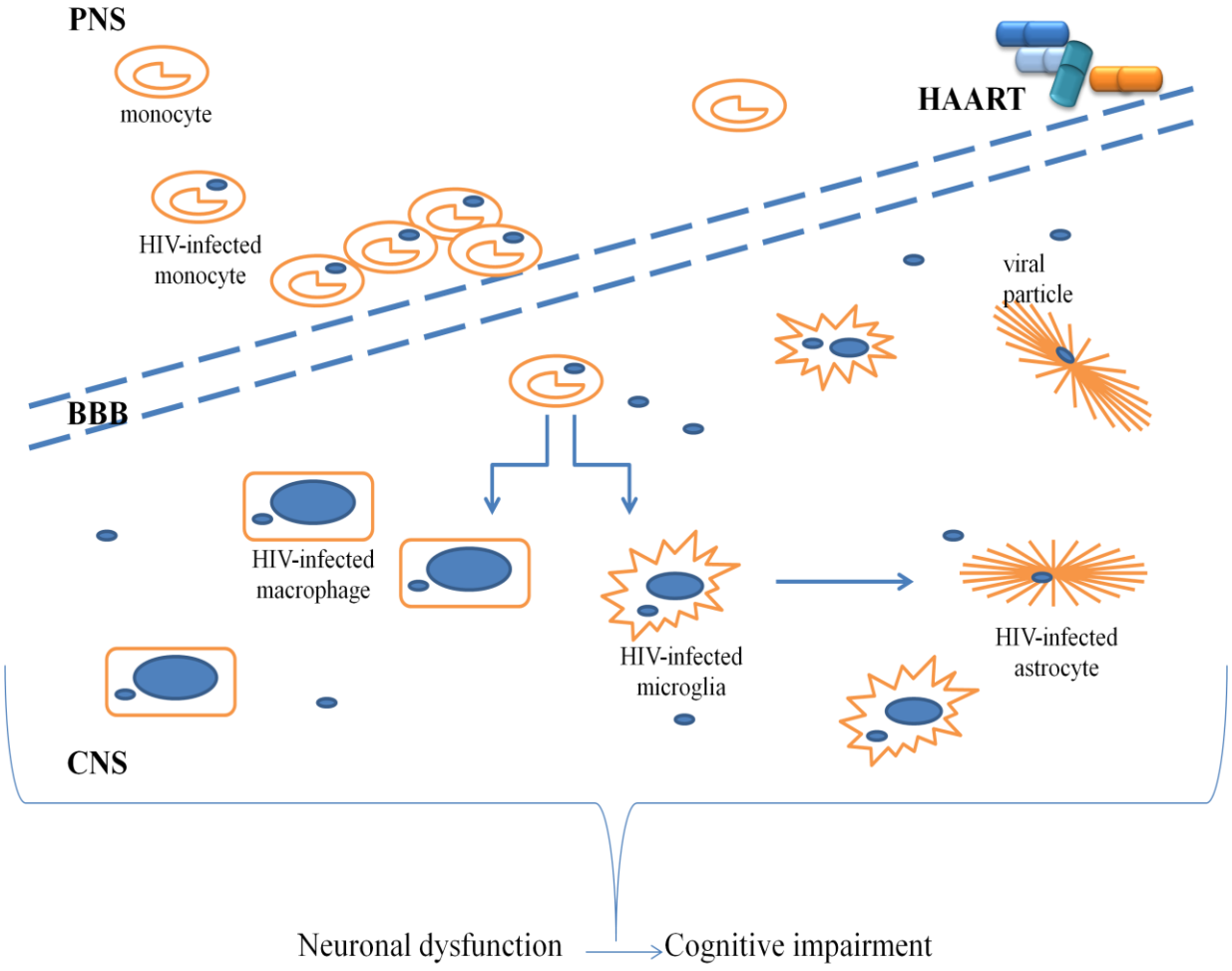

Figure 4 – Schematic representation of the "Trojan" horse mechanism and consequent HIV infection of the CNS

Potential HIV-specific mechanisms of CNS injury despite HAART. HIV virus infects circulating monocytes, some of which may cross the BBB. CNS virus may not be adequately suppressed by combination antiretroviral therapy (cART) due to decreased CNS Penetration Effectiveness (CPE). These mechanisms result in infection and inflammation of the brain, leading to astrocyte, macrophage, and microglial activation, neuronal dysfunction, and cognitive impairment.

There are two models, the direct and the indirect, that explain the neurodegeneration and the spectrum of neurological symptoms associated to HIV infection [6, 23, 24, 27]. The direct model suggests that viral proteins such as gp120, Tat and Vpr, are released from infected monocyte-derived cells (macrophages, microglia and astrocytes) and constitute the main source of neurotoxicity through direct interaction of viral proteins with neurons [5, 6, 23]. On the other hand, the indirect model relies on a cascade of inflammatory events initiated by infected macrophages and microglia that secrete a wide range of neurotoxins, such as chemokines, pro-inflammatory cytokines (tumor necrosis factor-alpha (TNF-α) and interferon-delta (IFN-d)), quinolinic and arachidonic acids, nitric oxide (NO), among other substances. Additionally, some inflammatory mediators impair the neuroprotective functions of astrocytes and increase the rates of astrocytic apoptosis, leading to defective glutamate re-uptake from synapses. This will induce the release of excessive glutamate, therefore creating an extended activation of N-methyl-D-aspartate glutamate receptors (NMDARs), and consequently, raised intraneuronal calcium concentrations, which can reach toxic levels. All these intracellular reactions may result in the production of reactive oxygen species (ROS) and nitric oxide (NO), contributing to neuronal death [5, 23, 26]. In summary, the indirect model suggests that neuronal injury is mediated by the inflammatory response of the host organism against HIV infection in the brain tissue. Both models may demystify the pathogenesis of HAND, as it has been being attributed an important role to viral particles and prolonged brain inflammation [28]. Thus, this may culminate in apoptosis of neuronal cells and correlate to the development of neurocognitive impairment associated to HIV [5, 23, 26] (Figure 5).

Recently, some studies in animal models using elevated concentrations of bacterial products such as LPS (involved in neuroinflammation and neuronal loss),

demonstrated a triggered cognitive decline through similar inflammatory processes observed in chronic HIV infection [23, 28]. Furthermore, systemic infections or inflammatory states in mice express higher levels of circulating pro-inflammatory cytokines, as observed in HIV infected patients with cognitive dysfunction [29]. Furthermore, it has been demonstrated that microglia can be activated in response to inflammatory stimuli from the periphery. Once activated, microglia produces neurotoxins that impair the function of neurons and other glial cells, resulting in neurodegeneration [28]. These data elucidate about the active communication between the peripheral immune system and brain and also about the significant effects of systemic inflammation on brain damage.

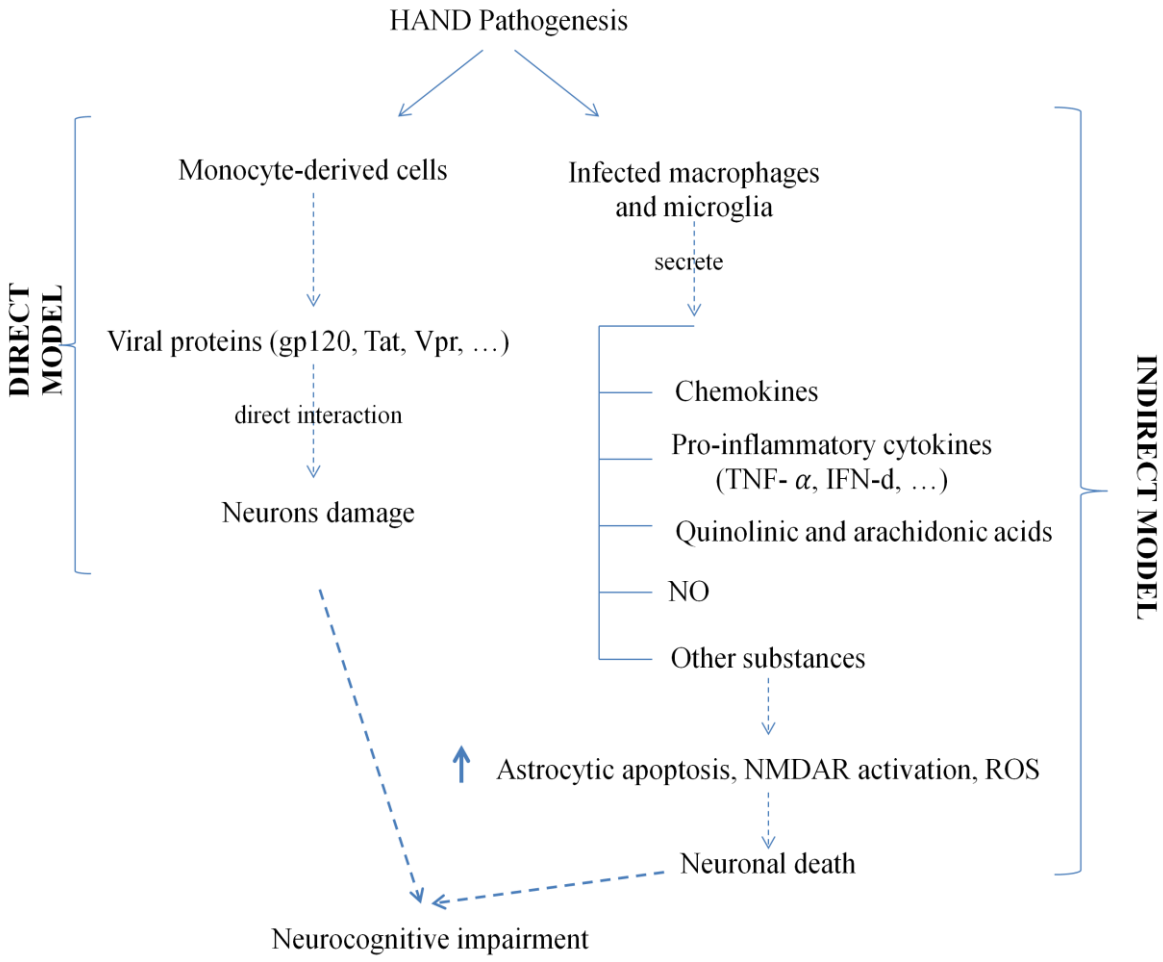

Figure 5 – The direct and the indirect models leading to neuronal injury and consequently neuronal death, a probabe explanation to HAND pathogenesis.

