

## Diagnosing HIV-associated cerebral diseases – the importance of Neuropathology in understanding HIV

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### Abstract

The study aims to compare two aspects concerning the diagnosis of acquired immune deficiency syndrome (AIDS)-associated central nervous system (CNS) pathology (neuroAIDS): clinical diagnoses issued *ante mortem* with pathology results issued *post mortem*. The group of 39 human immunodeficiency virus (HIV)-positive patients was created over 23 years and is limited by marked heterogeneity. The enrolled cases were treated at the "Prof. Dr. Matei Balș" National Institute for Infectious Diseases, Bucharest, Romania, deceased due to AIDS-related complications and underwent brain necropsies performed in the Pathology Laboratory at the "Colentina" Clinical Hospital, Bucharest. The level of superposition between clinical and the necroptic diagnoses of neurological AIDS-associated diseases was: 60% for progressive multifocal leukoencephalopathy (PML), 50% for cerebral cryptococcosis, 33% for cerebral toxoplasmosis, 20% for cerebral lymphoma, null for cerebral tuberculosis, HIV encephalopathy (HIVE), neurosyphilis and cytomegalovirus cerebral infection. Half of the cases without an AIDS-associated CNS lesion were previously clinically overdiagnosed. We observed that the rate of overdiagnosis concerning an AIDS-associated cerebral illness has risen from 36% in 1993 to 124% in 2015, an elevation with statistical relevance [ $p=0.037$ , confidence interval (CI) 95%]. The rate of underdiagnosis has slowly risen from 24% in 1993 to 40% in 2015, however, with no statistical relevance. The rate of clinical confirmation has been stagnant in linear regression from 1993 to 2015. The results of our study reveal a gap between *ante mortem* and *post mortem* diagnoses, with many instances of overdiagnosis and underdiagnosis of several major AIDS-associated CNS illnesses, highlighting the need for a more detailed, multidisciplinary approach of neuroAIDS.

**Keywords:** HIV, neuropathology, neuroAIDS, HIVE, PML.

### Introduction

A total number of 20 146 cases of human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) infection have been recorded in Romania, between 1985 and 2014, resulting in 6619 deaths. Patients had an average survival rate of 45.6 months [confidence interval (CI) 95% 44.3–46.9], one third of deaths occurring in less than 50 months after the confirmed diagnosis. In December 2014, there were 12 886 HIV-infected persons alive, more than half being long-term survivors, with parenteral HIV infection in the childhood, now 20–29 years old young adults. Eight thousand eight hundred and forty-six HIV-infected patients in Romania are currently receiving antiretroviral therapy, according to the evaluation of *The Compartment for Monitoring and Evaluation of HIV/AIDS Infection in Romania*, statistics made available on December 31, 2014 [1].

Although the combined antiretroviral therapy (cART) may efficiently control viral replication and normalize the

immune system function, HIV still remains a global health problem [2]. Treatment cessation is invariably associated with HIV viral load recurrence because of its persistence in latent viral pools, phenomena that stands in the way of discovering a fully functional treatment [3]. Viral pools spawn early during infection and are elusive in patients undergoing cART. Latent proviruses can be found in all CD4+ T-lymphocyte (CD4) subcategories including naïve T-lymphocytes, central T-lymphocytes with memory, effector T-lymphocytes with memory and mature T-lymphocytes, as well as in monocytes and macrophages. HIV-1's capacity of forming pools in different cells leads to the formation of heterogeneous CD4+ T-lymphocytes populations, with different lifespan and possibly different needs for cellular and viral activation. It was presumed that pools' heterogeneity has a key role in the formation and maintenance of the latent viral pool [4].

Anatomical sanctuaries (anatomic pools in which antiretroviral medication penetrability is variable, usually low) may be a source for viral relapses. One of the sanctuaries

is the brain, where cART tissue concentration is considerably low in comparison to plasmatic levels. The brain registers quasi-continuous minimum levels of viral replication, even with undetectable HIV serum viral loads [5]. This phenomenon has been analyzed by the quantification of the cerebrospinal fluid (CSF) viral level, considering CSF as serum surrogate. The correlation between the CSF viral load and the serum viral load tends to be variable, as the exact correlation between the two is still to be revealed [6].

