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HIV-Associated Neurocognitive Disorders (HAND): performance of "3 Questions Test" as screening test, prevalence, clinical correlates and therapeutic approach in the San Paolo Infectious Diseases' (SPID) cohort

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Summary

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Background

The prevalence of HIV-associated neurocognitive disorders (HAND) has not declined in recent years, except for most severe forms of dementia, and is estimated around 15-55% of treated HIV-positive patients. A brief, easy-to-perform and standardized screening test for cognitive impairment in HIV-infected subjects is not yet available and current European AIDS Society (EACS) guidelines for HIV treatment suggest the use of "Three Questions Test" for the first approach. Moreover, the interaction between HIV and the brain is complex and poorly understood; the virus itself, immunological and toxic phenomena are implicated in the pathogenesis of cognitive disorders in HIV-infected patients. Several CSF and plasma biomarkers have been studied, but no one is suitable in clinical practice. Finally, combination antiretroviral therapy (cART) is effective only in a subset of patients and complementary treatments, as well as cognitive rehabilitation training, are needed.

Aim

To shed light on this topic, we conducted 5 studies with the following aims:

- "Three Questions Test (3QT) study": we aimed to explore the performance of the "Three Questions Test", proposed by EACS guidelines for the screening of cognitive impairment in HIV-infected patients;
- "NeuroHIV study" and "CSF/plasma HIV-RNA ratio study": we investigated the prevalence, the clinical and viro-immunological correlates of HAND in a cohort of antiretroviral-naïve and cART-treated HIV-positive patients. We also studied the modification of HAND after at least 12 months of virological effective antiretroviral treatment;
- "Neuro MITO study": we explored a panel of plasma and CSF inflammatory and immune activation markers in naive HIV-infected patients and we studied the effects of 12 months of virally-suppressive cART, according to CSF/plasma HIV-RNA ratio;
- "Case report of cognitive rehabilitation": we studied the efficacy of a new computer-based program complementary to cART in improving cognitive functioning.

Material and methods

We enrolled antiretroviral-naive and cART-treated HIV-infected patients in active follow up at the SPID Cohort. Patients underwent a comprehensive neuropsychological

evaluation by a trained neuropsychologist consisting of 11 tests exploring 7 main cognitive domains; the neuropsychological assessment included also the Instrumental Activities Of Daily Living (IADL), the Hospital Anxiety and Depression Scale (HADS) and the SF-36 Health *Survey* (SF-36). Frascati's criteria were used for the diagnosis of HAND. According to the study, blood sampling and lumbar puncture were performed to detect HIV-RNA and a panel of inflammation and immune activation markers. Statistical analyses were performed with SPSS software (version 21.0).

Results

- "3QT study": we enrolled 191 antiretroviral-naive and 111 on treatment HIV-positive patients. 99/302 (33%) showed an "abnormal" 3QT (a3QT) and 82 (27%) had HAND. Sensitivity, specificity, positive and negative predictive value (PPV and NPV) were low and far from 80%, both in naïve and treated patients. HAND was not associated with a3QT, while an altered quality of life by SF-36 Health Survey was associated with a higher probability of a3QT in the multivariate model.
- "NeuroHIV study": 62/237 (26%) untreated and 44/116 (34%) cART-treated subjects presented HAND, with a prevalence of mild forms. In naïve patients low CD4+ nadir, not Italian origin and unemployment, while in treated subjects a longer length of HIV infection, non infectious comorbidities, symptoms of depression and detectable HIV-RNA were independently associated with a higher probability of HAND. No association with longer time on cART, CPE score and type of cART regimen was observed. 50 naïve and 10 treated patients repeated the same neuropsychological evaluation after 12 months of virally suppressive cART and most of the them did not show any improvement of cognitive functioning.
- "CSF/plasma HIV-RNA ratio study": 50/155 (32%) untreated HIV-positive patients presented HAND. AIDS-defining diseases and a CSF to plasma HIV-RNA ratio ≥1 were associated with a 3-fold higher risk of HAND, adjusting for CD4+ nadir.
- "Neuro MITO study": 61/70 patients (87%) showed low CSF/Plasma RNA ratio (<1, L-CSF) at baseline; L-CSF and H-CSF (high CSF/Plasma ratio, ≥1) patients showed comparable peripheral and CSF inflammation, T-cell activation/proliferation and maturation and HIV/CMV-specific response. After 12 months of cART both H-CSF and L-CSF patients showed a significant contraction of memory activated CD38+CD45R0+CD8+ T-cells, while maintaining stable neurocognitive performance.

Interestingly, only L-CSF patients showed a reduction in CSF sCD14, IL-6 and MCP-1 and in plasma TNF-α, an increase in naïve CD4+ and CD8+ and central memory CD4+ T cells, with a parallel reduction of terminally differentiated CD8+ T cells. With regard to cognitive performance, at baseline, 9/22 subjects presented altered neurocognitive evaluation: 5/16 (31%) L-CSF, 4/6 (67%) H-CSF, with no significant variation at T12.

- "Case report of cognitive rehabilitation": 3 treated patients with HAND underwent a computer-based rehabilitation program for 12 weeks. At the end (T1), the patients were tested again with the same neuropsychological battery. Patient 1 (current CD4+ count 405/mmc, HIV-RNA <40 copies/ml) had a diagnosis of mild neurocognitive disorders (MND) at enrolment (T0) and a normal evaluation at T1. Patient 2 (CD4 T-cells 366/mmc, HIV-RNA <40 copies/ml) had asymptomatic neurocognitive impairment (ANI) at T0 and a normal performance at T1. Patient 3 (CD4 T-cells 522/mmc, HIV-RNA <40 copies/ml) had MND at T0 and normal cognitive functioning at T1. Patients' adherence to the program was 100%.

Conclusions

Asymptomatic forms of HAND still have a relatively high prevalence, both in naïve and cART-treated patients. Given the low performance of 3QT in ascertaining cognitive impairment in HIV-infected patients, other quick screening tools for HAND are needed.

The association of HAND with low CD4+ nadir and CSF/plasma HIV-RNA ratio ≥1 supports the need of an early ART introduction, especially in foreigners and unemployed patients; the control of peripheral viral replication, the management of depression and non infectious comorbidities could probably prevent HAND in treated patients. Moreover, patients with high CSF/plasma HIV-RNA ratio showed a slower response in terms of peripheral and CSF pro-inflammatory/effector phenotypes under cART, supporting the need of a closer follow-up of these subjects, in order to rapidly reduce the viral burden and limiting the neuroinflammation and neurotoxicity. In a follow up period of 12 months of virological suppressive cART, the majority of HAND cases seemed to remain stable, suggesting that cognitive improvement requires a longer time or other approaches complementary to cART, such as cognitive rehabilitation. A computer-based restorative approach showed good results in term of improvement in neuropsychological performances in treated HIV-infected patients, thus requiring a validation in larger cohorts and with randomized controlled trials.

Introduction

Definition and Prevalence of the HIV-Associated Neurocognitive Disorders (HAND)

The HIV-Associated Neurocognitive Disorders (HAND) are a spectrum of cognitive deficits that could affect HIV-infected patients. Currently, there are four diagnostic criteria for the definition of HAND:

(i) Frascati's criteria or HAND criteria[1]

(ii) the clinical rating (CR)[2]

(iii) the global deficit score (GDS)[3]

(iv) the multivariate normative comparison (MNC)[4].

The HAND criteria, proposed by Antinori et al, recognize three different syndromes: Asymptomatic Neurocognitive Impairment (ANI), Mild Neurocognitive Disorders (MND) and HIV-Associated Dementia (HAD); these disorders are diagnosed with a standardized neuropsychological evaluation on the following cognitive abilities: verbal/language; attention/working memory; abstraction/executive; memory (learning; recall); speed of information processing; sensory-perceptual and motor skills. ANI is characterized by a cognitive impairment in at least two cognitive domains and of at least 1.0 standard deviation (SD) below the mean for age and education appropriate norms; it does not interfere with day by day functioning. MND is defined by an impairment in cognitive functioning in at least two cognitive domains and of at least 1.0 SD below the mean for age and education with a mild interference in everyday life, such as a reduced mental acuity, inefficiency in work, homemaking or social activities. Finally, HAD is a severe cognitive deficit in at least two cognitive domains, but typically in multiple domains, and of at least 2.0 SD below the mean for age and education; HAD is also characterized by a striking impact on daily life.

For the diagnosis of each of these disorders, other preexisting causes for cognitive impairment have to be excluded[1].

According to CR, neurocognitive impairment is defined as a score of five to nine in at least two cognitive domains; demographically corrected test scores (T-scores) are categorized by cognitive domain and then CR scores for all domains are assigned on a scale ranging from 1 (above average) to nine (severely impaired) with a cut off of four for borderline cognitive disorders and of five for mild impairment[2].

In GDS approach, T-scores are transformed in a deficit score that has a range from zero (no impairment) to five (severe impairment); the deficit scores are then averaged across all tests used for the neuropsychological evaluation to create the GDS[2]. The

work of Carey et al in 2004 supported the validity of the GDS with a cut off of ≥0,5 to classify neurocognitive impairment in HIV-positive subjects[3].

Finally, MNC is based on one-sided Bonferroni corrected univariate as well as the one-sided multivariate comparisons to correct the scores of the multiple tests that are administered for the complete neuropsychological evaluation[4].

Recently, the POPPY Study investigated the level of agreement between Frascati's criteria, GDS and MNC and tried also to correctly define cognitive impairment in HIV-infected people. The authors found that especially Frascati and GDS showed a good agreement; however, the validity of these criteria is hardly assessable given the lack of a gold standard for HAND diagnosis. They then proposed that an optimal definition of HAND could capture patients with true pathological cognitive impairment and has to include subjects with the lowest cognitive performance scores and the greatest number of cognitive symptom complaints; these definition has still to be validated in the POPPY cohort, in other cohorts and in longitudinal studies[5, 6].

Due to the use of different diagnostic criteria, the reported prevalence of HAND changes from 15 to 55% of HIV-infected patients on antiretroviral treatment and from 30-35% of patients before treatment introduction, depending on the criteria used[5, 7-10]. Approximately 20% of patients died for HAD before the introduction of combination Antiretroviral Therapy (cART) and most of the subjects who died for HAD presented encephalitis at autopsy. Nowadays HAD is observed only in a smaller proportion of patients (1-4%), while less severe, but potentially disabling forms are the prevalent ones and these manifestations occur earlier during the course of HIV infection[8, 11-13]. In the Central Nervous System (CNS) Anti-Retroviral Therapy Effects Research (CHARTER) Cohort, one of the most largest cohort studying neurocognitive impairment in HIV-positive subjects, HAND was diagnosed in 47% of patients and specifically ANI in 33%, MND in 12% and HAD in 2%[14].

Risk factors and confounders

Well-known risk factors for HAND are low CD4+ T cell count, high plasma and cerebrospinal fluid (CSF) HIV-RNA before antiretroviral therapy and AIDS-defining diseases[15, 16]. After the introduction of cART, significant changes in the clinical features of HAND have been reported.

In fact, severe immune depression, high viral replication, HIV-related medical symptoms (such as anemia, low weight and fatigue) and the presence of extrapyramidal signs on neurological examination were associated with an increased risk of HAD before cART,

while many of these factors are no longer associated with less severe forms of HAND in cART-treated subjects[9]. Nowadays risk factors for mild forms of neurocognitive disorders are advanced age, low CD4+ nadir and cardiovascular risk factors[7, 17-21]. Patients with AIDS diagnosis and CD4+ count <200 cells/mmc still have a higher risk of developing HAND before the age of 50 years. The association between viral load and MND or ANI however is less clear than before, as suggested by the appearance of HAND also in treated patients with undetectable plasma and CSF HIV-RNA[7, 22, 23].

Several comorbidities have to be considered in the diagnosis of HAND, such as psychiatric conditions like severe depression and anxiety, age-related cognitive deficits, HCV coinfection, alcohol and substance abuse, nutritional and vitamin deficiencies, traumatic brain injury, accelerated atherosclerosis, obstructive sleep apnea and sleep disorders[7]. Given the ageing of HIV-infected population, cerebrovascular disease risk factors, as well as hypertension, diabetes and hypercholesterolemia, play a major role in contributing to cognitive deficits[13, 24]. Currently, the highest prevalence of these disorders is in the age group over 50 years; thus, the differential diagnosis of HAND must consider all comorbidities, expecially age-related ones[7, 13, 25, 26].

Clinical oucotme and temporal progression

Mild forms of HAND can have significant functional impairment in activities of daily living and have impact not only on the quality of life, but also on the survival[27]. The mean survival for patients diagnosed with HAND has considerably improved recently: in 1993-1995 survival for HAD was only 5 months, while in 1996-2000 increased to 38.5 months and patients with HAND on current cART regimens have a quite normal life span[9]. However, according to a population-based study of 1602 HIV-positive patients mortality risk is three-fold higher in subjects with HAND compared to individuals without neurocognitive impairment[27]. Furthermore, also patients with asymptomatic forms (ANI), even with suppressed plasma viral load, displayed a two to six higher risk of developing symptomatic HAND during several years of follow up, in comparison to subjects without neurocognitive disorders, according to the CHARTER study[28]. Finally, neurocognitive disorders could reduce treatment adherence and could also worsen side effects of therapies with subsequent altered quality of life[27, 29].

The temporal modification of HAND also changed in the cART era. Before cART introduction, HAD was rapidly progressive; in HIV-positive patients on virological effective treatment most individuals with HAND generally seem remain stable with a

small proportion of patients showing deterioration, but rarely they improve or recovered[13]. In a 4-year recent study no modifications of HAND were reported in 77% of patients, while 13% progressed to a more severe form and 10% improved[7]. Similar results were described in a longitudinal study over 5 years[30].

Pathogenetic aspects

Entry of HIV in the CNS

HIV can infect brain very early in the course of infection and can persist in the CNS for years; neuroimaging techniques have demonstrated brain changes already in the first 100 days after infection and the virus has been found in CSF and in brain biopsies even in the first weeks after HIV transmission[31]. In a cross-sectional study on 96 HIV-infected patients with primary HIV infection (PHI) and median 77 days post infection, CNS infection and immune activation was similar to the levels found in the chronic phase[31]. The early neuro-invasion could also explain the neurological disorders that some patients manifest in the acute phase of infection (meningitis, encephalitis, myelopathy and polyneuropathy).

Neuropathogenesis of HAND is a complex mechanism that begins with the invasion of the CNS by the virus and a productive infection of perivascular macrophages and microglia[32].

It is thus well recognized that HIV can cross the blood brain barrier (BBB) and invade the CNS; three different mechanisms have been proposed for the entry of the virus in the CNS[7, 33-35] (Figure 1):

(i) HIV can directly infect endothelial cells and they could release viral particles in the CNS; in fact, endothelial cells could express chemokine receptors involved in the HIV entry, like CXCR4, CCR3, DC-SIGN and L-SIGN.

(ii) The virus may cross directly the BBB, especially if the BBB is damaged or has an increased permeability; some pro-inflammatory cytokines, such as TNF-α secreted by infected macrophages, could increase BBB permeability[36, 37]. HIV could alter the permeability of BBB damaging the stability of tight junctions proteins[38, 39]. Cell-free virus particles can penetrate brain microvascular endothelial cells (BMVECs) thanks to an up-regulation of the expression of the intercellular adhesion molecule ICAM-1.

(iii) HIV infected monocytes, leukocytes and perivascular macrophages can pass the BBB and release viral particles in the CNS[40]. Viral particles are then able to infect resident cells such as microglia, establishing a persistent infection

("Trojan horse" hypothesis)[41]. The most important actors of this process are $CD14_{low}CD16_{high}$ monocytes, that are an intermediate subset between monocytes, differentiated macrophages and dendritic cells, and seem to be more permissive to HIV replication and more easily cross the BBB[42].

Neuro-inflammation and neurotoxicity

After the invasion of the CNS by the virus, a process of neuro-inflammation starts. Infected monocytes and lymphocytes can produce other viral particles; cellular factors and resident CNS cells are infected by viral particles infiltrating the CNS or released from infected cells. Monocytes and T cells infected with HIV can then release pro-inflammatory cytokines, such as TNF-α and IL-1B, which in turn activate microglia. The activation of these cells leads to recall from the peripheral blood of other cytokines, chemokines, products of microbial translocation and infected cells. Activated microglia can also produce neurotoxic factors, such as inflammatory mediators, platelet-derived growth factor (PDGF), nitrogen oxide and quinolinic acid (QUIN) with subsequent activation of astrocytes[43, 44]. Astrocytes can not release virions under normal conditions, but it has been demonstrated that they can release non-structural proteins of HIV and promote inflammation[45]. Both viral and host factors are able to damage brain glutamate metabolism and neurotransmission; in patients with HAND increased CSF levels of glutamate have been reported, in comparison to subjects without neurocognitive disorders. Viral proteins, like Tat and gp120, are able to decrease glial and synaptic glutamate uptake, increase glutamate release from nerve endings and phosphorylate glutamate receptors leading to an increased toxicity of this neurotransmitter[46-48]. The final increased excitotoxicity causes dysfunction and neuronal death[7, 33, 49]. Finally, the release of different viral proteins in the CNS could amplify the BBB permeability with increase of neuroinvasion form the periphery[37] (Figure 2). A kind of vicious circle is thus established and could persist also when the initial stimulus, the viral replication, is suppressed by cART[7].

Role of CSF and peripheral viral load and immune activation

As regard the correlation between CSF viral load and impaired cognitive function, in antiretroviral-naïve patients high levels of HIV-RNA on cerebrospinal fluid (CSF) have been described in HAD, correlate with the severity of dementia and predict future worsening of cognitive functions[50]. However, still nowadays the correlation between CSF HIV-RNA levels and mild forms of HAND is controversial[23, 51-53]; in fact,

neurocognitive impairment could also occur with low CSF viral load and very occasionally with CSF HIV-RNA below the detection limit [54-56]. Previous studies on cohorts of untreated HIV-infected patients have reported median CSF viral burden between 1000 and 10.000 copies/mL, about 1 log lower than plasma viremia[31].

Systemic HIV replication plays also a role in the pathogenesis of cognitive impairment: HIV-RNA levels in plasma are independently associated with neurocognitive disorders and strongly correlate with CSF viral load[11, 12].

In addition to HIV-RNA, chronic intrathecal inflammation and immune activation have been demonstrated in patients with HAND[22, 57-60]; previous studies have shown that in the earliest period of infection median CSF to plasma HIV-RNA ratio is lower than during chronic infection and could indicate a clear distinction between plasma and CNS compartment, but the extent of normal BBB's disruption and intrathecal inflammation is similar, suggesting an early initiation of CNS immune activation with a subsequent possible neuronal dysfunction[31]. A further confirmation of this process could be that most individuals also during primary HIV infection, despite lower viral load in CSF compared to plasma, display high CSF concentrations of neopterin, an indicator of macrophage activation, CXCL10, a chemokine associated with CSF lymphocytosis, and neurofilament protein light chain (NFL), a marker of axonal injury[31]. During long term antiretroviral therapy, it is well demonstrated that levels of immune activation in blood and CSF are reduced[61], although elevated levels of these biomarkers and activated T cells could persist in CSF[43, 62-64]. The percentages of activated CD4+ and CD8+ T-cells increase in CSF, since the earliest phases of HIV infection[65]; in particular, detection of CSF IFN-gamma expressing CD8+ T cells, absence of cytolitic CD8+ cells and low CD4+ T cells responses to HIV are correlated with the severity of neurocognitive impairment[66].

The possible relationship between cognitive function and peripheral inflammation in HIV-infected patients has also been investigated[67]: in fact, non invasive and cost-effective plasma biomarkers of neurocognitive impairment would be more useful and easy to perform than current methods of invasive CSF analysis and neuroimaging. Severe immune depression plays a main role in the development of neurocognitive disorders: a clear association between low CD4+ lymphocyte count at nadir and cognitive impairment has been well demonstrated and, in the pre-cART era, HAD almost exclusively occurred in patients with current CD4+ T cell counts below 200 cells/mmc[66]. Conversely, during viro-immunological efficient cART even subjects with

normal or near-normal CD4+ T cells could develop neurological disorders[68]. More recently, the correlation between CD4/CD8 ratio's inversion and cognitive impairment has been investigated, considering that the inversion of CD4/CD8 ratio can be used as a marker of immune activation and age-associate disease: interestingly, HAND resulted independently associated with a CD4/CD8 ratio <1 in the group of virologically suppressed patients with MND, while this association was not confirmed in subjects with ANI[69-71]. Lower peripheral CD8+ counts at presentation and a higher proportion of CD4/CD8 ratio inversion were observed in impaired patients also in other studies[72].

A higher degree of neurocognitive deficits on cART has been associated not only with the inversion of CD4/CD8 ratio, but also with a higher immune activation of CD4+ and CD8+ cells and with a shift from naïve to effector memory T cells[73]. Thus, the low CD4/CD8 ratio with a skewed T cell differentiation could identify patients at a potential risk of developing neurocognitive impairment, despite immune-virological response to cART[71].

So far, however, the correlation between peripheral T-cells homeostasis, CSF HIV-RNA and neurocognitive deficits, especially in untreated patients, is not well known and deserves further studies.

Screening and diagnosis

Screening tests

The European AIDS Clinical Society (EACS guidelines, version 9, October 2017) states that neurocognitive impairment has to include (Figure 3):

(i) marked acquired impairment in cognitive functioning involving at least 2 cognitive domains, as documented by a performance of at least 1 SD below the mean for age-education appropriate norms on neuropsychological tests;
(ii) interference in daily functioning;
(iii) no evidence of another pre-existing cause for the dementia.

After the exclusion of obvious confounding conditions (severe psychiatric diseases, abuse of alcohol and psychotropic drugs and previous or current CNS opportunistic infections or other neurological diseases), cognitive deficits have to be screened in symptomatic HIV-infected patients at diagnosis and before cART introduction. The screening has to be done by the following 3 questions that may be used to guide physician assessment:

1. Do you experience frequent memory loss (e.g. do you forget the occurrence of special events even the more recent ones, appointments, etc.)?
2. Do you feel that you are slower when reasoning, planning activities, or solving problems?
3. Do you have difficulties paying attention (e.g. to a conversation, book or movie)?

For each question the answer could be. a) never, b) hardly ever, or c) yes, definitely. HIV-infected patients who answer “yes, definitely” on at least one question are considered to have an “abnormal” result and have to be evaluated for depression. If the diagnosis of depression is excluded or the disease is optimally treated, but the cognitive problem persists, the patients have to undergo a complete neuropsychological examination[74]. A comprehensive neuropsychological evaluation is the gold standard for the assessment of HAND. The administration of a complete neuropsychological testing is however time-consuming and requires trained staff, limiting its use in clinical settings and for screening purposes. There is therefore need for brief and easily accessible tools for the HAND screening. So far different screening tests have been proposed, but there is not consensus on the most adequate one[75-77].

Among screening tests, the Montreal Cognitive Assessment (MOCA) is frequently used, but it is not designed specifically for HIV-infected patients and is not culturally appropriate for global use[78-84]. International HIV Dementia Scale (IHDS) is another widely used tool, but has some limitations at the cut off score of ≤10 in discriminating between mild and more severe forms of HAND[75, 81, 85-87].

Several studies have therefore demonstrated that the combination of different screening tests or brief computerized screening batteries, including different combinations of standardized neuropsychological tests, could improve sensitivity and specificity and suggest this approach for the screening of HAND[81, 88-91].

Neuropsychological evaluation

The comprehensive neuropsychological examination has to include tests exploring the following cognitive domains: fluency, executive functions, speed of information processing, attention/working memory, verbal and visual learning, verbal and visual memory and motor skills. The examination is then completed by the assessment of daily functioning through the Instrumental Activities of Daily Living (IADL)[74] (Figure 4).

All tests are administered by trained neuropsychologists and scored in accordance with published manuals. Raw scores were converted to demographically adjusted normative T scores; all scores are also adjusted for age and length of education[1, 91] (Figure 4).

Neuroimaging

To diagnose and manage HIV-associated neurocognitive impairment, a neurological examination, brain MRI and CSF examination have to be performed also to exclude other diseases. The quantification of HIV-RNA on CSF and, where appropriate, genotypic drug resistance (GDR) in paired CSF and plasma samples have to be assessed[74]. Different neuroimaging techniques have been studied for the diagnosis and the monitoring of HAND[92]; beside standard MRI, magnetic resonance spectroscopy (MRS), volumetrics and diffusion tensor imaging (DTI), functional MRI (fMRI) and positron emission tomography (PET) have been used in HIV-infected patients in research settings. Most common radiological findings of HAND are brain atrophy, demyelinization and white matter alterations[93-97].

Lumbar puncture

Lumbar puncture for the determination of CSF viral load allows the identification of CSF viral escape, that is defined as CSF HIV-RNA ≥1 $\log_{10}$ copies/mL compared to plasma HIV-RNA or the presence of detectable CSF viral load when plasma viremia is undetectable[98]. Viral escape can occur in 4-21% of cART treated patients and is associated with CNS immune-activation[99, 100]. The mechanisms leading viral escape in CSF and its risk factors are not completely understood. Potential risk factors for viral escape could be inadequate CNS penetration by some cART regimens, persistent low-level viremia (LLV), length of time on cART, drug-resistance mutations in CSF and low CD4+ nadir[101]. Given the not exact correlation between CSF HIV-RNA levels and neurological symptoms, also the clinical features of patients with CSF viral escape do not seem so clear[70, 102, 103]. However, besides some cases of asymptomatic patients harbouring up to 200 copies/mL of CSF HIV-RNA, several case series describe subjects with new neurological symptoms, CSF viral escape and clinical improvement after switching cART regimens[55, 104-107].

Biomarkers

In research settings a lot of biomarkers have been identified, even if no one could be used in routine clinical practice[108]. Saylor D and colleagues proposed a classification for the available biomarkers in four groups: immune activation, metabolic or cellular stress, neuronal injury and neuroimaging markers[7]. Another possible classification includes monocyte activation markers, cerebral injury markers, inflammatory cytokines and proteins, chemokines and cardiovascular markers (Figure 5). The most widely studied biomarkers are the following:

Neopterin: CSF and plasma neopterin has been used as a soluble marker of CNS inflammation, as it is produced by activated macrophages and astrocytes, mainly in response to interferon-gamma[109]. It has been shown that 97.5% of neopterin in CSF is intrathecally produced, and it is not simply a passage from blood. Levels of CSF neopterin correlate with neurofilament light chain, a marker of neuronal injury[110].

Neurofilaments (NFLs) are structural proteins that are specific for neurons and are released into the CSF and blood following axonal disruption or degeneration[111]. In HIV infection, CSF NFLs levels are elevated since the earliest phases of infection[63], also in patients with mild forms of HAND[112]. Moreover, plasma and CSF NFLs levels are highly correlated, thus the plasma NFLs levels can be useful to assess the presence of active CNS injury[113].

LPS and sCD14. During HIV infection, the gastrointestinal mucosa undergoes a massive T cells depletion, leading to translocation of microbial products such as lipopolysaccharide (LPS) into the systemic circulation[114]. LPS is able to activate innate immune system cells thanks to Toll-like receptor-4 (TLR-4) and its binding with CD14. CD14 is expressed on monocytes, macrophages, and neutrophils, and upon stimulation by LPS, as well as other microbial products, CD14 is secreted and cleaved from the cells as soluble form (sCD14)[115] . Plasma sCD14 levels independently predict mortality and impaired neurocognitive test performance in HIV-infected subjects[116]. Thus, sCD14 is an excellent candidate biomarker of monocyte/macrophage activation associated with HIV-induced neurodegeneration. A recent study demonstrated, in untreated patients, a positive correlation between expression of CSF NFLs isoforms and

CSF sCD14, thus directly linking CNS monocyte/macrophage activation with neuronal injury[64].

CD163, a monocyte-associated hemoglobin/haptoglobin complex scavenger receptor, is cleaved and shed from activated monocyte/macrophages in a soluble form (sCD163) in inflammatory states. Plasma sCD163 levels are elevated in HIV-infected subjects, particularly those with cognitive impairment[117]. In addition, CD163+ monocytes/macrophages accumulate in perivascular brain regions in individuals with HIV encephalitis[118]. The number of perivascular CD163+ monocyte/macrophages positively correlates with plasma HIV load, suggesting trafficking of peripherally activated monocytes to perivascular areas in the brain[118]. Finally, increased plasma sCD163 levels have been demonstrated in cognitively impaired HIV-infected individuals compared with non-impaired individuals and plasma sCD163 highly correlates with CSF sCD163 levels[117, 119].

TNFα. Tumor necrosis factor-α is a pro-inflammatory cytokine produced by macrophages, circulating monocytes and microglia. Although there is evidence that TNFα may protect neuronal cells by promoting astrocytosis, activating NF-kB, stimulating antioxidant pathways and limiting the inflammatory response, TNFα also damages CNS cells through direct and indirect mechanisms. These pathogenic effects include oligodendroglial toxicity and demyelination, resident microglial activation and disruption of the BBB[120]. This cytokine is significantly increased in the HIV infected brain and has been shown to correlate with the HAD severity and the degree of immune activation[121].

IP-10 (CXCL10). Interferon-γ-inducible protein 10 is expressed by astrocytes, microglia and endothelial cells during inflammation and acts specifically on activated T cells and macrophages, attracting T lymphocytes into the CSF[122]. In the brain, IP-10 can be induced by HIV-1 viral gp120, Nef, and Tat. Cooperative interaction of HIV Tat and IFN-γ results in IP-10 over-expression, which in turn can amplify the inflammatory responses within the CNS by recruiting more lymphocytes into the brain[123]. IP-10 has been detected in the CSF of individuals with HIV-1 infection and in the astrocytes of individuals with HAD[124]. Yuan L. et al demonstrated that the expression of IP-10 was

significantly elevated in HIV-infected patients with neurocognitive impairment whether in CSF or in plasma[122].