HIV-ASSOCIATED NEUROCOGNITIVE DISORDERS

DEFINITION

HAND are clinically characterized by cognitive, motor and behavioural abnormalities [26]. In 2007, the American Academy of Neurology formulated new and improved criteria – the Frascati criteria [8, 25], which classified HAND into three subclasses: asymptomatic neurocognitive impairment (ANI), mild neurocognitive disorder (MND) and HIV-associated dementia (HAD), sorted in ascending order of neurological disability severity [18, 28, 30] (Figure 6). HIV-related neurocognitive impairments are usually confirmed by neuropsychological, neuroimaging and CSF tests. HAND postulates the absence of a pre-existing cause, and the associated neurocognitive debility is triggered in all or most cases by HIV infection [5]. The spectrum of cognitive disorders generated by HIV gives information about the presence and severity of cognitive impairment, as well as the presence and degree of the functional decline. The subclasses are based on dysfunctional neurological domains and the most frequently involved are language, memory, including learning and recall, information processing, simple or complex motor skills, attention, abstraction skills, sensory perception abilities, among others [5, 6]. ANI is defined by any level of neuropsychologic testing impairment in at least two cognitive domains mentioned above, but without any functional abnormalities in every day´s activities [30]. MND is diagnosed by mild to moderate impairment in at least two cognitive domains on neuropsychologic testing and is typically associated with mild to moderate impairment of function of daily activities. The category designated as HIV-associated dementia or HAD is defined by severe damage in at least two cognitive domains and is associated with severe functional impairment, resulting in a significant impact in life's quality and day-to-day, social and occupational functioning [13, 5, 24, 30].

The thought that HAD can be a later and severe stage of MND is common, however, there are no data supporting this theory. Indeed, lesser HAD outcomes in the HAART era and the fluctuating course of HAND with some patients showing cognitive, motor and behavioural improvements, do not suggest that HAD is a universal progressive event of MND [24]. Instead, a recent immunohistological study showed inflammation in brain tissue of HIV infected patients with different degrees of neurocognitive dysfunction that were under active ART [28]. Taking into account that HAND is a continuum of the same disease, one could infer that progressing into the severest subclasses over time, together with the inflammatory process, constitute the main reasons responsible for the progression of the disease, taking into account that viral replication is suppressed by ART [28]. Thus, it remains, unclear why some individuals develop the most severe forms of HAND, while the majority of HIV-infected people do not, because until the present, it is not known whether the pathogenic pathways of MND and HAD overlap or if they are really distinct [24].

Not all PLHIV develop dysfunctional cognitive abilities. There are other factors related to host and viral contributors that make a few number of infected people more susceptible to develop HAND [5, 23]. The major host factors that have been associated to an increasing risk of developing neurocognitive impairments refer to low CD4+ count and high plasma and CSF viraemia at the same or at greater levels compared to plasma [5, 6]. In addition, other factors such as hepatitis C virus (HCV) infection, which is an independent risk factor for developing HAND can also over activate infected microglia cells and consequently compromise some cognitive abilities [21]. Nevertheless, people co-infected with HIV and HCV display higher numbers of neurocognitive ineffectiveness than people infected by HIV or HCV alone [24]. Drug consumption of substances, which is a common practice in some HIV-infected

individuals, may cause persistent adverse effects in neurocognitive function. These drugs (e.g. methamphetamine and cocaine) increase BBB permeability to neurotoxic viral proteins and increase oxidative stress in the brain. On the other hand, drug abusers have usually lower adherence to ART [24]. Other host factors include genetic predisposition, like APOE (apolipoprotein E) 4 allele, MBL-2 O/O haplotypes and polymorphisms in the MCP-1 gene, which have been associated with neurocognitve impairment in HIV-infected patients [5, 23]. More studies are needed to clarify the role of host genotypes. Similarly, metabolic comorbidities such as insulin resistance and diabetes, together with other factors, namely cardiovascular risk factors, vascular disease, anaemia, malnutrition, immune reconstitution syndrome, neurotoxic treatments including ART, head injury, cerebral opportunistic infections and tumours, also appear to be associated to increased rates of cognitive impairment in HIV-infected individuals. [5, 23] Besides that, HAND can also result from mental instability caused by AIDS diagnosis, as this disease still continues to be a stigmatizing and life-threatening disorder often associated with fear, guilt, and uncertainty. Furthermore, AIDS is frequently correlated with intense stress, anxiety, depression and even suicidal ideation, which altogether have been shown as important factors to contribute negatively to cognitive functioning [21]. There is also a growing interest in unveiling HIV factors associated with the development of HAND, including HIV sub-types, their stage of infection and immune activation, HIV neuroadaptation and drug resistance [23, 25]. Although the role of the virus seems to be well established in the outgrowth of neurodegenerative features, in clinical practice is still difficult to assign the degree of the impact of clinical, social, and psychological factors that may contribute to HAND [20, 28, 30].

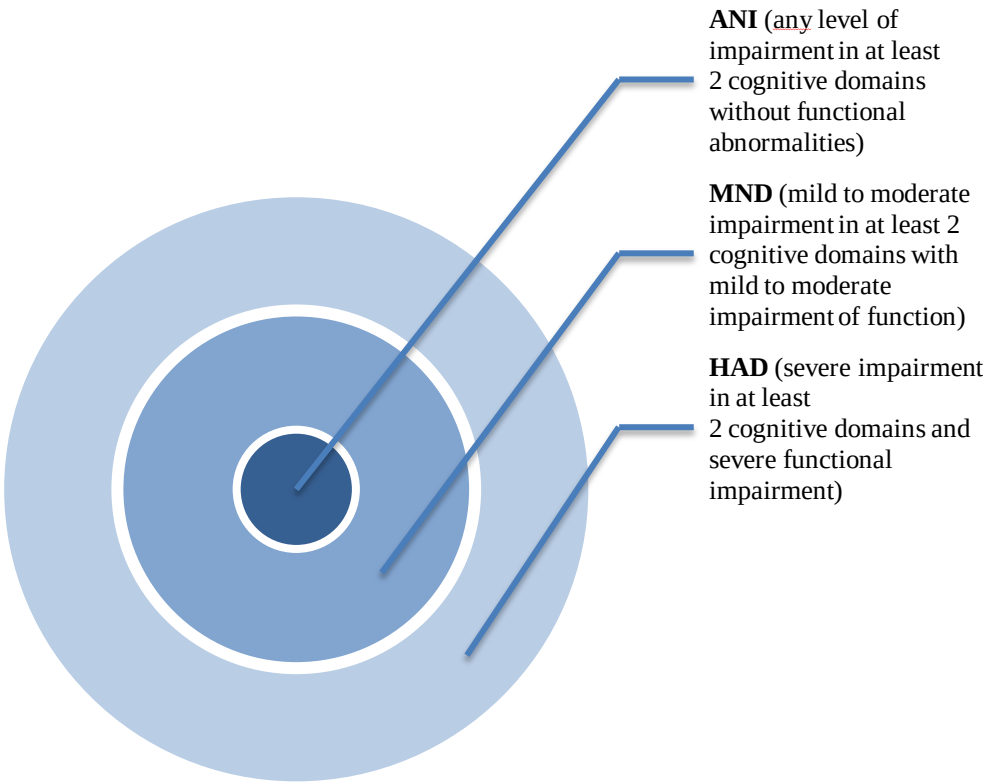

Figure 6 – HIV-associated neurocognitive disorders (HAND): Frascati criteria

HAND defines three categories of disorders: asymptomatic neurocognitive impairment (ANI), mild neurocognitive disorder (MND) and HIV-associated dementia (HAD).

EPIDEMIOLOGY

Regardless of the improved knowledge on the pathogenic mechanisms and the widespread distribution and effectiveness of HAART, complications associated to neurological and cognitive deficits still persist. The prevalence of HAND in HIV-infected patients has not changed significantly from pre- to potent ART era, remaining approximately in 50% of HIV-infected patients [23, 26, 30]. Before the introduction of ART, the prevalence of patients with HAD was around 14%, 5% for MND and 16% for ANI. During the HAART era, HAD was found to affect only 2% of the HIV-infected individuals, while MND and ANI affected approximately 10% and 32%, respectively

[23]. Dementia has been considered a late complication of HIV infection in the pre-HAART era but, nowadays, dementia still occurs mainly due to a great number of HIV-infected individuals living longer with the infection. However, the mild forms of HAND are prevalent, comparing to HAD. This suggests that HAD onset may be delayed and its severity reduced. Thus, HAART has dramatically changed the natural course of neurological manifestations of HIV infection [23].

DIAGNOSIS

HAND include a spectrum of diseases featured by cognitive, motor, affective, behavioural and social dysfunction with increasing severity. The clinical manifestations of HAND typically have an insidious course, appearing within weeks or months; the occurrence of acute symptoms points out to another etiology. The initial stage of neurodegenerative impairments may be subtle or present non-specific symptoms and therefore, they can be easily overlooked or difficult to distinguish from other disorders, leading to misdiagnosis. Moreover, the lack of awareness about cognitive difficulties presented by patients, which symptoms cannot always be easily disclosed by medical doctors, often lead to masked neurological deficiencies. Diagnosis is based on clinical history, neurological and cognitive examination, but there are no gold standard markers to confirm it, so other reversible and treatable causes should be excluded, through neuropsychological tests, neuroimaging methods and CSF analysis, before HAND diagnosis is correctly done. Therefore, HAND may be considered a diagnosis of exclusion. The differential diagnosis is broad and covers a variety of options such as CNS opportunistic infections; neoplastic, vascular, toxic-metabolic, nutritional and autoimmune brain disorders; mental retardation; depression or other psychiatric

disorders. The erroneous diagnosis of HAND can have a very significant negative impact in medical care and treatment, self-esteem, financial outcomes, functional independence and life planning of PLHIV and their closed family members [28].

