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Effectiveness of routine lumbar puncture in patients with HIV-associated Dementia (HAD) receiving suppressive antiretroviral treatment

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ABSTRACT:

— ***Background:*** The presence of active Human Immunodeficiency Virus (HIV) viral replication in the central nervous system (CNS) despite optimal plasma virological suppression is a largely unexplored issue, which is important for the understanding of HIV-associated neurocognitive diseases and development of drug resistance. We aimed to study the presence of detectable HIV RNA, as well as cerebrospinal fluid (CSF) drug levels, in a population of HIV-infected patients with HIV-associated dementia (HAD) and stable plasma virological suppression, in order to modify therapy and avoid further progression of the disease.

— ***Patients and Methods:*** HIV-positive patients attending the University Department of Infectious Diseases in Brescia (Northern Italy), with a previous diagnosis of HAD, on both stable Highly Active Antiretroviral Therapy (HAART) and plasma HIV RNA <37 copies/mL for at least 6 months underwent lumbar puncture (LP) in order to study HIV RNA and antiretroviral concentrations in the CSF.

— ***Results****:* Fifteen subjects were included in the analysis. Mean age was 51 years, 13 patients were males. Eight patients were injection drug users. Mean nadir CD4+ T-cell count was 99cells/μL. Mean time from HAD diagnosis was 9 years. Eight patients experienced more than 5 lines of HAART and 13 patients were on PI-boosted regimens. Only one patient had detectable HIV RNA in CSF (130 copies/mL) with a pattern of widespread drug resistance. As for CSF antiretroviral drug concentrations, allsamples showed detectable levels, but atazanavir concentrations were significantly lowerin the patient with CSF viral escape (1.1 ng/mL) compared to the other patients on the same PI (range: 3.5-40.3 ng/mL).

— ***Conclusions****:* In our study, only one out of fifteen patients had CSF viral escape. However, a cautious approach would suggest to perform screening LP in patients with previous HAD who could experience severe clinical deterioration. Indeed HIV may replicate in CSF despite low viral levels or viral suppression in the blood compartment, resulting in acute or subacute neurocognitive impairment.

— ***Key words:*** AIDS, Antiretroviral therapy, Central nervous system, HIV, Viral escape.

INTRODUCTION

The invasion of the central nervous system (CNS) by Human Immunodeficiency Virus (HIV) occurs early, during the phase of primary infection, and the virus remains detectable in cerebrospinal fluid (CSF) in most untreated individuals throughout the natural history of' HIV infection[1-3]. The sustained viral replication in the CNS compartment with (or without) immunological dysfunctions hampers neurocognitive functions, resulting

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in a spectrum of clinical conditions (known as HIV-associated neurocognitive disorders (HAND)) ranging from Asymptomatic neurocognitive impairment (ANI) to HIV-associated dementia (HAD)[4-6].

The introduction of Highly Active Antiretroviral Therapy (HAART) led to a marked decline in the incidence of severe forms of HAND, such as HAD and progressive multifocal leukoencephalopathy (PML)[7]. Nevertheless, the prevalence of milder forms of HAND is increasing due to the aging of the HIV-infected population[4,8-10].

High levels of HIV RNA in the CSF have been correlated with the severity of HAD[11]: therefore, it has been suggested that effective HAART may reduce the incidence and progression of neurological disorders by suppressing viral replication in the CNS compartment. The blood-CSF barrier may prevent the achievement of effective concentrations of antiretroviral drugs in the CSF favouring viral replication, the selection of multiresistant subpopulations and the phenomenon of compartmentalization[12,13].

Letendre et al proposed an ordinal categorization to account for the neuropenetration of antiretroviral agents (CNS penetration effectiveness (CPE) score), providing a tool to estimate the effectiveness of different antiretroviral regimens in the CNS[14-16]. There are reports of patients with neurological disease not otherwise explained, that showed an active viral replication in the CSF compartment despite a good control of plasma viremia: this phenomenon has been called CSF viral escape[2,17] and its prevalence and impact in clinical practice are still largely unknown.

We conducted a study in HIV-infected patients with previous diagnosis of HAD with stable plasma virological suppression, who underwent lumbar puncture (LP) to evaluate the effectiveness of HAART and the penetration of antiretroviral drugs in the CSF.

PATIENTS AND METHODS

In this observational study, we enrolled all patients with a previous diagnosis of HAD followed at the University Department of Infectious and Tropical Diseases, University of Brescia, and Division of Infectious Diseases, Spedali Civili General Hospital of Brescia. Patients underwent LP according to clinical indications as suggested in the Guidelines for HIV management[18]. We included all patients on stable antiretroviral therapy for at least 6 months with HIV RNA below the threshold of sensitivity of the PCR system (<37 copies/mL) for at least two consecutive determinations. We recorded the demographic and viro-immunological characteristics of the population since HIV diagnosis and HAART history. Patients underwent the following blood tests: HIV RNA (RT-PCR), blood cell count, T lymphocytes, TPHA, rapid plasma reagin (RPR) and the pharmacokinetics of antiretroviral drugs (PK).