IL-6 is an activator of acute phase responses and is involved in crosstalk with other inflammatory mediators. IL-6-mediated inflammation is known to cause a higher incidence of gliosis and dendritic damage in patients with Parkinson's disease, amyotrophic lateral sclerosis, multiple sclerosis and Alzheimer Disease[125]. High plasma levels of IL-6 are associated with worse cognitive function in HIV-infected patients, supporting a role for chronic inflammation in neurocognitive decline[126, 127].

MCP-1. The monocyte chemoattractant protein-1 is a chemokine that is expressed during inflammation and that, upon activation of its receptor (CCR2), can induce chemotaxis of monocytes to inflammatory sites. MCP-1 is expressed by monocytes, macrophages, dendritic cells, neurons, astrocytes, microglia and endothelial cells[128, 129].

MCP-1 levels in the brain and cerebrospinal fluid (CSF) are elevated in HIV-infected patients with encephalitis, AIDS dementia and HIV-positive patients with cerebral inflammation[130].

s100β is a calcium binding protein that is expressed almost exclusively by astrocytes in the brain and is a marker of astrocytosis[131]. The presence of s100β in peripheral blood is an useful indirect marker of BBB damage and its levels have been associated with cognitive impairment in HIV-infected patients[132].

HIV proviral DNA. In cART-treated patients the circulating peripheral HIV proviral DNA load in macrophage/monocytes (CD14/CD16) is associated with HAD and minor cognitive motor disorders, independently of HIV RNA levels[133]. The total amount of circulating HIV-DNA associates with an increased risk of HAND also in treatment-naïve subjects with advanced immune suppression[134]. This marker also correlates to MRS markers of brain injury and to neopterin levels[135].

Any single biomarkers is however insufficient to identify early stages and minor forms of HAND; thus, to date a validated biomarker for the diagnosis and the follow up of HAND has still to be found.

Clinical features

HAND is characterized by an insidious onset with a slow progression. Neurocognitive disorders are associated with several dysfunctions: decrease of attention, mood alterations, depression, psychomotor disturbs, alteration in the extrapyramidal movements and spasticity[33]. In the earliest stages difficulties in concentration and memory and impaired executive functions predominate, while psychomotor slowing, depression, irritability and subclinical motor signs are the most frequent characteristics with the progression of the disease. If untreated, a progression towards dementia with mutism and incontinence could be observed[13].

Clinical features of HAND have also changed in the cART era; extrapyramidal signs like bradykinesia, rigidity, tremor and motor signs are now less common. Regarding the neuropsychological profile, before cART cognitive impairment involved more commonly subcortical area with psychomotor slowing, motor dysfunction and memory deficit, while nowadays a mixed pattern of both cortical and subcortical features prevails with higher impairment in executive functions and working memory[14].

Therapeutic approaches

cART is the only therapeutic possibility in HIV-infected patients with HAND, but unluckily it is effective only in a subset of patients. Early initiation of cART seems to be essential to prevent the establishment of neurocognitive disorders, that are then very difficult to treat. However, in the START trial early cART initiation does not have a great impact on HAND; although early cART introduction results in fewer AIDS-related diseases in comparison to patients with deferred therapy, neurocognitive performance does not differ between groups after a mean of 3.3 years of follow up[136].

A recent study has not confirmed these results: according to Robertson et al, the cART start very shortly after HIV acquisition results in greater improvement in neurocognitive performance of HIV-infected patients compared to defer treatment after 24 weeks[9].

However, as possible explanations of these contrasting results we have to consider that in the START trial the mean follow up of patients was not so long, there was a high proportion of subjects on potentially neurotoxic efavirenz and low rates of HAND were found due to short disease duration and high CD4+ counts[137].

The CNS penetration effectiveness (CPE) score was developed by Letendre et al in order to rank drug penetration and efficacy in the CNS[138]; the initial and updated

ranking systems have then been validated in the CHARTER cohort[139] (Figure 6). In this ranking system, cART drugs are hierarchically assigned a value from 1 (low) to 4 (high) according to published data about:

(i) effectiveness in the CNS (pharmacodynamics),

(ii) drug concentrations in CSF (pharmacokinetics),

(iii) drug characteristics (i.e. protein binding).

The values for all drugs in a regimen are then summed up to yield a final CPE score. Higher CPE scores correlate with undetectable CSF viral loads, suggesting that this score may provide useful information to select cART regimens that better control HIV replication in the CNS and reduce HIV-related brain injury. A review on this topic found that the majority of published studies reported an association between regimens with higher CPE and either decreased CSF viral load or better neurocognitive outcomes[140]. However, the correlation between CPE score and neurocognitive performance is still controversial, given that other studies did not find any association or reported even a worse cognitive outcome associated with higher CPE scores[141-143] and thus the utility of the CPE score remains controversial. These contrasting results may be attributed to the study design and statistical power differences, the failure to adequately account for confounders and the neurotoxicity of some cART drugs. Indeed, the CPE score is limited by its categorical scoring, unclear weighting of each criterion (pharmacokinetic, chemical properties, etc.), and lack of consideration of toxic effects or drug interactions. Furthermore, the CPE score relies on CSF drug concentrations, which may not reflect the brain parenchymal pharmacokinetics, and efficacy in glial cells is not considered, primarily due to limited available data[76]. The efficacy of CNS-penetrating antiretrovirals in astrocytes and macrophage-lineage cells have been studied using *in vitro* models of infection: all the studied agents could inhibit HIV infection in macrophages with concentrations typically found in CSF and similar results were found in astrocytes, except for lamivudine, stavudine, and zidovudine, which required greater concentrations, suggesting that these drugs probably do not adequately treat astrocyte reservoirs[144].

Recent studies suggest that CCR5 receptor interactions with CNS reservoir cells are associated with HAND. Maraviroc, a CCR5 receptor antagonist, has adequate CSF penetration, inhibits CNS viral replication, including in monocyte/macrophage cells, and has anti-inflammatory properties in the CNS. A recent prospective, open-label, pilot randomized controlled trial in individuals with viral suppression and stable cART for 12

months found maraviroc-intensified cART improve global neurocognitive performance at both 6 and 12 months without significant side effects[145]. Current randomized controlled trials are investigating the effects of cART intensification with maraviroc or dolutegravir on neurocognitive performance.

Given the potentially confounding CNS effects of efavirenz (EFV) and the detrimental effects on neurocognitive function of EFV[146] and nucleoside sparing regimens, such as protease inhibitor monotherapy[147], current international guidelines recommend to avoid these regimens as first-line treatment in patients with HAND. Considering the uncertainty in literature about better neurocognitive performance with CNS-penetrating antiretrovirals, guidelines do not recommend to take in consideration CPE score in therapeutic decision for starting cART in HIV-infected patients with HAND; however, at least in subjects with symptomatic CNS disease, probably also CPE score has to be considered[13, 76] and EACS guidelines suggest to consider the inclusion of potentially CNS-active drugs. A CNS-active drug is characterized by:

(i) a demonstrated CSF penetration when studied in healthy HIV-positive populations (concentration above the IC90 in >90% examined persons)

(ii) proven efficacy on cognitive function or CSF HIV-RNA decay in a short-term period (3-6 months) when evaluated as single agents or in peer-reviewed controlled studies.

In patients already on cART the management of HAND is still more complicated. Generally, cART should optimized according to plasma and CSF GDR test[76]. In patients without CSF viral escape, the decision of switching cART or continuing the same regimen depends on each specific situation and other causes for neurocognitive impairment have to be searched[74] (Figure 3-Figure 7).

As a result, there is no definitive HAART guideline for HAND and further investigations with randomized controlled trials are needed[13, 77, 92, 144].

The absence of an optimal first-line pharmacotherapy for HAND has led researchers to explore cognitive and behavioral approaches. In the last years some pilot studies investigating computerized rehabilitation on HIV-infected patients have been conducted[148-151]. Even if their outcome was not the resolution of HAND, but the improvement of specific neurocognitive functions, such as learning memory and speed of processing, their initial results are encouraging, providing important information regarding a possible strategy for the future treatment of cognitive deficits[150, 152, 153].

Moreover, in 2015 Livelli et al. have shown data about the efficacy and stability over time of a cognitive rehabilitation protocol in treated HIV+ patients with HAND[154].

Indeed, non-antiretroviral treatments (intranasal insulin, IL-1 antagonists, paroxetine and complement inhibitors, as well as limiting the replication of viral transcripts by Tat monoclonal antibodies, antisense oligonucleotides and adjunctive neuroprotective strategies) have been tested for the treatment of HAND without a clear clinical benefit[13]. Non pharmacological interventions that may have a positive clinical effect are the correct management of concurrent subclinical and clinical diseases, such as HCV infection, major depressive disorders, cardiovascular and metabolic comorbidities; another crucial step has to be the improvement of cART adherence[76]. A comprehensive algorithm for the management of HIV-positive patients with HAND has been proposed by Calcagno A et al and includes the prevention and treatment of age, virus and drug-associated comorbidities[144] (Figure 8).

Aim

Mild forms of neurocognitive impairment are still quite common among HIV-infected patients, especially in cART treated patients, also in the context of suppressed viral replication in periphery. Besides traditional risk factors, ageing and vascular comorbidities are acquiring more and more evidence as cofactors in the pathogenesis of cognitive impairment in HIV-infected patients. Despite the gold standard for the diagnosis of HAND is a comprehensive neuropsychological evaluation, it is not always applicable in the clinical routine and thus the search for the perfect screening test is still ongoing. Finally, no effective treatment for HAND is currently available.

In this context, the aim of this study was:

-to explore the performance of the “Three Questions Test”, proposed by the European AIDS Clinical Society (EACS guidelines, version 9, October 2017), for the screening of cognitive impairment in HIV-infected patients;

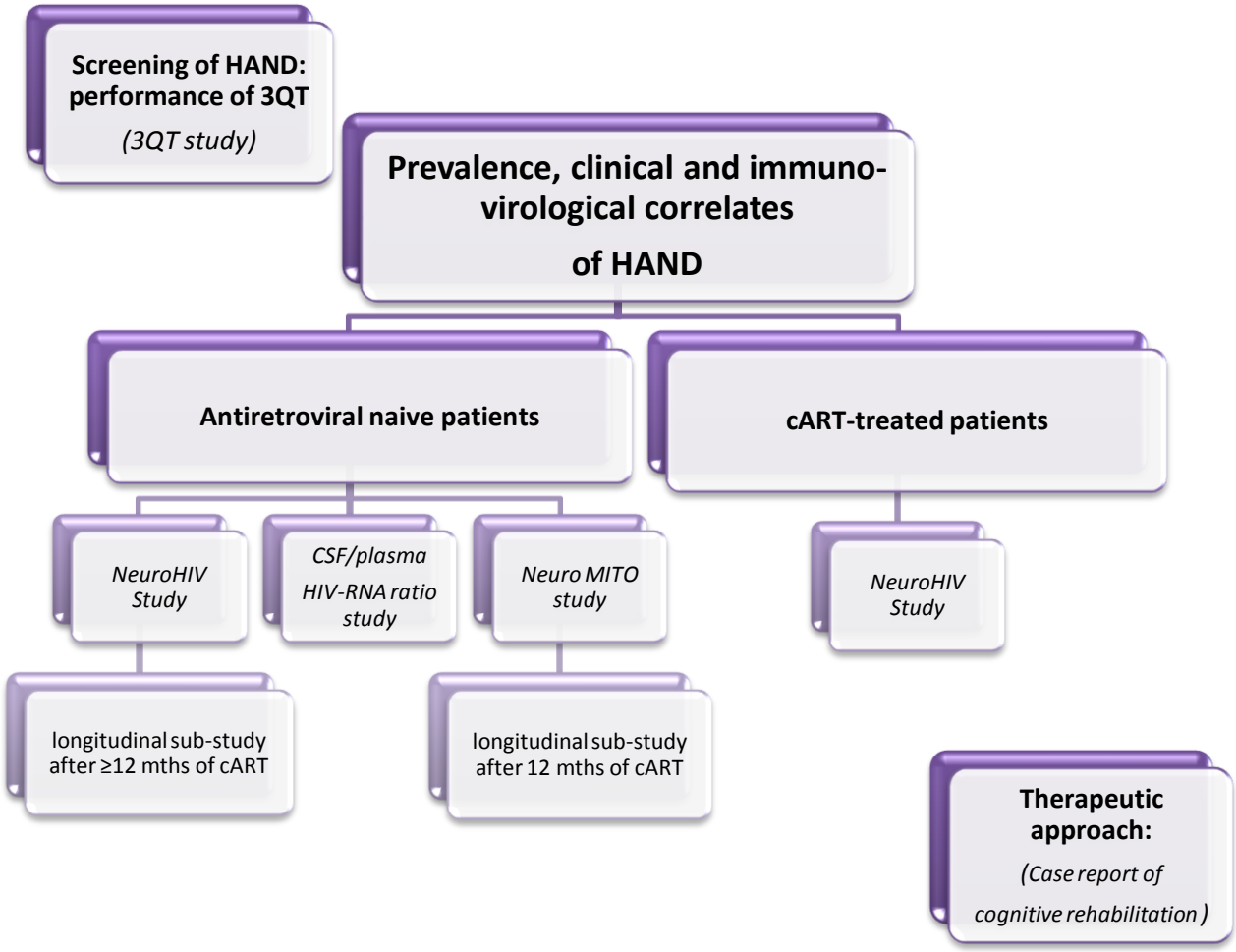
-to investigate the prevalence, the clinical and viro-immunological correlates of the HIV-associated neurocognitive disorders in a cohort of antiretroviral-naïve and cART treated HIV-positive patients;

-to observe the modification of HAND after at least 12 months of virological effective antiretroviral treatment;

-to explore a panel of peripheral and CNS inflammatory and immune activation markers in untreated HIV-infected patients and the effects of 12 months of virally-suppressive cART, according to CSF/plasma HIV-RNA ratio;

-to investigate the efficacy of a new computer-based program complementary to cART in improving cognitive functioning.

We thus performed the following studies:


Screening of HAND: performance of 3QT
(3QT study)
Prevalence, clinical and immuno-virological correlates of HAND
Antiretroviral naive patients
cART-treated patients
NeuroHIV Study
CSF/plasma HIV-RNA ratio study
Neuro MITO study
NeuroHIV Study
longitudinal sub-study after ≥12 mths of cART
longitudinal sub-study after 12 mths of cART
Therapeutic approach:
(Case report of cognitive rehabilitation)

Materials and methods

The San Paolo Infectious Diseases (SPID) Cohort

The study was conducted at the San Paolo Infectious Diseases Cohort (SPID cohort). The SPID Cohort has been established in 2006 with the objective to conduct clinical research's studies about HIV infection, hepatitis coinfection and sexually transmitted diseases; adult HIV-positive patients that are in active follow up at the Clinic of Infectious Diseases, San Paolo Hospital, ASST Santi Paolo e Carlo, Department of Health Sciences, University of Milan, Italy, are enrolled in the cohort after signing an informed consent. The data are collected during the routine blood exams and follow up visits; the database is reviewed once a year to correct eventual mistakes and missing data. 1143 patients are currently enrolled: 826 (76.6%) are males and median age is 48.4 (IQR 47.8-49) years. 1118 (98%) are on antiretroviral treatment.

Neuropsychological battery

HIV-infected patients enrolled in the study underwent a comprehensive neuropsychological evaluation. This evaluation was performed at our Centre by a trained neuropsychologist; routinely, in our centre this evaluation is proposed to all HIV-infected patients at the first diagnosis of infection before the introduction of antiretroviral treatment. cART-treated patients underwent the same neuropsychological assessment if they report cognitive symptoms during routine follow up visits. The neurocognitive battery includes 11 tests exploring 7 main cognitive domains (Table 4): speed of information processing [Trail Making Test Part A, Symbol Digit Modality Test, Stroop Color Test–Time]; learning and memory [Rey Auditory Verbal Learning Test Immediate Recall and Delayed Recall, Rey Osterrieth complex Delayed Recall]; abstraction/executive functioning [Stroop Color Test–Error, Trail Making Test Part B (TMT-B), Rey Osterrieth complex Figure Copy]; verbal fluency [Semantic and Phonemic Fluency]; attention/working memory [Corsi's block Tapping Test, Forward and Backward Digit span, Trail Making Test Part BA]; motor skills [Finger Tapping Test]; the neuropsychologist also performed a functional assessment through the Instrumental Activities Of Daily Living (IADL). Raw scores on each test were calculated. Scores were corrected for age, educational level and gender using Italian normative data. In particular, the raw scores were first adjusted and then transformed into new standardized scores (named Equivalent Scores) on an ordinal scale ranging from 0

(pathological performance) to 4 (best performance)[155]. Frascati's criteria were used for the diagnosis of HAND[1].

The neuropsychological assessment includes also evaluation for Anxiety and Depression symptoms with the Hospital Anxiety and Depression Scale (HADS) and evaluation for Quality of Life with the SF-36 Health Survey (SF-36).

Instrumental Activities Of Daily Living

Instrumental Activities Of Daily Living (IADL) is an eight-item questionnaire that includes functional evaluations about the ability of use the telephone, engage in cooking, shopping, housekeeping and laundry, use of transportation, medication and financial management[156, 157]. For each question there are three possible answers and the scoring for each response is: three points for independent, 2 points for any assistance needed, 1 point for totally dependent. The presence of any assistance in daily living is considered a positive score.

Hospital Anxiety and Depression Scale (HADS)

To assess psychological distress in non-psychiatric patients, the Hospital Anxiety and Depression Scale (HADS) is a frequently used self-rating scale to measure symptoms of anxiety and depression. It consists of two subscales, Anxiety and Depression, and includes 14 items, seven items for the anxiety subscale (HADS Anxiety) and seven for the depression subscale (HADS Depression). HADS Anxiety focus mainly on symptoms of generalized anxiety disorder and HADS Depression is focused on anhedonia, the main symptom of depression. Each item is scored on a response-scale ranging between 0 and 3. After adjusting for six items that are reversed scored, all responses are summed to obtain the two subscales. Recommended cut-off scores according to Zigmond AS are 8–10 for borderline cases and ≥11 for definite cases[158, 159].

The symptoms of depression could be also investigated through the Beck Depression Inventory-II (BDI-II) The BDI-II has a high validity and reliability in measuring depressive symptoms. It includes 21 symptoms from 0 to 3 according to how the patient felt during the past 2 weeks. The cut-off scores are: 0−13, minimal depression; 14−19, mild depression; 20−29, moderate depression; and 29−63, major depression. All patients with a BDI-II score of ≥ 20 have to be addressed to a psychiatric evaluation[160].

SF-36 Health Survey (SF-36)

SF-36 Health Survey is one of the most used instrument to assess quality of life. It is a questionnaire based on eight multi-item health domains (35 items): physical functioning (10 items), bodily pain (2 items), limitations due to physical health problems and personal or emotional problems (7 items), bodily pain (2 items), emotional well-being (5 items), social functioning (2 items), fatigue (4 items) and general health perceptions (5 items). These eight scales are then aggregated into two summary measures: Mental Health Index (MHI) and Physical Health Index (PHI). The 36th item, that provides an indication of perceived change in health, is not included in the summary scores. The responses are scored in a two-step process: first, precoded numeric values are recoded per the scoring validated key and each item is scored on a 0 to 100 range; then, in step 2, items in the same scale are averaged together to create the 8 scale scores. Higher scores define a more favorable health state. There is not a standardized cut off, but a database of normative data is available to calculate the norm-based score that identifies the pathological cases.[161-164].

According to the study in which patients were enrolled, after signing an informed consent, HIV-infected individuals could also undergo blood exams to detect plasma HIV-RNA and CD4+ T cells count and lumbar puncture to quantify CSF HIV-RNA by the ultrasensitive Abbott RealTime HIV-1 assay (Abbott Laboratories) on frozen samples (lower detection limit of <40 copies/mL). Demographic, clinical and other viro-immunological parameters were collected from the clinical records and the database of SPID cohort.

Results

Performance of the "3 Questions Test" as a screening test of HIV-Associated Neurocognitive Disorders: evaluation of Sensitivity, Specificity, Positive and Negative Predictive Value and association with Altered Quality of Life (*3QT study*).

Introduction

An adequate and reliable screening test for HAND is not yet available; thus, even if cognitive impairment in HIV-infected patients displays an unacceptable relatively high prevalence, these disorders are not routinely screened. In fact, several screening tools have been studied for HAND, but they display a low sensitivity and specificity in the range of 50-70%, in particular for milder forms of cognitive impairment, that are the prevalent ones nowadays[80, 165-167]. The overall performance of the available screening tests is thus not optimal and suggests the ongoing need for accurate and simple instruments to assess neurocognitive deficits in HIV-infected subjects.

The European AIDS Clinical Society (EACS guidelines, version 9, October 2017) recommends that the "Three questions test" (3QT) may be used to guide physician to screen HAND in HIV-infected patients complaining of cognitive problems. This test, also called the Simioni Symptom Questions, had been proposed for the first time by Simioni S and colleagues; they found that patients complaining cognitive symptoms were affected by HAND in the 84% of cases, but 64% of patients with cognitive impairment were without complaints. As expected, the majority of "non complainers" subjects presenting HAND showed ANI[168].

EACS guidelines suggest this screening test as the first approach in HIV-positive patients (Figure 3). After the exclusion of the confounding conditions, such as severe psychiatric illnesses, abuse of psychotropic drugs, alcohol abuse, *sequelae* from previous opportunistic infections of the CNS or other neurological diseases and current CNS infections or neurological conditions, the following simple 3 questions have to be asked to the patient:

1. Do you experience frequent memory loss (i.e. do you forget the occurrence of special events, even the more recent ones, appointments, ect)?
2. Do you feel that you are slower when reasoning, planning activities or solving problems?
3. Do you have difficulties paying attention (i.e. to a conversation, a book or a movie)?

For each question, answers could be: a) never, b) hardly ever, or c) yes, definitely. HIV-positive persons are considered to have an “abnormal” result when answering “yes, definitely” on at least one question. If the test results “abnormal”, an evaluation for depression and its eventual treatment have to be considered. If the problem persists, even when depression has been excluded or optimally managed, patients undergo a complete neuropsychological examination.

Neuropsychological evaluation will have to include tests exploring the following cognitive domains: fluency, executive functions, speed of information processing, attention/working memory, verbal and visual learning, verbal and visual memory, motor skills plus assessment of daily functioning through Instrumental Activities of Daily Living (IADL).

The association between self-reported cognitive symptoms and objective neurocognitive impairment is still under debate; in fact, while few published works reported an association between self-reported complaint and overall cognitive performance assessed by neuropshychological evaluation, several studies did not find any association[169]. It has been hypothesized that the lack of association reflects the changes in the pattern of cognitive deficits in the recent years, especially in subjects with mild disorders, with a shift from the impairment in executive functions (reasoning) and working memory (attention) to poorer verbal learning related to patients reported memory problems. The reasons for the poor performance of subjective measures of cognitive function in the screening of objective cognitive deficits could be the over-reporting of symptoms, the excessive subjective nature of some instruments, such as the 3QT, or the influence of depression and anxiety disorders[5].

Aim

In this context, we hypothesized that the self-reporting 3QT is not reliable for the screening of cognitive disorders in patients with frontal and temporal cortex impairment. The aim of our work was thus to investigate the sensitivity, specificity, positive and negative predictive value of 3QT in a cohort of antiretroviral naive and treated HIV-positive patients; we also explored factors associated with having an “abnormal” 3QT.

Methods

We conducted a cross-sectional study, including antiretroviral-naive and treated HIV-infected people in active follow up at our centre (SPID cohort).

Inclusion criteria were:

- >18 years old
- Antiretroviral-naive HIV-positive patients or HIV-positive patients on cART with any cART regimen
- Comprehension and speaking of Italian language
- Any CD4+ count and plasma HIV-RNA

Exclusion criteria were:

- Acute HIV infection
- Current or *sequelae* of CNS Opportunistic Infections
- Current alcohol or substance abuse
- Cirrhosis
- Current psychiatric or neurologic disorders

After enrolment, patients underwent 3QT, a complete neurocognitive evaluation (11 tests exploring 7 cognitive domains), performed by a trained neuropsychologist, SF-36 Health Survey and Hospital Anxiety and Depression Scale (HADS) on the same day.

Comorbidities were defined as cardiovascular risk factors (Framingham risk score>20%) or cardiovascular diseases, renal impairment (GFR<90 ml/min by CKD EPI equation) or history of malignancies.

According to 3QT results, patients were divided in two groups: subjects with abnormal 3QT (a3QT) if they answered “yes, definitely” to at least one question and patients with normal 3QT (n3QT).

HAND was diagnosed according to the Frascati’s criteria (neurocognitive impairment was defined as 1 SD below the mean for demographically corrected norms over at least 2 cognitive domains)[1]. We considered pathological a score >1 in the IADL, a score >11 in the HADS and the norm-based pathological scores for MHI and PHI in the SF-36 Survey (see section Materials and Methods).

Sensitivity, specificity, positive and negative predictive value (PPV and NPV) were calculated with the standard methods in comparison with the reference diagnosis (patients with HAND).

We considered a value of 80% as a good sensitivity and of 80% as a good specificity.

We performed receiver operating characteristic (ROC) curve analyses to identify the predictive accuracy of the 3QT. The area under the curve (AUC) and the 95% confidence interval were calculated. Differences between groups were evaluated

through Chi-squared test and Mann Whitney test, as appropriate. Factors associated with “abnormal” 3QT were investigated through univariable and multivariable logistic regression analyses. We also explored the association between altered quality of life assessed by SF-36 Health Survey and HAND.

All the analyses were performed on the whole study population and on untreated and treated patients separately. Statistical analyses were carried out with SPSS software, version 21.0.

Results

Performance of 3QT in the study population

We enrolled 302 HIV-infected patients; 191/302 (63%) were antiretroviral-naive subjects, 111/302 (37%) were on treatment. Table 1 shows demographic and viro-immunological characteristics of study population. Median age was 44 (35-51) years and 244 (81%) were males. CD4+ T cells nadir was 311 (163-469) cells/mmc and 52 (17%) subjects presented an AIDS-defining disease.

99/302 (33%) of the patients presented an “abnormal” 3QT (a3QT), 82/302 (27%) showed HAND (Figure 9a-b). As regards quality of life, 121 (40%) and 52 (17%) patients displayed altered MHI and PHI, respectively; 38 (12%) were symptomatic for depression and 92 (30%) for anxiety. Among patients with HAND, we observed, as expected, a prevalence of mild forms of cognitive impairment: 71 (86%) cases of ANI, 10 (12%) of MND and only 1 case (2%) of HAD (Figure 9b).

Analyzing the performance of 3QT on the whole population, we observed that among 99 patients complaining of cognitive decay, only 35 (35%) had HAND diagnosis; similarly, 47/82 (57%) HAND diagnoses would have been missed relying only on 3QT (Figure 9c).

Sensitivity, specificity, PPV and NPV of 3QT resulted low and <80% in the population: sensitivity was 43% (32-53%), specificity 71% (65-77%), VPP 35% (26-45%) and VPN 77% (71-83%). (Table 2).

The ROC curve is presented in Figure 10: area under the curve (AUC) was 0,568, 95%CI 0,494-0,642. Optimal cut off for 3QT cannot be investigated given the binary score of the test (normal/abnormal) and the lack of data regarding patients’ answers at each of the three questions of the test.

Given that 3QT did not show an adequate sensitivity and specificity for the diagnosis of HAND, we asked which factors were associated with answering positively to the 3QT

(a3QT). By fitting an univariable logistic regression analysis, females, heterosexual contacts, a longer time since HIV diagnosis, being on cART and comorbidities were associated with having a3QT. Furthermore, HAND, but also impaired SF-36 PHI and MHI and altered anxiety and depression scores by HADS resulted associated with a3QT. In the multivariable model, being on treatment was confirmed associated with a3QT; interestingly, HAND was not independently associated with a3QT, while SF-36 PHI and MHI displayed, respectively, a 4- and 3-fold higher probability of a3QT (Table 3).

Performance of 3QT in untreated and on treatment HIV-infected patients

We then explored the performance of 3QT in the group of untreated and on treatment patients separately. The population of untreated and on cART patients presented some differences that reflect the effect of antiretroviral treatment and the epidemiological characteristics of the SPID cohort. In fact, in comparison with treated HIV-infected patients, antiretroviral naïve subjects were younger, more frequently males and MSM. They were less commonly affected by HCV coinfection and other comorbidities. As regards HIV-related characteristics, untreated patients presented a shorter time since HIV diagnosis and a higher CD4+ nadir. 94 (85%) patients on treatment displayed undetectable plasma viral load (Table 1).

Finally, untreated subjects also showed a lower proportion of a3QT, anxiety and depression symptoms and HAND (Table 1): prevalence of a3QT was 23% in untreated patients and 49% in treated subjects, HAND was found in 22% and 35% of antiretroviral naïve and on treatment patients, respectively.

Sensitivity, specificity, PPV and NPV were confirmed low also dividing patients in antiretroviral naïve and on treatment subjects (Table 2). ROC curves are presented in Figure 11: AUC was 0,56, 95%CI 0,448-0,672 for untreated subjects and 0,543, 95%CI 0,443-0,643 for patients on cART.