Treatment of an HIV-patient is considered successful if the CD4 count is elevated over 500 cells/mm<sup>3</sup> (immunological success), the serum viral load is undetectable (viremic success) and clinically, the patient does not present any complications (clinical success). Even so, frequent follow-ups are needed, as viremic blips (intermittent low-level viremic levels) and some opportunistic diseases may occur [7]. Considering the variability between the serum viral load and the CSF viral load, as well as the clinical tendency to overlook CSF viremic loads in serum viremic undetectable patients, brain HIV activity is frequently underdiagnosed or misdiagnosed (*e.g.*, HIV-associated dementia). Thus, considering the lack of corresponding development of HIV in the brain *versus* other anatomical areas, better understanding of HIV neuropathology provides important data in the pursuit of a better treatment. Critical HIV-patients are frequently diagnosed with a neurological HIV-associated pathology, involving both meningeal and encephalitic disturbances [8]. In our study, we took into consideration both clinical and pathological aspects in order to better understand the accuracy of AIDS-associated central nervous system (CNS) pathology (neuroAIDS) diagnosis and the magnitude it conveys upon the patient's prognosis.

Our aim is the evaluation of the *ante mortem* clinical diagnosis accuracy in neuroAIDS, by comparison with CNS autopsy results. Although limited by a small cohort and, as such, having a low statistical power, our study may shed light upon the subject of overdiagnosis and underdiagnosis concerning neuroAIDS. We have reached the conclusion that a larger study may prove difficult to implement. The process of recruitment is extremely slow, as for example, in Bucharest (Romania), an average of two brain necropsies of AIDS patients are being performed each year. The strength of our study relies on a long time span of recruitment (23 years) – creating the possibility of calculating diagnosing trends.

## Materials and Methods

The analyzed material consisted of human brain tissue collected during complete autopsies performed in the Pathology Department of the "Colentina" Clinical Hospital of Bucharest, between 1993 and 2015. The group is composed of 39 HIV+ inpatients of the "Prof. Dr. Matei Balș" National Institute for Infectious Diseases, Bucharest. All cases were simultaneously examined by two pathologists and quantified after common criteria, ensuring a unitary evaluation of the research data.

Protocols regarding the collection of tissue samples during necropsy comprised the macroscopic examination of the organs and the storage of at least one sample from each organ, from the presumed injured sites. Tissues with

a highly/moderately autolysis status and disrupted staining were discarded, whereas those with a low autolysis status had a reserved diagnosis.

Data selected from the medical history is represented by general quantification (sex, age), HIV-infection status, clinical diagnosis and/or the presumption of brain damage, antiretroviral treatment and, where possible, CD4 levels and viral loads. All needed legal approvals for this research have been given beforehand.

## Results

The study group sums up 39 cases, HIV+ C3 patients, between three months and 54 years old and a high male to female sex ratio of 25:14. Fourteen brain necropsies were performed between 1993 and 2000, 14 between 2000 and 2007 and 11 between 2008 and 2015. The Pathology Laboratory issued 47 diagnoses; 32 cases with one type of AIDS-associated cerebral illness, six cases with two different diagnoses and one with three diagnoses. *Ante mortem*, 44 clinical diagnoses were issued for the study group.

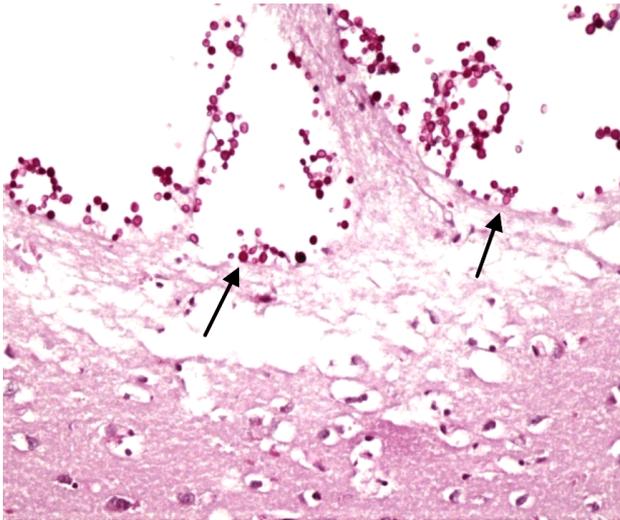
The most frequent necroptic diagnosis was hyperemia and cerebral edema, in 13 cases; eight cases were diagnosed with non-specific meningoencephalitis, five cases with cryptococcal meningoencephalitis (Figure 1), four cases with HIV encephalopathy, toxoplasmic meningoencephalitis (Figure 2) and progressive multifocal leukoencephalopathy (PML) (Figure 3, A and B); three cases were diagnosed with cerebral infarction, two with cerebral lymphoma, and one with cerebral cytomegalovirus (CMV) infection (Figure 4), non-specific encephalitis, carcinomatous meningitis and pontocerebellar scarring.