CLINICAL MANIFESTATIONS

HAND show a highly variable spectrum of signs and symptoms, ranging from subtle neurocognitive changes to evident dementia [30]. Although rare, but not impossible, characteristics from unexplained dementia may be the first presenting feature of HIV infection [20]. According to anatomopathological and neuroimaging studies, HAD is a sub-cortical dementia similar to Parkinson's dementia, contrasting to the cortical dementia frequently characteristic of AD [5].

In early stages, the patient may complain about poor concentration and attention, apathy, forgetfulness, psychomotor slowing and decline in motor skills. With the disease progression, appears memory loss, impaired reading, global cognitive deterioration, poor movements, diminished handwriting, loss of coordination, personality change, mutism, incontinence, severe confusion and tremors resembling to parkinsonian features. Moreover, psychiatric symptoms such as irritability, anxiety, agitation, mania, hallucinations and paranoia can also occur in the later stages of HAND. The clinical course can be progressive, floating or static, depending whether the patients show stability for several months or years and can improve with HAART, or get worse in the presence of concomitant diseases [20, 30]. A study comparing improving, declining or fluctuating changes in the cognitive status of HIV-infected and uninfected individuals revealed that 30% of HIV-infected patients showed their

cognitive abilities altered over time, more less about twice compared to what is usually observed in HIV-uninfected patients [30].

HIV-associated neurocognitive dysfunctions can affect negatively the patients' quality of life, leading to serious personal, social, labour and economic problems. Neurocognitive impairment is associated with emotional disequilibrium, difficulty to perform complex tasks such as driving and inefficiency to perform typical work functions, resulting in higher rates of unemployment, incapacity to managing finances and decreased ability to adhere to medication [6, 20, 23, 30]. Therefore, direct or indirectly, increased rates of morbidity and mortality in HIV-infected adults may have a strict relation with HAND. Hence, clinicians are aware of the benefits of an early screening of all these patients who may present an increased risk of developing this condition [5, 23].

ANTIRETROVIRAL THERAPHY

INTRODUCTION

Since 1996, the year in which the ART became available to HIV-infected patients [17], we have observed a significant decrease in the number of deaths from this infection and, in the meantime, a longer survival rate similar to the general uninfected population [31]. Therefore, the global expansion of HIV treatment has been allowing a higher and growing number of PLHIV, even those with advanced age [32]. Nevertheless, more than 50% of the world population who is HIV-infected, still do not have access to ART, particularly in low and middle-income countries [31].

ART has been successful in diminishing the magnitude of people acquiring HIV infection and in decreasing HIV incidence however, the prevalence is increasing due to

the aforementioned reasons. In the HAART-era, HIV-infected adults display lower plasma viral load, which means, a lower viral replication and a higher CD4+ cell count. Consequently, this leads to the suppression of the viral infectiousness and transmissibility of HIV [4] and allows the human body to restore the immune system [32], which, in turn, decreases the occurrence of opportunistic infections and AIDS serious complications [33]. Approximately, more than 70% of patients on antiretroviral drugs therapy have suppressed viral loads to undetectable concentrations (<50 virus particles/ml blood) and normal CD4+ count (>500 cells/mm3 blood) [8]. In these cases, HIV-related deaths occur mostly due to non HIV-related diseases and comorbid conditions, since patients live longer [33].

Currently, ART is implemented as a preventive strategy, covering pre-exposure prophylaxis and post-exposure prophylaxis and reducing the transmission of the virus by HIV-infected people [1, 34].

Taking into account that ART changed the infection outcomes, AIDS has now become a chronic and treatable medical condition with much better prognosis instead of a fatal illness with rapid evolution [4, 23, 35]. In particular, in recent years, there were quite important scientific advances in antiretroviral regimens, which became more efficacious, with less side-effects and lower burden of tablets. Thus, ART is better tolerated by patients, although HIV infection remains an incurable disease [4, 36].

WHEN SHOULD ART START?

As mentioned before, the viral load and CD4+ cell count translate the infectiousness of the virus, besides being markers of progression, morbidity and mortality of the disease. Moreover, they are also used as parameters in the decision when to start the treatment and evaluate its effectiveness [7, 17].

The amount of CD4+ T cells bellow which the antiretroviral intervention should be started remains under discussion. Nevertheless, the actual guidelines suggest that ART should be started in patients with AIDS-defining illness, symptomatic HIV-positive patients, patients with CD4+ < 200 cells/mm3, infected pregnant women, patients co-infected with HIV/HBV, HIV-associated nephropathy and infected subjects in a serodiscordant relationship independently of CD4+ values [15, 31, 34, 37]. The WHO recommends initiating ART for HIV asymptomatic individuals with a CD4+ cell count $\leq$ 500 cells/mm3 [1, 38], because it was demonstrated that the treatment significantly reduces the morbidity and mortality rates [39] and HIV incidence [38]. One of the main reasons to avoid starting ART with a low CD4+ count is the greater likelihood of the virus to affect the CNS and the probability of the patients to develop HIV-related neuropathologies [5].

CLASSES OF DRUGS

When two or more antiretrovirals are administrated at the same time to HIV-infected patients, this is called combination antiretroviral therapy (cART). Taking a set of three or more anti-HIV drugs is sometimes referred to as HAART and has become the pillar of HIV care [27].

More than twenty-five antiretroviral drugs are presently available for HIV treatment and they mainly act by dysregulating HIV replication in different processes during the virus life cycle in human cells [4, 8, 17, 36]. Some pharmaceutical agents may interfere with RT and inhibit the synthesis of dsDNA from viral RNA – nucleoside, nucleotide and non-nucleoside RT inhibitors (NRTIs, NtRTIs and NNRTIs); others are protease inhibitors (PIs) and block the protein cleavage in the last phase of generation of mature virions; there are also the fusion inhibitors – suppress the fusion and entry of virus into the cell; CCR5 and CXCR4 antagonists are also administrated, which are inhibitors of the co-receptors CCR5 and CXCR4 necessary for HIV entry [40]; and finally the integrase inhibitors that prevent the integration of the genetic material of the virus into the host chromosomal DNA [17].

In general, the initial regimen includes 2 NRTIs associated with a NNRTI, a ritonavir-boosted PI or an IN inhibitor, which are equally well tolerated and effective [4]. The administration of only one drug to a patient may lead to potentiate a resistance mechanism by the virus, which will interfere in the intended effect of the treatment. Instead, the cART greatly reduces the resistance rate [31]. Thus, long-term treatment can also contribute to drug-resistant viral variants [31]. When treatment fails, it is usually replaced by a new combination of drugs based on resistance testing [4].

ART is a chronic treatment, which means it is necessary for the resting life. Therapeutic intervention should be individualized, taking into account the lifestyle, behavioural factors, comorbidities and other medications of each patient.

ALZHEIMER´S DISEASE

DEFINITION

AD is a complex age-related neurodegenerative disease [41, 42]. AD is the principal cause of dementia in world population, mostly affecting elderly people [41, 43]. Dementia incidence has an exponential growth with age [13], especially in the 7th and 8th decades of life, and demential population is estimated to quadruple in 2050 [13, 44]. AD clinical presentation has a great diversity and normally slow progression. The principal cognitive deficit is memory impairment, first characterized by an episodic memory, but numerous other domains are affected, such as language and nomination, visuospatial and executive functions [13]. Furthermore, this disease is accompanied by the deterioration of the capacity to perform daily functions and simple or complex tasks [13, 45]. Some patients display aberrant behaviours, namely hallucinations and delusions, occurring psychomotor excitement, dysphoria, irritability and aggression in more than 30% of the patients. Depressed mood, anxiety, anergia, anhedonia and sadness are common features of these patients and occur in 86% of the cases; however, major depression is only present in less than 15% of the cases. In the final stage of Alzheimer's dementia the patient becomes bedridden, without sphincters control, with absence of speech and unresponsive to stimulus [13]. Recently, it has been defined a pre-clinical stage known as mild cognitive impairment that corresponds to the time interval between normal cognitive functions and the presence of dementia [46].

ETIOLOGY

AD exact etiology still remains unclear [13, 44, 47]. However, it is assumed that two main proteins are responsible for initiating the neuropathological process: amyloid-β (Aβ) and Tau. Tau protein is a constituent of neurons and is linked to the stabilization of axon microtubules. Typically, in AD, Tau proteins are hyperphosphorylated and form filaments which produce intraneural insoluble deposits, called neurofibrillary tangles. Aβ proteins can undergo aggregation and originate extracellular amyloid plaques. Indeed, the pathological conformation of the Amyloid and Tau protein contributes to neuronal and synapse degeneration, mainly in the limbic and paralimbic regions [13]. The time line and the real relation between the main components of plaques and tangles, constituted by Aβ and Tau, respectively, are not fully understood. A recent hypothesis proposes that the amyloid cascade predates the events of Tau protein for many years and is responsible for its phosphorylation and initiation of Tau cascade [45, 48]. Furthermore, it may be possible that Tau and Aβ act synergistically, potentiating their neurodegenerative effects [49]. Diverse biological pathways seem to be involved in the neuropathological process of AD, although currently, the amyloid hypothesis is considered the main molecular pathogenic phenomenon [49]. According to several researches, genetic and environmental factors play an etiological role that conducts to the neurotoxicity and neurodegeneration observed in Alzheimer's dementia [47]. Besides Amyloid and Tau neurotoxicity and acetylcholine (ACh) dysfunction, other factors were associated to progressive decline in cognitive functions leading to dementia, namely the natural aging process, oxidative stress, mitochondrial dysfunction, inflammation, vascular risk factors (e.g. hypertension, diabetes, smoking, obesity and high total cholesterol levels), physical inactivity, psychosocial disorders, toxic occupational exposures, between others [13, 44, 48, 49, 50].