In the group of untreated patients, we observed that a3QT was associated with female gender and impairment in PHI and MHI, performed by SF-36 survey.

By fitting a multivariable analysis, antiretroviral naïve patients with altered SF-36 MHI presented a 2,3-fold higher probability of a3QT, adjusting for gender, SF-36 PHI and HAND (Table 4).

No demographic and viro-immunological parameters were associated with a3QT in treated patients; in the univariable model, a higher reporting of anxiety, depression and altered physical and mental health were associated with a3QT. Adjusting for HAND,

altered HADS anxiety and depression scores and SF-36 PHI and MHI, only SF-36 PHI remained independently associated with a3QT (Table 5).
HAND was not associated with a3QT both in untreated and on treatment patients.

Association between self-reported quality of life and HAND

Given that the evaluation of quality of life by PHI and MHI of SF-36 Health Survey is more complete and detailed than the simple three questions of 3QT, we investigated if SF-36 Health Survey was associated with HAND; we found that altered PHI was associated with HAND in the study population (OR 2,17 versus normal PHI, 95%CI 1,14-4,11, p=0,018) and in the subgroup of antiretroviral naïve patients (OR 2,5 versus normal PHI, 95%CI 1,07-5,85, p=0,034). Adjusting for age, months since HIV diagnosis, CD4+ nadir, plasma HIV-RNA, AIDS-defining diseases, hepatitis coinfection and comorbidities, PHI was not confirmed associated with HAND (AOR 2,63 versus normal PHI, 95%CI 0,85-8,1, p=0,09). No association between MHI and HAND was reported.

Discussion

Even if the need of screening for cognitive disorders in HIV-positive patients is more and more evident, a validate and accurate screening test for HAND is still not available. An adequate screening test would be useful especially for asymptomatic and mild forms of HAND, considering that they are associated with a decrease in the quality of life, reduced treatment compliance and increased mortality. Indeed, it is known that asymptomatic forms are at increased risk of future deterioration[89]. On the other hand, several risks in ascertaining ANI cases have to be taken into account, such as possible over-diagnosis, distress to asymptomatic individuals receiving a diagnosis, lack of an effective treatment and resources used for the detection of this disorder[81].
The “Three Questions Test” is a screening test proposed by Simioni S et al and still recommended by EACS guidelines; its advantages are that is an easy-to-perform test, is very quick and could be administered by a physician without need of specific training or adjunctive costs. In the study by Simioni S et al, only cognitive complaint according to 3QT was relatively predictive of HAND, but the negative result at the test did not rule it out; they however reported that the majority of non complainers presenting HAND had ANI[168].
In our cohort we reported low performance of the “Three Questions Test” as a screening tool for HAND: both sensitivity and specificity resulted lower than 80% in a cohort of

antiretroviral naïve and treated patients. In a previous study by Sacktor et al 3QT did not perform better (in detecting HAND the test had a sensitivity of 77.6%, a specificity of 32%, a PPV of 69% and a NPV of 35%)[81]. One of the possible reason of the low accuracy of the 3QT could be that it is based on a very simple self-reported evaluation of cognitive symptoms; in previous works the association of HIV-associated cognitive impairment with self-reported cognitive symptoms was generally poor[5]. A dissociation between subjects' self-complaint of neurocognitive impairment and objective performance was already found in a study by Hinkin and colleagues in 1996: in 46 HIV-infected males that underwent a neuropsychological battery to investigate episodic memory, metacognition and depression, more than half of the patients presented a reduced awareness of their disorders reporting an impairment that exceeded memory testing's deficits or denying impairment that was evident on memory testing[170]. Similar results were obtained also in a larger group of patients and with more complex evaluation of cognitive complaint: even the Cognitive Failures Questionnaire, a 25-item self-report questionnaire, failed to find an association between patient's complaint and the objective results of the neuropsychological assessment, suggesting that asymptomatic HIV-infected subjects are not able to perceive their cognitive decline[171].
On the contrary, using a self-report test that is very similar to 3QT, an association between self-reported cognitive functional status and objective neuropsychological performance was reported; this test, a simplified version of the MOS-HIV cognitive functional scale, includes 4 questions (how often during the previous 4 weeks: 1. Did you have difficulty reasoning and solving problems, for example, making plans, making decisions, learning new things? 2. Did you forget things that happened recently, for example where you put things, appointments? 3. Did you have trouble keeping your attention on any activity for long? 4. Did you have difficulty doing activities involving concentration and thinking?) and provides a measure of general health assessing 10 specific dimensions of health. The authors found that this measure of cognitive functional status predicted overall neurocognitive performance assessed by a brief standardized battery in 85 HIV-positive males; however, not all enrolled subjects underwent the same complete neurocognitive evaluation and possible selection bias were reported: in fact, all subjects in this study were in the early phase of infection and volunteered to participate in a longitudinal research project that evaluated the effect of a supportive group program on clinical disease progression, suggesting that enrolled patients probable displayed a high grade of education. Furthermore, even if the authors

hypothesized the use of the 4-item MOS-HIV scale for the screening of cognitive deficits after validation in larger cohorts, the sensitivity of this instrument for cognitive decline over time has to be ascertain[172]. We probably were not able to replicate the same association because of the larger number of enrolled patients, the less selection of patients (patients were consecutively enrolled in the study, including also individuals with low education and in the chronic phase of infection) and the easier nature of 3QT (results are not normally transformed as in MOS-HIV test). However, the previous work could suggest to investigate the utility of SF-36 Health Survey, that originated from MOS-HIV questionnaire, or a quicker form such as SF-12 survey as a screening test for HAND. In the work by Underwood J et al, both SF-36 summary score of mental and physical health were lower in patients with cognitive impairment than in subjects without deficits, but they were significant only for cognitive impairment identified with Multivariate Normative Comparison (and not for impairment defined by Global Deficit Scores or Frascati's criteria)[169]. In our study, no association between SF-36 and HAND was found, but a study better designed for this outcome with the investigation of sensitivity and specificity of SF-36 Health Survey could provide more useful information.

We described that self-reported mental and physical health measures are associate with having an "abnormal" 3QT, but both of this subjective evaluations are not associated with HAND in a cohort of antiretroviral naïve and on treatment patients. The association between SF-36 summary scores and positive 3QT reflects the subjective nature of these two tests.

We also reported a probable influence of anxiety and depression symptoms in answering to 3QT, even if this association was not confirmed in multivariate analysis; similarly, previous works have underlined that depressive symptoms could affect both subjective and objective cognitive function and exclusion or optimal management of depression has always to be considered in diagnosing cognitive problems[5, 171].

As already hypothesized by other authors, the lack of association between 3QT and HAND could be explained by an over-reporting of symptoms, also by patients that are not affected by objective cognitive impairment, the subjectivity of the questions regarding especially memory loss and the exploration of only some cognitive domains using these questions.

Differences in neuropsychological performance between cART-naïve and experienced subjects may exist and thus we have performed the analysis also dividing the two

groups of patients, but 3QT maintains a low accuracy in detecting objective cognitive impairment.

Our study has some limitations: we have not included a group of HIV-seronegative control subjects and the cross sectional design of the study could not allow a prospectively assessment of the validity of this test. Information about comorbidities were obtained by clinical records, thus they could be underestimated due to missing data and this could have introduced confounders in the analysis.

To conclude, the “Three Questions test” in our cohort showed a low sensitivity and PPV with a high number of false negatives both in antiretroviral-naïve and on cART patients, suggesting a lower self-awareness in patients with frontal and temporal cortex impairment and confirming the lack of association between subjective cognitive symptoms and objective cognitive impairment. The usefulness of this test as a screening tool for HAND should thus be revised.

Oral presentation at 6 th International Meeting on HIV Infection of the Central Nervous System, Matera, Italy 2015

Less severe HIV-Associated Neurocognitive Disorders (HAND) are still common among antiretroviral (cART)-naive and cART-treated people living with HIV: association with not Italian origin, unemployment and non AIDS-related comorbidities (*NeuroHIV Study*)

Introduction

HIV-associated neurocognitive disorders (HAND) still affect 15-55% of HIV-infected patients and with the increased longevity of the HIV-infected population this prevalence is likely to increase[5]. While HIV-Associated Dementia (HAD) has declined, milder forms such as Asymptomatic Neurocognitive Impairment (ANI) and Mild Neurocognitive Disorders (MND) predominate and account for more than 70% of cases[28]. The persistence of these forms also in treated individuals could be explained by several factors: advancing age and the known lower cognitive reserve in older subjects, longer time since HIV diagnosis with the establishment of irreversible CNS damage prior to the cART introduction, persistent HIV viral replication and immune activation in the CNS despite effective control of replication in the plasma compartment, the presence of non-infectious comorbidities and finally the toxic effect of some antiretroviral drugs[24, 99, 173]. One current matter of debate is the clinical impact of these mild forms of cognitive impairment, especially for the asymptomatic ones; previous works have demonstrated that these less severe disorders are still associated with a significant impairment in activities of daily living[27] and with an increased risk of evolution in more severe deficits[76]. Grant I and colleagues found that individuals with ANI from the longitudinal CHARTER cohort study display a three-fold higher risk of developing everyday life problems, compared to patients without cognitive impairment, and suggested that patients diagnosed with ANI deserve regular monitoring of cognitive status[28].

Aim

We aimed to evaluate the prevalence and factors associated with HAND in a cohort of cART-naive and cART-treated HIV-positive patients. We also explored the modification of HAND after at least 12 months of virologically effective cART.

Matherials and methods

We conducted a cross-sectional study enrolling antiretroviral naïve and on cART HIV-infected patients in active follow up at our Clinic. Exclusion criteria were:

-the presence of neurocognitive comorbidities (brain injury, mental retardation, psychiatric disorders)
-active alcohol or substance abuse
-cirrhosis
-not comprehension of Italian language.

Patients underwent blood withdrawal to assess plasma HIV-RNA and CD4+ T cells count and a comprehensive neuropsychological evaluation including 11 tests that explore 7 cognitive domains to diagnose HAND according to the Frascati's criteria[1]. Quality of life was assessed by self-reported SF-36 Health Survey and symptoms of anxiety/depression were investigated by Hospital Anxiety and Depression Scale (HADS) (see section Materials and Methods).

A subgroup of cART-treated patients underwent also lumbar puncture to detect CSF HIV-RNA; we then identified patients with viral escape (VE), defined as CSF HIV-RNA >50 copies/mL when plasma HIV-RNA <50 copies/mL or CSF HIV-RNA ≥1$\log_{10}$ copies/mL than plasma viremia[55].

Comorbidities were defined as non-AIDS-related conditions: cardiovascular risk factors (Framingham risk score >20%), renal subclinical impairment (Glomerular Filtration Rate, GFR<90 ml/min by CKD EPI equation) and presence of malignancies. These data were collected from clinical records; Framingham risk score and CKD EPI equation were calculated using parameters measured in a range of 3 months since the neuropsychological evaluation.

In a subgroup of unselected patients we perform also a longitudinal study: patients were re-evaluated after at least 12 months of virologically effective ART (T12) with the same neuropsychological battery.

To explore factors associated with neurocognitive impairment in untreated and on cART patients and to take in account possible confounders we performed univariable and multivariable logistic regression models. Statistical analyses were carried out with SPSS software, version 21.0.

Results

Prevalence and factors associated with HAND in antiretroviral-naïve patients

We enrolled 237 untreated patients; table 6 shows the baseline characteristics of the study population. Median age was 39 (IQR 32-47) years and 211 (89%) were males. Median time since HIV diagnosis was 2 (IQR 1-13) months and 42 (18%) subjects presented AIDS-defining diseases. Hepatitis C and B co-infection were found in 23

(10%) patients and comorbidities in 41 (17%). Patients showed a median CD4+ count of 368 (IQR 220-526) cells/mmc, while median plasma viremia was 4,71 (IQR 4,09-5,34) log_{10} copies/mL. 24 (10%) patients were not Italian and 19 (8%) were unemployed.
62/237 (26%) patients presented HAND and the majority of subjects were diagnosed with mild forms of cognitive impairment (ANI in 93% and MND in 5%). HAD was reported only in 2% of subjects (Figure 12). 45 (19%) patients displayed impaired quality of life assessed by SF-36 Health Survey.
In cART-naive patients, as expected, we found that patients with severe immune suppression presented a higher risk of HAND; in fact, in univariate anaylsis lower CD4+ T cells nadir and AIDS events were associated with HAND. Furthermore, we reported an association between HAND and non-Italian origin, unemployment, shorter duration of education and alterated SF-36 physical health score. By fitting a multivariable logistic regression analysis, HAND was independently associated with lower CD4+ nadir, non-Italian origin and unemployment (the model was adjusted for age, months since HIV diagnosis, hepatitis coinfection, AIDS diseases, education and the presence of non infectious comorbidities) (Table 7).

Prevalence and factors associated with HAND in cART-treated patients

We enrolled 116 cART-treated patients: median age was 50 (43-56) years, most of them were males (81, 70%) and they showed a long history of HIV infection (median time since HIV diagnosis was 200, IQR 81-311 months). cART-treated patients were also characterized by a relatively advanced immune suppression: 29 (25%) subjects had AIDS-defining diseases and median CD4+ T cells nadir was 252 (IQR 150-399) cells/mmc. Patients presented a median plasma viremia of 3,26 (IQR 1,59-4,71) log_{10} copies/mL; 27 (23%) displayed a detectable HIV-RNA. Hepatitis C and B co-infection were found in 50 (43%) patients and comorbidities in 41 (35%). Only 4 (4%) patients were not Italian and 16 (14%) were unemployed.

HAND was found in 44/116 (34%) subjects: mild forms were the most prevalent ones (75% of ANI and 20% of MND), but, in comparison with untreated patients, we observed higher proportion of mild neurocognitive disorders (20% in treated versus 5% in cART-naïve patients, p=0,004). Low prevalence of HAD was confirmed (5%) (Figure 12). More than half of the patients (54, 46%) reported an impaired QoL.
39 patients performed also lumbar puncture and 7 (18%) had a viral escape.

A higher probability of HAND was observed in patients with AIDS-defining conditions and detectable plasma HIV-RNA. We found also an association between cognitive impairment and the presence of comorbidities, unemployment and symptoms of depression. Conversely, no association with longer time on cART, CPE score and type of cART regimen was observed. In the multivariable model, adjusting for age, CD4+ nadir, AIDS diseases, hepatitis, years of education and employment, a longer length of HIV infection, the presence of comorbidities, symptoms of depression and the presence of detectable HIV-RNA were confirmed associated with a higher probability of cognitive impairment (Table 8).

Longitudinal sub-study

50 cART-naïve patients (with or without HAND at the first evaluation) repeated the complete neuropsychological evaluation after at least 12 months of virologically effective treatment (T12): 11 (22%) patients presented HAND (all cases of ANI). 33 (66%) patients had a normal cognitive function both at enrolment and T12; among the 17 patients presenting HAND at baseline, 8 (47%) subjects were improved from baseline and 9 (53%) maintain the same grade of cognitive impairment from baseline.

10 treated patients (with or without HAND at the first cognitive assessment) were re-tested at 12 months: in 6 subjects cART had been changed to a higher CPE score regimen at baseline and 7 underwent a second LP. In all subjects plasma and CSF HIV-RNA were undetectable at T12. Half of these patients presented normal cognitive evaluation (5, 50%) and half presented ANI at T12 (5, 50%). 3 patients presented no cognitive impairment at baseline and at T12; among the 7 patients with HAND at baseline, 3 (43%) subjects displayed an improvement from baseline and 4 (57%) patients failed to restore their cognitive function.

Discussion

In our cohort of HIV-positive patients the prevalence of HAND is 26% in untreated subjects and 34% in cART-treated individuals; the prevalence rates of cognitive impairment in previous cohorts, usually assessed in antiretroviral treated populations, vary widely, probably due to differences in normative data sets, in the used definition of cognitive impairment and in the enrolment of asymptomatic or symptomatic patients[8, 174].

Our study adds information about the limited data regarding cognitive impairment in antiretroviral-naive subjects; in these patients we assessed cognitive performance in all subjects entering the study and not only in those with symptoms of cognitive impairment.

Prior to the initiation of cART we found an association of traditional demographic and HIV-related risk factors with cognitive performance, as reported also by other cohorts: fewer years of education, low CD4+ T cells nadir and AIDS-defining diseases have been associated with poorer cognitive performance in several previous studies[19, 175-177].

We also reported an association between not Italian origin and cognitive impairment: all foreigner patients that have been enrolled in the study spoke and understood Italian language and thus were able to undergo the neuropsychological evaluation. We did not find a difference in years of education between Italian and not Italian patients (data not shown) and the association with cognitive performance was confirmed in the multivariable model, adjusting for education. The association with ethnicity has already been described previously and could probably be related to different cultural background and the lack of normative data for different ethnic groups, as suggested by Winston A et al[176, 178].

Unemployed HIV-infected patients presented a higher risk of cognitive impairment: also in this case, possible confounders of this association have to be taken in account; a lower education was associated with unemployment in our cohort (data not shown), but surprisingly the association between unemployment and HAND was found also in the multivariable model after correction for years of education.

However, in our cohort the number of non Italian and unemployed patients entering the study was limited and a possible interaction between these social factors, education and HAND has to be better explored.

In cART treated patients we found a higher proportion of mild cognitive impairment, defined as MND by Frascati's criteria, compared to untreated patients, probably reflecting the older age and a longer time since first HIV diagnosis of the cohort of treated subjects. We confirm the association of AIDS diseases, duration of known HIV infection and detectable plasma HIV-RNA with poorer cognitive performances, in line with other previous cohorts[7, 20, 21, 175, 177]. Current plasma viral replication could reflect poor cART adherence, that is another demonstrated risk factor for HAND[29]. The presence of non infectious comorbidities was associated with HAND: recently, MACS, CHARTER and other cohort studies have found a strong association between

comorbidities, especially cardiovascular risk factors, and cognitive impairment [7, 21, 179-181]. Regarding the association between symptoms of depression and HAND, we have to underline that in our cohort a small number of individuals reported depressive symptoms that were well-managed pharmacologically and none had a formal diagnosis of mild or severe depression. It has been widely demonstrated that depression could affect cognitive performance and mood disorders could be one of the causes or a consequence of cognitive impairment [23, 182].

We did not find an association between cART regimen, duration of cART or CPE score: some previous reports have found that regimens yielded higher CPE values were associated with better neurocognitive outcomes, while others have found evidence of worse outcomes; disagreement between studies could reflect differences in study design and power account[99]. Furthermore, the role of antiretroviral neurotoxicity on cognitive function is still uncertain[183].

Finally, the lack of association between HCV co-infection and HAND could instead reflect the successful treatment of HCV by Direct Acting Antivirals (DAAs). Unluckily, we have no data about plasma HCV-RNA in these patients at the moment of the neuropsychological evaluation, but most of them have probably already received antiviral treatment and reached undetectable HCV-RNA, reflecting the current guidelines.

After one year of suppressive cART we did not observe modifications in the cognitive performance with the persistence of mild forms. The results from the MACS and CHARTER cohorts are similar and confirm that the majority of individuals with ANI, in a range between 61 and 77%, remain at the same HAND stage over a 4-year time period[12].

The limitations of this study are represented by the small number of patients enrolled, in particular in the longitudinal sub-study; however, our results confirm previous findings on larger cohorts. The presence of missing data about comorbidities and hepatitis coinfection's treatment and the small numbers of foreigners, patients reporting unemployment and symptoms of depression and treated subjects with detectable plasma HIV-RNA could affect the associations reported in the multivariable analyses and thus have to be confirmed in larger cohorts of patients.

In conclusion, we found a relatively high prevalence of asymptomatic forms of cognitive impairment in HIV-positive patients. The association of HAND with low CD4+ nadir,

AIDS diseases and longer duration of infection supports the need of an early ART introduction, especially in foreigners and unemployed individuals. The control of peripheral viral replication, improving cART adherence, and the optimal management of comorbidities could probably prevent HAND in cART-treated patients. In a follow up period of ≥12 months of virologically suppressive cART, the majority of HAND cases seems to remain stable, suggesting that cognitive improvement requires a longer time or other approaches complementary to cART.

Poster at 7th International Meeting on HIV Infection of the Central Nervous System, Pollenzo Italy, 2017.

Clinical and viro-immunological correlates of HIV-Associated Neurocognitive Disorders in a cohort of antiretroviral-naive patients (*CSF/plasma HIV-RNA ratio study*)

Introduction

HIV spreads early into the CNS, causing inflammation and HIV-associated neurocognitive disorders (HAND)[7, 35]. In untreated subjects HIV-associated dementia (HAD) currently represents a rare diagnosis, yet minor neurocognitive disorders affect 30-35%[1, 8, 15, 184, 185] of patients.

HIV *per se* plays a crucial role in the multifactorial pathogenesis of HAND[15, 16, 19, 43, 186-188], however, while high CSF HIV-RNA levels feature subjects with HAD[50], studies have still to establish the precise link between CSF viral load and milder forms of HAND[14, 23, 52, 53, 189-191]. In this respect, also peripheral HIV replication has been described as a risk factor for cognitive impairment[7, 11] and the CSF/plasma HIV-RNA ratio was found to predict encephalitis better than CSF viral load[192]. Further, immune abnormalities both in the peripheral blood (a low CD4+ nadir[66], CD4/CD8 inversion[69, 72], T-lymphocyte activation together with the shift from naïve to effector memory T-cells[71]) and CNS (intrathecal immune activation[22, 58, 59, 193], activated T-lymphocytes and monocytes migrating to the CNS[66, 193]) have been associated with HAND.

These findings suggest a complex relationship between viral burden and immune cells in the development of HAND and entail the need to identify markers other than CSF HIV-RNA for the management of this condition.

Aim

We explored the clinical and viro-immunological correlates of HAND in a cohort of untreated HIV-infected individuals.

Methods

We prospectively enrolled HIV-infected, antiretroviral-naive subjects at our centre. Exclusion criteria were:

-the presence of neurocognitive comorbidities (brain injury, mental retardation, psychiatric disorders)

-active alcohol or substance abuse

-cirrhosis.

Participants underwent simultaneous lumbar puncture and blood sampling to detect CSF and plasma HIV-RNA (ultrasensitive Abbott RealTime HIV-1 assay). The CSF/plasma HIV-RNA ratio was calculated as $\log_{10}$ CSF HIV-RNA divided by $\log_{10}$ plasma HIV-RNA copies/mL.

Peripheral T-cell activation and maturation subsets were measured by flow cytometry using the following fluorochrome-labeled antibodies: CD38-PE/CD45R0-APC-H7/CD45RA-FITC/CD127-BV421/CD4-PerCPCy5.5/CD8-V450 (FACSVerse, BD Bioscience).

A neuropsychological evaluation exploring seven cognitive domains was performed by a trained neuropsychologist within one month from enrolment. The Instrumental Activities Of Daily Living (IADL) and the Hospital anxiety and depression scale (HADS) were also assessed. HAND was defined according to the Frascati's criteria[1] (see section Materials and Methods). Logistic regression analysis was used to identify the independent predictors of HAND.

Results

Study population

189 HIV-infected individuals were enrolled. In our cohort, 18 participants (9%) were female and the median age was 39 (31-48) years. Of the 39 (21%) subjects presenting an AIDS-defining event, 3 had CNS involvement (neurotoxoplasmosis, cryptococcal meningitis) and thus underwent the neuropsychological evaluation upon complete resolution of the CNS diseases (i.e. >1 month from enrolment). The median time since HIV diagnosis was 2 (1-12) months. The median CD4+ nadir was 307 (126-443) cells/mmc with 60 (32%) subjects displaying <200 cells/mmc. The median plasma HIV-RNA was 4.71 (4.09-5.34) $\log_{10}$ copies/mL, with CSF HIV-RNA values approximately 1 log lower than those in plasma (3.63, 3.02-4.16 $\log_{10}$ copies/mL). The median CSF/plasma HIV-RNA ratio was 0.77 (0.61-0.92) and 30 (16%) subjects presented a ratio ≥1.

Comparison between patients with HAND and subjects without cognitive disorders

HAND was identified in 53 (28%) participants: 1 (2%) patient presented HIV-Associated Dementia (HAD), 10 (19%) a Mild Neurocognitive Disorder (MND) and 42 (79%) an

Asymptomatic Neurocognitive Impairment (ANI). Subjects with HAND were comparable to those without cognitive defects in terms of age, sex, viral hepatitis co-infections and time since HIV diagnosis (Table 9). Patients with HAND more commonly presented AIDS-defining events and a lower CD4+ nadir. Of note, despite comparable median plasma and CSF HIV-RNA load as well as median CSF/plasma HIV-RNA ratio, the former were more frequently diagnosed with a CSF/plasma ratio ≥1. Further, subjects with HAND displayed higher CD8+, lower central memory CD127+CD4+ and naïve CD45RA+CD4+ T-cell percentages, compared to individuals without cognitive impairment.

AIDS-defining diseases and CSF to plasma HIV-RNA ratio ≥1 were independently associated with HAND

We next performed a multivariate logistic regression analysis to identify the independent predictors of HAND (covariates: AIDS, CD4+ nadir <200 cells/mmc, CSF/plasma HIV-RNA ratio) and found that, both a diagnosis of AIDS and a CSF/plasma RNA ratio ≥1 were associated with a 3-fold and 2.7-fold higher risk of HAND (respectively, AOR 3.441, IC95% 1.38-8.58, p=0.008 and AOR 2.707, IC95% 1.122-6.531, p=0.027; Table 10).

Discussion

The present study was conducted to explore the clinical and viro-immunological correlates of HAND in a cohort of HIV-infected, antiretroviral-naïve subjects, given the need, stemming from the controversial association between CSF viral burden and development of HAND, to identify possible novel markers of neurocognitive impairment in this setting.

In our study, mild forms of HAND were reported in 28% of enrolled subjects and, in the univariate analysis, associated with AIDS, a CSF to plasma HIV-RNA ratio >1, a CD4+ T-cell nadir <200 cells/mmc and lower CD4+ naïve and central memory T-cells. In accordance with literature evidence suggesting a role of blood and CSF T-lymphocytes in the development of cognitive disorders in treated individuals[66, 71, 72], our findings put forward severe skewing of peripheral T-cell subsets as a pathogenic mechanism underlying minor forms of HAND in naïve subjects with advanced immune-depression[7, 43]. Impairment of adaptive immunity in late stages of HIV disease may indeed favour

viral penetration in the brain and trigger a neuroinflammatory response that drives cognitive defects in this setting[7, 43].

The understanding of the precise mechanisms by which peripheral immune cells infect those residing in the CNS, hence generating compartmentalized viral replication is of interest also in light of the independent association we found, in the multivariate analysis, between HAND and a high CSF/plasma HIV-RNA ratio. The present finding highlights, in fact, the role of replication sources in the CNS rather than in plasma in the pathogenesis of minor forms of HAND in antiretroviral-naïve individuals. In contrast, we failed to observe a correlation between neurocognitive impairment and CSF HIV-RNA; this result may be explained by the general characteristics of our study population, which included subjects with minor forms of neurocognitive impairment[50, 194] and a moderate degree of immune-suppression[14].

Adding to previous findings in mixed HIV settings, our research is novel in clearly identifying increased CSF/plasma HIV-RNA ratio as a predictor of HAND in a large cohort of untreated HIV-infected individuals. These findings set the basis for mechanistic investigation of HIV replication sources in the CNS, and, most importantly, put forward CSF/plasma HIV-RNA ratio as candidate biomarker to be used in the initial clinical assessment of antiretroviral-naïve patients, possibly guiding treatment and follow-up decisions.

Research letter published in AIDS journal (Bai F, Iannuzzi F, Merlini E, Borghi L, Tincati C, Trunfio M, Bini T, d'Arminio Monforte A, Marchetti G, AIDS 2017; 31 (2):311-314.

Peripheral and Cerebrospinal Fluid Immune Activation and Inflammation in Chronically HIV-Infected Patients Before and After Virally-Suppressive cART (*Neuro MITO Study*)

Introduction

While the current antiretroviral treatments of HIV infection have dramatically reduced the incidence of opportunistic infections [195-197], the onset of non-infectious co-morbidities, such as cardiovascular, bone, renal and neurologic disease remains an issue in the everyday clinical practice [198-201].

HIV infects the central nervous system (CNS) within days of initial exposure and induces neuroinflammation that includes invasion of infected mononuclear cells and subsequent activation of localized inflammatory cells [202-204], possibly leading to neurocognitive decline [205, 206]. Many studies have investigated whether such an early seeding forms the basis for an independent viral replication in the brain, or whether the virus is cleared and penetrates at other points during the infection,without reaching a clear consensus [43, 203, 207, 208].

Several authors have associated HIV-RNA levels in the cerebrospinal fluid (CSF) with the degree of HIV encephalitis (HIVE), cognitive impairment and severity of dementia [209-211]. Indeed, HIV viral load in the CSF has been generally considered a better predictor/correlate of neurocognitive impairment than HIV-RNA in blood [212, 213]. Interestingly, CSF/plasma HIV-RNA ratio has been recently demonstrated a better predictor of encephalitis and HIV-associated neurocognitive disorders (HAND) than CSF viral load alone [214, 215], leading us to assume a close interplay between the two anatomical compartments in the pathogenesis of neurological disorders in HIV infection. In support of this hypothesis, both peripheral and central immune impairment have been associated with HAND [57-59, 69, 72, 73, 115, 216-218].

