Tiltle: Cerebrospinal Fluid HIV-1 Escape According to Different Thresholds and Underlying Comorbidities: Is It Time to Assess the Definitions?

Authors: Mattia TRUNFIO[1], Carmela PINNETTI[2], Emanuele FOCÀ[3], Francesca BAI[4], Gaetano MAFFONGELLI[5], Luigi CELANI[6], Paola CINQUE[7], Anna CELOTTI[3], Massimo ANDREONI[5], Antonella D'ARMINIO MONFORTE[4], Gabriella D'ETTORRE[6], Andrea ANTINORI[3], Andrea CALCAGNO[1]

Affiliations:

1. Unit of Infectious Diseases, Department of Medical Sciences, University of Torino, Torino, Italy
2. Clinical Department, National Institute for Infectious Diseases "Lazzaro Spallanzani," Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS), Rome, Italy
3. Division of Infectious and Tropical Diseases, Department of Clinical and Exeprimental Sciences, University of Brescia and Spedali Civili General Hospital, Brescia, Italy
4. Department of Health Sciences, Clinic of Infectious Diseases and Tropical Medicine, S.Paolo Hospital, ASST Santi Paolo e Carlo, University of Milan, Milan, Italy
5. Department of Medicine of Systems, Infectious Diseases, University of Rome "Tor Vergata", Rome, Italy
6. Department of Public Health and Infectious Diseases, Azienda Policlinico Umberto I, Rome, Italy
7. Department of Infectious Diseases, San Raffaele Scientific Institute, Milano, Italy

Total Number of Words: 970 words

Total number of figure/table: 1 figure + 1 supplementary figure

Corresponding Author/ Author to whom requests for reprints should be made: Mattia Trunfio, Unit of Infectious Diseases, Department of Medical Sciences, University of Torino, Torino, Italy; e-mail: mattia.trunfio@edu.unito.it; phone: +393383309546.

Funding sources: No Funding was received

Summary 74/75 words:

No consensus has been reached on how to define cerebrospinal fluid HIV-1 escape (CSF-E). We describe its prevalence in 1095 paired CSF-plasma HIV-RNA measurements from antiretroviral-treated patients according to several definitions and neurological affections. CSF-E prevalence varied substantially (9.0-38.9%) and was higher in patients with cerebrovascular disorders, HIV-associated dementia and white matter abnormalities. Considering the variability in HIV-RNA quantification assays, the biological relevance of viral escape at different thresholds needs to be accurately assessed.

Manuscript 970/1000 words:

BACKGROUND

In the combination antiretroviral treatment (cART) era, HIV-related comorbidities and eradication of the virus from reservoirs represent major challenges. In view of this, the study of selective HIV replication observed in the cerebrospinal fluid (CSF) of treated and suppressed patients plays an eminent role. This phenomenon, called CSF viral escape (CSF-E), has in fact been associated erratically with central nervous system (CNS) complications [1-4] and represents a rare opportunity to understand the establishment and persistence of the virus in compartmentalised reservoirs [3]. CSF-E prevalence varies widely from 0,7% to 42,3%, as recently reviewed [3,5]; this difference may be explained by variability in methods and clinical features of patients' samples and by the surprisingly large range of adopted CSF-E definitions [3-5]. An International Consortium has been recently founded: [3] one of its aims is to find consensus on CSF-E through the identification of clinically meaningful CSF HIV-RNA thresholds and level of discordance with plasma HIV-RNA [3]. To date, without a shared CSF-E definition both clinical management and research opportunities are debatable. Aiming at unravelling these issues, we described and compared the prevalence of CSF-E in treated patients according to different escape definitions and several underlying CNS conditions.