The APOE4 allele is considered a susceptible allele for the development of AD and it has been demonstrated to originate about 65– 75% of the cases of sporadic AD [40]. APOE4 favours the accumulation of amyloid plaques in the brain by interfering with Aβ peptides clearance. In addition, neurodegenerative mechanisms via APOE4 may be independent of Amyloid protein through BBB disruption, destruction of the brain blood vessels by dysregulation of cyclophilin A signalling mechanisms and, ultimately, the production of APOE4-induced neurotoxic metabolites [49]. The presence of APOE4 does not imply the appearance of this mental illness, but increases its probability. A great number of alleles also predispose to a greater risk to develop AD [44]. Mutations in genes coding for amyloid precursor protein (APP), presenilin 1 (PSEN1) or presenilin 2 (PSEN2) commonly lead to early-onset cases or familial AD [50, 52]. The first one (APP) is an integral transmembrane protein present in neurons and glial cells and is involved in the improvement of learning and memory processes, neuronal defense against oxidative and biological stresses, synaptic plasticity and control of neuronal excitability [52]. APP is the precursor of Aβ small peptides and when mutated, it may change Aβ cleavage and promote its aggregation. PSEN1 and PSEN2 are the catalytic subunits of the β and γ secretases, respectively, which cleave APP. Abnormal presenilins result in wrong cleavage of APP and consequently in production of toxic Aβ (1-42) peptides, accountable for senile plaques generation [49].

ACh is the most affected neurotransmitter in AD, as well as the enzyme choline acetyltransferase (ChAT). Evidences show that in the *nucleus basalis* of Meynert, the master source of ACh in neocortex, more than 90% of cholinergic neurons are damaged in AD. On the other hand, in the brain stem region, the cholinergic nuclei seem to be largely untouchable [13, 45]. The decrease in the release of ACh normally translates in an impaired memory processing [52].

TREATMENT

AD is yet an incurable disease. At the present, only 2 classes of drugs are used in clinical practice: cholinesterase inhibitors (AChEIs) – donepezil, rivastigmine, and galantamine, and the glutamate antagonist – memantine. The first ones enhance ACh in the synaptic cleft [52], leading for a short period of almost a year, to the maintenance of neurocognitive capacity and regular functioning. The glutamate antagonist memantine is commonly applied in monotherapy or in association with AChEIs and has revealed profits in the treatment of moderate-to-severe dementia [49]. Nevertheless, these drugs are incapable of slowing down AD progression or even reverse its natural history [52] and the current treatment has only palliative effects. Due to the genetic and non-genetic nature of AD, the therapeutic approach should include a healthy diet, avoiding contact with risk factors, physical and mental exercise and treatment of comorbid diseases associated to pharmacological therapy.

PARKINSON´S DISEASE

DEFINITION

PD is the second most frequent age-related neurodegenerative disorder after AD [53, 54]. It is a progressive multisystemic disease, affecting the elderly population and leading to motor impairment [55]. PD patients display a risk of developing dementia six times higher than the normal population [56]. Its clinical picture and pathophysiology are complex and diverse [57]. PD symptoms include motor and non-motor features [58]. These last ones are related with cognitive abnormalities, including disturbances in memory, attention, language, execution and visuospatial function [45, 53]. Other non-

motor features persist during the course of the disease, such as psychiatric symptoms, including depressed and anxious mood, hallucinations; autonomic dysfunction comprising gastrointestinal symptoms [57], abnormal sense of smell, sexual dysfunction, sleep difficulties, among others [55, 58, 59, 60]. It is believed that motor characteristics begin approximately 20 years after the first non-motor manifestations [61], being this interval considered as the "pre-motor" period [56, 58]. In turn, typical motor features arise at an advanced stage of PD, when the *substantia nigra* lost at least half of its dopaminergic neurons [58], and they are represented by resting tremor, postural imbalance, muscular rigidity and bradykinesia [55,58, 62].

ETIOLOGY

PD is considered a multifactorial disease. There are several environmental and genetic factors that predispose to the risk of developing this disorder [63]. Indeed, an increased number of genes has been reported to cause this disorder and are usually related with early onset cases of familial PD. Sporadic PD is usually associated to exposure to environmental factors. Nevertheless, old age is the most important risk factor [56].

The etiological basis of this disorder is characterized by a gradual depletion of dopaminergic neurons in the *substantia nigra*, specifically in the *pars compacta* and low levels of dopamine in the basal ganglia, namely in the striatum. Neuropathologically, PD is characterized by the presence of Lewy bodies mainly composed by α–synuclein protein aggregates [45, 54, 60, 64]. Thus, these PD hallmarks are exacerbated in the compromised brain areas by an inflammatory component via microglia, nitrosative and oxidative stress and mitochondrial and lysosomal dysfunction [54, 58, 60, 64, 65].

Genetic mutations have been helping in the comprehension of the mechanisms involved in PD pathogenesis [57]. Mutations in α-synuclein gene (SNCA) and leucine-rich repeat kinase 2 gene (LRRK2) originate insoluble α-synuclein aggregates constituting Lewy bodies. The mechanisms that lead to neuronal degeneration are not fully understood, however, it is suspected that α-synuclein causes cell dysfunction at mitochondrial levels, protein synthesis and transport and fusion of synaptic vesicles, inducing intraneuronal stress and thereby neurotoxicity and cell death [53, 59, 60,64, 65].

TREATMENT

PD has currently no cure, leading clinicians to focus on symptomatic therapy [57] and the main purpose became the enhancement of dopamine neurotransmitter concentrations [59]. Although pharmacological treatment does not delay the progression of the disease [58], this neurodegenerative disorder can be controlled for decades [56]. At the present, antiparkinsonian drugs are dopaminergic drugs (levodopa); central cholinergic receptor blocker (benzhexol); type B monoamine oxidase inhibitors (selegiline, rasagiline, safinamide); dopaminergic agonists (ropinirole, pramipexole and rotigotine); catechol-O-methyl transferase (COMT) inhibitors (entacapone and tolcapone) and amantadine, among other agents [56, 59]. Levodopa is the drug with most impact on PD treatment because it improves dopaminergic motor symptoms [66]. However, levodopa has serious side-effects, namely increased liver enzymes and the development of cardiovascular and gastrointestinal symptoms. Its chronic use leads to the loss of the effect in the treatment of motor symptoms, therefore, requiring higher doses, leading to the appearance of on–off syndrome. Finally, it can contribute to neurotoxic effects, which is translated in dopaminergic neuron death [59]. Nevertheless,

early treatment with levodopa should be the initial therapy and can be combined with all the others anti-PD drugs as adjunct therapies in advanced PD [56, 59]. When the disease turns into refractory, other therapeutic options can be applied, such as deep brain stimulation [49, 56].

ART AND NEUROCOGNITIVE IMPAIRMENT

There are evidences of neurodegeneration in HAART-treated patients with at least efficient systemic viral control [19]. HAART cannot prevent individuals infected with HIV from developing psychocognitive impairments, however, the most severe forms, as HAD are often not observed [23].

Data from several studies point out that antiretroviral CSF concentrations are at odds with levels in the blood, and are generally lower than in the plasma [5]. This can be explained by the poor penetration ability of the BBB by the antiretroviral agents [6, 23]. The diffusion into the CNS depends on the features of the BBB and the properties of the molecules that can restrain the accessibility to therapeutics. BBB presents astrocytic foot processes and tight junctions that hamper the circulation of the antiretroviral agents across the BBB together with efflux pumps that eliminate pharmaceutical molecules from the brain [5, 6, 24]. Indeed, there are certain drug features that facilitate its entry into the CNS such as low molecular weight, lipophilic properties, weak protein binding and ionized molecules [6, 23]. A patient under an antiretroviral regimen with an undetectable viral load in plasma, is frequently detected with a persistent viral activity in the brain, as it has been shown in some studies, probably due to incomplete penetration of antiretroviral drugs across BBB [24, 28]. However, only 3% to 10% of treated people with optimal viral suppression in the

plasma, present viral load in CSF [24]. The CNS Penetration Effectiveness (CPE) Score was created from numerous analysis of CSF viral load from individuals being treated for HIV infection. This score determines the bioavailability of antiretroviral drugs in the brain tissue [23]. The CPE parameter classifies ART in non-penetrators (score of 1), poor penetrators (score of 2), good penetrators (score of 3) and super penetrators (score of 4). Different classes of antiretrovirals have different potentials in reaching the brain [5]. When CPE score is > 7, this means that a great number of individuals has undetectable HIV RNA levels in the CSF [23].

It has been demonstrated that elevated CPE score, meaning that antiretroviral easily penetrate the BBB, results in lower CSF viral loads and neuropsychological improvement in patients with HAND. Nevertheless, other studies associated high scoring system to neurological impairment, instead of showing improvement in cognitively impaired individuals, suggesting that ART neurotoxic effects can be part of HAND pathogenesis [5, 23].

To reduce the risk of neurodegeneration, ART penetration should be ideally optimized, because recent studies show that CNS infiltration has a better clinical outcome regarding neurological functions [19]. However, some of the data demonstrated to be controversial. During HAART, some patients presented a good cognitive performance, while others had cognitive deterioration [5, 23]. Thus, the findings of the previous reports suggest that ART has a protective role against neurological imbalance in HIV infection, although it can also be neurotoxic. It is assumed that there should be an appropriate concentration of antiretroviral drugs in the CNS, above or below which, it may become harmful. Ideally, it should exist a narrow therapeutic range in which the drugs could prevent or even reverse HIV-neurocognitive damage by inhibiting HIV replication in the brain and enhancing the immune system

through increasing CD4+ count. Whether HIV-directed drug levels in CNS are low, HIV replication will raise and consequently it will take place a cascade of events that ultimately will result in neuroinflammation, as well as in a great risk of drug resistance. On the other hand, if drug levels in the CNS are elevated, it will occur a cumulative action of adverse effects. Both culminate in neuropsychological injury [5, 6, 23]. Antiretroviral medications can dysregulate the normal cerebral physiology due to their adverse effects, which, depending on their severity can lead to a decrease in the quality of patient's life, and in rare cases, they can even be life-threatening. NRTIs have been associated with anaemia, leukopenia, peripheral neuropathy, lactic acidosis and pancreatitis, while NNRTIs are related to CNS toxicity, rash and sleep problems [21]. Nucleoside analogues and NRTIs are substantially linked to mitochondrial toxicity, mainly by inhibiting the mitochondrial DNA polymerase gamma and, therefore, by inhibiting the synthesis of mitochondrial DNA [32]. Finally, the majority of the PIs present insulin resistance that can cause type 2 diabetes, diarrhoea, gastrointestinal disorders, abnormal fat distribution (lipoatrophy/lipohypertrophy) and hyperlipidaemia as negative side effects [21]. In conclusion, one can infer that HAART is associated with metabolic, cardiovascular, renal, hepatic and neurological disorders, which may be mentioned as "serious non-AIDS events" that occur in this population group. Antiretrovirals have well documented iatrogenic effects in CNS [24]. In addition, these complications occur more frequently in older patients for two main reasons [22]: first because these patients have a weakened immune system and they are less resistant to damage caused by ART. The second reason is related with the population age, which *per se* has a higher drug burden due to their elevated number of comorbidities and therefore, a higher chance of pharmacological interactions [15, 21, 32].