The introduction of combination antiretroviral therapy (cART) resulted in a decline of HIV-associated dementia (HAD) [8, 143, 219] and a reduction of markers of intrathecal inflammation, such as neopterin and β-2 microglobulin [101, 220-223] and of active neural injury, such as neurofilament light chain protein (NFL) and tau protein [224-227]. However, the incidence of HIV-related mild, yet insidious forms of cognitive impairment persists with a significant impact on mortality and quality of life [8, 23, 139, 219, 228]. Partial explanation for the persistence of HAND despite successful cART may reside in the ongoing high levels of chronic systemic immune activation, associated with microbial

translocation products and residual viral replication, sustained glia cell activation and markers of brain damage in the CNS of cART-treated individuals [223, 229-232]. Other potential mechanisms of pathogenesis include antiretroviral drug neurotoxicity, poor access of antiretroviral agents to the CNS, the legacy effect of CNS damage due to sustained HIV replication before cART initiation and indirect effects linked to aging and comorbid conditions, such as cerebrovascular disease and hepatitis C co-infection [8, 23, 233, 234].

Aim

Given our recent finding of an association between neurocognitive impairment and CSF/plasma HIV-RNA ratio [214], we wanted to assess whether HIV-infected patients with high CSF/plasma ratio (≥1) might display specific peripheral and/or CNS immune features, worthy of further investigation as possible players in promoting neurocognitive impairment.

We therefore sought to explore a panel of peripheral and CNS inflammatory and immune activation markers in untreated HIV-infected patients and the effects of 12 months of virally-suppressive cART, according to CSF/plasma HIV-RNA ratio. We also aimed at investigating whether specific peripheral and/or CNS immune features in HIV-positive patients with diverse CSF/plasma HIV-RNA ratio might be associated with patients' performance at neurocognitive screening tests.

Materials and methods

Study Population

HIV-positive patients were consecutively enrolled at the Clinic of Infectious Diseases, University of Milan - ASST Santi Paolo e Carlo, Milan and at the Amedeo di Savoia Hospital, Turin (Italy) after providing written, informed consent. The study was approved by the Ethical Committees of both hospitals. Inclusion criteria were:

-age >18 years old

-naïve to cART

-sufficient knowledge of Italian language to undergo neurocognitive evaluation.

Exclusion criteria were:

-past and/or current presence of psychiatric or neurological diseases,

-drug/alcohol abuse
-CNS opportunistic infections in the 6 months prior to the enrollment
-cirrhosis.
HIV-positive patients underwent lumbar puncture and blood sampling prior to cART (T0) and after at least one year of therapy (T12).
Patients were stratified according to CSF/plasma HIV-RNA ratio in:

(i) CSF/plasma HIV-RNA <1, defined as Low Cerebrospinal Fluid (L-CSF)
(ii) CSF/plasma HIV-RNA ≥1 defined as High Cerebrospinal Fluid (H-CSF).

Neuropsychological Assessment

The presence and the severity of depression were assessed through the Beck Depression Inventory-II (BDI-II) and we excluded from the study patients with severe depression (score ≥30). A short screening neurocognitive battery, consisting of Mini Mental State Examination (MMSE)[79], Frontal Assessment Battery (FAB)[235] and International HIV Dementia Scale (IHDS)[85, 86], was administered by a physician to explore the cognitive performances at T0 and T12 in an unselected subgroup of patients. A pathological neurocognitive performance was defined as at least one altered test.

Plasma and CSF Immune Activation/Inflammation Markers

Plasma levels of sCD14, IL-6, TNF-α and IFN-γ were quantified by commercially available ELISA assay (R&D Systems), according to the manufacturer's protocol. CSF levels of IL-6, TNFα, sCD14, MCP-1 and IP-10 were measured by LUMINEX technology (R&D Systems), whereas CSF levels of Neopterin and S100β were measured by commercially available ELISA assay, according to manufacturer's instructions (IBL international; ALPCO).

T-Cell Immune Phenotypes

T lymphocyte surface phenotypes were evaluated on cryo-preserved samples by flow cytometry. The following fluorochrome labeled antibodies were used: CD4 PE-Cy7, CD8 PE-Cy5, CD38 PE, CD45R0 APC, CD45RA FITC, CCR7 PE, HLA-DR FITC and Ki67 FITC (BD Biosciences). The following combination of antibodies were used: CD8 /CD4/CCR7/CD45RA (maturation), CD8/CD4/CD38/HLA-DR/CD45R0 (activation),

CD8/CD4/Ki67 (proliferation). For the evaluation of the intracellular Ki67 a fixation/permeabilization step was required. Cell viability was assessed by 7-aminoactynomycin D (7-AAD; BD Biosciences): only samples with viability greater than 70% were used for the experiments.

PBMC stimulation

Cryo-preserved PBMCs ($1x10^6$) were thawed and incubated in the presence of a pool of gag-env peptides (5uM), or CMV (2 µg/mL,) or Staphylococcal Enterotoxin B (2,5 µg/mL) as positive control. Brefeldin A (10 µg/mL, Sigma-Aldrich) and the co-stimulatory antibodies CD28 (1 ug/ml, Becton Dickinson) were added and cells were incubated over night at 37°C with 5% CO_2.

Intracellular cytokine staining

Cells were harvested and stained with CD8 PE-Cy5 and CD4 PE-Cy7 (Beckman Coulter). After PFA fixation (1%, Sigma-Aldrich), cells were permeabilized with Saponin 0.2% (Sigma-Aldrich) and stained with IL-2 PE and IFNg FITC (Beckman Coulter) for 30min at RT. The values of IL-2 and IFN-γ assessed in medium alone were subtracted from SEB, HIV or CMV stimulation to reduce any possible bias due to the experimental procedure.

Total HIV-DNA detection

A pellet of $1x10^6$ PBMC were digested with lysis buffer over/night at 55°C and the lysate was used directly in a nested PCR in order to quantify both HIV and CD3 gene copy numbers, as previously described.

Statistical Analysis

Continuous variables were expressed as median and interquartile range (IQR), whereas categorical variables were expressed as absolute numbers and percentages. The groups of patients and the different time points were compared using Chi-square or Fisher's exact test, Mann-Whitney, Kruskal-Wallis or Wilcoxon paired test as

appropriate and the correlations among variables were tested by Spearman Rank correlation's coefficient. p values <0.05 were considered statistically significant. Data were analyzed with GraphPad 5 Prism (GraphPad Software Inc).

Results

Patient population at baseline

We enrolled 70 HIV-infected antiretroviral-naive patients, stratified according to the CSF/plasma HIV-RNA ratio (see material). 61/70 (87%) were L-CSF, whereas 9/70 (13%) were H-CSF.

Figure 13 shows patients' assignments to peripheral blood (PB) and CSF analyses and to neurocognitive screening tests.

H-CSF group featured lower CD4 count and CD4/CD8 ratio at time of analysis, as compared to L-CSF group (Table 11). As per definition, H-CSF showed lower plasma HIV-RNA levels and higher CSF HIV-RNA levels, yet similar peripheral total HIV-DNA (Table 11). No other differences were found among the two study groups.

We found a positive correlation between plasma and CSF HIV-RNA (r=0.34, p=.004), and between CSF HIV-RNA and total plasma HIV-DNA (r=0.43, p=.027) .

Baseline peripheral and CNS immunological features of L-CSF and H-CSF patients

Before cART initiation, L-CSF and H-CSF patients showed comparable levels of peripheral T-cell activation, proliferation and maturation, as well as HIV- and CMV-specific response (Table 12). Interestingly, the baseline frequency of circulating activated HLA-DR+CD38+CD8+ positively correlate with both CSF and plasma HIV-RNA (CSF: r=0.35, p=.046; plasma: r=0.53, p=.005 Figure 14a-b).

At baseline, L-CSF and H-CSF displayed comparable levels of pro-inflammatory markers both in peripheral blood (i.e. IL-6, TNF-α, sCD14, IFN-ɣ: Table 12) and in CSF (i.e. sCD14, IL-6, TNF-α, MCP-1, IP-10, neopterin, Table 13).

Interestingly, we did find a positive association between CSF HIV-RNA and intratechal pro-inflammatory markers (IL-6: r=0.39, p=.004; MCP-1: r=0.30, p=.031; IP-10: r=0.31, p=.033; neopterin: r=0.35, p=.005; Figure 14c-f).

Peripheral and CSF viro-immunologic parameters in H-CSF and L-CSF following 12 months of cART.

In an unselected subgroup of 33/70 HIV-positive patients (26 L-CSF and 7 H-CSF) who agreed to undergo CNS and blood sampling after 12 months of cART, we investigated the effect of virally-suppressive cART on markers of immune activation/inflammation according to CSF/plasma HIV-RNA ratio (Figure 13). No differences in cART regimens between L-CSF and H-CSF patients were reported (Figure 15a). As expected, in both groups we observed viral suppression, with all patients reaching undetectable plasma and CSF HIV-RNA despite stable total HIV-DNA, and a parallel rise in CD4 count and in CD4/CD8 ratio (Figure 15a).

i) Peripheral blood pro-inflammatory cytokines

Upon 12-month cART, HIV-infected patients as a whole significantly reduced plasma TNF-α (p=.008; Figure 15b), with no other differences following cART introduction (Figure 15b-e). Similarly, following the stratification of patients according to CSF/plasma HIV-RNA ratio, only L-CSF group showed a reduction of circulating TNF- α (p=.048; Figure 15b).

ii) T-cell activation and maturation

At T12, HIV-positive patients significantly reduced activated CD38+CD8+ (p<.0001, Figure 15f) and memory activated CD38+CD45R0+CD8+ (p<.0001; Figure 15g). In particular, H-CSF and L-CSF significantly reduced the proportion of CD38+CD8+ to a median of 2% (IQR 2-3) (p=.063, p=.003 respectively; Figure 15f) and the proportion of CD38+CD45R0+CD8+ to a median of 1% (IQR 1-2) (p=.062; p=.003 respectively; Figure 15g). Similarly, we observed a non-significant decrease in HLA-DR+CD38+ CD4 (H-CSF: 3.3% [1.03-6.85] vs 1% [0.30-1.87] p=0.13; L-CSF: 1.90% [0.98-5.05] vs 1.80% [0.50-2.56], p=0.57) and CD8 T-cells (H-CSF: 8.35% [5.58-18.55] vs 3.48% [2.30-5.22] p=0.25; L-CSF: 8.35% [4.45-13.73] vs 3.42% [1.20-8.97] p=0.32) in both study groups. No differences in proliferating Ki67-expressing T-cells were found neither in HIV+ patients as whole nor in H-CSF and L-CSF, following 12 month of cART (data not shown).

With regard to T-cell maturation, in HIV+ patients as a whole we observed increased proportion of naïve CCR7+CD45RA+CD8+ (p=.022; Figure 16d), with a decrease in effector memory CCR7-CD45RA-CD8+ (p=.007; Figure 16d).

Stratifying the patients according to CSF/plasma HIV-RNA ratio, we found that while H-CSF did not show any modification following cART introduction (figure 16b, 16e), in L-CSF 12 months of cART partially redistributed naïve and memory T-cell subsets. Indeed, L-CSF significantly increased the proportion of naïve CCR7+CD45RA+CD4+ and CCR7+CD45RA+CD8+ (p=.044; p=.005 respectively; Figure 16c, 16f) and central memory CCR7+CD45RA-CD4+ (p=.005; Figure 16c) and significantly reduced effector memory CCR7-CD45RA-CD8+ (p=.018; Figure 16f). Moreover, at T12, L-CSF showed higher proportion of central memory CD4+ (p=.054) and lower proportion of effector memory CD8+ (p=.045) as compared to H-CSF.

iii) Intracellular Cytokine production following HIV and CMV challenge

We next assessed HIV- and CMV-specific intracellular IL-2 and IFN-ɣ production (see Figure 17a for flow cytometry profiles of cytokine-producing CD4 T-cell distribution).

The percentage of both HIV- and CMV-specific single-positive IL-2+CD4+ significantly decreased following cART introduction in HIV+ patients as a whole (p=.008, p=.001, respectively, Figure 17b-e) as well as in L-CSF group (p=.007; p=.001; respectively, Figure 17b-e), with no changes in IFN-ɣ and IL-2/IFN-ɣ producing CD4+ (Figure 17b-e). No major differences in HIV- and CMV-specific CD8+ response were observed before and after cART introduction in both L-CSF and H-CSF (Figure 18).

iv) CSF pro-inflammatory markers

In HIV-infected patients as a whole, cART was efficacious in reducing CSF pro-inflammatory cytokine/chemokine levels, reaching significance for sCD14 (p=.001; Figure 19a), IL-6 (p=.025; Figure 19b), MCP-1 (p=.012; Figure 19d) and IP-10 (p=.014; Figure 19e). Interestingly, following cART introduction, only L-CSF patients significantly contracted sCD14 (p=.006, Figure 19a), IL-6 (p=.037, Figure 19b) and MCP-1 (p=.017, Figure 19d).

No significant differences in pro-inflammatory TNF-α, IP-10, neopterin as well as in astrocyte damage marker S100beta were found in both H-CSF and L-CSF following 12 months of suppressive cART (Figure 19c,e-g).

Neurocognitive performance in H-CSF and L-CSF before and after 12 months of cART.

An unselected group of 22/33 patients (16 L-CSF and 6 H-CSF subjects) were willing to undergo a neurocognitive evaluation at baseline and after 12 months of virologically-effective cART (see Figure 13). At baseline, 9/22 subjects presented altered neurocognitive evaluation: 5/16 (31%) L-CSF, 4/6 (67%) H-CSF; p=0.178. Following 12 month of suppressive cART, we failed to observe significant changes in neurocognitive performance with persistent neurocognitive deficits in 4/16 (25%) L-CSF and 4/6 (67%) H-CSF (p=0.137).

In this subgroup of patients, both peripheral and CSF markers paralleled the trends described in the longitudinal population of 33 patients. Indeed, at T12 only L-CSF patients significantly decreased circulating pro-inflammatory markers and the proportion of activated T-cells, with a partial redistribution of naïve/memory CD8 T-cell subset (Table 14). Similarly, CSF inflammatory markers such as MCP-1 and IP-10 significantly diminished following 12 months of cART only in L-CSF (Table 15). At the opposite, no significant changes in circulating and CSF immune activation/inflammation were evident in H-CSF (Table 15).

Discussion

We have recently demonstrated that a CSF/plasma HIV-RNA ratio of at least 1 was independently associated with HIV-associated neurocognitive disorders (HAND), after adjustment for CD4 nadir, suggesting a role for active CNS viral replication in the pathogenesis of neurocognitive impairment[214]. Nonetheless, whether and how the presence of a high CSF/plasma HIV-RNA ratio is linked with specific immunological alterations in both systemic circulation and CNS is still under investigated.

Hence, this study aimed to explore (i) a panel of peripheral and central immune activation/inflammation markers in HIV-infected untreated patients with high and low CSF/plasma HIV-RNA ratio, (ii) the effects of 12 months of suppressive cART and (iii) the possible associations between peripheral/CNS immune features and neurocognitive performance at a first-line/screening neurocognitive assessment.

In our cohort, we found that 13% (9/70) of patients displayed a CSF/plasma HIV-RNA ratio≥1, possibly reflecting a compartmentalization of the virus that actively replicates in the CNS[202]. Research investigating the pathways underlying neuropathogenesis suggest the mixed contribution of both free virus and cell-mediated HIV entry, seemingly dependent on the precocity of HIV infection: in earliest infection, HIV seems to mainly invade the CNS through circulating infected cells[203, 236], whereas

during chronic infection the virus might freely pass into the CNS, as consequence of inflammation-mediated damage of BBB integrity. In such context, our finding of a positive association between CSF and plasma HIV-RNA irrespective of CSF/plasma ratio coupled to the association between CSF HIV RNA and CSF pro-inflammatory cytokines, would altogether lend support to passive viral flow from blood to CSF, through a highly damaged blood-brain barrier rather than active *in situ* replication[204, 236] in our cohort of chronically-infected patients. In fact, previous data have shown an association between BBB altered permeability and markers of neuronal damage and astrocytosis, in late presenter cART-treated subjects with suppressed CSF HIV RNA[237]. Nonetheless, we did not find any association with S100beta possibly reflecting the earlier diagnosis and treatment of our cohort. In our cohort of chronically HIV-infected antiretroviral-naïve patients, we hereby describe similar levels of CNS and peripheral immune activation, function and inflammation irrespective of the levels of CSF/plasma HIV-RNA ratio, pointing to a well-established disruption of blood-brain barrier[238], in sharp contrast with data by Spudich et al[31], showing significantly lower CNS inflammation in acutely HIV-infected patients with minimal CSF HIV-RNA replication (CSF HIV-RNA<100 cp/ml) compared to participants with higher intrathecal HIV-RNA.

Along with the expected decline of both plasma and CNS HIV-RNA, 12 months of virally-suppressive cART, resulted in a reduction of peripheral and CNS immune activation and inflammation in the entire cohort, with no recovery of HIV/CMV-specific polyfunctional IL-2/ /IFNγ-secreting T-cells, in line with the massive impairment of T-cell-mediated control of chronic viral infections[239-241]. Interestingly, following 12 months of virally-suppressive cART, while L-CSF patients partially restored peripheral and CNS immune activation/inflammation, individuals with high CSF/plasma HIV-RNA ratio maintained a skewed T-cell homeostasis, toward a more effector/exhausted immune phenotypes and failed to reduce intrathecal inflammatory levels. The persistence of disrupted T-cell homeostasis and function and of a pro-inflammatory *milieu* in the systemic circulation, are highly likely to impair immune patrolling over HIV, possibly favoring CNS virus entry and further exacerbating neuro-inflammation and damage, as clearly indicated by persistently heightened levels of pro-inflammatory mediators in the CSF of these patients. At the opposite, HIV+ individuals featuring low CSF/plasma HIV-RNA ratio showed a trend toward recovered peripheral and CNS immune abnormalities, supporting the hypothesis that HIV+ individuals with low viral burden, by achieving and

maintaining a better viral suppression, possibly prevent further injurious CNS processes, including viral invasion, cellular trafficking, and immune activation[204, 222]. Another possible explanation of the observed amelioration may reside in the more preserved immune competence at baseline. Indeed, the higher CD4 count and CD4/CD8 ratio might explain why L-CSF partially recovered peripheral and CNS immune abnormalities; this could also probably suggests that H-CSF patients might benefit of a cART regimen with a higher neuropenetration or might not be the best candidates for simplification strategies.

Lastly, we asked whether CSF and peripheral activation and inflammation might be associated to neurocognitive impairment as assed by first-line neurocognitive evaluation in our cohort of chronically-infected individuals. Previous studies have demonstrated higher CSF and peripheral HIV-RNA levels, as well as activation/inflammation markers in individuals with HIV-associated neurocognitive disorders as compared to individuals without dementia [116, 211, 242-246], demonstrating the pathogenic role of HIV virus and immune activation in shaping neurocognitive outcome.

In our cohort, while the proportion of individuals with neurocognitive impairment assessed by a short battery of screening tests was higher in H-CSF (67%) than L-CSF patients (31%), this did not reach statistical significance possibly due to a small number of subjects in study. Most interestingly, after 12-months suppressive cART, H-CSF patients, while failing to contain intratechal pro-inflammatory burden, did not show a worsening in cognitive performance as assessed by screening tests, possibly suggesting that longer exposure to high intrathecal viral load is required to induce cognitive decline as previously shown by Ellis et al [212].

We should acknowledge that the screening neurocognitive battery used in this study consent the sole identification of patients with high degree of cognitive decline and is not powerful enough to highlight more subtitles disorders. However, a short screening cognitive battery has the advantage of being easier to perform allowing the administration by physicians during routine visits, instead of a complete neuropsychological evaluation that is time- and resource-consuming and needs a trained neuropsychologist, and is supported by both Italian and EACS guidelines [247, 248]. Given the lack of a validated and universally accepted screening battery, we decided to use IHDS and MMSE, two of the first published screening tools for HIV-Associated Dementia, that have been used more generally for HIV-associated neurocognitive disorder in several previous studies [79, 86], coupled with FAB, that has

been recently associated with high specificity [235, 249]. Despite the efforts in validating the clinical use of both IHDS and MMSE as screening for HAND, a clear consensus is still lacking, with no other options than combine different tests, in order to gain more accuracy and reliability. This absence of a complete battery of neuropsychological test might have prevented us from finding associations between CSF inflammation and neurocognitive performance.

Other caveats in the experimental design of the present study must be acknowledged including (i) the limited sample size for the group of H-CSF patients and for the longitudinal analysis that might have influenced the statistical power, (ii) the absence of a cART follow up longer than 12 months and (iii) the lack of a direct BBB damage marker.

In conclusion, CNS damages persist even in HIV+ individuals on virally-effective cART [205, 222] through mechanism(s) still poorly understood.

Aside from immune activation/inflammation and HIV viral load, other conditions are now emerging in the setting of long-term survival with chronic HIV infection that may influence the integrity of the CNS and the subsequent cognitive impairment. In particular, accelerated/premature aging and cardiovascular risk factors, such as hyperlipidemia, hypertension and carotid intima-media thickness have been recently identified as associated with reduced neurocognitive performance in HIV infected subjects [250-252].

To our knowledge this is the first study trying to explore the association between both peripheral and CNS inflammation/activation and neurocognitive screening tests.

Although the nature of our study did not allow us to find a “cause and effect” relationship, our observations that HIV+ patients with high CSF/plasma HIV-RNA ratio are not able to reduce and contain both peripheral and central pro-inflammatory/effector phenotypes despite suppressive cART, might point to the need of a closer follow-up of these subjects and strongly support the urgency of early treatment, in order to rapidly and massively reduce CNS viral burden, in turn limiting the neuroinflammation and neurotoxicity. These data may provide the rationale to use CSF/plasma HIV-RNA ratio as biomarker for monitoring and possibly identifying patients that maintain higher CNS pro-inflammatory *milieu* and that for this reason might be at higher risk of neurocognitive impairment.

Oral Poster at 8th IAS Conference on HIV Pathogenesis, Treatment and Prevention, Vancouver, BC, Canada, 2015.

Poster at 7th International Meeting on HIV Infection of the Central Nervous System, Pollenzo Italy, 2017.

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Cognitive Neuro-Rehabilitation of HIV-Associated Neurocognitive Disorders: Case Reports of A New Computer-Based Restorative Approach In 3 HIV-Positive cART-Treated Patients

Introduction

HIV-infected patients undergoing long-term cART present an increased risk of morbidity and mortality in comparison to HIV-negative individuals due to clinical complications typically associated with aging, including cardiovascular disease, cancer, osteoporosis and cognitive impairment[253]. HAND, that is still a relatively common problem in the management of HIV-infected patients, is characterized by impairments in several cognitive domains leading to poor cART adherence, regardless of other neuropsychiatric factors[254]. Considering the consequences of HAND, novel strategies to handle cognitive impairment still remain an unmet clinical need and should be a priority.

Due to the lack of an optimal pharmacotherapy for HAND, researchers have investigated the possibility of cognitive rehabilitation. In the last years pilot studies investigating computerized rehabilitation on HIV-infected patients have been published[148-150]: all these studies have adopted a restorative approach. Even if their outcome was not the resolution of HAND, but the improvement of specific neurocognitive functions, their initial results were encouraging. Given a recent report by Livelli et al[154] showing the efficacy and stability over time of a cognitive rehabilitation protocol in treated HIV-positive patients with HAND, we hereby describe our experience on the efficacy of a new computer-based program in 3 HIV-positive patients on cART.

Aim

We investigated the efficacy of a new computer-based program in improving cognitive functioning in 3 HIV-infected patients on stable effective cART.

Materials and methods

We consecutively enrolled 3 HIV-positive patients on stable cART from a larger cohort who underwent a complete neuropsychological battery in the context of a dedicated outpatient service for the diagnosis and follow-up of HAND. Inclusion criteria were:

-≥ 18 years of age

-history of HAND according to Frascati's criteria[1]

-being on stable cART (>6 months) with plasma HIV-RNA <40 UI/ml, regardless of CD4+ T-cells.

Exclusion criteria were:

-history of CNS diseases and psychiatric disorders

-drug addiction or alcohol abuse in the last 12 months.

The Institutional Review Board approved the study and all the patients signed a written informed consent. At enrolment (T0), the patients underwent a complete neuropsychological battery composed by tests covering seven different cognitive domains. The functional assessment was evaluated through the instrumental abilities in daily living (IADL) and Short Form-36 (SF-36) for quality of life; anxiety and depression symptoms were explored by the Hospital Anxiety and Depression Scale (HADS) (see section Matherials and Methods). Frascati's criteria were used for the diagnosis of HAND[1].

The patients underwent 12 sessions of a computer-based rehabilitation program, a novel brain plasticity-based computerized cognitive training (https://dynamicbrain.brainhq.com/), adopting a restorative approach. Previous studies demonstrated the efficacy of this program in improving generalized measures of memory and attention and in maintaining long-term effects among the elder general population[255, 256]. Given these encouraging data, the neuropsychologist selected a panel of exercises from the training program; the selected exercises stimulated the skills of the cognitive domains that are most frequently impaired in HAND[1]. The 17 selected exercises were: divided attention, target tracker, double decision, mixed signals, freeze frame, hawk eye, visual sweeps, eye for detail, memory grid, scene crasher, syllable stacks, face facts, card shark, juggle factor, right turn, mental map and optic flow. Each exercise was automatically administered in different steps with progressively increasing difficulty. Each session lasted one hour once a week for three months and it was performed under the supervision of a physician. The physician scheduled each session according to patient's availability, helped patients to use the program and explained how to perform the exercises. Two weeks after the end of the program (T1), the patients were re-tested with HADS, IADL, SF36 and the same neuropsychological battery using parallel forms for those tests which test-retest reliability is over three months, in order to control for the potential confounding 'practice effect'.

Results

All patients were Caucasian males; two patients had previous AIDS-defining events without central nervous system involvement. One subject had diabetes mellitus with good glycemic control on oral hypoglycemic therapy; no other known comorbidities, including viral hepatitis co-infection, were present. Table 16 shows features of the performances at T0 and T1 for each patient. Table 17 shows the functional assessment for each patient at T0 and T1.

Patient 1 was 58 years old; at the time of examination CD4+ T-cells were 405/mmc, with undetectable HIV-RNA. Nadir CD4+ T-cells was 5/mmc. Time from the first HIV diagnosis was 174 months and the patients was on cART for 173 months. At T0 the patient complained of cognitive decline and he was diagnosed with MND: he showed deficit in two different cognitive domains (attention/working memory and motor skills) and an altered IADL score. After 12 weeks of cognitive rehabilitation (T1) the patient improved, according to Frascati's criteria: only one cognitive domain (motor skill) remained altered. He also presented a normal IADL and HADS anxiety score.

Patient 2 was 40 years old; he presented a CD4+ T-cells count of 366/mmc with a CD4+ T-cells nadir of 183/mmc and a HIV-RNA <40 UI/ml; time from the first HIV diagnosis was 14 months, while cART duration was 12 months. He had ANI at T0: two different cognitive domains were altered (memory and executive functioning) in absence of symptoms. At T1 he restored normal neurocognitive performance, reaching the normal equivalent score in each neuropsychological test.

Finally, patient 3 was 36 years old with CD4+ T-cells of 522/mmc, CD4+ T-cells nadir of 96/mmc and HIV-RNA <40 UI/ml. Time from the first HIV diagnosis was 19 months and he was on cART for 15 months. This patient complained of cognitive decline and displayed a diagnosis of MND at T0: he had deficits in 3 different cognitive domains (speed of information processing, motor skills and executive functioning) and an altered IADL score. At T1 the patient improved reaching normal performance according to Frascati's criteria: he showed only one altered domain (speed of information processing), without symptoms, and he had normal IADL score. Moreover, patient 3 had an improvement of SF36 score. Patients' adherence to the program was 100%.

Discussion

The absence of an effective treatment for HAND has led researchers to explore cognitive and behavioral approaches. The neuro-rehabilitation is known to be useful in the management of other forms of dementia, like Alzheimer's and Parkinson's disease,

and to prevent senile cognitive decline[257]. Typically, the cognitive rehabilitation approaches are divided in two categories: restorative and compensatory. Compensatory rehabilitation aims to minimize the cognitive deficits by helping the patient to develop and learn new strategies to overcome the impairment (i.e. people with poor memory can have a small slip to write down what they need to remember). The compensatory approach is mainly used for rehabilitating deficits after a stroke or a traumatic brain injury, by stimulating perilesional or controlateral homologous areas and their corresponding functions. Restorative approaches rely upon the principle of neuroplasticity and propose that "drill-and-practice" of cognitive skills will encourage more effective neural organization and ultimately improve impaired cognitive abilities. In fact, restorative rehabilitation aims to enable people to develop the lost function through specialized computerized and manual cognitive exercises.

All three previous pilot studies investigating cognitive rehabilitation on HIV-infected patients adopted a restorative approach[148-150], while Livelli at al. used a compensatory approach[154]. In our study we chose the brainHQ program (Posit Science Inc, San Francisco, US), a restorative approach tool, given previous data in preventing senile cognitive decline in a long time follow-up[257]. Furthermore, this program is available in eight different languages and can therefore be reproduced in different geographical settings.

In the previous studies on HIV-positive patients, the interventional program was self-administered at the research center or at patient's home, resulting in limited adherence. In order to improve patients' adherence, we chose a one session-per-week formula under the supervision of a physician in a short time period (12 weeks). We obtained 100% of adherence with this scheme. Importantly, all patients were very motivated to participate to the program suggesting the importance of a good motivational pre-intervention counselling. Although in a small group of 3 patients, with no power to detect statistically significant differences, we hereby describe an improvement in HAND through a new computer-based tool of cognitive rehabilitation in the setting of treated HIV infection. In particular, we show an improvement in the executive functioning, attention/working memory, memory and motor skills. Future research with larger samples should be performed to evaluate the efficacy of our restorative computer-based program in improving other cognitive domains. Moreover, we observed an improvement in daily life activities, with a better quality of life and a reduction of anxiety symptoms. This outcome suggests that improving cognitive functions could result in a better emotional

wellbeing and that a frequent interaction between patients and physician can reduce psychological apprehension of people living with HIV.