Moreover, the following tests were performed on the CSF: biochemical examination; HIV RNA; JCV DNA; Herpesviridae DNA (EBV, CMV, HSV1-2, HSV-6); CSF pharmacokinetics (PK) of antiretroviral drugs.If CSF HIV viral load was positive, resistance genotyping test and viral tropism (also in the plasma proviral DNA) were performed.

VIRAL ESCAPE DEFINITION

Viral escape was defined as the presence of detectable HIV RNA in CSF despite undetectable plasma viremia (<37 copies/mL).

PRIVACY AND APPROVAL OF THE ETHICS COMMITTEE

Data managementwas performed using procedures designed to protect privacy (Law 31/12/1996, No 675). The study protocol was approved by the Ethics Committee of the Hospital of Brescia. All patients (or legal guardians in case of incapacitated patients) signed a written informed consent for the study and a specific consent for LP.

STATISTICAL ANALYSIS

Data were collected and analysed with Microsoft Office Excel® (2010, Microsoft INC, Redmont, USA). Categorical variables are expressed as numbers and proportions, continuous variables are expressed as mean ± standard deviation (SD).

RESULTS

Fifteen patients were included in the analysis: all baseline clinical and demographical characteristics are showed in Table 1. Mean age was 51 years, male gender was more prevalent (13/15). The main risk factor for HIV infection was injection drug use (IDU), recorded in 53% of patients (8/15). The average time since HAD diagnosis was 9 years. The majority of patients (8/15) had more than 5 previous lines of therapy. Other tests performed on CSF (JCV DNA, EBV DNA, CMV DNA, HSV 1-2, HSV-6, TPHA and RPR) were all negative.

We only identified one case of viral escape. This 53-year-old patient, who was HIV-infected since 1990, received a diagnosis of HAD in 1998 and started HAART in 1992 with zidovudine monotherapy. Since 1992, he changed more than 5 different antiretroviral regimens. CD4+ T cell count at enrollment was 577 cells/μL and HIV viraemia was <37 copies/mL. At the time of LP, the patient was taking tenofovir (TDF), emtricitabine (FTC) and unboosted atazanavir (ATV), with a CPE score of 4. The nadir CD4 + T-cell count was 179 cells/μL, with a zenithviraemia of 125,000 copies/mL.LP showed CSF protein = 67 mg/dL, glucose = 53 mg/dL, cells = 12/µL. HSV1-2, syphilis, JCV, CMV tests were negative. We found detectable HIV RNA in CSF (130 copies/mL) with a picture of widespread drug resistance (Protease: L10I; Transcriptase: M41L-L74V-V106I-M184V-H208Y-L210W-T215Y), showing high-level resistance to

Table 1. Demographic and viro-immunological characteristics of the study population.

Variable	N. (%)
Male gender	13 (87)
Age, years [mean (SD)]	51 (7)
Trasmission Risk- IDU- Heterosexual contacts- Homo/bisexual contacts- Other/unknown	8 (53)2 (13)4 (27)1 (7)
Nadir CD4+cells/µL[mean (SD)]	99 (70)
Nadir CD4+cells<200 cells/µL>200 cells/µL	14 (93)1 (7)
Zenith HIV RNA copies/mL [mean (SD)]	198,701 (226,218)
Zenith HIV RNA< 100.000 copies/mL> 100.000 copies/mL	6 (40)9 (60)
CD4+ at the time of HAD diagnosis cells/µL[mean (SD)]	254 (231)
HIV RNA at the time of HAD diagnosis copies/mL [mean (SD)]	68,312 (203,183)
Latest CD4+ cells/µL[mean (SD)]	508 (173)
Latest CD4+<350 cells/µL>350 cells/µL	3 (20)12 (80)
CSF HIV RNA DetectableUndetectable	1 (7)14 (93)
CPE Score of ongoing HAART [mean (SD)]	8.4 (1.8)
Time since HAART initiation, years [mean (SD)]	12 (5)
Previous lines of therapy[number]<33-5>5	3 (20)4 (27)8 (53)

CPE: cerebrospinal fluid penetration effectiveness; CSF: cerebrospinal fluid; HAART: Highly Active Antiretroviral Therapy; HAD: HIV-Associated Dementia; HIV: Human Immunodeficiency Virus; IDU: intravenous drug use; SD: standard deviation

lamivudine, thymidins analogue and abacavir. No previous drug-resistance tests were performed; therefore, it was impossible to compare this resistance profile to a previous one. ATV level in CSF, while detectable, was lower (1.1 ng/mL) compared to the other patients treated with the same protease inhibitor (PI) (range: 3.5-40.3 ng/mL). Table 2 shows the CSF concentrations of antiretroviral drugs.