The confluence of aging with the cumulative toxic effects in CNS in infected patients treated for decades, contribute to a more vulnerability to early brain aging and, hence, to an accelerated development of neurodegenerative diseases-age related [22, 24]. The several adverse effects associated with chronic HAART medication together with HIV-mediated inflammation and T cells activation, among other factors, may contribute to brain aging-induced toxicity and neuropsychological deficits [32, 67]. ART besides the aforementioned lypodystrophic effects including diabetes and hyperlipidaemia, also induces coronary artery disease and immune reconstitution inflammatory syndrome (IRIS) [19, 68, 69]; and may propitiate the development of AD as these iatrogenic effects are well established risk factors for this disorder [70]. Furthermore, IRIS is an autoimmune consequence of ART. IRIS is a common condition and has been reported to occur in approximately 15% to 25% of HAART-treated patients [6]. It results when cell-mediated immunity presents normal values obtained by HAART and can face pathogenic antigens [4, 8]. The pathogen-specific immune response promotes inflammation, metabolic defects, such as dyslipidaemia, diabetes, abnormal fat distribution in the body, coronary disease, connective tissue disease, and all are also known risk factors to develop AD. [68, 70] In some cases it has been reported that asymptomatic HAND may turn into symptomatic dementia, being IRIS the main cause. In the case of IRIS, the functional immune system worsens the underlying sub-clinical pathology [71].

The majority of antiretroviral agents are metabolized by hepatic cytochrome P (CYP) 450 isoenzymes, as well as other drugs that are very often required such as antacids, statins, antidepressants, hypoglycaemic drugs, cardiovascular drugs and antiplatelet agents or anticoagulants. Furthermore, anti-HIV drugs interfere with the activity of CYP450 enzyme system, suppressing some isoenzymes while boosting the

activity of others, thus, tending to cause significant drug interactions that may lead to additional ART toxicity [67]. Chronic antiretroviral drugs increase the probability of neurocognitive impairments occurrence, possibly by mechanisms related with mitochondrial toxicity that is often associated with neuronal and synaptic loss [8]. In addition, antiretroviral agents' abnormal absorption and metabolism can also induce neurotoxicity [33]. These medications have a small therapeutic interval, therefore, if for any reason, the absorption is decreased or the CYP450 metabolism is modified, the improvement or preventive ART effect of neurologic deficits is compromised. Highlighting what has been stated above, when anti-HIV drug plasma levels are elevated, the risk of toxicity and less tolerability increase, while low plasma concentrations of these agents do not inhibit viral replication and easily enhance drug resistance; in either cases the action of HIV treatment has no effect [67].

In patients treated with ART there is evidence that inflammation and immune activation are reduced, although not abolished in the brain, may be because low levels of HIV replication persist. The role of these residual phenomena in the pathogenesis of neurological impairments is receiving increased recognition [4, 39]. It has been shown *in vivo* that despite the capacity of ART in minimizing HIV RNA in blood to almost undetectable concentrations, there are other reservoirs such as gastrointestinal tract, lymphoid tissue, CNS (monocytes, macrophages and astrocytes) [4], ovaries and testicles that still present HIV viral levels [17]. Likewise, infected resting memory T cells and residual replication contribute to the persistence of immune activation and neuroinflammation [4], particularly in the hippocampus and in the temporal cortex, which are involved in the working memory [72]. ART can only delay HAND progression and not extinguish the outcome of dementia, because the underlying mechanisms driving the development and evolution of neuropathy persist [27, 28].

Some groups have addressed the connection between P-glycoprotein function and the neurocognitive decline. This P-glycoprotein, encoded by the ABCG-1 gene, is an important ATP dependent efflux pump located in the cell membrane and has a wide variety of substrates including PIs, chemotherapy agents, steroid hormones, loperamide and imodium [73]. It exists in many types of cells, including the BBB. It has been described a poor absorption by the BBB of PIs and NNTRIs, such as efavirenz, through pumping out these drugs [19, 73]. Therefore, avoiding the penetration of the molecule into the brain, will not prevent the neurologic effects caused by AIDS. Moreover, suppression of P-glycoprotein expression is intentionally achieved in HIV treatment improving the efficiency of the anti-HIV drugs as PIs [19]. Additionally, it was found that P-glycoprotein is involved in Aβ clearance from cerebral tissue. Whether ART inhibits Amyloid removal, this will probably lead to neuronal injury due to the growing Amyloid levels in the brain. Thus, P-glycoprotein can play a role in the pathogenesis of neurocognitive disorders in HIV-treated patients due to the depletion of neurotoxins clearance that has already been associated to PD pathogenesis. [19]

There is evidenced that patients on antiretroviral regimens show increasing beta-amyloidosis and decreasing Aβ clearance by macrophages, chiefly in the hippocampus, when compared to non-treated patients. This can be explained by the intervention of some PIs in Aβ degradation. Some examples include nelfinavir, which inhibits the activity of Aβ degrading enzyme, insulin degrading enzyme (an Aβ degrading enzyme) [70], and ritonavir, which prevents Amyloid removal from the CNS [71]. AD-like Aβ aggregates have been reported in this study to be proportional to the number of patients treated chronically for HIV-associated disease. Other HIV-related medications may modify the synthesis, the clearance and extracellular Aβ accumulation. The same

observations were noticed for phosphorylated Tau (p-Tau), and high levels of p-Tau are usually related to the evolution of HAD [70].

One study from Xiqian Lan *et al.* (2012), aimed to clarify the effects of several PIs in the Aβ peptide clearance in macrophages. The authors observed that ritonavir, saquinavir and atazanavir slightly suppressed peptide degradation; ritonavir, nelfinavir and lopinavir increased the number of undegraded Aβ peptides. Furthermore, they found that all the aforementioned HIV-PIs, except atazanavir, reduced Aβ biosynthesis in neurons through inhibition of the activity of Beta-site APP Converting Enzyme 1 (BACE1) and γ-secretase enzyme [69]. However, when the performance of these drugs regarding purified BACE1 activity was evaluated *in vitro*, it showed a slight suppression, thus indicating that ART medications may have an indirect action on neurons BACE1 activity [69]. Using the APP SCID mice model (with a double mutant form of APP 695 (KM670/671NL + V717F) in homozygosity for the scid allele of Prkdc), which were administrated with nelfinavir or lopinavir/ritonavir during 1 month, no changes were observed in Aβ accumulation, although there was evidence throughout this study of the decrement of Aβ synthesis in neurons and its removal in macrophages [69]. In general, PIs do not readily cross the BBB because they have high levels of protein-bound in the plasma. The interpretation of these insignificant effects of antiretroviral drugs on beta-amyloidosis in the brain may possibly reside on the low infiltration in the CNS. Other group of researchers has associated PIs to neuroprotective effects due to their capacity in restraining mitochondrial apoptosis [69].

In some clinical studies, the development of parkinsonic symptoms at early age, mean 48 years old, has been associated with HIV-infected patients with maxim viral suppression at the systemic and CNS level [74]. The authors postulated that chronic inflammation, mitochondrial toxicity and proteasome dysfunction constitute the main

mechanisms leading to the early onset of Parkinsonism in long-term treated HIV-infected patients. In general, antiretrovirals cause mitochondrial toxicity and some PIs can disrupt the proteasome function [74].

There seems to exist growing evidence that cART does not revert or prevent CNS impairment. Interestingly the only means available up to now used to evaluate HIV-associated neurocognitive deficits are related to HAART [5, 23, 24]. The battle in the treatment of HIV-infected individuals with neurocognitive complications will be to avoid the neurotoxic effects through proper concentrations of these drugs that do not affect the brain [23].

RELATIONSHIP BETWEEN HIV AND NEUROCOGNITIVE IMPAIRMENT

The exact pathogenesis of AIDS-related neurocognitive disorders remains unknown, however, emerging improved biochemical markers, neuroimaging and genomic studies have extended the knowledge about this subject.

The implementation of HAART and their correct application culminate in great outcomes that have been described in HIV-infected patients. Currently, these patients live approximately the same number of years, when compared with non-infected people, and, therefore, become propitious to develop age-related neurodegenerative disorders as older general population [68, 74, 75]. Other perspective exploits that HIV can cause identical routes to the ageing process in brain cells and synapses, initiating an early ageing of the brain and enhancing the risk of neurodeterioration [76].