Our experience supports the need to explore innovative ways to manage HAND in HIV-infected patients. A study with a large cohort should be designed to assess the real effectiveness and feasibility of a computer-based neurocognitive approach in the management of this HIV-related comorbidity. Furthermore, a short and long-term follow-up will be necessary to determine the durability of the rehabilitation program's outcome.

Case report published in Journal of Neurophysiology and Neruological Disorders (Iannuzzi F, Bai F, Borghi L, Trunfio M, Vegni EAM, d'Arminio Monforte A, Marchetti G, Journal of Neurophysiology and Neurological Disorders 2016; 3: 1-5.

Conclusions

HIV-associated neurocognitive disorders still need to be evaluated in the clinical management of both untreated and treated HIV-positive patients, given their relatively high prevalence. We have reported cognitive impairment in symptomatic HIV-positive patients on active treatment that display a long history of HIV infection with a low CD4+ nadir. Moreover, in our cohort, also asymptomatic naive patients, with a short time since first HIV diagnosis and with median CD4+ T cells higher than 200 cells/mmc could display cognitive impairment. We confirm that nowadays the majority of these cognitive disorders are asymptomatic or mild forms, while dementia is now a rare finding.

However, the definition of a universally validated screening test is still a matter of debate; the “Three Questions test”, proposed as first approach for cognitive deficits by EACS guidelines, showed a low performance both in antiretroviral-naïve and on cART patients in our cohort. These data confirm the lack of association between subjective, self-reported cognitive symptoms and the objective measure of cognitive impairment and suggest the need of a new, easy-to-perform and quick test for the screening of HAND.

Both traditional and new risk factors for HAND have been identified: besides the well recognized factors such as severe immune depression, long length of HIV infection and detectable plasma HIV-RNA, also social parameters, as well as foreign origin and unemployment, and non infectious comorbidities could participate in the development of HIV-associated cognitive disorders. Therefore, early diagnosis and prompt treatment of HIV infection thanks to screening services, programs aimed to improve retention in care and cART adherence and a proper management of non infectious comorbidities are required to reduce the prevalence of HAND, especially in high risk groups of patients.

Minor forms of HAND are also associated with a skewing of peripheral T-cell subsets and an increased CSF to plasma HIV-RNA ratio in naive HIV-positive patients; furthermore, subjects with increased CSF/plasma ratio fail to reduce CNS pro-inflammatory *milieu* under effective antiretroviral treatment.

Before the introduction of antiretroviral treatment, the measurement of CSF viral load and the assessment of the CSF/plasma ratio could be useful in identifying subjects at high risk of developing HAND. In fact, these subjects could probably benefit from an antiretroviral treatment with an adequate CNS penetration and possibly cART complementary treatments. However, lumbar puncture is an invasive procedure and it is associated with possible complications. This consideration suggests the need for other non-invasive peripheral biomarkers.

Most of the patients with HAND did not show any improvement in their cognitive function even after the introduction of virally suppressive cART, confirming that antiretroviral treatment alone is not enough to determine a complete resolution of these disorders.

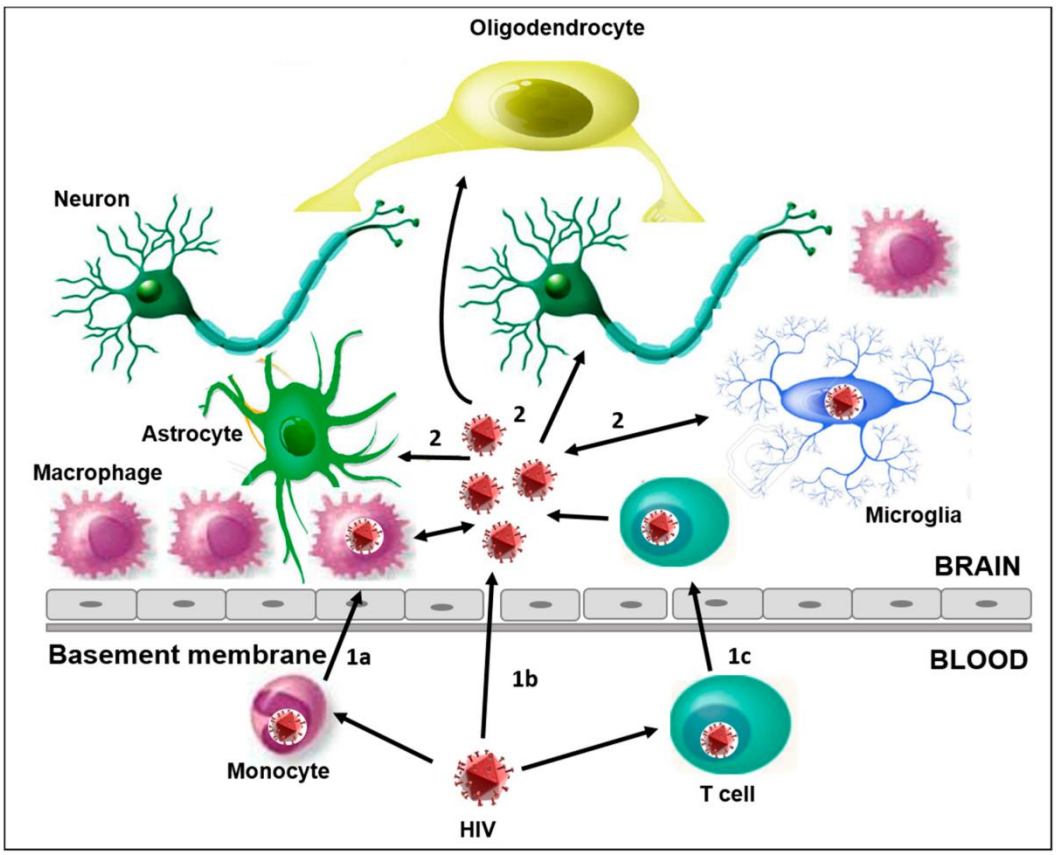
The lack of improvement despite suppressive cART is also seen in peripheral and central T cells phenotypes that maintain a pro-inflammatory pattern in subjects with high CSF to plasma HIV-RNA ratio. All these data underline the need of a closer follow-up of these patients and again support the possible benefit of early treatment to reduce neuroinflammation.

An attempt to restore cognitive function in these patients could be a computer-base program of neurorehabilitation: the first results of this approach seem encouraging, even though the stability over time has to be yet demonstrated and larger randomized controlled trials are needed.

Figures and tables

Figure 1

Mechanisms of the HIV entry in the Central Nervous System



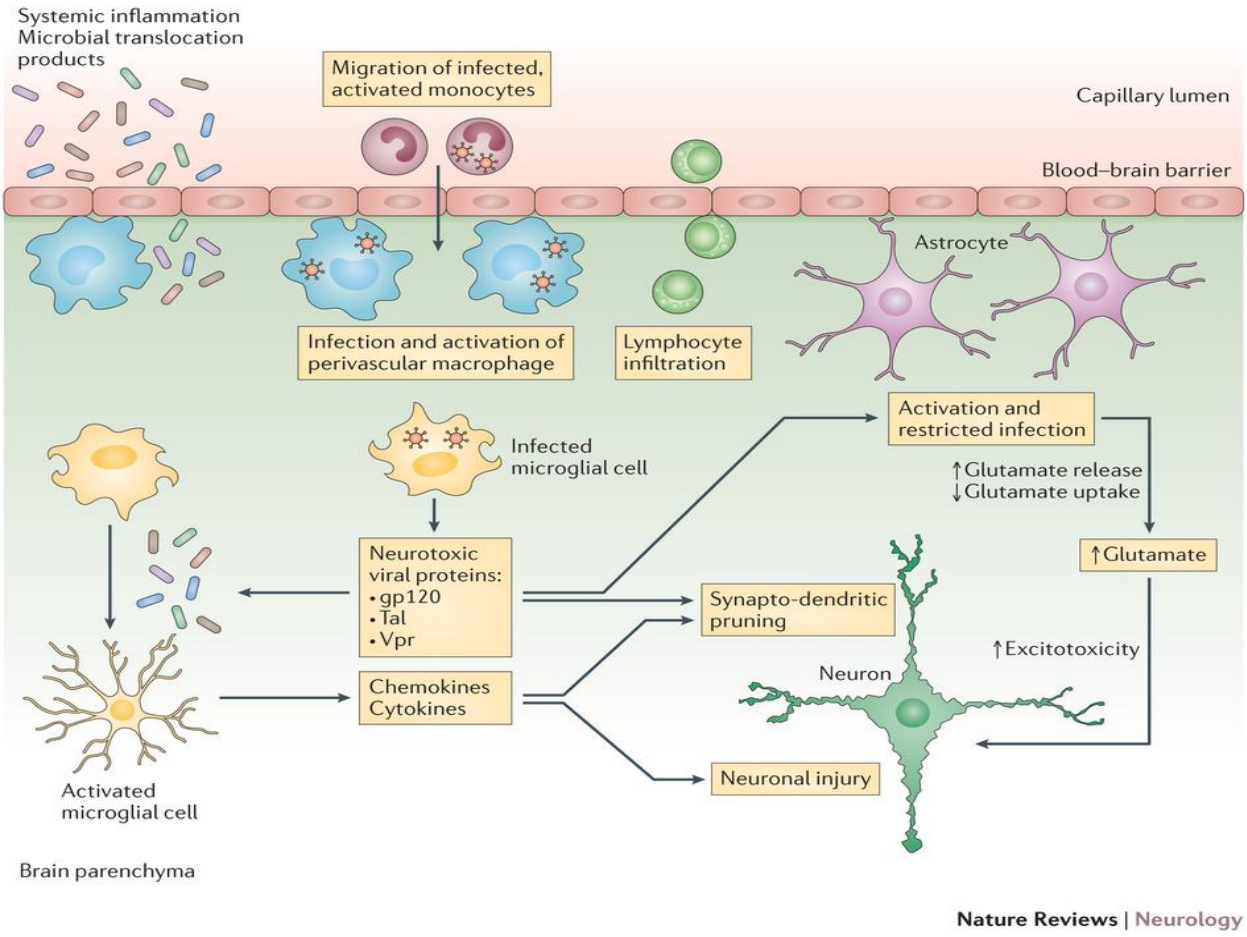
LEGEND

The proposed mechanisms of the HIV entry in the CNS. 1a) The Trojan horse mechanism, 1b) entry of cell-free viral particles, 1c) migration of HIV infected monocytes, leukocytes or perivascular macrophages.

2) CNS resident cells that are susceptible of HIV infection: microglia, astrocytes, oligodendrocytes and neurons.

Figure 2

Patogenetic mechanisms of neuro-inflammation



LEGEND

The proposed mechanisms of neuro-inflammation leading to neuronal injury.

Figure 3

Algorithm for Diagnosis and Management of HIV-Associated Neurocognitive Impairment (NCI) in persons without obvious confounding conditions

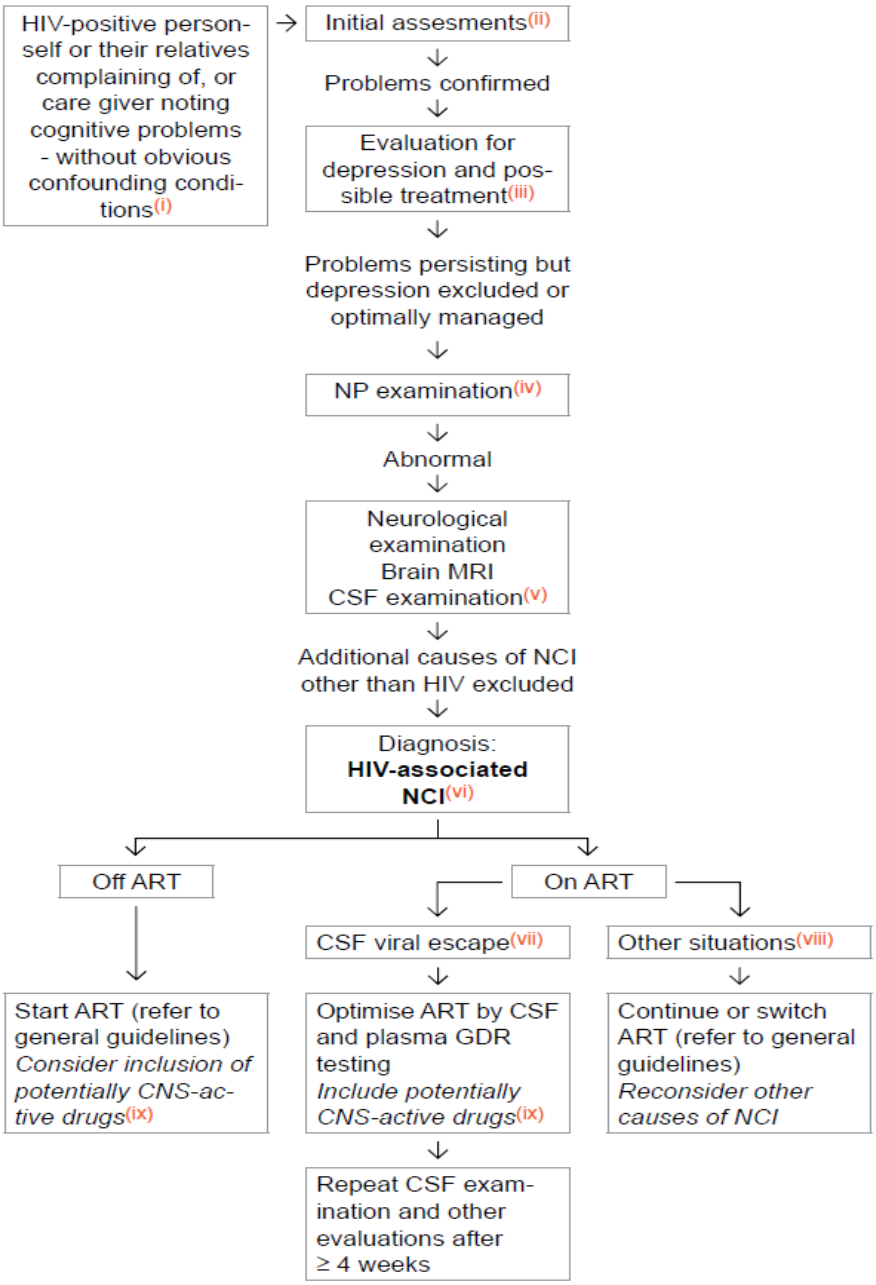
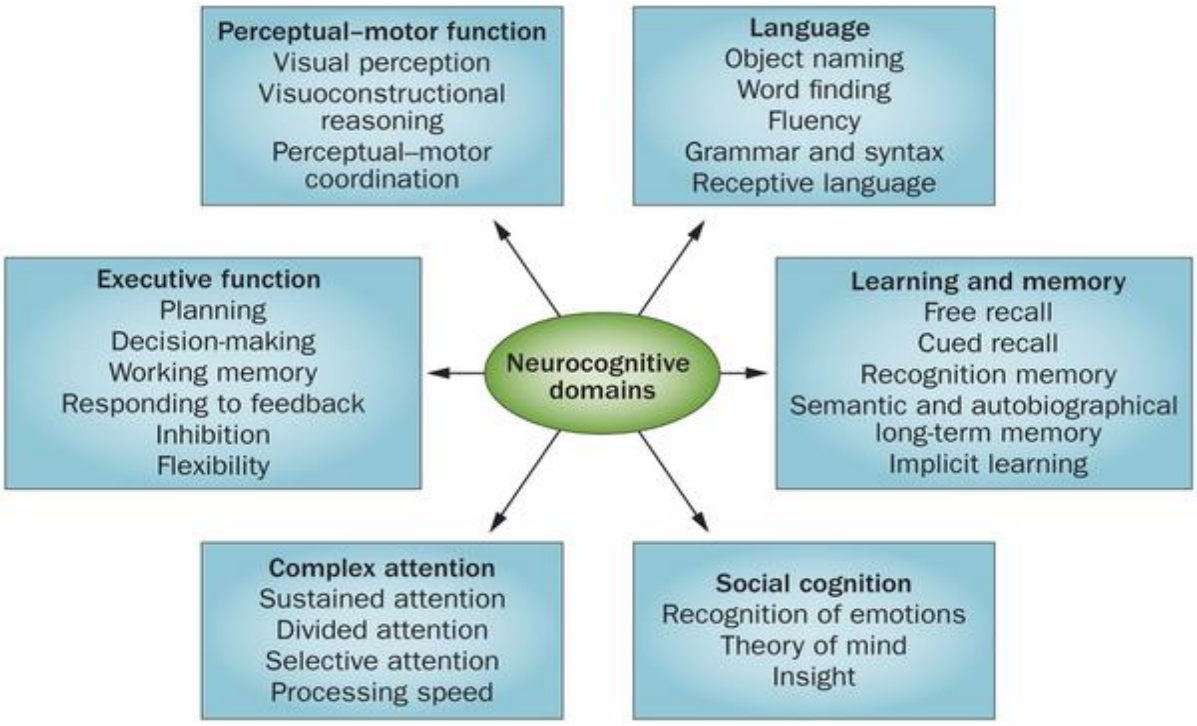


Figure 4

Neurocognitive domains and neuropsychological tests for the diagnosis of HAND



Neuropsychological test	Cognitive domains
1)Forward and Backward Digit span	Attention/working memory
2)Corsi's block Tapping Test	
3)Trail Making Test Part A-B (TMT A-B)	
4)Trail Making Test Part A (TMT A)	Speed of information processing
5) Stroop Color Test–Time	
6)Symbol Digit Modality Test	
7) Rey Auditory Verbal Learning Test Immediate Recall	Learning and memory
8)Rey Auditory Verbal Learning Test Delayed Recall	
9)Rey Osterrieth complex Delayed Recall	
10)Rey Osterrieth complex Figure Copy	Abstraction/executive function
11)Trail Making Test Part B (TMT-B)	
12)Stroop Color Test–Errors	
13)Semantic and Phonemic Fluency	Verbal fluency
14) Finger Tapping Test	Motor skills
15)Instrumental Activities of Daily Living Questionnaire (IADL)	Functional

LEGEND

Table shows the neuropsychological tests used in this study (first column) and the explored cognitive domains (second column).

Figure 5

Most common biomarkers for HAND (by Blokhuis C et al, Dove Press 2016; 7:1-13).

Laboratory markers		HIV-related findings
Monocyte-activation markers	sCD14, sCD163, neopterin	Elevated plasma sCD14 and neopterin have been reported in children.[79,80] In adults, elevations in plasma and CSF are associated with cognitive impairment, MRS neurometabolite alterations and elevated CSF NFL.[2,46,74,75]
Cerebral injury markers	NFL, tau, amyloid precursors	Elevated in CSF of adults, most prominently in those with HAD; NFL is associated with monocyte-activation markers and MRS metabolite alterations.[74,76,98,99]
Inflammatory cytokines and proteins	TNFα, IL-1β, IL-6, hsCRP	hsCRP and IL-6 predict cardiovascular morbidity and all-cause mortality in adults. hsCRP was reported elevated in children, in association with vascular dysfunction; IL-6 is associated with poor lipid profiles in adolescents.[44,80,112]
Chemokines	MCP-1, MIP-1α, MIP-1β, IP-10, RANTES, fractalkine	Often elevated in plasma and/or CSF of adults, several in association with MRS neurometabolite alterations.[46,81] In children, CSF chemokine levels were not associated with plasma inflammation or neurological symptoms.[82]
Cardiovascular markers	sVCAM, sICAM, P-selectin, fibrinogen	Elevations have been reported in cART-treated children. Adhesion molecules were associated with inflammation and poor lipid profiles, P-selectin and fibrinogen with poor cognitive performance.[44,45,80,112]

Figure 6

The CPE score (by Letendre S et al, Arch Neurol 2008; 65 (1):65-70).

CNS Penetration-Effectiveness (CPE) Ranks (2010)

Table 1.	4	3	2	1
NRTIs	Zidovudine	Abacavir	Didanosine	Tenofovir
		Emtricitabine	Lamivudine	Zalcitabine
			Stavudine	
NNRTIs	Nevirapine	Delavirdine	Etravirine	
		Efavirenz		
PIs	Indinavir-r	Darunavir-r	Atazanavir-r	Nelfinavir
		Fosamprenavir-r	Atazanavir	Ritonavir
		Indinavir	Fosamprenavir	Saquinavir-r
		Lopinavir-r		Saquinavir
				Tipranavir-r
Fusion/Entry Inhibitors		Maraviroc		Enfuvirtide
Integrase Inhibitors		Raltegravir		

Letendre S. et al. , 17th Conference on Retroviruses and Opportunstic Infections, poster n°430

Figure 7

Algorithm for the management of HAND in HIV-positive patients on stable cART (proposed by Underwood J and Winston A, Curr HIV/AIDS Res 2016; 13:235-240).

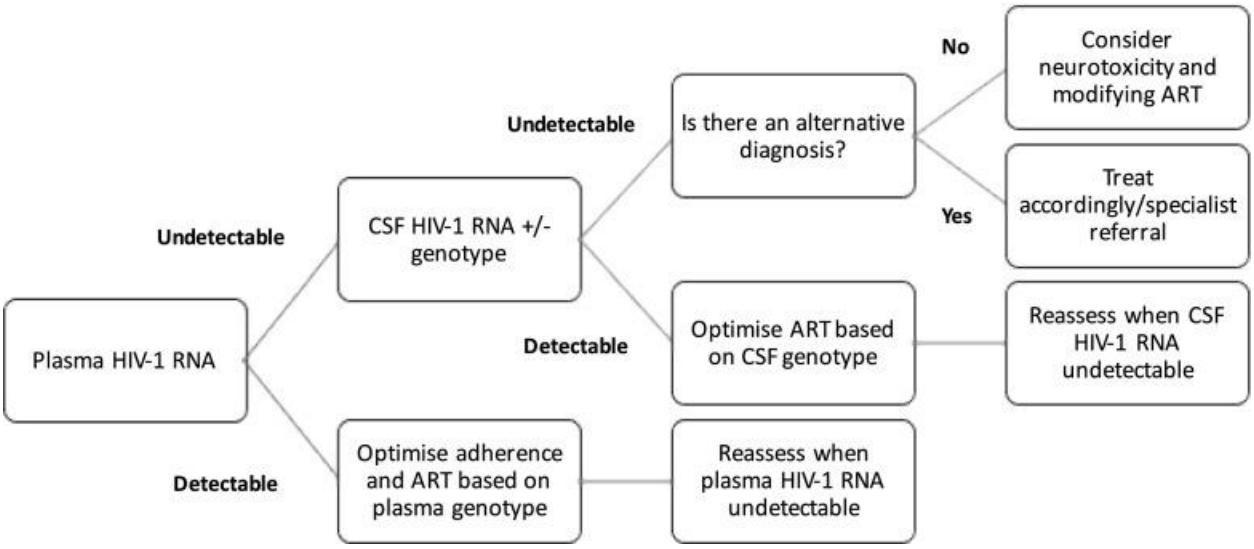


Figure 8

Algorithm for the management of HAND in HIV-positive patients on stable cART (proposed by Calcagno A et al, Drugs 2017; 77:145-157).

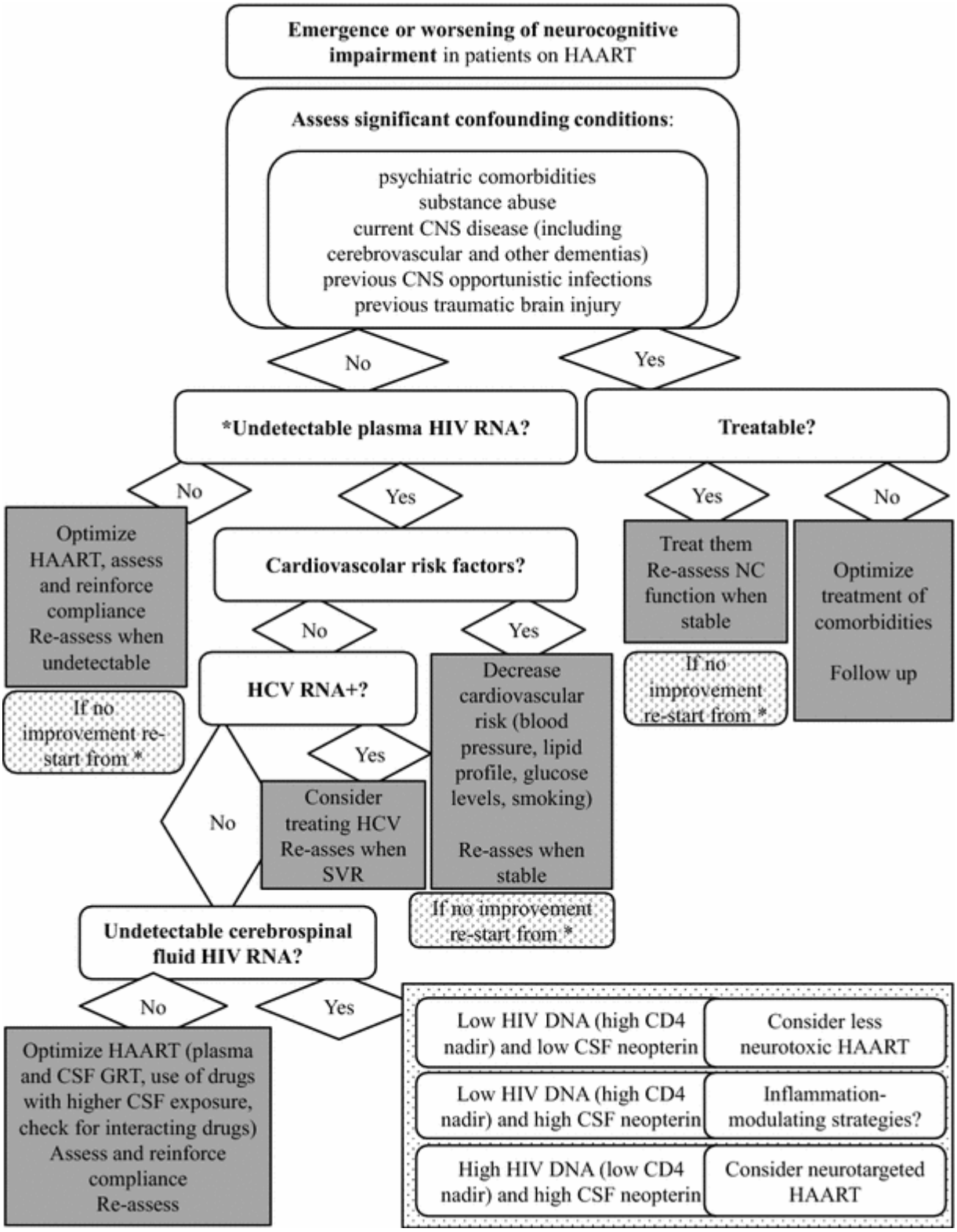
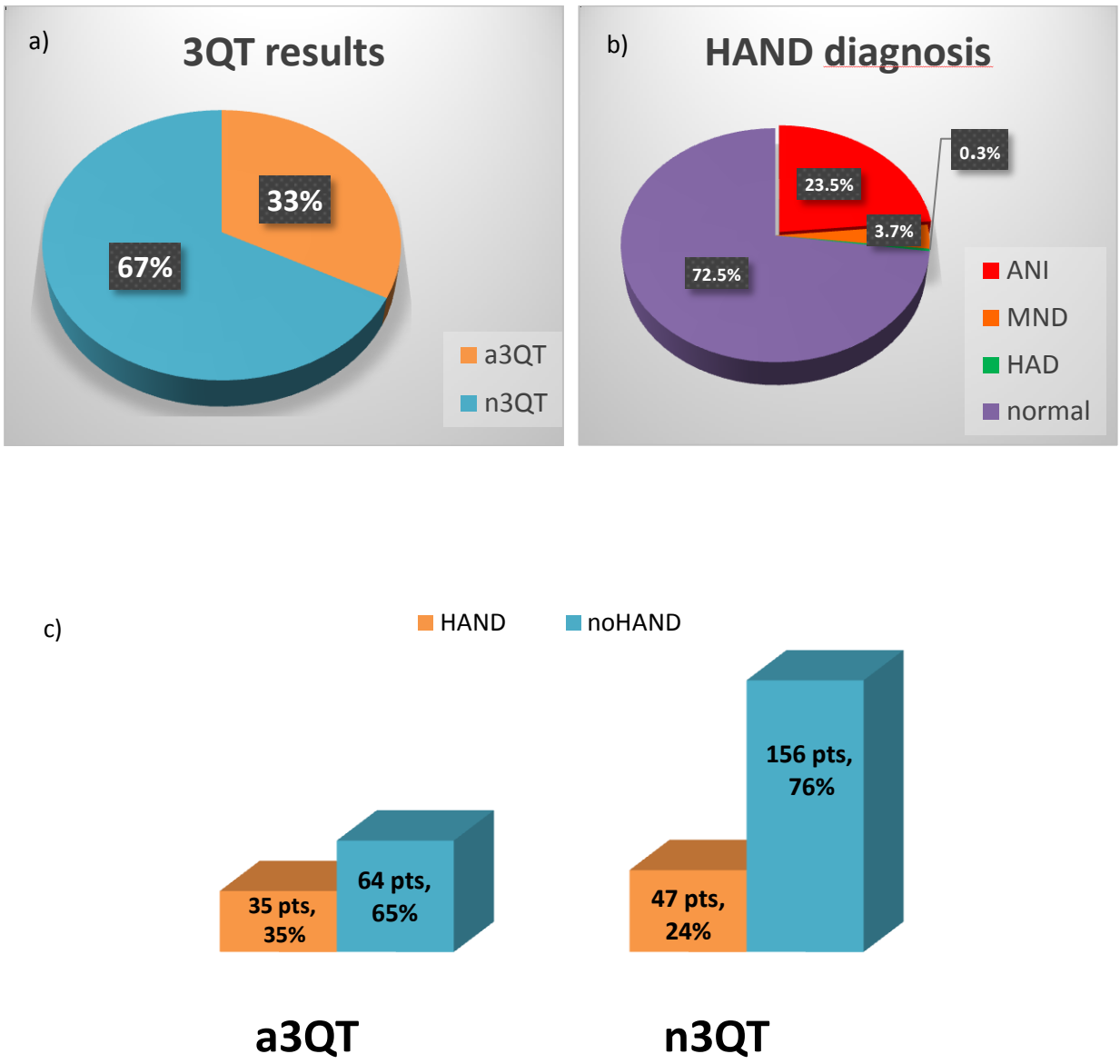