The most frequent clinical diagnosis was cerebral tuberculosis, in seven cases, followed by HIV encephalopathy, in six cases; five cases of meningoencephalitis, four cases of cryptococcal meningoencephalitis, PML, cerebral lymphoma and cerebral toxoplasmosis, two cases of cerebral infarction, cerebral tumor and neurosyphilis and one diagnosis of CMV cerebral infection, hydrocephaly, cerebral Kaposi, carcinomatous meningoencephalitis and cerebral hemorrhage.

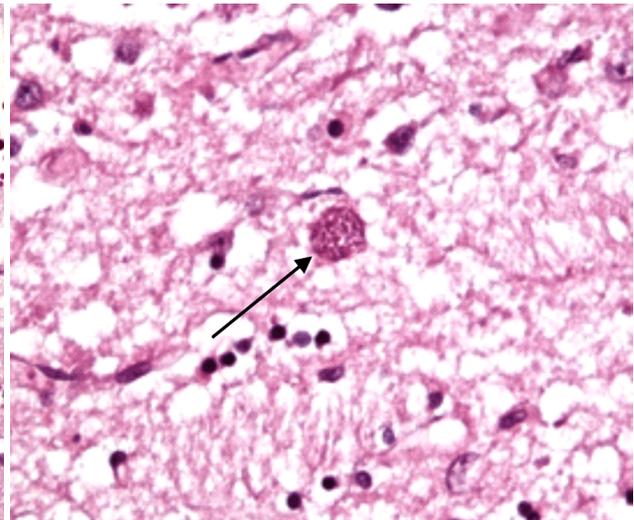
The superposition of clinical and the necroptic diagnoses of neurological AIDS-associated diseases was: 60% for PML (3/5 cases), 50% for cerebral cryptococcosis (3/6 cases), 33% for cerebral toxoplasmosis (2/6 cases), 20% for cerebral lymphoma (1/5 cases), null for cerebral tuberculosis (0/7 cases), HIV encephalopathy (0/10 cases), neurosyphilis (0/2 cases) and CMV cerebral infection (0/2 cases).

Half of the cases without an AIDS-associated CNS lesion were previously clinically diagnosed with several diseases, as CMV CNS infection (one case), cerebral tuberculosis (two cases) or HIV encephalopathy (two cases).

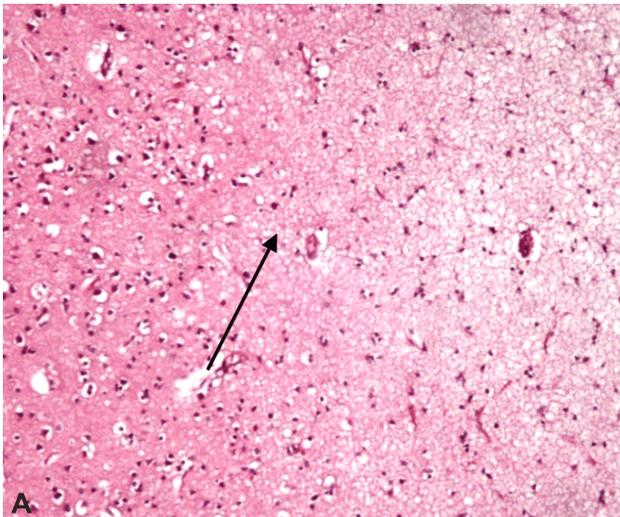
Studying the timeline of necropsies and applying linear regression, we observed that the rate of overdiagnosing an AIDS-associated cerebral illness has risen from 36% in 1993 to 124% in 2015 (considering 100% equal to one overdiagnosed disease per patient), an increment with statistical significance ( $p=0.037$ , CI 95%). The rate of underdiagnosis has slowly risen from 24% in 1993 to 40% in 2014, with no statistical relevance. The rate of clinical confirmation has been stagnant by linear regression from 1993 to 2015 at a level of 78%.