Available information about the neuropathology behind HIV-associated neurocognitive disorders, indicates that HIV neuroinvasion induces an inflammatory process in the brain, mainly through microglia. When activated, microglia outputs pro-

inflammatory agents as chemokines and cytokines including IL (interleukin) -6, IL-1β and TNF-α [29]. Consequently, neuroinflammation may increase oxidative stress, which in turn, leads macrophages, astrocytes and microglia to release viral proteins and other neurotoxic products. Furthermore, activated microglia manufactures ROS in exaggerated levels, being one more cytotoxic factor to enhance the damage of DNA in brain cells [29, 76]. At this point, neuroinflammatory condition is exacerbated by activation of redox-sensitive transcription factors, including nuclear factor-κB (NF-κB), triggered by increased ROS. All these inflammatory mediators and toxic products contribute to microglia ageing and, therefore, to premature ageing of the brain. The finding that microglia ageing is linked to a speedup reduction of cognitive functions since middle age, is receiving increased support [29]. This is in agreement with epidemiological observations showing that people with HIV-associated neurologic disorders are normally younger when they become cognitively impaired, comparing to individuals without HIV disease [77]. Several studies have disclosed that neuronal death arise especially in people in advanced stages of the disease. This usually coincides with the patient advanced age, but over the course of the disease there is also accumulation of slight damages, particularly in subcortical brain regions, culminating in neuronal death and consequently, cognitive impairment [78].

MicroRNAs (miRNAs) are short-length RNA molecules which connect to mRNA, generally inhibiting gene expression [79]. miRNAs have been widely investigated, due to its important role in the pathophysiology of numerous diseases, including neurodegenerative disorders. An overexpression of miR-21 subsequent to chronic NMDAR stimulation (a neurotoxic process active in AIDS and other neurodegenerative illnesses such as PD and AD) was found in neurons from individuals with HIV encephalitis (HIVE) and in its animal model (monkeys with simian

immunodeficiency virus encephalitis (SIVE)) [79]. The neuropathological findings in HIV-infected brain are together known as HIVE and include BBB disruption; leukocyte infiltration into the CNS; microglia, macrophages and astrocyte activation and damage and/or death of neurons [70]. The enhanced signalling of NMDAR was shown to be caused by macrophages, low weight non-peptide molecules and HIV proteins, including Tat, gp120 as well as other substrates implicated in HIV neuropathy pathogenesis [79]. In neurons of HIV-infected people, the induction of miR-21 can lead to neuronal damage and death through the stimulation of these receptors [79]. Each miRNA has diverse mRNA targets, which induces gene repression; miR-21 has as target-gene, the myocyte enhancer factor 2C (MEF2C) which is an essential transcription factor in CNS [79]. This study showed that MEF2C was suppressed by miR-21, and had low expression in HIV infected neurons [79]. Furthermore, it was demonstrated in mice that MEF2C is involved in memory and learning processes, as well as in neuronal cells survival mechanisms [79]. Humans with only one copy of MEF2C present deep mental retardation [79]. In addition, the down regulation of MEF2C leads to an increased CNS excitatory input, possibly by a self-sustainable cycle that begins in NMDARs augmented excitatory input. This may result in increased miR-21 and reduced MEF2C, amplifying the excitatory input, and possibly contributing to excitotoxic neuronal damage and death [79]. There are other genes possibly implied in cognitive dysfunction in CNS-related HIV infection.

When infected, brain monocyte-derived cells release HIV proteins, generating brain toxicity, one of the important mechanisms of the pathophysiology leading to deficits associated with HAND [80]. Previous reports have given the expanding insights about HIV proteins, which alter the autophagy process in monocyte-derived cells, mainly in aged individuals. However, the machinery underlying this abnormal

autophagy is not completely understood. A more recent study has hypothesized that HIV proteins, specifically Tat, may modify autophagy in CNS. Briefly, autophagy is the process by which the cell degrades and recycles cellular components [81]. This pathway involves the formation of a double membranes structure – the autophagosome that after fusion with the lysosome, will degrade or recycle the cell components. Microtubule-associated protein-1 light chain β II (LC3II) and sequestosome 1 (SQSTM1) are located in the autophagosome membrane and are considered typical markers of autophagy. Both change concentrations during the autophagy process; SQSTM1 is inversely related to autophagy activity, while LC3II is reduced during autophagy initiation and degradation [81]. It was reported that Tat diminishes autophagy markers, LC3II and SQSTM1, depending on its dose, which suggests an enhanced autophagic activity [81]. *In vitro*, but also *in vivo* studies using GFAP-Tat tg mice, replicating the neurotoxic environment observed in HIV brains, proved that Tat induces neurodegeneration due to the increase in the number of damaged autophagosomes and its accumulation in neurons [81]. Furthermore, these last studies revealed that Tat can interact with lysosomal-associated membrane protein 2A (LAMP2A) [81]. This interaction seems to promote abnormal autophagosome and lysosome fusion, resulting in dysfunctional autophagolysosomes and consequent dysregulated autophagy in neurons. In turn, Tat-mediated neuronal toxicity is decreased by LAMP2A higher-expression [81] which reinforces once again that Tat can cause altered autophagic degradation. This premise in combination with other injurious Tat effects, may lead to HAND. Some data in the context of HIV infection are consistent, showing high levels of autophagy components in younger patients and low levels in patients aged over 50, supporting the concept that ageing in HIV individuals turns them more vulnerable to neurocognitive impairments. Furthermore, current findings that HIV proteins alter intracellular clearance mechanisms

as autophagy, have also been reported in neurodegenerative disorders such as AD and PD [81]. LAMP2A may be involved in the pathophysiology of PD, taking into account that LAMP2A is a target of α-synuclein. Additionally, HIV Nef and gp120 have been linked to autophagy deficiencies. Nef, which is a catalyst of viral replication, restrains the autophagy machinery maturation in neurons, preventing the degradation of viral-related toxic products [81]. *In vitro* studies using HIV-infected microglial supernatants or in gp120-transgenic mice assays, it was described a decline in the autophagic activity, may be due to an inhibition of Beclin activity and its respective levels. Beclin plays a critical role in the regulation of both autophagy and cell death mechanisms. Hence, we can assume that dysregulated autophagy is involved in the neuropathology associated to HIV infection [81].

Furthermore, HIV Tat has been associated to neurotoxicity through activation of several genes transcription, induction of pro-inflammatory mediators and dysregulation of cellular proteins, even after its degradation or when far away from its place of origin. *In vitro* experiments with regular concentrations of Tat in HIV-positive patients CSF, ranging between 10 to 1000 nM, revealed to be neurotoxic and led to HIV-associated neuropathology [82]. This protein can interfere with glial cells function because it induces pro-inflammatory cytokines release and gliosis, both responsible for the loss of synaptic density and neuronal dysfunction [82]. Furthermore, HIV Tat can directly damage neurons, which entry into the cells is supported by low density lipoprotein receptor (LRP)-mediated endocytic transport [82]. Intraneuronal Tat augments cytoplasmic Ca^{2+} levels through secretion of Ca^{2+} from neuronal compartments by changing 1,4,5-triphosphate receptors or promoting diffusion by plasmatic membrane of extracellular Ca^{2+} via voltage-gated Ca^{2+} channels and NMDAR. The elevated cytoplasmic Ca^{2+} concentration mediated by Tat causes mitochondrial influx of Ca^{2+},

leading to its dysregulation and mitochondrial ROS production that generate oxidative stress and signalling events that may lead to neuronal apoptosis [82].

Chronic drug addiction, in particular cocaine and marijuana, are typically consumed by HIV-positive patients and may lead to more pronounced neurological dysfunctions in these people [82, 80]. In addition, numerous data propose cocaine and methamphetamine (meth) abusers with HIV infection to display accelerated onset of neurocognitive debilities [80]. There is a synergistic effect in HIV-related disease progression when HIV and cocaine consumption are present concomitantly, as their interaction accelerates immunodeficiency due to increased viral reproduction and loss of CD4+ cells [82]. This recreational drug ruptures BBB structure, by cleavage of BBB intercellular junctions [80], allowing the virus to spread easily into the brain, and turning CNS more susceptible to viral proteins neurotoxicity, even under antiretroviral treatment [82]. Thus, HIV-related neurocognitive disorders are exacerbated in cocaine addicts [82]. The anatomical substrates mainly affected in this situation are frontal/prefrontal cortex (PFC), striatum and hippocampus. Morphologic changes in PFC, as well as atrophy and tissue loss, are similar in HIV patients and cocaine users. One study involving tasks that required attention and executive function, showed in PFC of HIV-positive adults, a low activation of these functions, which was also reported in cocaine consumer individuals [82]. In addition, a study using blood oxygen levels dependent (BOLD) functional magnetic resonance imaging (fMRI) in women throughout verbal learning tasks was associated with hypo-activation of PFC in both current and former cocaine-abusing HIV-positive individuals comparing with HIV-infected non-users. These results demonstrated a possible overlap between neuropathological mechanisms in cocaine abuse and HIV infection [82].

Cocaine blocks the reuptake of monoamine neurotransmitters from the synapse, such as norepinephrine, serotonin and dopamine, because it connects and competes with its transporters. Thus, high neurotransmitters concentrations will remain in the synapses, making synaptic and peri-synaptic receptors hyper-reactive [82]. However, long exposure to this drug reduces the reactivity of postsynaptic neurons, with loss of function of monoamine neurotransmitters (like dopamine) in the nucleus accumbens (NAc). Additionally, it occurs internalization of receptors, decrease of neurotransmitter synthesis and damage in synaptic connections. Subsequent to the end of cocaine abuse, the side effects can be maintained for long periods of time [82]. Likewise, cocaine promotes the expression of macrophage activation marker human leukocyte antigen (HLA)-DR and a co-receptor of the virus, the dendritic cell-specific intercellular adhesion molecule-3- grabbing non-integrin (DC-SIGN), enhancing HIV-infection. *In vitro*, viral replication in astrocytes and monocytes seems to be augmented in the presence of cocaine [80]. Consumers have lower adherence to therapeutics compared to non-consumer individuals. It was demonstrated that abnormal dendritic network can be increased when HIV gp120 and cocaine abuse co-exist. These evidences support the idea that cocaine facilitates and amplifies HIV infection and neuronal dysfunction, enhancing impaired neurocognitive functions [80].