Table 1
Characteristics of study population

Characteristics	Population (N 302)	Antiretroviral naïve patients (N 191)	On cART patients (N 111)	p value
Demographic parameters				
Age, years*	44 (35-51)	40 (32-48)	49 (42-55)	0,0001
Males°	244 (81%)	167 (87%)	77 (69%)	0,0001
Months since HIV diagnosis*	16 (1-119)	2 (1-14)	208 (84-322)	0,0001
Risk factor for HIV° MSM Heterosexuals IDUs	 180 (60%) 110 (36%) 12 (4%)	 140 (74%) 43 (22%) 8 (4%)	 40 (36%) 67 (60%) 4 (4%)	0,0001
HCV coinfection°	60 (20%)	14 (7%)	46 (41%)	0,0001
HBV coinfection°	7 (2%)	3 (1.5%)	4 (4%)	0,263
AIDS diseases°	52 (17%)	28 (15%)	24 (22%)	0,114
cART regimen° PI NNRTI INSTI Other	 - 	 - 	 32 (29%) 36 (32%) 31 (28%) 12 (11%)	
Comorbidities°	82 (27%)	41 (21%)	41 (37%)	0,004
Unemployment°	35 (11%)	19 (10%)	16 (14%)	0,15
Viro-immunological parameters				
CD4+ T cells nadir, cells/mmc*	311 (163-469)	368 (183-500)	259 (155-400)	0,002
Current CD4+ T cells, cells/mmc*	455 (277 (626)	399 (233-551)	567 (398-760)	0,0001
Current CD4/CD8 ratio*	0,55 (0,32-0,8)	0,37 (0,22-0,64)	0,69 (0,44-0,94)	0,0001
Log_{10} HIVRNA cp/mL*	4,62 (4,01-5,29)	4,64 (4,08-5,3)	3,7 (1,67-5)	0,0034
Undetectable HIVRNA°	-	-	94 (85%)	
Neuropsychological parameters				
Altered SF-36 MHI°	121 (40%)	82 (43%)	39 (35%)	0,243
Altered SF-36 PHI°	52 (17%)	30 (16%)	22 (20%)	0,299
Altered HADS depression°	38 (12%)	47 (25%)	45 (40%)	0,004
Altered HADS anxiety°	92 (30%)	18 (9%)	20 (18%)	0,03
A3QT°	99 (33%)	45 (23%)	54 (49%)	0,0001
HAND° ANI MND HAD	82 (27%) 71 (86%) 10 (12%) 1 (2%)	43 (22%) 39 (90%) 3 (7%) 1 (3%)	39 (35%) 32 (82%) 7 (18%) 0	0,017

LEGEND
MSM, Men who have sex with men; IDUs, Intravenous Drug Users; cART, combination antiretroviral therapy; PI, Protease Inhibitors, NNRTI, Non Nucleoside Reverse Transcriptase Inhibitors, INSTI, Integrase Inhibitors, Other, other regimens (monotherapies or dual therapies). Comorbidities, GFR <90 ml/min, cardiovascular diseases, cancers. SF-36 MHI, Mental Health Index by SF-36 survey; SF-36 PHI, Physical Health Index by SF-36 survey.
HADS, Hospital Anxiety and Depression Scale, score >11 was considered pathological.
*Data are presented as Median (Interquartile Range), p values by Mann Whitney test.
°Data are presented as absolute numbers (percentages), p values by Chi-squared test.

Figure 9
Proportion of "abnormal" 3QT (a) and HAND (b) in the study population



LEGEND
a3QT, patients with "abnormal" 3QT; n3QT, patients with normal 3QT.
HAND, HIV-Associated neurocognitive Disorders; ANI, Asymptomatic Neurocognitive Impairment, MND, Mild Neurocognitive Disorders, HAD, HIV-Associated Dementia.

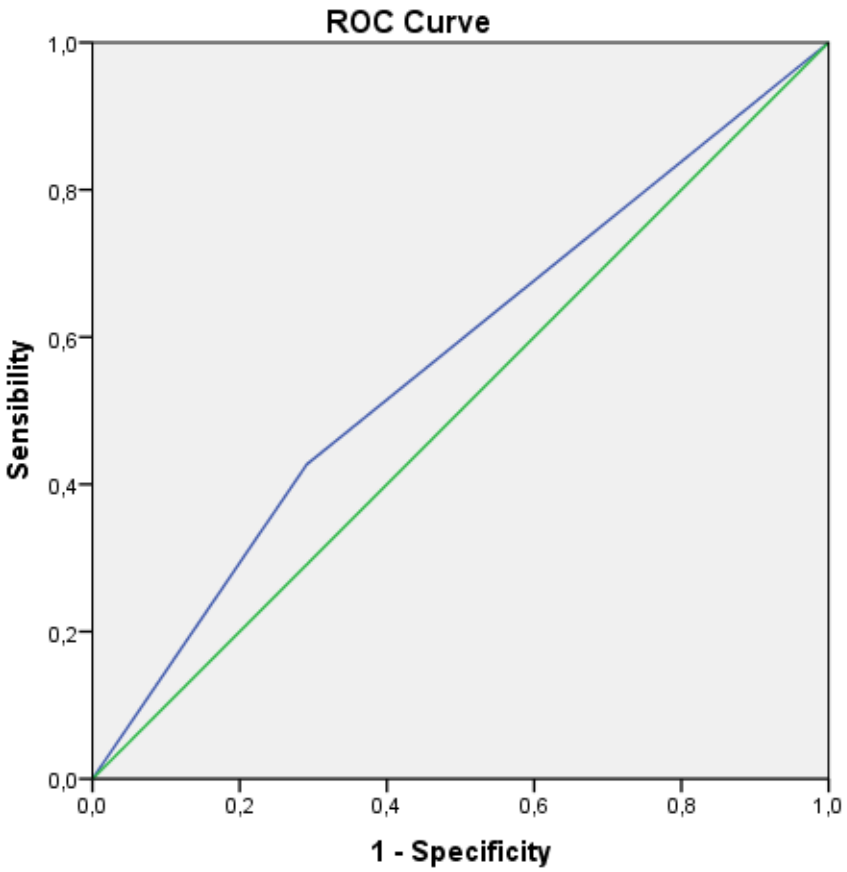
a) Proportion of patients with a3QT and n3QT
b) Proportion of patients with forms of HAND and of patients without neurocognitive impairment
c) Proportion of HAND cases in patients with a3QT and n3QT

Table 2
Sensitivity, specificity, negative and positive predictive value of 3QT.

Statistical Parameters	Population (N 302)	Antiretroviral naive patients (N 191)	On cART patients (N 111)
Sensitivity	**43 (32-53)**	**30 (16-44)**	**56 (41-72)**
Specificity	**71 (65-77)**	**78 (72-85)**	**44 (33-56)**
Positive Predictive Value	**35 (26-45)**	**29 (25-33)**	**38 (26-51)**
Negative Predictive Value	**77 (71-83)**	**79 (73-85)**	**70 (58-82)**

LEGEND
Data are presented with 95% Confidence Interval.
Sensitivity is the probability that a test will indicate 'disease' among those with the disease, **Specificity** is the fraction of those without disease who will have a negative test result. **Positive predictive value** is the probability that subjects with a positive screening test truly have the disease, while **Negative predictive value** is the probability that subjects with a negative screening test truly don't have the disease.

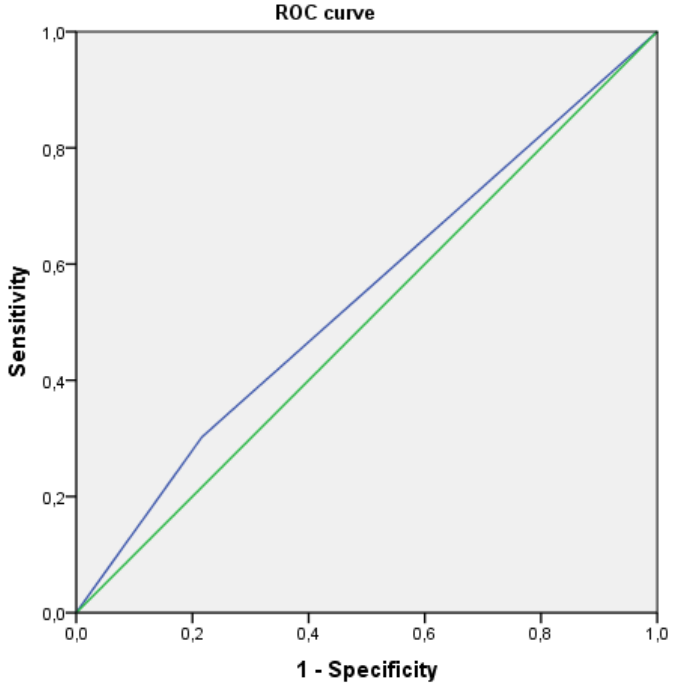
Figure 10
Receiver operating characteristic curve, 3 Questions Test.



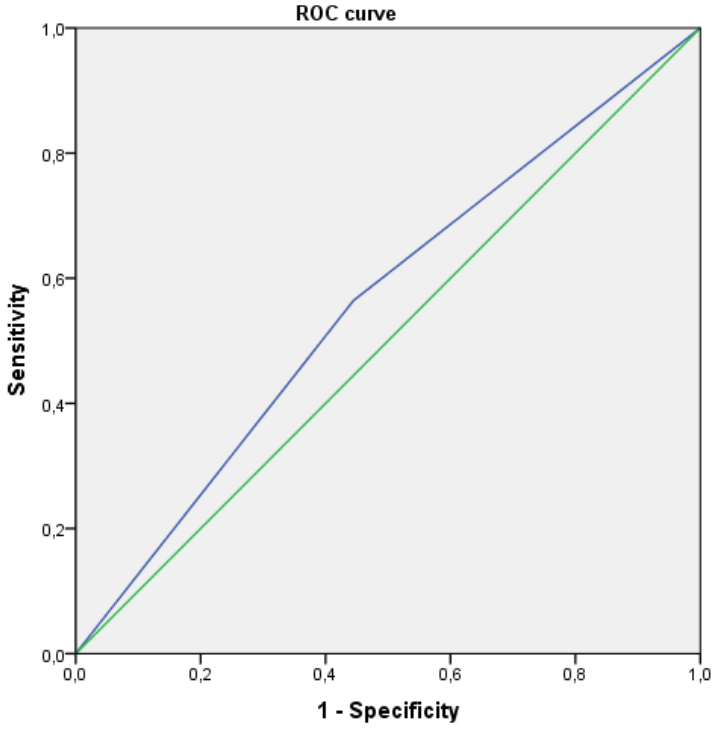
a

LEGEND
Receiver operating characteristic curve, ROC curve, for the “3 Questions test”. Participants with HAND versus those without cognitive impairment. Area under the curve=0,568, 95%CI 0,494-0,642.

Figure 11
Receiver operating characteristic curve, 3 Questions Test in antiretroviral naïve and on treatment HIV-positive patients.
a)



b)



LEGEND
Receiver operating characteristic curve, ROC curve, for the "3 Questions test": a) antiretroviral naïve patients, Area under the curve=0,56, 95%CI 0,448-0,672. b) treated patients, Area under the curve=0,543, 95%CI 0,443-0,643. Participants with HAND versus those without cognitive impairment.

Table 3
Factors associated with "abnormal" 3QT by fitting an univariable and multivariable logistic regression model

Characteristics	Crude OR (95% CI)	p value	Adjusted OR (95% CI)	p value
Age, each year more	1,019 (0,997-1,042)	0,087	0,999 (0,97-1,03)	0,209
Males	Ref		Ref	
Females	2,717 (1,513-4,88)	**0,001**	1,718 (0,739-3,993)	0,957
Months since HIV diagnosis, each month more	1,003 (1,001-1,005)	**0,004**	0,999 (0,995-1,002)	0,518
Risk factor for HIV°				
Heterosexuals	Ref	**0,026**	Ref	0,983
MSM	0,514 (0,31-0,852)	**0,01**	0,936 (0,421-2,081)	0,871
IDUs	1,444 (0,438-4,766)	0,546	1,04 (0,185-5,833)	0,965
Hepatitis coinfection (yes vs no)	1,304 (0,734-2,318)	0,366		
AIDS diseases (yes vs no)	1,513 (0,818-2,801)	0,187		
On cART patients	Ref		Ref	
Antiretroviral naïve patients	0,325 (0,197-0,537)	**<0,0001**	0,249 (0,103-0,602)	**0,002**
Comorbidities (yes vs no)	1,61 (0,949-2,733)	0,078	1,374 (0,708-2,666)	0,348
Unemployment (yes vs no)	1,584 (0,771-3,256)	0,211		
CD4+ T cells nadir, each cells/mmc higher	1 (0,998-1,001)	0,405		
Current CD4+ T cells, each cells/mmc higher	1 (1-1,001)	0,361		
Current CD4/CD8 ratio, Each unit higher	0,967 (0,431-2,167)	0,935		
Log_{10} HIVRNA cp/mL, Each log_{10} cp/mL higher	0,893 (0,628-1,269)	0,527		
Altered SF-36 MHI, (yes vs no)	2,4 (1,438-4,005)	**0,001**	2,86 (1,427-5,73)	**0,003**
Altered SF-36 PHI, (yes vs no)	3,124 (1,683-5,797)	**<0,0001**	4,073 (1,879-8,828)	**<0,0001**
Altered HADS depression, (yes vs no)	2,114 (1,261-3,545)	**0,005**	0,504 (0,181-1,406)	0,191
Altered HADS anxiety, (yes vs no)	2,593 (1,298-5,178)	**0,007**	0,802 (0,386-1,6659	0,533
HAND, (yes vs no)	1,815 (1,073-3,07)	**0,026**	1,216 (0,629-2,352)	0,56

LEGEND
OR, odds ratio; 95% CI, 95% confidence interval.
MSM, Men who have sex with men; IDUs, Intravenous Drug Users; cART, combination antiretroviral therapy; Comorbidities, GFR <90 ml/min, cardiovascular diseases, cancers. SF-36 MHI, Mental Health Index by SF-36 survey; SF-36 PHI, Physical Health Index by SF-36 survey. HADS, Hospital Anxiety and Depression Scale, score >11 was considered pathological. Multivariable analysis included the following

variables: age, gender, months since HIV diagnosis, risk factor for HIV, being antiretroviral naïve, comorbidities, altered HADS depression and anxiety score, altered SF-36 MHI and PHI score and HAND.

Table 4
Factors associated with "abnormal" 3QT by fitting an univariable and multivariable logistic regression model in untreated HIV-infected patients

Characteristics	Crude OR (95% CI)	p value	Adjusted OR (95% CI)	p value
Age, each year more	0,994 (0,9963-1,026)	0,691		
Males **Females**	Ref 2,694 (1,103-6,579)	 **0,03**	Ref 1,765 (0,665-4,683)	0,254
Months since HIV diagnosis, each month more	1,003 (0,998-1,009)	0,277		
Risk factor for HIV° **Heterosexuals** **MSM** **IDUs**	 Ref 0,518 (0,242-1,108) 1,243 (0,259-5,956)	 0,158 0,09 0,786		
Hepatitis coinfection (yes vs no)	0,998 (0,309-3,229)	0,997		
AIDS diseases (yes vs no)	1,362 (0,555-3,344)	0,5		
Comorbidities (yes vs no)	1,082 (0,482-2,429)	0,849		
Unemployment (yes vs no)	1,163 (0,394-3,433)	0,785		
CD4+ T cells nadir, each cells/mmc higher	1 (0,998-1,001)	0,589		
Current CD4+ T cells, each cells/mmc higher	1 (0,999-1,001)	0,987		
Current CD4/CD8 ratio, Each unit higher	0,599 (0,121-2,962)	0,53		
Log_{10} HIVRNA cp/mL, Each log_{10} cp/mL higher	1,025 (0,681-1,542)	0,907		
Altered SF-36 MHI, (yes vs no)	2,684 (1,309-5,501)	**0,007**	2,309 (1,104-4,83)	**0,026**
Altered SF-36 PHI, (yes vs no)	2,644 (1,15-6,083)	**0,022**	2,056 (0,855-4,941)	0,017
Altered HADS depression, (yes vs no)	1,658 (0,583-4,714)	0,343		
Altered HADS anxiety, (yes vs no)	1,283 (0,603-2,728)	0,518		
HAND, (yes vs no)	1,571 (0,735-3,357)	0,244	1,277 (0,552-2,953)	0,567

LEGEND
OR, odds ratio; 95% CI, 95% confidence interval.
MSM, Men who have sex with men; IDUs, Intravenous Drug Users; Comorbidities, GFR <90 ml/min, cardiovascular diseases, cancers. SF-36 MHI, Mental Health Index by SF-36 survey; SF-36 PHI, Physical Health Index by SF-36 survey. HADS, Hospital Anxiety and Depression Scale, score >11 was considered pathological. Multivariable analysis included the following variables: gender, altered SF-36 MHI and PHI score and HAND.

Table 5
Factors associated with "abnormal" 3QT by fitting an univariable and multivariable logistic regression model in treated HIV-infected patients

Characteristics	Crude OR (95% CI)	p value	Adjusted OR (95% CI)	p value
Age, each year more	1,014 (0,975-1,054)	0,495		
Males	Ref			
Females	1,807 (0,797-4,093)	0,156		
Months since HIV diagnosis, each month more	0,999 (0,995-1,002)	0,415		
Risk factor for HIV°				
Heterosexuals	Ref	0,568		
MSM	1,103 (0,5-2,432)	0,808		
IDUs	3,484 (0,345-35,223)	0,29		
Hepatitis coinfection (yes vs no)	0,654 (0,306-1,396)	0,272		
AIDS diseases (yes vs no)	1,359 (0,548-3,369)	0,508		
Comorbidities (yes vs no)	1,661 (0,762-3,62)	0,202		
Unemployment (yes vs no)	1,707 (0,568-5,128)	0,34		
CD4+ T cells nadir, each cells/mmc higher	1,001 (0,999-1,003)	0,476		
Current CD4+ T cells, each cells/mmc higher	0,999 (0,998-1,001)	0,438		
Current CD4/CD8 ratio, Each unit higher	0,461 (0,149-1,429)	0,18		
HIVRNA < 40 c/mL (yes vs no)	1,623 (0,57-4,627)	0,365		
Altered SF-36 MHI, (yes vs no)	3,028 (1,317-6,964)	**0,009**	2,827 (0,899-8,89)	0,075
Altered SF-36 PHI, (yes vs no)	3,917 (1,384-11,084)	**0,01**	3,613 (1,519-11,263)	**0,027**
Altered HADS depression, (yes vs no)	3,09 (1,084-8,807)	**0,035**	0,707 (0,167-2,981)	0,636
Altered HADS anxiety, (yes vs no)	2,721 (1,229-6,023)	**0,014**	1,314 (0,475-3,63)	0,599
HAND, (yes vs no)	1,618 (0,738-3,548)	0,23	1,6125 (0,631-4,183)	0,314

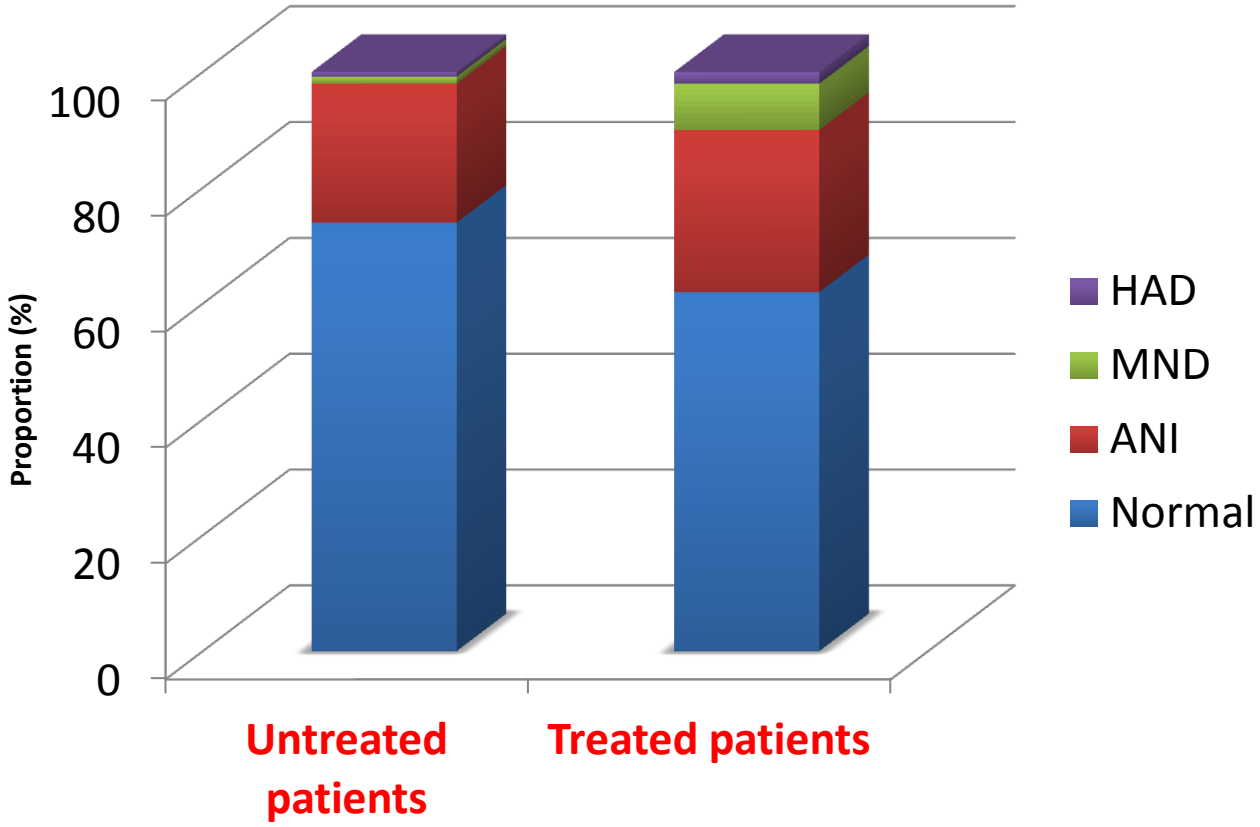
LEGEND
OR, odds ratio; 95% CI, 95% confidence interval.
MSM, Men who have sex with men; IDUs, Intravenous Drug Users; Comorbidities, GFR <90 ml/min, cardiovascular diseases, cancers. SF-36 MHI, Mental Health Index by SF-36 survey; SF-36 PHI, Physical Health Index by SF-36 survey. HADS, Hospital Anxiety and Depression Scale, score >11 was considered pathological. Multivariable analysis included the following variables: altered HADS depression and anxiety score, altered SF-36 MHI and PHI score and HAND.

Table 6
Baseline characteristics of the study population.

Characteristics	Untreated HIV+ patients (N 237)	Treated HIV+ patients (N 116)
Age, years, median, IQR *	39 (32-47)	50 (43-56)
Males, n (%) °	211 (89%)	81 (70%)
Months since HIV diagnosis, median, IQR *	2 (1-13)	200 (81-311)
Hepatitis coinfection, n (%) °	23 (10%)	50 (43%)
Risk factor for HIV, n (%)° **MSM** **Heterosexuals** **IDUs**	 173 (73%) 55 (23%) 9 (4%)	 41 (35%) 68 (59%) 7 (6%)
Not Italian, n (%) °	24 (10%)	4 (4%)
AIDS events, n (%) °	42 (18%)	29 (25%)
CD4+ T-cells nadir, cells/mmc, median, IQR *	368 (220-526)	252 (150-399)
CD4/CD8 ratio, median, IQR *	0,37 (0,22-0,64)	0,68 (0,44-0,94)
Plasma Log_{10} HIV-RNA, cp/mL, median, IQR *	4,71 (4,09-5,34)	3,26 (1,59-4,21)
CSF Log_{10} HIV-RNA, c/mL , median, IQR * **(N of patients)**	3,64 (3,02-4,16) (N 189)	4,22 (3,54-5,09) (N 39)
Plasma HIV-RNA >50 cp/mL, n (%) °	-	27 (23%)
CSF HIV-RNA >50 cp/mL, n (%) °	-	13/39 (33%)
Viral Escape, n (%) °	-	7/39 (18,4%)
cART regimen, n (%) ° **PI-based** **NNRTI-based** **INSTI-based** **Dual therapies**	 - 	 34 (29%) 36 (31%) 33 (28%) 13 (12%)
CPE score, median, IQR *	-	7 (6-8)
Years of education, median, IQR *	13 (8-17)	8 (8-13)
Unemployed pts, n (%) °	19 (8%)	16 (14%)
Comorbidities, n (%) °	41 (17%)	41 (35%)

LEGEND
HAND, HIV-Associated Neurocognitive Disorders; MSM, Men who have sex with men; IDUs, Intravenous Drug Users; Pts, patients; CSF, Cerebrospinal fluid; cp/mL, copies/mL; cART, combination antiretroviral therapy. Comorbidities: GFR <90 ml/min, Framingham risk score >20%, cancers.
*Data are presented as Median (Interquartile Range) - °Data are presented as absolute numbers (percentages).

Figure 12
Prevalence of HAND in untreated and cART-treated patients.



LEGEND
Proportion of patients with normal neurocognitive performance and HAND in antiretroviral-naïve and cART-treated patients.
HAD, HIV-Associated Dementia; MND, Mild Neuocognitive Disorders; ANI, Asymptomatic Neurocognitive Impairment.

Table 7
Factors associated with HAND in antiretroviral-naïve HIV-infected patients by fitting an univariate and multivariate logistic regression model.

Parameters	Univariate		Multivariate	
	OR (95% CI)	p values	AOR (95% CI)	p values
Age, each year more	1,02 (0,99-1,05)	0,145	1,002 (0,959-1,047)	0,927
Females	1	0,135		
Males	0,523 (0,224-1,224)			
Months since HIV diagnosis, each month more	1,001 (0,996-1,006)	0,752	1,002 (0,994-1,01)	0,602
AIDS events		0,0001		0,262
No	1		1	
Yes	3,756 (1,873-7,534)		1,958 (0,606-6,328)	
Hepatitis coinfection		0,325		0,975
No	1		1	
Yes	1,58 (0,635-3,933)		1,022 (0,257-4,06)	
Italian nationality	1	0,001	**1**	**0,0001**
Not Italian nationality	4,567 (1,871-11,15)		**7,27 (2,535-20,873)**	
Normal SF-36 physical health score	1	0,034		
Impaired SF-36 physical health score	2,502 (1,071-5,846)			
Normal SF-36 mental health score	1	0,256		
Impaired SF-36 mental health score	1,511 (0,741-3,082)			
Normal HADS-A	1	0,784		
Impaired HADS-A	1,117 (0,507-2,462)			
Normal HADS-D	1	0,533		
Impaired HADS-D	1,418 (0,473-4,246)			
Education		0,036	0,938 (0,832-1,058)	0,296
Each year more	0,904 (0,822-0,993)			
Comorbidities		0,906		0,557
No	1		1	
Yes	0,951 (0,414-2,187)		0,748 (0,283-1,973)	
Employed patients	1	0,0001	**1**	**0,018**
Unemployed patients	5,801 (2,158-15,59)		**4,103 (1,27-13,248)**	
CD4+ T-cells nadir, each cell/mmc more	0,996 (0,995-0,999)	0,0001	**0,998 (0,995-0,999)**	**0,044**
Log_{10} HIV-RNA copies/mL each copies/mL more	0,939 (0,675-1,305)	0,708		

LEGEND
Univariable and multivariable logistic regression analysis. OR, odds ratio; 95% CI, 95% confidence interval. AOR, adjusted odds ratio. Comorbidities: GFR <90 ml/min, Framingham risk score >20%, cancers. SF-36 MHI, Mental Health Index by SF-36 survey; SF-36 PHI, Physical Health Index by SF-36 survey. HADS, Hospital Anxiety and Depression Scale, score >11 was considered pathological. Multivariable analysis included the following variables: AIDS diseases, CD4+ T cells nadir, age, months

since HIV diagnosis, hepatitis coinfection, years of education, comorbidities, nationality and employment.

Table 8 Factors associated with HAND in cART-treated HIV-infected patients by fitting an univariate and multivariate logistic regression model.