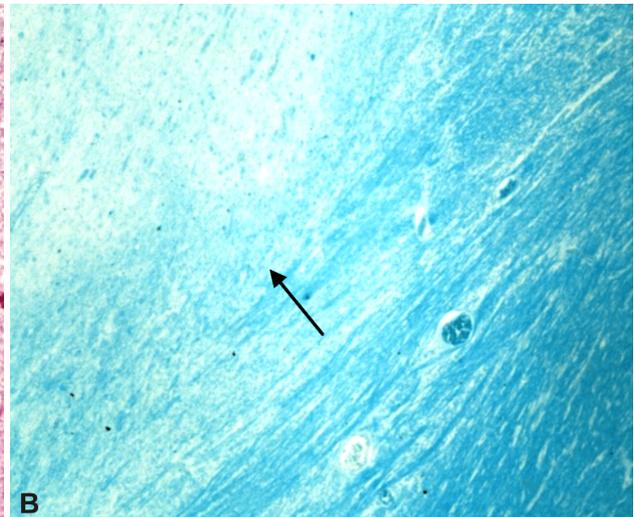
**Figure 1 – Cerebral cryptococcosis.** Yeasts in areas of tissue destruction in parietal lobe. [Periodic Acid-Schiff (PAS) staining,  $\times 200$ ]. Luciana Nichita, “Colentina” Clinical Hospital, Bucharest.



**Figure 2 – Cerebral toxoplasmosis.** Pseudocyst with bradyzoites in frontal lobe. [Hematoxylin-Eosin (HE) staining,  $\times 200$ ]. Luciana Nichita, “Colentina” Clinical Hospital, Bucharest.

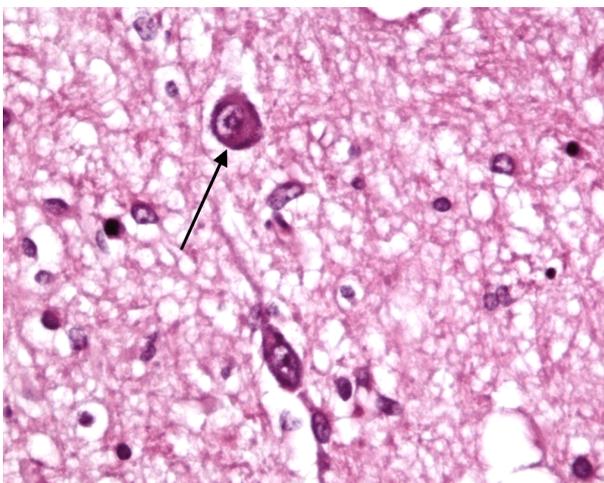


A



B

**Figure 3 – Progressive multifocal leukoencephalopathy:** (A) Irregular area of myelin loss (HE staining,  $\times 100$ ); (B) Poorly defined area of demyelination (Luxol staining,  $\times 100$ ). Luciana Nichita, “Colentina” Clinical Hospital, Bucharest.



**Figure 4 – Cytomegalovirus cerebral infection.** Prominent intranuclear inclusion with clear halo (HE staining,  $\times 200$ ). Luciana Nichita, “Colentina” Clinical Hospital, Bucharest.

## Discussion

The most frequent necroptic diagnosis was cerebral hyperemia with edema, in 12 cases. This central neurological diffuse lesion is non-specific and may appear in various non-AIDS CNS afflictions, thus a correlation with neuroAIDS cannot be stated clearly. Therefore, all cases in which this lesion was present were considered non-AIDS related in this study, especially considering that HIV encephalopathy – the most unspecific AIDS-related injury in our study – was not viewed in the same category as hyperemia and edema, and classified separately.