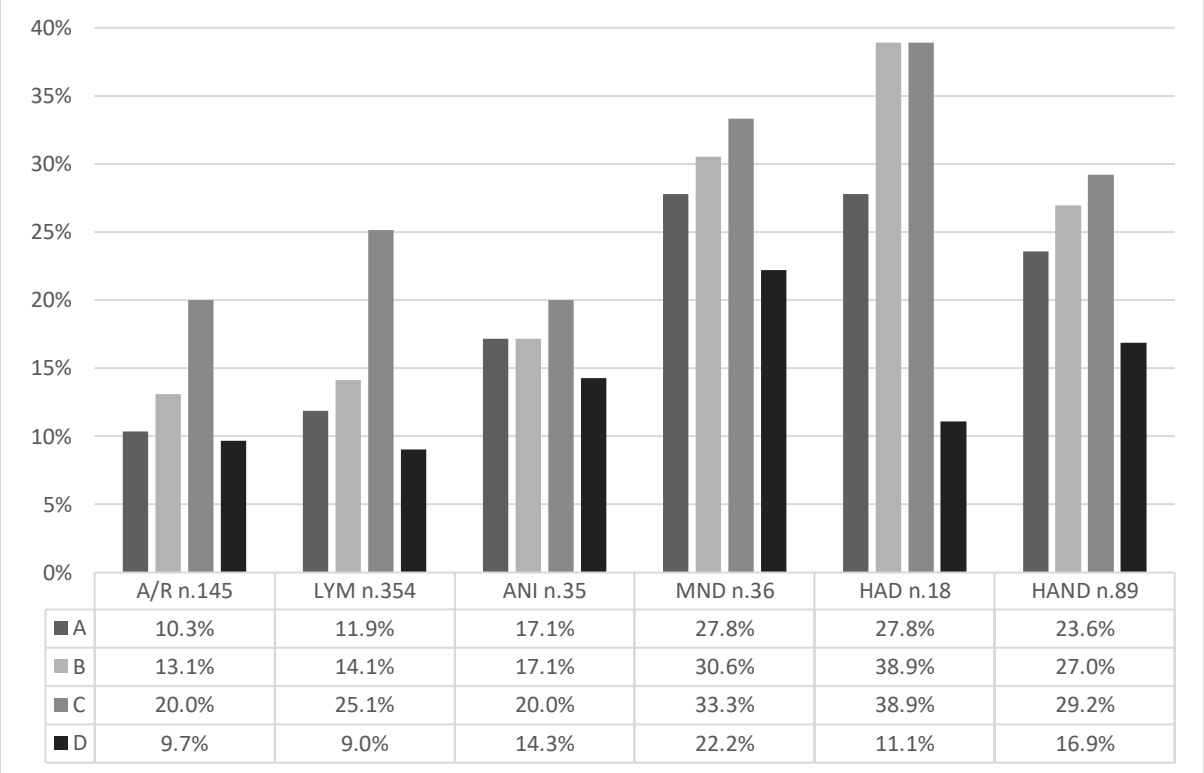
METHODS

We conducted a retrospective (1993-2018) multi-centric observational study (Rome, Milan, Brescia, Turin). We included data on HIV-positive adult patients on cART with available paired measurement of plasma and CSF HIV-RNA. Asymptomatic patients/participants enrolled in research studies (A/R) and those with lymphomas without CNS involvement (LYM) were included for comparisons. The ethics approval was obtained at each Institutions in the context of other ongoing studies**.** The used CSF-E definitions included:

A. Detectable CSF HIV-RNA with undetectable plasma HIV-RNA or CSF HIV-RNA 1 Log_{10} higher than plasma if the latter was detectable;
B. Detectable CSF HIV-RNA with undetectable plasma HIV-RNA or CSF HIV-RNA 0.5 Log_{10} higher than plasma if the latter was detectable;
C. Detectable CSF HIV-RNA with undetectable plasma HIV-RNA or CSF HIV-RNA higher than plasma if the latter was detectable;
D. Detectable CSF HIV-RNA with undetectable plasma HIV-RNA