An extensive study was conducted to further strengthen the possible link between HIV Tat and cocaine in neurodegenerative processes. The authors have reported direct and indirect effects in neurological homeostasis with both [82]. Astrocytes regulate many functions that contribute to synaptic transmission. However, there is some evidence, obtained through *in vitro* experiments showing that when Tat is in contact with brain components, activates astrocytes and approximately 7 days later astrogliosis emerges. In fact, astrogliosis is a common feature of neurodegenerative

disorders. In mammalian brains, it was found an extended astrogliosis, particularly in medial prefrontal cortex (mPFC), after exposure to Tat [82]. Furthermore, astrocytes structural protein, glial fibrillary acidic protein (GFAP), has a higher expression in about 14 days after Tat intracerebral administration [82]. Tat-amplified GFAP expression may be due to the interaction between Tat and specific proteins, such as activator protein 1 (AP1) and specificity protein 1 (SP1). Regarding cocaine, short administrations or even abstinence for a few weeks also augment GFAP in PFC and NAc. Tat alters astrocytes status through complex bonds; when cocaine co-exists in these situations, there is a higher probability of the neurological capacities to be abolished, leading the brain to be more susceptible to Tat effects [82].

Recent observations revealed that both Tat and cocaine promote ROS generation, although this psychostimulant also raises the production of antioxidants, creating an equilibrium between oxidative stress and antioxidant products, thus preventing neuronal apoptosis [82]. Tat-induced increase intracellular Ca^{2+} to cytoplasmic and mitochondrial levels, contributes to the production of mitochondrial ROS. When cocaine and Tat co-exist, cocaine has a stimulating role in this protein effects, possibly enhancing ROS production [82]. In cultured rat cortical neurons, it has been shown that Tat protein-induced Ca^{2+} intracellular uptake, driving to excitability of neurons through membrane depolarization. This phenomenon is controlled by NMDAR and L-type Ca^{2+} channels; when the first receptor is blocked, Ca^{2+} influx remains untouched. On the other hand, if Ca^{2+} channels are inhibited, it will occur a decline in Ca^{2+} influx mediated by Tat. Low Tat concentrations in rat CNS, similar to physiological human concentrations, augments L-channel expression in the mPFC [82]. Recent studies indicated that higher expression of L-type Ca^{2+} channels in mPFC neurons membrane is observed following a couple of weeks after cocaine removal [82].

Regarding meth, which is another psychostimulant drug, it has been associated to neurological damage due to BBB dysregulation and enhancement of pro-inflammatory cytokines in the brain. Meth affects dopaminergic neurons and as a result, dopamine and norepinephrine concentrations decrease in the CNS [80]. Beyond that, in HIV meth consumers, raised levels of HIV replication in astrocytes were observed, concomitantly with higher CXCR4 and CCR5 co-receptors expression [80]. In the presence of meth, lesser hippocampal volume is associated to a severe cognitive deficit [80]. In astrocytes, meth and gp 120 act together enhancing cytochrome P450 2E1 (CYP2E1) and IL-6 activity, which leads to ROS production. Similarly, there is an increase in DNA degradation and apoptosis in astrocytes due to increased activity of caspase 3 [80].

The combination of cannabinoids and HIV infection has also been described to intensify the pathology of neurocognitive disorders [80]. Assays using HIV gp120 confirmed CXCR4 activation and subsequently secretion of IL-1β. This mediator induces an ubiquitin ligase and the NMDAR activation causing deterioration of synaptic network in the hippocampus [80]. A protective role for cannabinoids has been demonstrated, since the cannabinoid receptor full agonist (Win55212-2) prevented neurodegenerative pathways, however, this was only reported for the CB2 cannabinoid receptor, and not for the CB1 [80]. Moreover, exposure to Tat protein instead of gp120, in the presence of Win55212-2 reduced the number of synapses. It was also found that cannabinoid CB2 receptor agonist (AM1241) decreased gliogenesis and astrogenesis in the hippocampal areas of GFAP/Gp120 transgenic mouse model and increased neurogenesis *in vivo* [80]. HIV-infected patients commonly abuse of marijuana as a common practice. Evidences from convergent effects between HIV infection and marijuana are only described in advanced stages of the infection, while in early stages

of the disease or in non-HIV marijuana patients, it has an insignificant action [80]. However, there is little research on this matter and more studies are needed to confirm these effects.

In general, there is an increased loss of synaptic networks caused by drug consumption together with HIV Tat, gp120 proteins and reduction in synapses, which are related with the outcome of HAD. However, a recent observation pointed out that drug dependence related to HIV disease did not raise the abnormal cognitive functions. Thus, it was suggested that after a period of absence of drug consumption, the effects of these drugs in the neuropathological outcomes of HIV-infected individuals can be total or partially reversible. These results demonstrated that drugs can assist neurological impairment while patients are active consumers, exacerbating inflammatory and neurotoxic processes associated to neurodegeneration. In addition, HIVE is less observed in HIV-infected people, who do not consume drugs, compared to HIV drug consumers [80].

Neurodegeneration progress in HAND in older people is exacerbated by chronic drug abuse concerning the neurotoxic mechanisms reached by HIV proteins, such as Tat, Nef, and gp120. This also includes neuroinflammation routes, oxidative stress, mitochondrial function, signalling pathways and autophagy [81, 83].

In HIV-positive population there is an increased incidence of neurological degenerative illnesses, such as AD and PD, mediated by common domains of the pathological HAND mechanisms. In other words, their biopathogenesis share common fields, namely relating to neuroinflammation, oxidative and nitrosative stress and cellular degradation pathways [19].

Immune activation in the CNS by HIV infection primarily occurs in the *substantia nigra* and other basal ganglia, such as *putamen* and also in areas like the

hippocampus, which are involved in PD and AD, respectively [78, 84]. A genomic profile analysis of HIV contaminated peripheral blood mononuclear cells (PBMCs), monocytes and macrophages, highlighted diverse pathways linked to neurodegeneration that can explain the neurological decline in AIDS. PBMCs were used to investigate gene expression due to their implication in immunological responses in many diseases and because they play an important role in the evolution of HAND. This specificity of dysregulated genes make the infected individuals neurologically susceptible to the development of AD or PD-related dementia. HIV DNA in PBMCs seems to be directly proportional to the severity of cognitive deterioration. Thus, we can question whether these cells, which are able to infiltrate the CNS are already programmed at the sub-genomic or sub-cellular level, leading to an early specific neurocognitive defect [77]. There are consistent evidences that HIV, AD, PD and other brain disorders exhibit impaired autophagy and ubiquitin-proteasome system and, ultimately lead to a dysfunctional BBB [19].

In terms of histopathology, HAD is considered a subcortical pathology, while AD is mainly cortical. However, in quite clinical observations, it has been reported AD old patients, who are HIV-positive for a long number of years [68].

The chronic inflammation-activated microglia cascade favours Aβ accumulation in the brain. There is a substantial overlap in neurological phenomena and neurotoxicity between HIV and AD patients, which makes us believe that HIV neuropathological pathways may induce pathogenesis related to AD. Some experimental findings in animal models involving the following sequence: chronic systemic inflammation, neuroinflammation and microglia aging, resulted in Tau protein phosphorylation, deposits of Aβ, neuronal and synaptic damage, which constitute main hallmarks of AD [29].

CSF analysis of individuals with HAD showed identical values to those observed in AD, namely, low concentrations of Aβ 1-42 deposited in the brain tissue as amyloid plaques [19], high levels of total Tau (t-Tau), and p-Tau, suggesting that cognitively impaired HIV-positive patients may correlate with AD pathophysiologic processes. Years later, in new CSF tests performed in a larger sample, the same results were described, with the exception of normal concentrations of p-Tau instead of high levels, as referred before [19]. Therefore, the increased parameters for Tau that are typically observed in AD patients, seem not to correlate with HIV-demented population. This differences in p-Tau concentration may be due to the higher number of individuals participating in the tests [70]; possibly this may be a rare finding in this group of HIV-infected individuals who are in the process of developing AD [19], or otherwise this may be explained by the absence of therapeutic intervention for viral infection or even advanced stage of the disease [70, 85]. The pathology of Tau protein, a cytoskeletal-microtubule axon protein, may be justified by the Aβ theory, which hypothesizes that Aβ pathology predates and enhances Tau hyperphosphorylation, this way leading to the amyloidogenic process in HIV infection and clarifying the elevated p-Tau concentrations in CSF [70]. As aforementioned, the amyloidogenic process is induced by cytokines and chemokines released from activated-microglia, especially IL-1 and also by HIV proteins [70]. Curiously, activated-microglia assists Tau phosphorylation by IL-1 receptor antagonist (IL-1ra) and anti-IL-1β antibody-sensitive manner in neuronal cells [70]. There are neuropathological evidences that in HIV-associated Alzheimer dementia occur similar modifications in the metabolic pathways of Aβ, however, the same changes are not found in Tau pathology. Thus, the results given by CSF measurements are essential for the distinction between neurologic impairment from HIV infection and AD [19, 85].

In the year of 1998, Aβ clusters were detected in the brain of patients with HIV, with the same Amyloid type (Aβ 42) found in AD patients and further several studies were consistent with these observations [78]. Aβ aggregates have been found in extracellular perivascular deposits, inside neurons and in neuronal axons. In HIV-infected patients, approximately 4–13% were extraneuronal plaques and 30–40% represented intraneuronal Aβ [70].

In general, the more advanced age of the individuals with AIDS, the greater number of patients with Amyloid deposition. In the fourth and seventh decades of life, 18% and 50% of HIV-infected people present Aβ accumulation, respectively [19]. These numbers are higher when compared to non-AIDS patients. Interestingly, it is not well established whether the duration of the infection is more important than the age of onset, or if is the intricate interaction between both – age-duration event – that has a significant impact on the predisposition to develop AD. Some of the anatomical areas affected by Aβ deposition in the HIV infected brain are also frequently altered in AD, namely the hippocampus, while others areas, such as the frontal lobe are commonly not involved in this mental illness [19]. Furthermore, new available information points out that when HIV is present, hippocampus is the preferential brain area where occurs greater astrogliosis, microglia activation, inflammatory mediators activation, loss of synaptic plasticity and density and especially neuronal apoptosis induced by Tat protein. Indeed, hippocampus is hypersensitive to HIV-associated damage [78]. A minority of neuropathological observations showed neuritic plaques, a specific AD marker, only in the hippocampus, entorhinal cortex, and parahippocampal gyrus of HIV-infected patients. On the contrary, diffuse plaques are observed in many of these studies when compared with Alzheimer's patients, however, most investigators think that diffuse plaques are the precursors of the neuritic ones [19].