Parameters	Crude OR (95%CI)	p values	AOR (95%CI)	p values
Age, each year more	0,995 (0,957-1,034)	0,78	0,944 (0,888-1,003)	0,063
Females **Males**	1 0,627 (0,28-1,407)	0,258		
Months since HIV diagnosis, each month more	1,0 (0,999-1,006)	0,119	**1,006 (1-1,013)**	**0,05**
AIDS events **No** **Yes**	 1 3,756 (1,873-7,534)	0,0001	 1 0,5019 (0,081-3,121)	0,459
Hepatitis coinfection **No** **Yes**	 1 1,57 (0,737-3,352)	0,242	 1 1,151 (0,279-4,747)	0,846
Italian nationality **Not Italian nationality**	1 1,892 (0,256-13,98)	0,532		
Normal SF-36 physical health score **Impaired SF-36 physical health score**	1 1,686 (0,633-4,486)	0,296		
Normal SF-36 mental health score **Impaired SF-36 mental health score**	1 1,132 (0,48-2,667)	0,777		
Education **Each year more**	0,962 (0,872-1,062)	0,446	0,862 (0,729-1,019)	0,082
Comorbidities **No** **Yes**	 1 2,273 (1,01-5,108)	0,047	 **1** **4,757 (0,916-9,612)**	**0,07**
Normal HADS-A **Impaired HADS-A**	1 1,131 (0,502-2,546)	0,766		
Normal HADS-D **Impaired HADS-D**	1 3,875 (1,41-10,65)	0,009	**1** **5,221 (1,095-24,885)**	**0,038**
Employment patients **Unemployed patients**	1 4,823 (1,52-15,3)	0,008	1 2,898(0,527-15,938)	0,221
CD4+ T-cells nadir, each cell/mmc more	1 (0,998-1,003)	0,755	1,004 (0,999-1,008)	0,092
Log10 HIV-RNA copies/mL each copies/mL more	0,939 (0,675-1,305)	0,708		
Plasma HIV-RNA >50 c/mL **No** **Yes**	 1 4,125 (1,419-11,991)	0,009	 **1** **16,28 (2,225-117,64)**	**0,006**
CPE score, each score more	0,397 (0,115-1,379)	0,146		
Months of cART treatment, each month more	1 (1-1,001)	0,213		
cART **3 drugs** **Mono-dual therapies**	 1 2,926 (0,889-9,626)	0,077		

LEGEND

Univariable and multivariable logistic regression analysis. OR, odds ratio; 95% CI, 95% confidence interval. AOR, adjusted odds ratio. Comorbidities: GFR <90 ml/min, Framingham risk score >20%, cancers. SF-36 MHI, Mental Health Index by SF-36 survey; SF-36 PHI, Physical Health Index by SF-36 survey. HADS, Hospital Anxiety and Depression Scale, score >11 was considered pathological. Multivariable analysis included the following variables: AIDS diseases, CD4+ T cells nadir, proportion of patients with detectable HIV-RNA, age, months since HIV diagnosis, education, hepatitis, comorbidities, symptoms of depression and employment.

Table 9

Comparison between subjects with normal neurocognitive function and patients with neurocognitive impairment

Total population n=189	No HAND (n=136)	HAND (n= 53)	*p value*
Demographic characteristics			
Female, n (%) *	10 (7.4)	8 (15.)1	0.164
Age (years), median (IQR)°	38 (30-45)	39 (33-48)	0.105
Time since first HIV diagnosis (months), median (IQR)°	2 (1-12)	2 (0.5-24)	0.295
AIDS events, n (%)*	18 (13.2)	21 (40)	**0.0001**
Risk factor for HIV, n (%)			0.456
Heterosexuals	25 (18.4%)	14 (20.6%)	
MSM	104 (76.5%)	37 (69.8%)	
Ex IDUs	7 (5.1%)	2 (3.8%)	
HCV Ab positive, n (%)	9 (6.6%)	4 (7.5%)	0.76
HBsAg positive, n (%)	4 (2.9%)	2 (3.8%)	0.674
Viro-immunological parameters			
Plasma HIV-RNA, Log_{10} cp/ml median (IQR)°	4.63 (4.09-5.39)	4.79 (4.21 -5.3)	0.806
CSF HIV-RNA, Log_{10} cp/ml median (IQR)°	3.61 (3-4.11)	3.67 (3.19-4.4)	0.839
CSF to plasma HIV-RNA ratio, median (IQR)°	0.76 (0.61-0.86)	0.79 (0.62-1.01)	0.371
Pts with CSF to plasma HIV-RNA ratio ≥1, n (%)*	15 (11.3)	15 (28.3)	**0.004**
Nadir CD4+ T-cells, cell/mmc median (IQR)°	381 (249-492)	274 (74-411)	**0.0001**
Pts with nadir CD4+ T-cells <200 cell/mmc, n (%)*	34 (25.4)	26 (52)	**0.001**
CD8+% T-cells, median (IQR)°	56 (48-65)	59 (49-70)	**0.005**
CD4/CD8 ratio, median (IQR)°	0.38 (0.21-0.5)	0.3 (0.15-0.47)	0.455
T cell activation			
CD38+CD8+%, median (IQR)°	12 (7-20)	12 (8-20)	0.094
CD45R0+CD38+CD8+%, median (IQR)°	7 (4-15)	8 (4-15)	0.059
T cell maturation/differentiation			
CD127+CD4+%, median (IQR)°	13 (8-17)	10 (5-14)	**0.001**
CD127+CD8+%, median (IQR)°	29 (24-37)	28 (23-37)	0.182
CD45RA+CD4+%, median (IQR)°	8 (5-12)	6 (2-9)	**0.0001**
CD45RA+CD8+%, median (IQR)°	19 (13-24)	18 (14-25)	0.777
CD45R0+CD8+%, median (IQR)°	23 (17-31)	21 (16-33)	0.055

LEGEND

HAND, HIV-Associated Neurocognitive Disorders; MSM, Men who have sex with men; IDUs, Intravenous Drug Users; Pts, patients; CSF, Cerebrospinal fluid; cp/mL, copies/mL.

CSF to plasma HIV-RNA ratio was calculated as log_{10} CSF HIV-RNA divided by log_{10} plasma HIV-RNA copies/mL.

*Data are presented as Median (Interquartile Range), p values by Mann Whitney test.

°Data are presented as absolute numbers (percentages), p values by Chi-squared test or Fisher exact test as appropriate.

Table 10

Factors independently associated with HIV-Associated Neurocognitive Disorders (HAND) by fitting a multivariable logistic regression analysis.

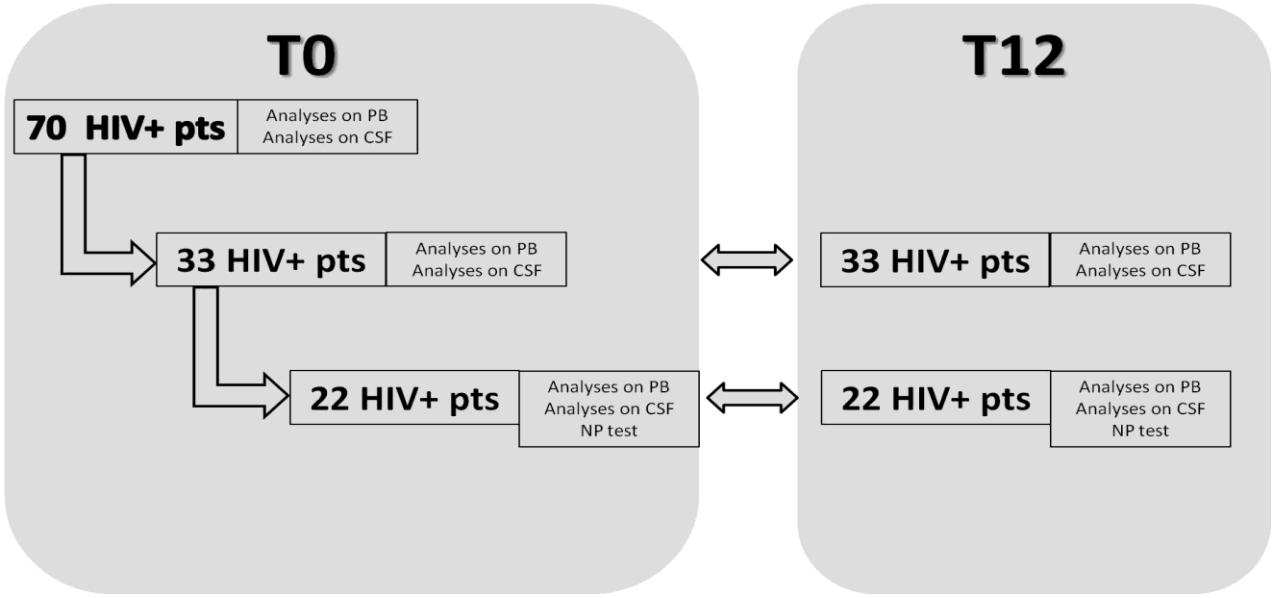
Total population n=189	*Multivariate analysis*		
	AOR	**95%CI**	***p value***
AIDS			
No	Ref		
Yes	3.441	1.38-8.58	**0.008**
Pts with CSF to plasma HIV-RNA ratio <1	Ref		
Pts with CSF to plasma HIV-RNA ratio ≥1	2.707	1.122-6.531	**0.027**
Nadir CD4+ T-cells ≥200 cells/mmc	Ref		
Nadir CD4+ T-cells <200 cells/mms	1.803	0.8-4.06	0.155

LEGEND

AOR, adjusted odds ratio; 95% CI, 95% confidence interval. Each variable is mutually adjusted.

Pts, patients; CSF to plasma HIV-RNA ratio was calculated as $\log_{10}$ CSF HIV-RNA divided by $\log_{10}$ plasma HIV-RNA copies/mL.

Figure 13.
Diagram illustrating patients' assignments to peripheral blood (PB) and CSF analyses and to neurocognitive screening test.



LEGEND
Seventy HIV-infected cART naïve subjects were enrolled and assigned to underwent blood and CSF collection. Thirty-three patients underwent lumbar puncture and blood withdrawal at T12 as well. Twenty-two patients agreed to performed a short screening neurocognitive battery, consisting of Mini Mental State Examination (MMSE), Frontal Assessment Battery (FAB) and International HIV Dementia Scale (IHDS) both at T0 and T12.

Table 11
Baseline demographic and viro-immunological features of the study population.

	Total (n=70)	L-CSF (n=61)	H-CSF (n=9)	p values
Females, yes (%)*	5 (7)	4 (6.5)	1 (11)	0.508
Age, years, (IQR)°	39 (32-46)	38 (31-45)	39 (37-55)	0.136
HCV co-infection, yes (%)*	3 (4)	3 (5)	0	1
HBV co-infection, yes (%)*	3 (4)	3 (5)	0	1
CMV IgG levels, (IQR)°	102 (36-141)	101 (42-139)	131 (22-180)	0.755
Mode of HIV transmission, (%)*				0.188
Homo/Bisexual contact	47 (67)	43 (70)	4 (45)	
Heterosexual contact	7 (10)	11 (18)	4 (45)	
IDU/Other	16 (23)	7 (12)	1 (10)	
Time since 1st HIV diagnosis, months (IQR)°	2.5 (1-8)	2 (1-8.5)	3 (1.5-6)	0.978
AIDS-defining conditions, yes (%)*	12 (17)	10 (16)	2 (22)	0.646
Plasma HIV-RNA, Log cp/ml (IQR)°	5,06 (4,22-5,62)	5,07 (4,51-5,64)	4,04 (2,59-4,90)	**0.009**
CSF HIV-RNA, Log cp/ml (IQR)°	3,57 (2,92-4,06)	3,52 (2,91-3,87)	4,42 (3,04-5,72)	**0.034**
Total plasma HIV-DNA, cp/10*6 PBMC, (IQR)°	987 (205-2380)	1480 (246-3226)	189 (93-556)	0.064
CD4 T-cell count, mmc/ml (IQR)°				
Nadir CD4	166 (38-340)	179 (32-347)	123 (39-309)	0.0667
At time of analyses	326 (218-416)	339 (242-438)	183 (123-326)	**0.050**
CD8 T-cell count, mmc/ml (IQR)°	992 (785-1197)	996 (715-1184)	895 (841-1756)	0.695
CD4/CD8 ratio, (IQR)°	0,33 (0,17-0,41)	0,35 (0,20-0,47)	0,14 (0,10-0,36)	**0.026**

LEGEND
°Data are median (IQR). Statistical analyses: Mann-Whitney U Test. *Data are n (%). Statistical analyses: Pearson Chi squared or Fisher Exact Test. IQR: Interquartile range; CSF: cerebrospinal fluid; H-CSF: High cerebrospinal fluid (CSF/plasma HIV-RNA ≥1); L-CSF: Low cerebrospinal fluid (CSF/plasma HIV-RNA<1). IDU: intravenous drug users.

Table 12
Plasma Inflammation/Activation, Maturation and Cytokines Production.

	Total (n=70)	L-CSF (n=61)	H-CSF (n=9)	p values
Peripheral blood markers				
T cell activation, median (IQR)				
CD8+38+, %	12,0 (9,00-20,30)	12,00 (9,00-20,00)	12,00 (5,00-32,00)	0.613
CD8+CD38+CD45R0+, %	7,00 (5,00-13,30)	7,00 (5,00-13,00)	6,00 (4,00-16,00)	0.634
HLA-DR+CD38+CD4+, %	2,10 (1,00-5,10)	1,90 (0,95-5,05)	3,30 (1,03-6,85)	0.522
HLA-DR+CD38+CD8+, %	8,35 (4,50-14,50)	8,35 (4,45-13,73)	8,35 (5,58-18,55)	0.734
T-cell proliferation, median (IQR)				
KI67+CD4+, %	3,10 (1,75-6,60)	3,15 (1,80-6,55)	1,90 (1,60-9,40)	0.579
KI67+CD8+, %	2,90 (1,55-5,75)	2,95 (1,75-5,68)	2,00 (0,80-6,50)	0.386
T-cell maturation, median (IQR)				
CD4+CCR7+CD45RA+ (naive), %	2,00 (1,05-3)	2,00 (1,20-3,10)	1,25 (0,28-4,20)	0.406
CD4+CCR7+CD45RA- (CM), %	3,80 (2,40-5,10)	3,90 (2,43-5,43)	2,65 (2,35-4,70)	0.494
CD4+CCR7-CD45RA+ (TD), %	36,9 (27,3-46,30)	36,80 (27,53-45,13)	42,30 (14,68-52,68)	0.692
CD4+CCR7-CD45RA- (EM), %	56,3 (45,90-66,7)	56,30 (45,83-66,55)	51,75 (42,15-82,13)	0.885
CD8+CCR7+CD45RA+ (naive), %	1,20 (0,70-2,40)	1,15 (0,70-2,45)	1,50 (0,80-2,40)	0.717
CD8+CCR7+CD45RA- (CM), %	1,50 (1,00-2,10)	1,50 (1,03-2,08)	1,50 (0,80-2,10)	0.908
CD8+CCR7-CD45RA+ (TD), %	42,10 (32,1-47,7)	43,40 (33,83-47,60)	32,10 (26,90-48,70)	0.172
CD8+CCR7-CD45RA- (EM), %	54.10 (47-64,80)	53,95 (44,30-62,50)	64,90 (49,70-70,30)	0.126
Plasma Cytokines, median (IQR)				
IL-6, pg/ml	2,30 (0,80-4,00)	1,82 (0,68-3,76)	3,50 (1,54-6,27)	0.086
TNFa, pg/ml	1,30 (0,60-3,50)	1,33 (0,60-3,60)	1,49 (0,62-4,08)	0.921
sCD14, ug/ml	3,50 (2,60-5,00)	3,53 (2,64-4,92)	3,86 (2,78-5,36)	0.544
IFN-γ, pg/ml	59,1 (41,1-67,70)	52,14(39,72-66,02)	65,85 (57,42-88,58)	0.068
HIV-specific T-cell Response, median (IQR)				
CD4+IL2+IFNγ+, %	0 (0-0)	0 (0-0,075)	0 (0-0)	0.939
CD4+IL2+IFNγ-, %	0,8 (0,1-1,8)	0,9 (0,4-1,6)	0,75 (0,025-1,898)	0.815
CD4+IL2-IFNγ+, %	0 (0-0,5)	0,2 (0-0,5)	0 (0-0,575)	0.713
CD8+IL2+IFNγ+, %	0 (0-0,1)	0,1 (0-0,2)	0 (0-0,1)	0.099
CD8+IL2-IFNγ+, %	0 (0-0,1)	0,1 (0-0,4)	0 (0-0,113)	0.406
CMV-specific T-cell Response, median (IQR)				
CD4+IL2+IFNγ+, %	0 (0-0,1)	0 (0-0,15)	0 (0-0,1)	0.8
CD4+IL2+IFNγ-, %	0,9 (0,2-2,3)	2 (1,3-3,3)	0,66 (0,12-2,1)	0.065
CD4+IL2-IFNγ+, %	0,4 (0-0,7)	0,5 (0,08-0,85)	0,4 (0-0,73)	0.782
CD8+IL2+IFNγ+, %	0 (0-0,1)	0 (0-0,1)	0 (0-0,095)	0.907
CD8+IL2-IFNγ+, %	0 (0-0,3)	0,2 (0-0,8)	0 (0-0,235)	0.442

LEGEND

All data are median (IQR). Statistical analyses: Mann-Whitney U Test. IQR: Interquartile range; CSF: cerebrospinal fluid; H-CSF: High cerebrospinal fluid (CSF/plasma HIV-RNA ≥1); L-CSF: Low cerebrospinal fluid (CSF/plasma HIV-RNA<1). CM: central memory, EM: effector memory, TD: terminally differentiated

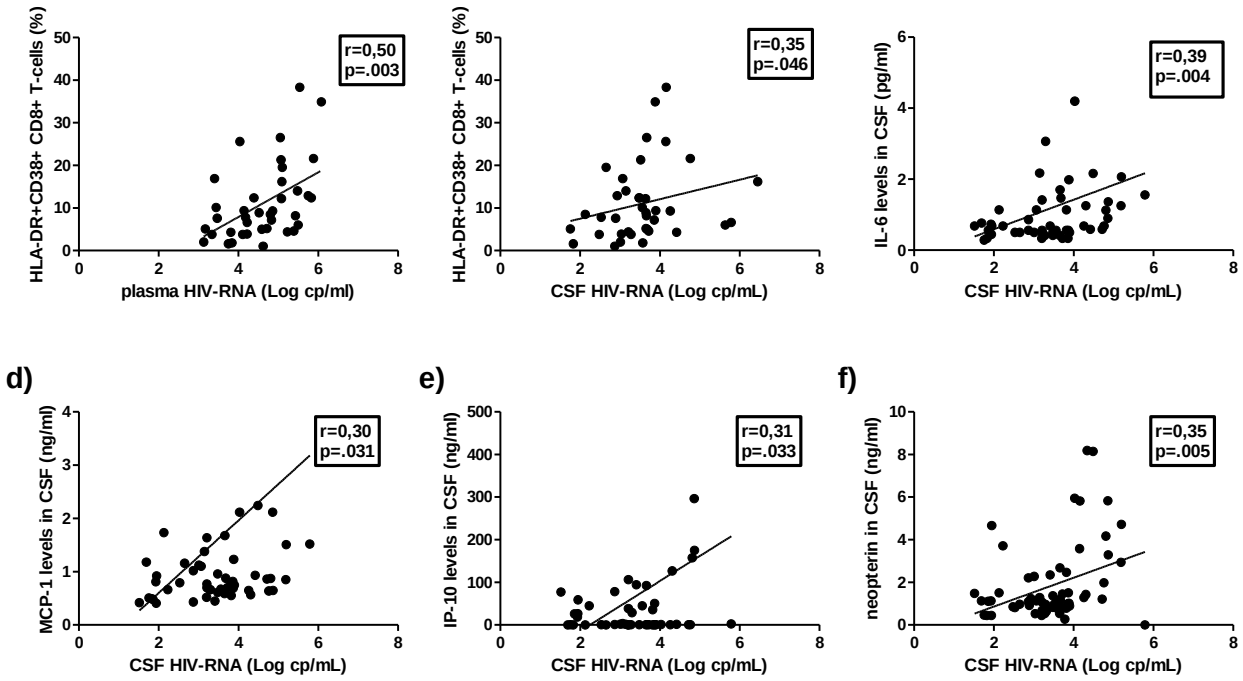
Table 13
CSF soluble markers.

	Total (n=70)	L-CSF (n=61)	H-CSF (n=9)	p values
CSF Soluble Markers				
CSF Cytokines, median (IQR)				
sCD14, ng/ml	147 (78-264)	144,2 (72,91-232)	670 (78,44-1195)	0,204
IL6, pg/ml	0,59 (0,5-1,14)	0,68 (0,5-1,25)	1,07 (0,48-1,94)	0,57
TNF-α, pg/ml	0,86 (0,78-0,94)	0,86 (0,76-0,94)	1,17 (0,78-2,16)	0,123
MCP-1, ng/ml	0,77 (0,63-1,11)	0,79 (0,64-1,12)	1,22 (0,54-1,52)	0,539
IP-10, ng/ml	1,16 (0,47-39,9)	1,33 (0,49-68,1	2,41 (1,34-26,34)	0,798
Neopterin, ng/ml	1,1 (0,77-1,50)	1,13 (0,83-2,21)	0,8 (0,22-4,15)	0,499
CSF S100 Beta Protein, pg/ml median (IQR)	241 (99-380)	203,5 (76,75-376,5)	293 (78,5-343)	0,939

LEGEND
All data are median (IQR). Statistical analyses: Mann-Whitney U Test. IQR: Interquartile range; CSF: cerebrospinal fluid; H-CSF: High cerebrospinal fluid (CSF/plasma HIV-RNA ≥1); L-CSF: Low cerebrospinal fluid (CSF/plasma HIV-RNA<1).

Figure 14

Correlations between CSF/plasma HIV-RNA and peripheral and CNS markers of immune activation/inflammation.



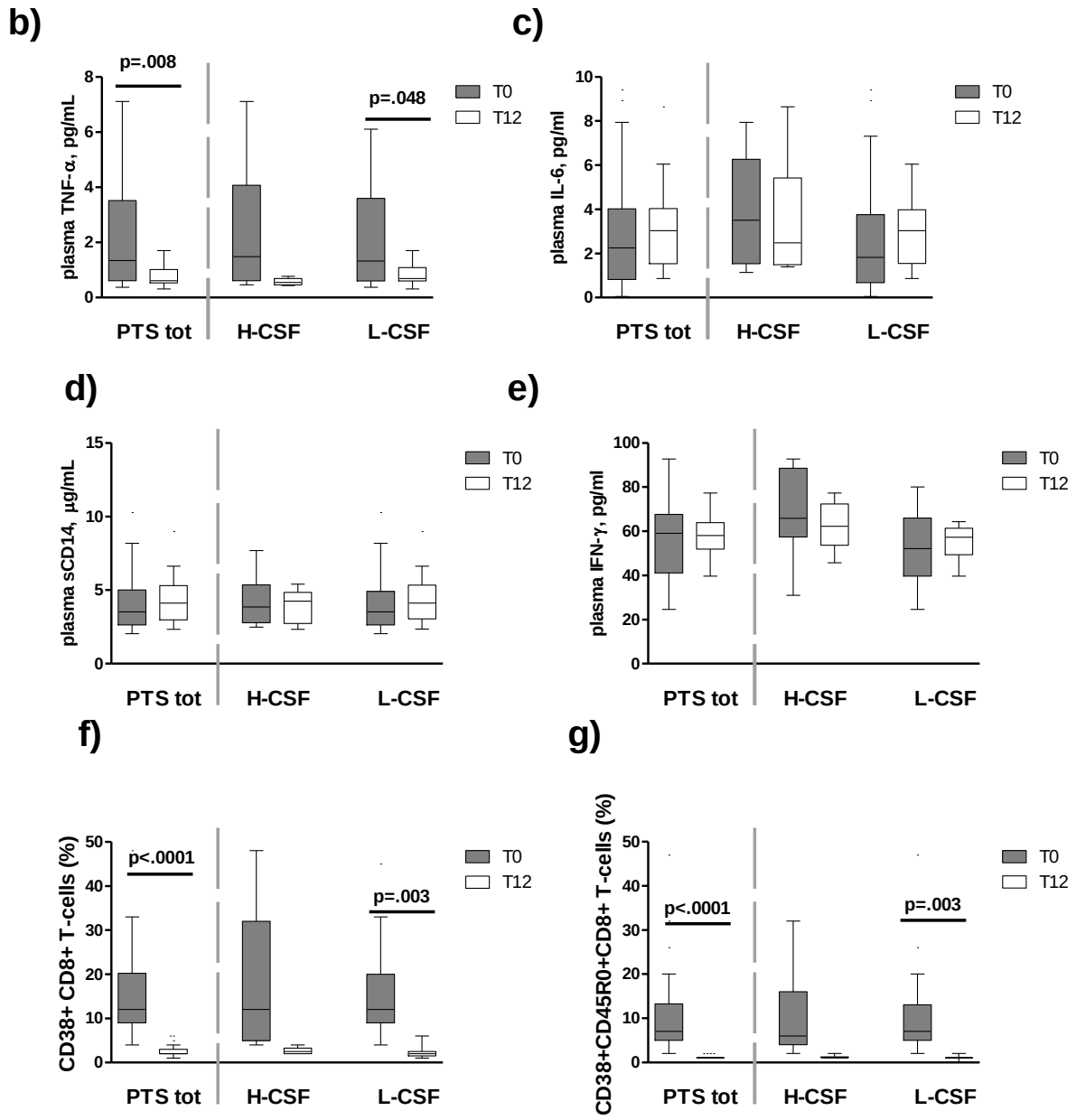
LEGEND

We investigated whether activation/inflammation markers might sustain high CSF HIV-RNA in untreated HIV-infected subjects. **a)** Plasma HIV-RNA positively correlates with HLA-DR+CD38+CD8+ (r=0.50, p=.003). **b)** We observed a positive association between CSF HIV-RNA and HLA-DR+CD38+CD8+ (r=0.35, p=.046). **c)** CSF HIV-RNA positively correlates with IL-6 (r=0.39, p=.004). **d)** CSF HIV-RNA positively correlates with MCP-1 (r=0.30, p=.0.31). **e)** CSF HIV-RNA positively correlates with IP-10 (r=0.31, p=.033). **f)** CSF HIV-RNA positively correlates with neopterin (r=0.35, p=.005).