Half of the patients with cerebral hyperemia and edema (six out of 12) were diagnosed with various CNS illnesses, elevating the rate of overdiagnosis within neuroAIDS.

## Cryptococcosis

The most frequent opportunistic CNS infection was cryptococcal infection, with five confirmed cases. The rate of a successful clinical diagnosis was 50%, with a

slight tendency towards underdiagnosing. Difficulties in the oversight of this affliction arise in the first stage of the disease, as clinical presentation is frequently non-specific and with a slow progression. Efficacy may rise from screening methods involving HIV patients with low CD4 counts, considering the good specificity and sensitivity levels cryptococcal serum antigen [9].

Cryptococcosis is the most common systemic fungal infection that occurs on the background of HIV-associated immunosuppression. Before using cART, it had a prevalence of about 5–10%, sometimes even higher in certain geographical areas, but, with the introduction of anti-retroviral medication, the frequency of cryptococcosis has dropped dramatically. Positive diagnosis of cryptococcosis should be excluded from all patients showing a level of CD4+ lymphocytes less than 200 cells/ $\mu$ L and specific symptoms [10]. According to Kisenge *et al.* (2007), CD4 levels less than 100 CD4+ cells per microliter was proved to have the highest sensitivity for diagnosis (93%). Also, the cryptococcal meningitis' lethality was higher than other conditions that were present in patients with a level of CD4+ lymphocytes/ $\mu$ L less than 100 [11]. To diagnose disseminated cryptococcosis, plasmatic cryptococcal antigen assay is performed. A negative result generally excludes the disease, although there are cases in literature in which disseminated cryptococcosis was present in this context also. The presence of the rheumatoid factor, heterophile antibodies and anti-idiotypic antibodies may cause false-positive results. In contrast, the cryptococcal antigen may be negative in the isolated pulmonary cryptococcosis; in this case, microscopic examination and microbial cultures of respiratory samples have diagnostic purposes [10]. This may explain both overdiagnosis and underdiagnosis, as well as the clinical suspicion encountered in these cases. Patients who tested positive for the cryptococcal antigen need to be evaluated with computed tomography (CT) or magnetic resonance imaging (MRI) and then with a lumbar puncture, not before excluding intracranial hypertension by manometry. The positive results of cryptococcal antigen, India ink test and CSF cryptococcal cultures confirm the diagnosis of meningitis.

### Cerebral tuberculosis

We observed a complete disjunction between clinical and pathology views on cerebral tuberculosis (TB). Necroptic analysis did not confirm any of the seven cases of CNS TB infection suspicion, calling in question the clinical diagnosis protocol. Many of the patients had non-specific CNS acute symptoms and, considering the probability of cerebral TB in the context of AIDS, were given anti-TB empiric treatment. The risk/benefit ratio is not clear – foresight of a TB infection treated before the diagnosing process is completed *versus* intake of anti-TB drugs without having the disease and suffering the consequences of drug interactions and side effects. A discrete presence of TB in the CNS, invisible for pathology, contradicts the presence of acute symptomatology, making false-negative results in the necroptic results improbable. Therefore, the rate of overdiagnosis of cerebral TB in AIDS patients, considering neuropathology as a confirmation factor, is very high in our study.

Globally, it is estimated that 14.8% of the new

tuberculosis cases in adults could be attributed HIV infection. This rate is higher in Africa, where approximately 79% of all HIV/TB coinfections are found. Consequently, all tuberculosis-diagnosed patients must be tested for HIV. In comparison with the immunocompetent population, HIV/TB patients with active pulmonary tuberculosis may show normal pulmonary X-rays and negative sputum exam, but positive for bacillus Koch (BK) after microbial cultures are performed. Clinicians that care for HIV-infected patients must always take precaution regarding the suspicion of TB co-infection [12].