The Amyloid deposition observed in HIV patients may be due to HIV regulatory protein Tat, known to suppress an enzyme necessary in Aβ degradation, called neprilysin [19, 85]. Tat can block LRP-responsible brain Amyloid clearance through its connection to the LRP-related protein, preventing the link to this receptor. Likewise, it may lead to augmented brain Amyloid accumulation [85]. When APOE allele 4 is present in AIDS subjects, it propitiates the development of AD, especially in aged patients, even when there is only the presence of one allele. Apart from being an AD-genetic inducer, APOE4 facilitates the virus entry into the cells and the development of AIDS-associated AD. Thus, it probably can contribute to a faster disease course and premature death of HIV-infected people [19, 70, 86]. The presence of APOE4 genotype in infected adults increases the susceptibility to Aβ extracellular accumulation in brain tissue, due to the fact that APOE can interact with soluble Aβ peptides, impairing its removal by the BBB and manipulating Aβ agglomeration and distribution. Furthermore, APOE 4 allele is not the only factor responsible for Aβ accumulation, although it has been associated to an early onset and raised distribution of Amyloid aggregates. [87]

Chronic inflammation is indirectly responsible for the accumulation of Aβ because this inflammatory state alters mononuclear phagocytes, which have an essential role in the clearance and intracellular destruction of Aβ through phagocytosis. Furthermore, brain Amyloid removal can be disrupted in many steps besides phagocytosis by mononuclear phagocytes, including matrix metalloproteases, insulin degrading enzyme (IDE), Aβ cleavage enzymes, such as neprilysin and, finally the transport by brain blood vessels [69]. In addition, APP accumulation is also observed in HIV brains. High levels of TNF-α, IL-1β and Interferon-γ in AIDS patients induce astrocytic BACE1 amounts and activity, causing APP degradation and Aβ formation. Thus, TNF-α increases β-secretase action and enhances Amyloid genesis [70].

There is increasing evidence that high levels of TNF-α in peripheral blood of AIDS patients, correlate with an increased progression of Alzheimer's dementia, as well as IL-13 and G-CSF can be associated to a fast progression of Alzheimer's evolution [84]. In HIV-associated AD, it has been well documented the presence of Amyloid precipitation in brain tissue, together with gp120 and Tat protein neurotoxic action, besides HIV proteins and cytokines toxic patterns. In the presence of HIV, the immune system is weakened, leading these patients to get easily predisposed to non-HIV risk factors that may help the establishment of AD, as is the case of infection with certain agents, including Chlamydia pneumonia, Helicobacter pylori, herpes virus 1, Borrelia burgdorferi, cytomegalovirus and sepsis among other factors [68, 84]. HIV infection can cause imbalance of Aβ biogenesis and clearance, accounting for the accelerated β-amyloidosis observed in HAND patients [70].

There is increasing evidence that in individuals predisposed to PD, there are some infections, particularly HIV infection that may increase the risk of suffering from this mental illness as the population ages [84]. Only 1% of AIDS patients present symptoms related to movement and about 50% of those have HIV-related PD [75]. Although not very frequent, the first HIV clinical presentation may be related to cognitive and movement impairments, because HIV has a preference for the frontal lobe white matter and basal ganglia, in particular the *substantia nigra*, which is also the main anatomical area injured in PD. HIV-positive population can present a specific biopathological pathway leading to frontostriatal deterioration, a common degeneration process in PD [88]. Both HIV and PD patients show deposition of α-synuclein in the *substantia nigra* and its amounts have been described to be increased with age. Some data reported that α-synuclein aggregation is partly due to alterations of tyrosines in proteins through the substrate peroxynitrite formed from nitrosative stress. The key neurotransmitter

involved in PD, dopamine, is also decreased in HAND; moreover, testosterone insufficiency has been observed in both diseases [19, 77]. The TNF-α concentrations in blood are associated with Parkinson non-motor symptoms, such as impaired cognition, sleep disturbances and depression [84].

In conclusion, it seems to exist an overlap between identical dysregulated biological pathways involved in the neuropathogenesis of HIV and neurocognitive diseases [89]. Indeed, in infected individuals with cognitive disabilities the probability to develop neurodegenerative disorders as a consequence of AIDS is increased [74].

DISCUSSION AND CONCLUDING REMARKS

30 years after the first description of the HIV virus, AIDS pandemic continues to be a worldwide significant burden in human, medical care and financial resources, without cure in sight. The knowledge of the structure and replication of HIV in host cells enabled the development of antiretroviral drugs, which proved to be substantially effective in extending the patients' longevity and improving quality of life. The natural history of AIDS disease has drastically changed, allowing infected people to get older, which, before treatment, was impossible, as these patients died within a few years after acquiring the infection. Hence, diseases that hitherto have never been observed in HIV-positive patients are emerging, including HIV-associated neurocognitive diseases and age-related neurodegenerative illnesses, such as AD and PD. With these new evidences is imperative to collect the existing information to better understand how HIV and ageing, in the era of HAART, can induce neuropsychological impairment.

It has been well demonstrated that HAART patients display cognitive difficulties, mainly those related with mild forms. These drugs do not have a 100%

penetrance into the CNS due to the BBB properties and the constituent molecules themselves. The diffusion capacity of the antiviral agent through the BBB may be assessed by CNS Penetration Effectiveness (CPE). However, some observations have shown that individuals who are receiving high CPE drugs still develop neurological problems because viral activity remains in the brain. Thus, HAART can be a double-edged sword. On one hand, the virus continues to reproduce or even offers resistance to treatment, taking into account that these drugs display low levels in the brain. On the other hand, although present in CSF, the side effects may result in neurocognitive damage over time. An additional reason to be vulnerable to the development of neurological diseases in old HIV-infected patients is the increased drug interactions due to the polypharmacy characteristic of this population group. Besides this, ART lypodystrophic effects such as diabetes, hyperlipidaemia and coronary artery disease, IRIS an antiretrovirals autoimmnune outcome, are also risk factors to AD, for example. Mitochondrial toxicity, chronic inflammation and proteasome dysfunction are common effects of ART that can cause neuronal and synaptic death. These reports allow us to conclude that HIV treatment slows down the disease progression for several years, however, HAND turns out to be an inevitable outcome.

There are several factors that can contribute to the development of neurologic disorders relate with HIV or at least to its treatment. Viral activity induces through monocyte-derived cells a neuroinflammatory cascade of events, leading to oxidative stress, ineffective autophagy process, dysfunction of ubiquitin-proteasome system, BBB impairment and finally neuronal death. HIV proteins and other neurotoxic molecules have been described to activate NMDARs, which in turn cause overexpression of miR-21. This microRNA suppresses the MEF2C gene, a transcription factor implicated in neuronal survival pathways, and can also lead to neurodegeneration. Furthermore, HIV-

Tat protein has been suggested to reduce autophagy markers: LC3II and SQSTM1, and impair LAMP2A function, indicating increased abnormal autophagic activity in neuronal cells. Other viral proteins including Nef and gp120 have also been associated to autophagy deficiency. Thus, data relative to drug abuse, an addiction behaviour very frequent in PLHIV, demonstrated to accelerate and aggravate neurological impairments. In fact, cocaine, which inhibits the monoamine neurotransmitter transporters from the synaptic cleft to the presynaptic neurons impairing normal synaptic function, enhances HIV replication in astrocytes and monocytes, as shown in *in vitro* assays. The modifications caused by cocaine consumption inclusively remain for a period of time after its consumption cessation.

The finding that there are common mechanisms of action shared by cocaine and Tat, suggests that both reinforce each other effects, contributing to a greater vulnerability, fast progression and severity of the neuropathy characteristic if cocaine users, which are HIV-positive patients. In the case of Tat, it directly damages neurons by increasing cytoplasmic Ca^{2+} using NMDAR and L-type Ca^{2+} channels, thereby increasing Ca^{2+} at mitochondria and inducing mitochondrial ROS production. Thus, both cocaine and Tat enhance the neurotoxic process called astrogliosis characterized by increased levels of GFAP. Indeed, meth, another psychostimulant drug and the cannabinoid marijuana were described to speed up the onset of neurocognitive debilities and their severity, although after the end of drug abuse, the neuropathological effects seemed to partial or totally revert.

Regarding a possible link between AIDS, treatment and neurodegenerative disorders, there is increasing evidence that AD and PD may have higher incidences in HIV-infected population. Hippocampus and *substantia nigra*, which are the brain anatomical areas mainly affected in these neurodegenerative disorders, respectively, are

also involved in HAND. There are consistent observations that HIV infection, AD and PD share pathogenic mechanisms, including neuroinflammation, oxidative and nitrosative stress, inhibition of proteasome system and autophagy pathway and dysfunction of BBB. These anomalous mechanisms favour the accumulation and decline in clearance of Aβ, as well as hyperphosphorylated Tau, the principal AD hallmarks. Furthermore, HIV-positive individuals displaying APOE4 allele, present faster and severe AD onset with premature death. Hence, there are sufficient evidences suggesting that HIV infection provides a favourable and susceptible environment to the occurrence of neurocognitive disorders, especially AD.

Surely, the main aim in the context of HIV is to end with the pandemic. New strategies to prevent, control and treat properly the viral infection are currently being investigated. More studies in HIV- infected population are needed to measure the real consequence of HIV addictive behaviours, virulence particles and drugs on ageing and vice-versa. The prospects for the development of new and more powerful antiretroviral agents are increasingly promising, since these are being produced and tested with impressive results. Obtaining an optimal therapeutic concentration in the CNS is a strong objective in HIV treatment, together with a greater CPE score and less adverse effects, but this work is still in progress.

Understanding the mechanisms involved in HIV and neuronal impairment, in the future, will assist the development of methods and therapeutic approaches that may block, suppress or ameliorate the symptoms regarding this interaction between the virus and neurodegeneration.

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