Figure 15
Circulating pro-inflammatory markers and T-cell activation in L-CSF and H-CSF following 12 months of cART

a)

	L-CSF (N=24)			H-CSF (N=9)		
	T0	**T12**	**p values**	**T0**	**T12**	**p values**
Plasma HIV-RNA, log_{10} cp/mL, (IQR)	4,81 (4,15-5,47)	1,59 (1,59-1,59)	<0,0001	5,45 (4,75-5,79)	1,59 (1,59-1,59)	**0,0002**
CSF HIV-RNA, cp/mL, (IQR)	3,25 (2,56-3,65)	1,59 (1,59-1,59)	<0,0001	4,74 (4,32-5,12)	1,59 (1,59-1,59)	**0,0002**
Total HIV-DNA, cp/10^6 PBMC, (IQR)	987 (176-211)	225 (16-598)	0,125	668 (195-3718)	891 (290-1097)	0,5
CD4+ T-cells, cells/mmc (IQR)	353 (268-447)	573 (390-713)	<0,0001	168 (123-326)	497 (293-612)	**0,016**
CD8+ T-cells, cells/mmc (IQR)	991 (769-1138)	794 (647-1275)	0,978	1069 (841-1789)	1176 (815-1462)	0,848
CD4/CD8 ratio, (IQR)	0,37 (0,25-0,47)	0,75 (0,48-0,92)	0,001	0,14 (0,1-0,27)	0,4 (0,28-0,51)	**0,007**
cART regimen, n (%)						0,693
2NRTI+PI/r	15 (57)			5 (72)		
2NRTI+NNRTI	8 (31)			1 (14)		
	3 (12)			1 (14)		

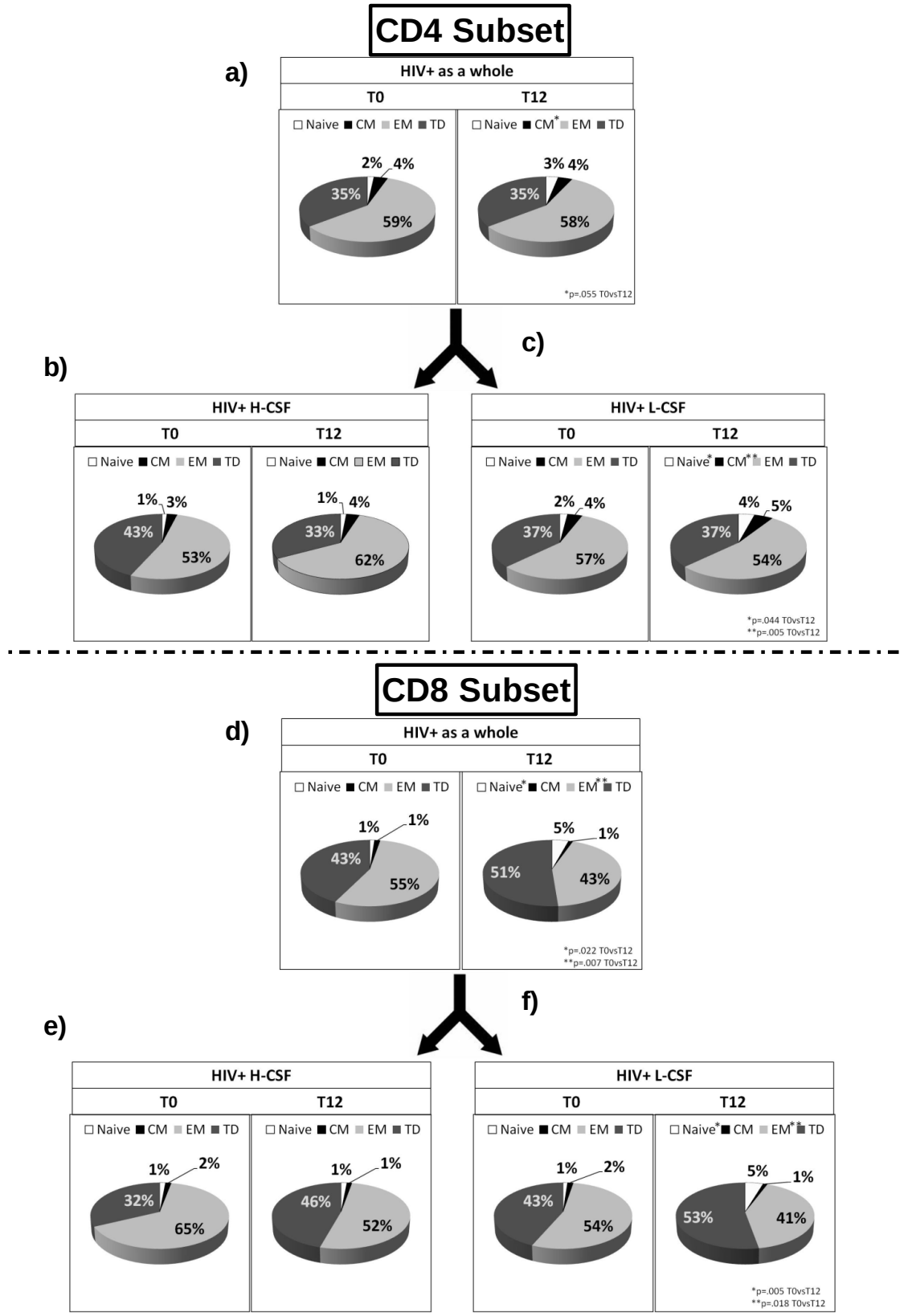


LEGEND

Data are presented as median (IQR). We defined HIV+ patients as (i) H-CSF: CSF/plasma HIV-RNA ratio ≥1 and (ii) L-CSF CSF/plasma<1.

a) Table summarizing the modifications of viro-immunological markers following 12 months of cART in both H-CSF and L-CSF patients. **b)** HIV+ as whole significantly reduced plasma TNF-α (1.35 pg/ml [0.62-3.52] vs 0.61 pg/ml [0.54-1.02]; p=.008). Only L-SCF group showed a reduction of circulating TNF-α (1.33 pg/ml [0.6-3.6] vs 0.69 pg/ml [0.6-1.09], p=.048). **c)** At T12, no differences in plasma IL-6 levels were observed in the study populations. **d-e)** We failed to find any differences after cART introduction in circulating sCD14 and IFN-γ in HIV+ as a whole, as well as in H-CSF and L-CSF. f**)** At T12, HIV+ patients as a whole significantly reduced activated CD38+CD8+ (p<.0001). Similarly, both H-CSF and L-CSF significantly reduced the proportion of CD38+CD8+(p=.0625; p=.003 respectively). **g)** Following 12 months of cART HIV+ patients as a whole showed a significant decrease of CD38+CD45R0+CD8+ (p<.0001). In line with this observation, both H-CSF and L-CSF significantly reduced the proportion of CD38+CD45R0+CD8+ (p=.0625; p=.003; respectively).

Figure 16
T-cell maturation in L-CSF and H-CSF following 12 months of cART.

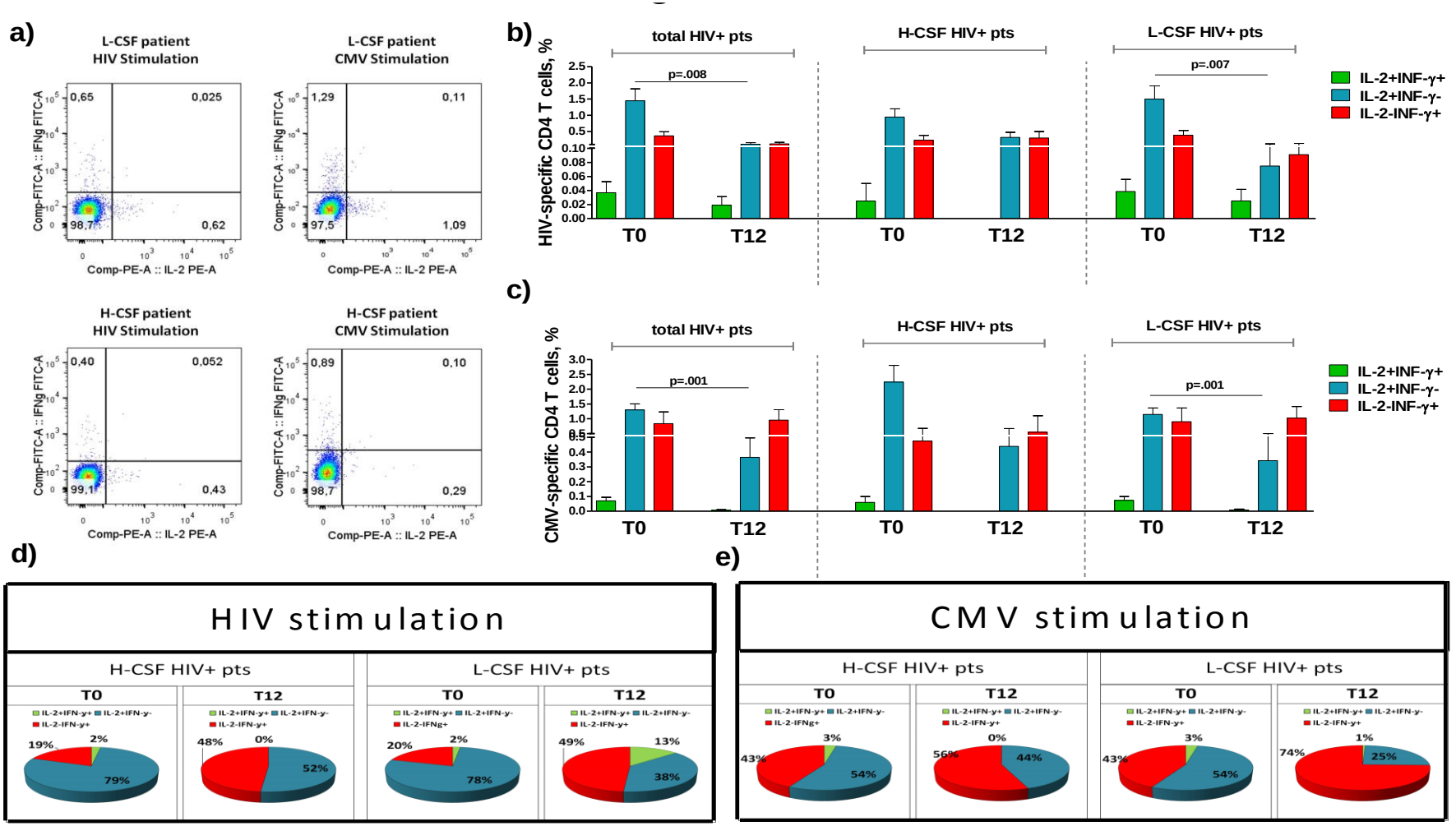


LEGEND

a) In HIV+ patients as a whole we observed an increasing trend in central memory CCR7+CD45RA-CD4+ (p=.055). **b)** H-CSF did not show any modification following cART introduction. **c)** L-CSF significantly increased the proportion of naïve CCR7+CD45RA+CD4+ (p=.044) and central memory CCR7+CD45RA-CD4+ (p=.005). **d)** HIV+ as a whole displayed a significant increase of naïve CCR7+CD45RA+CD8+ (p=.022), and a decrease of effector memory CCR7-CD45RA-CD8+ (p=.007). **e)** H-CSF did not show any modification following cART introduction in CD8 T-cell maturation. **f)** L-CSF significantly increased the proportion of naïve CCR7+CD45RA+CD8+ (p=.005) and significantly reduced effector memory CCR7-CD45RA-CD8+ (p=.018).

Figure 17

Analysis of functionally distinct populations of HIV- and CMV-specific CD4 T-cells in L-CSF and H-CSF following 12 months of cART



LEGEND

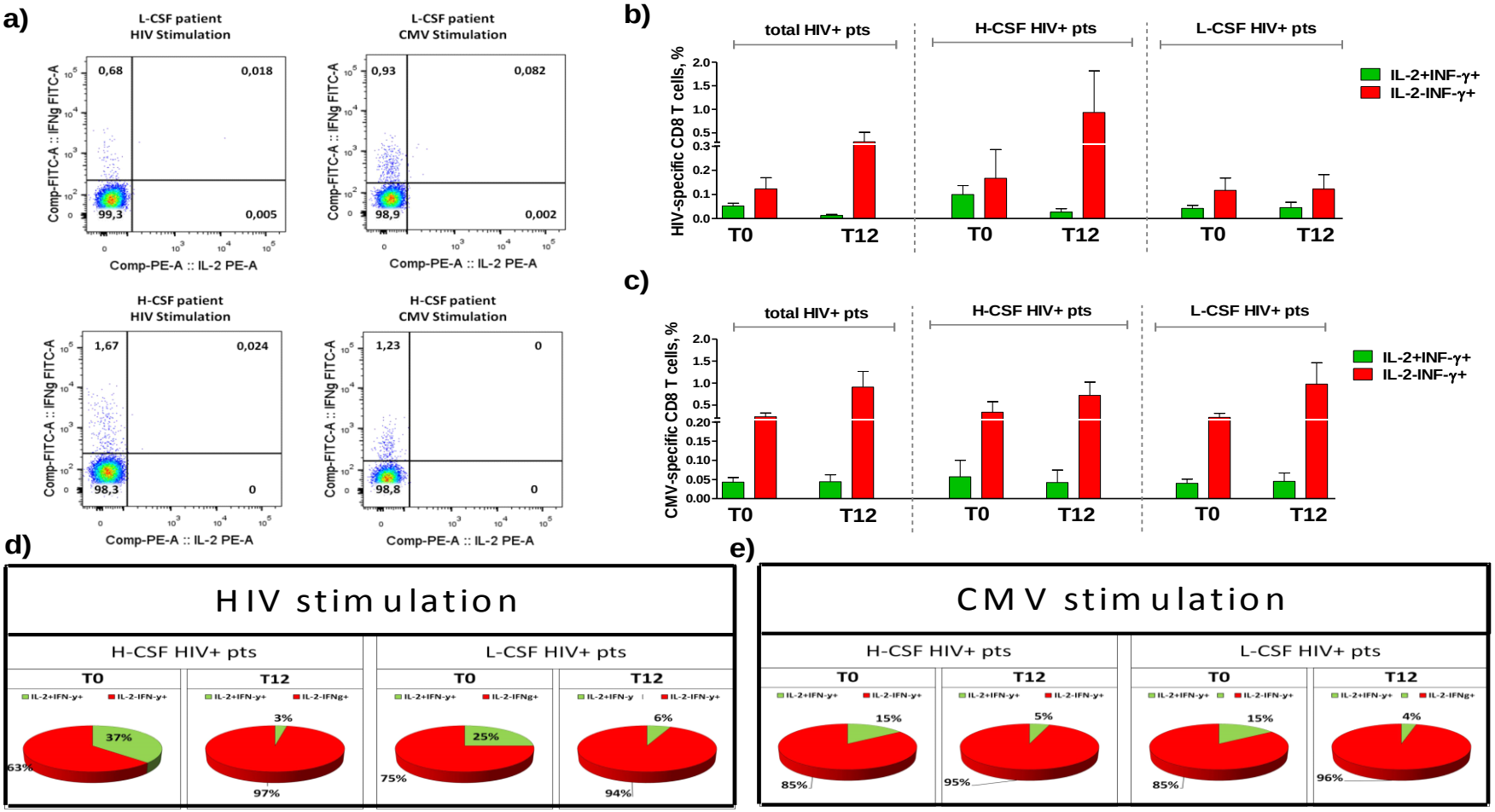
We next assessed CD4 ability to respond to HIV and CMV stimulation. We defined HIV+ patients as (i) H-CSF: CSF/plasma HIV-RNA ratio ≥1 and (ii) L-CSF CSF/plasma<1.

a) Flow cytometry profiles of the distribution of blood HIV and CMV-specific CD4 T-cells within IL-2-, IFN-γ- and IL-2/IFN-γ-secreting cell population in representative H-CSF and L-CSF. **b)**

Data are presented as median (IQR). Following cART introduction, HIV+ patients as a whole displayed a significant reduction of IL-2+IFN-γ-CD4+ upon HIV exposure (p=.008). Interestingly, when we stratified patients according to CSF HIV-RNA levels, we observed a significant decrease in IL-2-expressing CD4 T-cells in L-CSF alone (p=.007), but not in H-CSF (p=.250). **c)** 12 months of cART resulted in a significant decrease of IL-2+IFN-γ-CD4+ even after CMV challenge (p=.0007) in the total population. Similarly, at T12 L-CSF showed a significant decrease of IL-2-expressing T-cells following CMV exposure (p=.0005). **d-e)** Proportion of HIV- and CMV-specific CD4 T cells within the different cytokine-producing cell populations in H-CSF and L-CSF, prior and after 12 months of cART.

Figure 18

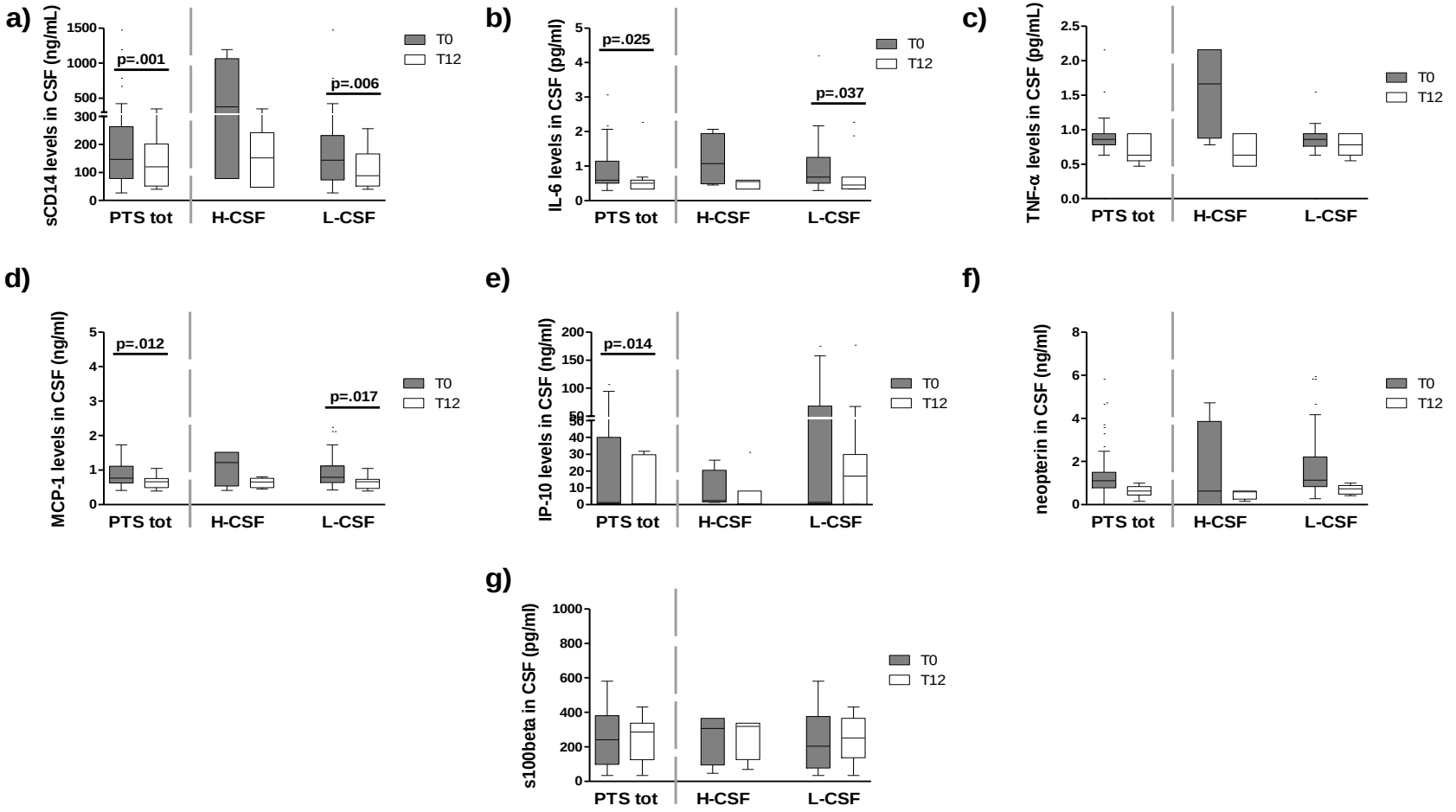
Analysis of functionally distinct populations of HIV- and CMV-specific CD8 T-cells in L-CSF and H-CSF following 12 months of cART



LEGEND

a) Flow cytometry profiles of the distribution of blood HIV and CMV-specific CD4 T-cells within IFN-γ- and IL-2/IFN-γ-secreting cell population in representative H-CSF and L-CSF. **b)** Data are presented as median (IQR). Following cART introduction, we failed to observed significant changes in HIV-specific cytokine-producing CD8. **c)** Similarly, no major differences were observed in CMV-specific response. **d-e)** Proportion of HIV- and CMV-specific CD8 T cells within the different cytokine-producing cell populations in H-CSF and L-CSF, prior and after 12 months of cART.

Figure 19
CSF markers in L-CSF and H-CSF following 12 months of cART



LEGEND

We next assessed the levels of cytokine/chemokine and astrocyte damage marker within the CSF following 12 month of cART. We defined HIV+ patients as (i) H-CSF: CSF/plasma HIV-RNA ratio ≥1 and (ii) L-CSF CSF/plasma<1. **a)** HIV+ as whole displayed significant reduction of sCD14 (p=.001). Similarly, L-CSF alone showed a significant reduction of CSF sCD14 (p=.008). **b)** HIV+ as a whole displayed significant reduction of IL-6 (p=.025), confirmed by L-CSF only (p=.037). **c)** No differences in CSF TNF-α levels were found. **d)** At T12, HIV+ as whole displayed significant reduction of MCP-1 (p=.012). Similarly, significant changes in L-CSF were noted (p=.017). **e)** HIV+ as whole displayed significant reduction of IP-10 (p=.014), not seen in H-CSF and L-CSF. **f)** We failed to find significant variation in neopterin levels. **g)** No significant differences in astrocyte damage marker S100beta were found in the study population.

Table 14 Peripheral markers in 22 patients with neurocognitive screening tests available.

	H-CSF			L-CSF		
	T0	**T12**	**p values**	**T0**	**T12**	**p values**
Peripheral Blood Markers						
CD8/38 %	12 (5-32)	2 (2,5-3,25)	0.063	15 (10-21)	2 (1,25-2)	**0.005**
CD8/38/RO %	6 (4-16)	1 (1-1,25)	0.063	8,5 (5-15,25)	1 (1-1)	**0.005**
DR/38/4 %	3,3 (1,02-6,85)	0,30 (1-1,87)	0.125	2,2 (1-5,3	1,35 (0,45-2,37)	0.203
DR/38/8 %	8,35 (5,57-18,55)	2,3 (3,48-5,21)	0.25	9,3 (5,05-19,10)	3,06 (1,12-7,73)	0.16
IL-6 pg/ml	3,5 (1,37-7,54)	1,46 (1,84-5,89)	0.812	1,30 (0,37-2,02)	1,57 (1,37-2,69)	0.461
TNFa pg/ml	1,79 (0,56-6,10)	0,455 (0,490-0,635)	0.125	3,44 (1,29-4,73)	0,6 (0,46-0,89)	**0.008**
sCD14 ug/ml	3,51 (2,64-5,26)	2,60 (3,89-4,63)	0.812	3,04 (2,58-4,14)	5,24 (2,95-5,86)	**0.008**
IFN-γ, pg/ml	67,36 (62,67-89,81)	50,99 (59,05-74,06)	0.063	66,86 (57,32-74,65)	55,36 (45,23-62,67)	0.625
CD4+CCR7+CD45RA+ (naive), %	1,25 (0,27-4,2)	0,45 (1,50-4,34)	1	1,65 (1,02-2,85)	3,40 (2,31-7,71)	0.079
CD4+CCR7+CD45RA- (CM), %	2,65 (2,35-4,70)	1,65 (3,50-3,53)	0.875	4 (2,55-4,65)	4,15 (2,56-6,925)	0.104
CD4+CCR7-CD45RA+ (TD), %	42,30 (14,68-52,68)	21,75 (32,80-52,96)	0.25	30,9 (25,3-42,7)	34,35 (28,38-46,15)	0.951
CD4+CCR7-CD45RA- (EM), %	51,75 (42,15-82,13)	39,63 (62,2-75,65)	0.5	59 (49,75-67,90)	50,95 (41,40-58,18)	0.241
CD8+CCR7+CD45RA+ (naive), %	1,50 (0,80-2,40)	0,95 (1,50-4,13)	0.625	1,1 (0,65-2,40)	4,6 (1,47-16,49)	**0.011**
CD8+CCR7+CD45RA- (CM), %	1,5 (0,8-2,1)	0,64 (1,30-1,65)	0.312	1,4 (1,05-1,95)	1,09 (0,85-1,71)	0.626
CD8+CCR7-CD45RA+ (TD), %	32,10 (26,90-48,70)	33,26 (45,10-53,60)	0.312	40,50 (29,75-47,10)	53,30 (35,47-60,28)	0.209
CD8+CCR7-CD45RA- (EM), %	64,90 (49,70-70,30)	43,65 (51,01-62,75)	0.312	54,60 (48,70-67,25)	42,80 (31,37-48,25)	**0.035**
HIV-specific T-cell Response, Median (IQR)						
CD4+IL2+IFNγ+, %	0,0 (0,0-0,07)	0,0 (0,0-0,0)	1	0,0 (0,0-0,0)	0,0 (0,0-0,03)	0.625
CD4+IL2+IFNγ-, %	0,90 (0,52-1,42)	0,10 (0,05-0,70)	0.62	0,75 (0,025-1,89)	0,0 (0,0-0,11)	**0.007**
CD4+IL2-IFNγ+, %	0,20 (0,0-0,50)	0,30 (0,10-0,50)	0.173	0,0 (0,0-0,57)	0,05 (0,0-0,15)	1
CD8+IL2+IFNγ+, %	0,10 (0,0-0,20)	0,01 (0,0-0,06)	0.25	0,0 (0,0-0,10)	0,0 (0,0-0,015)	0.136
CD8+IL2-IFNγ+, %	0,10 (0,0-0,40)	0,10 (0,0-2,70)	0.7518	0,0 (0,0-0,11)	0,06 (0,0-0,17)	1
CMV-specific T-cell Response, Median (IQR)						
CD4+IL2+IFNγ+, %	0,0 (0,0-0,15)	0,0 (0,0-0,0)	1	0,0 (0,0-0,10)	0,0 (0,0-0,002)	0.625
CD4+IL2+IFNγ-, %	2,0 (1,30-3,30)	0,20 (0,0-1,00)	0.174	0,66 (0,12-2,1)	0,05 (0,0-0,53)	**0.0005**
CD4+IL2-IFNγ+, %	0,50 (0,07-0,85)	0,55 (0,0-1,10)	0.197	0,40 (0,0-0,73)	0,34 (0,15-1,57)	0.812
CD8+IL2+IFNγ+, %	0,0 (0,0-0,10)	0,0 (0,0-0,11)	0.875	0,0 (0,0-0,09)	0,0 (0,0-0,09)	0.752
CD8+IL2-IFNγ+, %	0,20 (0,0-0,80)	1,0 (0,0-1,30)	1	0,0 (0,0-0,23)	0,12 (0,0-1,42)	0.625

Table 15
CSF markers in 22 patients with neurocognitive screening tests available.

	H-CSF			L-CSF		
	T0	**T12**	**p values**	**T0**	**T12**	**p values**
CSF soluble markers						
sCD14 (ng/ml)	932,5 (670,3-1195)	208 (137-294)	0.25	270,5 (197,3-353,3)	165,5 (116,3-202)	0.125
IL6 (pg/ml)	1,56 (0,59-2,079	0,59 (0,33-0,59)	0.057	0,59 (0,50-1,31)	0,50 (0,33-0,68)	0.125
TNF-α (pg/ml)	1,17 (0,78-2,16)	0,63 (0,47-0,78)	0.063	0,94 (0,745-1,015)	0,78 (0,63-0,94)	0.875
MCP-1 (ng/ml)	1,51 (0,93-1,52)	0,74 (0,53-0,77)	0.063	0,86 (0,735-1,15)	0,61 (0,49-0,73)	**0.031**
IP-10 (ng/ml)	1,87 (1,34-2,41)	0,25 (0,19-0,37)	0.125	0,585 (0,36-0,99)	0,22 (0,17-0,32)	**0.031**
S100BETA	321 (170-365)	298 (69-337)	0.5	290 (183-406)	251,5 (136,8-365,3)	0.547
Neopterin	0,80 (0,0-4,15)	0,35 (0,13-0,61)	0.625	1,15 (0,79-1,35)	0,73 (0,48-0,88)	0.207

LEGEND
All data are median (IQR). Statistical analyses: Mann-Whitney U Test. IQR: Interquartile range; CSF: cerebrospinal fluid; H-CSF: High cerebrospinal fluid (CSF/plasma HIV-RNA ≥1); L-CSF: Low cerebrospinal fluid (CSF/plasma HIV-RNA<1).CM: central memory, EM: effector memory, TD: terminally differentiated.

Table 16
Patients' neurocognitive performances at T0 and T1.

PATIENT 1	RAW SCORE (T0)	EQUIVALENT SCORE (T0)	RAW SCORE (T1)	EQUIVALENT SCORE (T1)
Digit Span Test direct/reverse	3/3	0/0	7/4	**4/1**
Rey Auditory Verbal Learning Test/ recalling	41/7	4/3	48/7	4/3
Trial making test A/B	26.5/106.48	4/4	38/91.09	4/4
Trial making test B-A	79.98	4	53.09	4
Symbol Digit Modality Test	38	2	43	2
Finger tapping test right/left	40.6/30.2	0/0	42.8/39.8	0/0
Fluency test (phonemic)/(semantic)	23/50	2/4	33/47	4/4
Stroop Color Word Interference Test (time)/error	31.58/1	2/3	20.15/1.5	4/3
PATIENT 2	**RAW SCORE (T0)**	**EQUIVALENT SCORE (T0)**	**RAW SCORE (T1)**	**EQUIVALENT SCORE (T1)**
Digit Span Test direct/reverse	8/8	4/4	7/5	4/2
Rey Auditory Verbal Learning Test/recalling	46/5	3/0	57/7	4/**1**
Trial making test A/B	27.12/50.62	4/4	20.76/58.23	4/4
Trial making test B-A	23.5	4	37.47	4
Symbol Digit Modality Test	59	2	66	2
Finger tapping test right/left	63.4/51.4	>1/>1	58.2/46.6	>1/>1
Fluency test (phonemic)/ (semantic)	48/62	4/4	32/63	3/4
Stroop Color Word Interference Test (time)/ (error)	15/3	2/0	9.71/0	4/**3**
PATIENT 3	**RAW SCORE (T0)**	**EQUIVALENT SCORE (T0)**	**RAW SCORE (T1)**	**EQUIVALENT SCORE (T1)**
Digit Span Test direct/reverse	4/4	1/1	6/4	4/1
Rey Auditory Verbal Learning Test/ recalling	44/10	3/3	47/7	4/1
Trial making test A/B	39,32/139.56	4/3	32.56/60.37	4/4
Trial making test B-A	100,24	2	27.81	4

Symbol Digit Modality Test	29	0	26	0
Finger tapping test right/left	39.2/46	0/0	43.8/52	**>1/>1**
Fluency test (phonemic)/ (semantic)	30/48	3/4	20/43	1/4
Stroop Color Word Interference Test (time)/(error)	23.9/7	2/0	12.5/0	4/**3**

LEGEND
Equivalent score: 0= pathologic, ≥1=normal. T0, baseline, T1, end of the rehabilitation program.

Table 17
Functional assessment for each patient.

PATIENT 1	SCORE AT T0	SCORE AT T1
IADL	7	8
HADS A/D	12/3	7/1
SF-36 PHI/MHI	1/0	1/0
PATIENT 2	**SCORE AT T0**	**SCORE AT T1**
IADL	8	8
HADS A/D	1/0	0/0
SF-36 PHI/MHI	0/0	0/0
PATIENT 3	**SCORE AT T0**	**SCORE AT T1**
IADL	7	8
HADS A/D	9/10	5/11
SF-36 PHI/MHI	0/1	0/0

LEGEND
IADL altered if <8, HADS A altered if ≥12, HADS D altered if≥12, SF-36 PHI and MHI altered if 1. HADS A=anxiety score; HADS D=depression score; SF-36 PSS= physical health index; SF-36 MSS= mental health index.

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