### Cerebral toxoplasmosis

The pathology laboratory issued four cerebral toxoplasmosis diagnoses, two of which confirmed clinical suspicion, while two had no clinical diagnosis beforehand. Another two patients have been issued a cerebral toxoplasmosis clinical suspicion, without a pathology confirmation. The group sums up six cases, with two correct and four incorrect diagnoses, half classified as overdiagnosed and half underdiagnosed. Research into the patients' history revealed that *Toxoplasma gondii* serum serology had no clinical significance, three of the confirmed patients having the IgG result positive and IgM negative, while the forth having both IgG and IgM results negative. The two patients that had clinical suspicion without confirmation revealed *T. gondii* IgG positive result. Moreover, all confirmed patients only had *T. gondii* lesions in the brain necropsies, with no other AIDS related opportunistic infections present, implying the hypothesis of a monopolistic nature of *T. gondii* as an opportunist in the brain.

*T. gondii* is a protozoan, strictly intracellular, whose final hosts are members of felines family. The primary infection, in case of immunocompetent patients, is usually asymptomatic or paucisymptomatic.

Cerebral toxoplasmosis is the most common etiology for mass-effect lesions in immunodeficient HIV seropositive patients, especially in those with a level of CD4+ cells/ $\mu$ L lower than 200. It is mainly caused by reactivation of the chronic disease previously contracted. A patient infected with HIV, untreated, with positive IgG for *T. gondii* has a risk of approximately 25% of developing toxoplasmic encephalitis [10].

Differential diagnosis of cerebral toxoplasmosis abscesses includes primary CNS lymphoma, tuberculous abscesses and PML. MRI is more sensible in establishing the diagnosis, especially in detecting lesions of the posterior fossa. If this investigation is not accessible, the patient requires a CT scan. Usually, the abscesses are represented by hyperdense, ring-shaped lesions, at the limit between white and dark matter [10].

### Progressive multifocal leukoencephalopathy

The pathology laboratory issued four diagnoses of PML, with five clinical suspicions registered. There were three confirmed cases, one overdiagnosed (revealed to be a cryptococcal infection) and one underdiagnosed. From the four confirmed cases, three had underlying HIV encephalopathy. The overall correct diagnosis rate was 60% (3/5).

Progressive multifocal leukoencephalopathy is caused by polyomavirus JC (John Cunningham) and is one of

the most severe complications of the HIV-1 infection. Regardless of other opportunistic infections, this disease may appear when patients have less than 200 CD4 count and even when undergoing cART, short time after initiation, or during long-term efficient therapy. From the examined patients, two had the CD4+/ $\mu$ L level investigated, 43, respectively 237. PML suspicion arises with the presence of focal neurological signs and demyelinating lesions at MRI. Diagnosis confirmation is made through viral identification in CSF or brain tissue. Even if a specific treatment does not exist, lowering immunosuppression using cART stabilizes patients in 50–60% of the cases. A significant rate of patients who were under treatment may suffer inflammatory lesions, which can be associated with variable prognostic [13].

Lesions in PML have a tendency to involve the white matter, without mass-effect or signal alterations [10].

### HIV encephalopathy

As in the case of cerebral TB clinical diagnosis, we observed a complete disjunction between suspicion and pathology observations in HIV encephalopathy (HIVE). We have registered six clinical suspicions of HIVE and four pathological diagnoses of this disease. All clinical suspicions were not confirmed, while all HIVE pathology diagnoses had no previous clinical insight. These findings highlight the need for better implementation of HIVE clinical diagnosis.

The pathogenesis of HIV encephalopathy began to be deciphered recently. The virus reaches the brain through CD4 lymphocytes and perivascular monocytes. Microglia seizes free viral particles by receptor-mediated endocytosis or by phagocytosis of the infected senescent lymphocytes. The only cells harboring the HIV in the brain are perivascular monocytes and microglia. Even though HIV has been detected in neurons and astrocytes by immunohistochemistry, the significance of this observation is not entirely clear. There is no active infection in neurons and neither at the glial cells level.

Therefore, the brain damage is not caused by the direct effect of HIV on neurons or oligodendroglia and the neuronal dysfunction is not correlated with viral load. Brain injuries are caused by cytokines such as tumor necrosis factor (TNF) and by neurotoxins, such as glutamate and nitric oxide (NO), which are produced by activated monocytes and microglia. The gp120 protein from the HIV envelope also activates N-methyl-D-aspartate (NMDA) receptors and, thus, inducing a cascade of neurotoxic reactions. The pathology of HIV myelopathy resembles the subacute combined degeneration of the spinal cord. This fact suggests that the metabolic changes induced by HIV could contribute to myelin degeneration [14].