DISCUSSION

In our study CSF viral escape occurred in only 1 out of 15 (7%) HIV-infected patients with previous HAD diagnosis and under stable and effective antiretroviral treatment.

Several studies have described the phenomenon of CNS viral escape: 2 case series reported, respectively, 10 and 11 patients who developed neurocognitive symptoms and had detectable CSF HIV RNA despite stable HAART and plasma virological suppression[17,19].

Moreover, in a study of 40 HIV-infected patients with detectable CSF HIV RNA, 18 (45%) patients were found to have greater levels of resistance in the CSF virus compared with plasma virus for at least one drug[12] but, differently from our study, not all the patients had undetectable plasma HIV RNA.

Our results are in keeping with those of other recent retrospective studies that found a prevalence of detectable CSF HIV RNA (>50 copies/mL) ranging from 5 to 23% in patients with plasma virological suppression with[17] or without neurological symptoms[20,21].

Therefore, there are still uncertainties regarding the frequency of CNS viral escape and its impact on neurocognitive function[22]. In fact, neurocognitive disorders in HIV-infected patients may be the result of CSF chronic immune activation, possibly determined by persistent viral replication, previously established tissue damage, or other unknown factors[1,23,24]. The use of an effective HAART, even in the CNS compartment, aims to inhibit viral replication in this sanctuary. The CPE score is a useful tool to estimate antiretroviral neuropenetration

Table 2. Monitoring of antiretroviral drugs on CSF and serum (ng/mL).

Patient	NVP Serum	NVP CSF	ATV Serum	ATV CSF	LPV Serum	LPV CSF	DRV Serum	DRV CSF	RTV Serum	RTV CSF	RAL Serum	RAL CSF	EFV Serum	EFV CSF
1			272.0	3.5										
2	4333.0	1109.0					6206.0	14.0				3.5		
3							5056.0	62.3	510.0	1.0				
4			971.0	6.2										
5											3219.0	21.7		
6	4097.0	959.0			8025.0	22.5			587.0	0.9				
7	4844.0	1543.0												
8														
9													3397.0	18.2
10			2821.0	40.3										
11			100.0	1.1										
12			545.0	4.8					<200	1.2				
13					4917.0	48.2			518.0	3.2				
14					4970.0	7.0			<200	0.4				
15			805.0	3.5					421.0	0.2				

ATV: atazanavir; CSF: cerebrospinal fluid; DRV: darunavir; EFV: efavirenz; LPV: lopinavir; NVP: nevirapine; RAL: raltegravir; RTV: ritonavir;

and neuro-effectiveness[15,16,25,26], but the optimal management of HIV-infected patients with neurocognitive disorders should probably include LP and appropriate investigations of CSF, in order to verify the effectiveness of HAART regimen in the CNS, as suggested by different guidelines[18,27]. LP, although invasive, is the only procedure able to identify the compartmentalization of the virus in the CNS and the presence of viral escape. However, tests to evaluate CNS viral escape are not widely available: viral load testing on the CSF is not performed in many laboratories, as well as genotypic drug resistance testing and therapeutic drug monitoring.

In the only patient presenting a viral escape, ATV concentration, although detectable in CSF, was significantly lower compared to the other patients, thus potentially allowing a viral replication within the CFS and a selection of multidrug-resistant *quasi species*: in fact, CSF genotypic resistance test showed the presence of multiple mutations on the reverse transcriptase gene. However, a comparison between CSF and blood resistance test was not possible since patients started therapy in 1992, when a genotypic testing was not recommended and therefore not performed.

The treatment prescribed to the patient with viral escape was not the standard of care according to international guidelines: unboosted ATV may not be the best choice in terms of neuropenetration and the backbone drugs (TDF/FTC) are not indicated in association with unboosted ATV[28]. Moreover, the current therapy with a CPE score of 4 points did not probably have a sufficient degree of neuropenetration. However, the regimen was prescribed due to profuse diarrhea experienced by the patient during ritonavir-boosted ATV therapy: ritonavir was therefore stopped but patient refused to change the remaining drugs.

The remaining 14 patients showed CSF therapeutic drug concentrations and undetectable CSF HIV-RNA.

This study has some limitations: first, the small sample size, secondly the absence of a control group (all patients were selected according to a previous HAD diagnosis) and finally the lack of longitudinal assessment in order to verify sustained CSF viral suppression. However, in our clinic such an invasive procedure is not routinely performed in healthy even if HIV-infected patients.

In conclusion, our data suggest the importance of an *in vivo* confirmation of HAART effectiveness in the CSF, particularly in patients with previous mild or moderate neurocognitive disorders, in order to optimize antiretroviral therapy on the basis of genotypic resistance tests and PK.

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