RESULTS

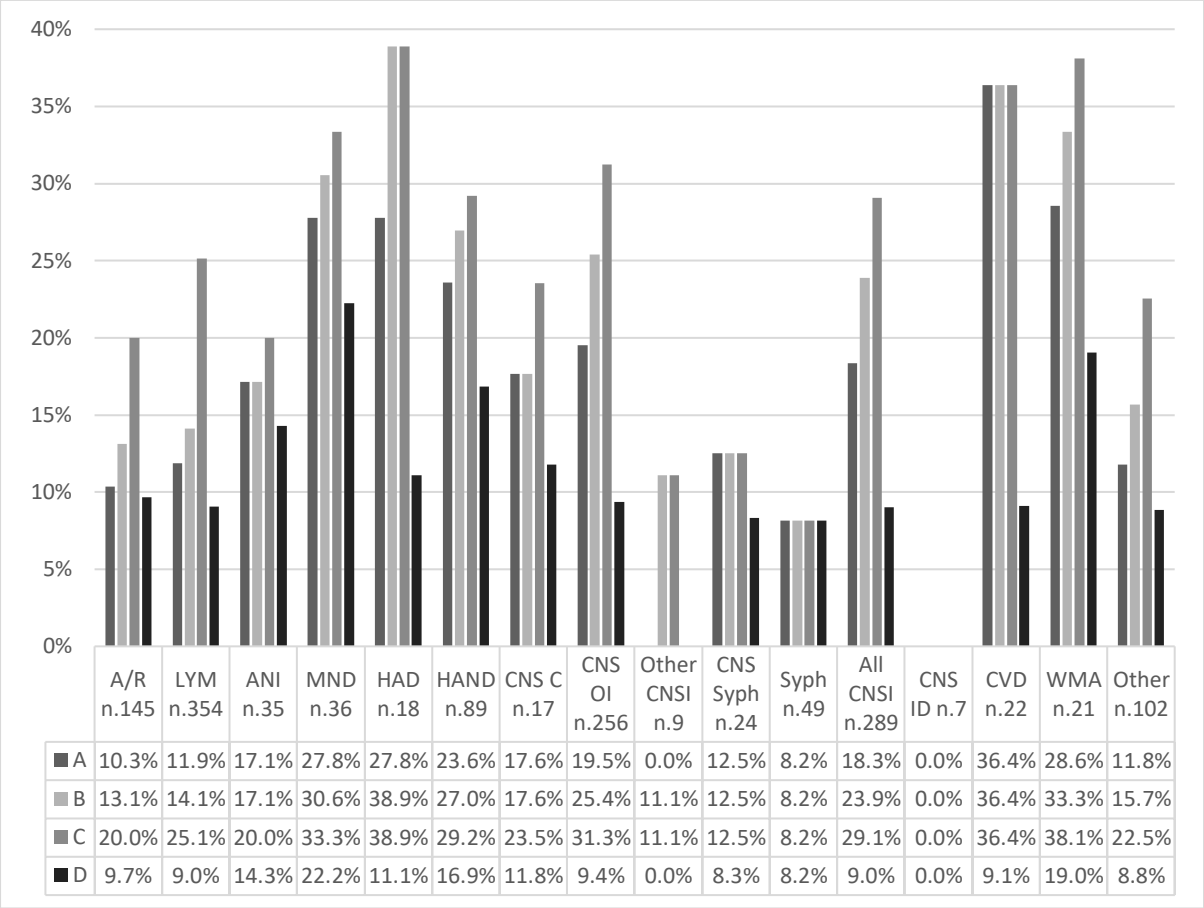
We included 1095 paired measurements of CSF and plasma HIV-RNA from 774 patients. Median age was 47 years (40-53); 601 (77.6%) were male; 523 (47.7%) and 556 (50.8%) plasma and CSF HIV-RNA measurement were below the lower limit of quantification (LLoQ). CSF-E cases overall were: 164 (15.0%; A), 200 (18.3%; B), 275 (25.1%; C) and 108 (9.9%; D). Similar CSF-E prevalence was observed according to calendar years. CSF-E prevalence according to the four definitions among A/R, LYM and those with HIV-associated neurocognitive disorders (HAND) is depicted in Fig.1, while according to all the different underlying clinical conditions is shown in Supplementary Figure 1.