A study published in 2014 stated that, with the introduction of the cART, the incidence of cerebral toxoplasmosis remained stable or decreased, the incidence of CNS lymphomas has decreased dramatically, the incidence of PML discretely increased and the incidence HIV encephalopathy has increased significantly. Although the combined therapy has reduced the mortality and the prevalence of the opportunistic infections, these therapies are less effective in preventing the appearance of direct effects of HIV-1 at brain level [15].

According to the pathological diagnosis which correspond to this group, both toxoplasmosis and PML had a higher prevalence in the treated patients than in the untreated ones, fact which is still statistically irrelevant ( $p=0.120$  and  $p=0.968$ , respectively).

Frontal cortex neuronal populations from HIV-infected patients, without presenting opportunistic infections or CNS neoplasms, but having only minimal modifications or HIV encephalitis, are having 38% less neuronal mass than the control group. This discovery had an important role in understanding the mechanism of development of HIV-associated dementia (HAD) and the antiretroviral therapy guidance [16].

Currently, combined antiretroviral therapy transformed the clinical presentation of chronic HIV infection-related complications, especially in the case of ones regarding the central nervous system. Severe, progressive forms of HAD, correlated with neuropathological diagnoses such as HIV encephalitis and opportunistic CNS infections, are currently rare, but instead, mild forms of neurocognitive disease have a continuous-rising prevalence, because of the cumulated immune-activation effect, the effects of ageing and the antiretroviral therapy's toxicity. Epstein-Barr and cytomegalic silent viral cerebral infections, with asymptomatic viremic loads, are of current concern, their activity, in parallel with the main HIV infection, being summative for cortex progressive damage [17–19]. Regarding patients who received cART, it has been observed through neuropsychometric evaluations that cortical damage, including learning problems and memory loss, is frequently predominant, in contrast with subcortical and motor function damage, found in patients who did not receive cART [20].

Continuously high rates of HAD, regardless of cART, have numerous possible explications, such as the presence of brain damage which preceded the cART initiation, the possible neurotoxicity of some antiretroviral drugs, the persistence even of minimal HIV replication in the CNS and the effect of chronic immune activation, which may cause metabolic disturbances and vascular degeneration in the brain tissue.

The length of the HIV infection may be an important predictor of CNS damage levels, even if the viral load and CD4+ cell levels are under control. It is important for clinicians to grasp the notion of viral sanctuary pools, especially in the central nervous system, for a better understanding of HIV mechanics in the pursuit of a proper functional cure.

### ☐ Conclusions

Clinical overdiagnosis and underdiagnosis within neuroAIDS brings forth the need for a better implementation of HIV clinical diagnosis protocols. The overdiagnosing rate within neuroAIDS in our Clinic has risen four fold between 1993 and 2015, phenomenon with statistical relevance (linear regression,  $p=0.037$ , CI 95%), while underdiagnosis remained stable (24% in 1993, 40% in 2015, no statistical difference). The correct diagnosis, including no clinical diagnosis when no pathological lesions were found in the CNS, remained stable from 1993 until 2015, at a 78% rate. The best superposition of

clinical and pathological overview was registered for PML (60%, 3/5 cases), followed by cerebral cryptococcosis (50%, 3/6 cases), cerebral toxoplasmosis (33%, 2/6 cases) and cerebral lymphoma (20%, 1/5 cases). Complete disjunction was registered for cerebral TB, HIVE, neurosyphilis and CMV infection. Half of the patients with no AIDS-related CNS afflictions have been overdiagnosed. In all, we conclude that analyzing necroptic findings in patients otherwise clinically diagnosed with several AIDS-associated CNS afflictions may raise awareness for better implementation of protocols and lower overdiagnosis, the latter with potentially harmful effects on patients treated with unnecessary medication. However, further research is needed for a better portrayal of the clinicopathological superposition in neuroAIDS.

#### Conflict of interests

The authors declare that they have no conflict of interests.

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