	A/R n.145	LYM n.354	ANI n.35	MND n.36	HAD n.18	HAND n.89
A	10.3%	11.9%	17.1%	27.8%	27.8%	23.6%
B	13.1%	14.1%	17.1%	30.6%	38.9%	27.0%
C	20.0%	25.1%	20.0%	33.3%	38.9%	29.2%
D	9.7%	9.0%	14.3%	22.2%	11.1%	16.9%

Figure 1. Prevalence of Cerebrospinal Fluid HIV-1 Escape according to different Definitions and Index clinical conditions among on cART-treated patients

Legend: A/R Asymptomatic patients/participants enrolled in Research studies; LYM Lymphomas without central nervous system involvement; ANI, Asymptomatic Neurocognitive Impairment; MND, Mild Neurocognitive Disorders; HAD, HIV-Associated Dementia; HAND HIV-Associated Neurocognitive disorders



	A/R n.145	LYM n.354	ANI n.35	MND n.36	HAD n.18	HAND n.89	CNS C n.17	CNS OI n.256	Other CNSI n.9	CNS Syph n.24	Syph n.49	All CNSI n.289	CNS ID n.7	CVD n.22	WMA n.21	Other n.102
A	10.3%	11.9%	17.1%	27.8%	27.8%	23.6%	17.6%	19.5%	0.0%	12.5%	8.2%	18.3%	0.0%	36.4%	28.6%	11.8%
B	13.1%	14.1%	17.1%	30.6%	38.9%	27.0%	17.6%	25.4%	11.1%	12.5%	8.2%	23.9%	0.0%	36.4%	33.3%	15.7%
C	20.0%	25.1%	20.0%	33.3%	38.9%	29.2%	23.5%	31.3%	11.1%	12.5%	8.2%	29.1%	0.0%	36.4%	38.1%	22.5%
D	9.7%	9.0%	14.3%	22.2%	11.1%	16.9%	11.8%	9.4%	0.0%	8.3%	8.2%	9.0%	0.0%	9.1%	19.0%	8.8%

Supplementary Figure. Prevalence of Cerebrospinal Fluid HIV-1 Escape according to different Definitions and Central Nervous System Affections among on cART-treated patients

Legend: A/R Asymptomatic patients/participants enrolled in Research studies; LYM Lymphomas without central nervous system involvement; ANI, Asymptomatic Neurocognitive Impairment; MND, Mild Neurocognitive Disorders; HAD, HIV-Associated Dementia; HAND HIV-Associated Neurocognitive disorders; CNS C, Central Nervous System Cancers (lymphomas and other cancers involving central nervous system); CNS OI, Central Nervous System Opportunistic Infections; Other CNSI, Other Central Nervous System Infections (non-opportunistic bacterial and viral meningitis and encephalitis); CNS Syph, Central Nervous System Syphilis; Syph, Syphilis not involving central nervous system; All CNSI, Central Nervous System opportunistic and non-opportunistic infections, including central nervous system syphilis; CNS ID, Central Nervous System Inflammatory disorders (multiple sclerosis); CVD, Cerebrovascular disorders (stroke and vascular dementia); WMA, White Matter Abnormalities; Other includes neurocognitive deficits not fulfilling Frascati Criteria, Headache, Hepatic encephalopathy, Differential diagnosis in patients without CNS infections, Seizures, Psychiatric symptoms, Neuropathy, Polyneuropathy, Myelitis, Drug-drug

Interactions and Paraneoplastic encephalopathy.

CSF-E prevalence in cART-treated patients significantly varied according to escape definitions and CNS affections. Definition C, recently adopted by EACS guidelines [6], was associated with the highest rate of escape in any considered category (Fig.1 and Supplementary Figure). The lowest prevalence was described by definition D in any group. The prevalence of asymptomatic CSF-E among A/R and LYM ranged significantly from 9.7 to 20.0% and from 9.0% to 25.1% (definition D-C), as well as for all other included clinical categories. The highest prevalence was observed in patients with cerebrovascular diseases (9.1-36.4%), white matter abnormalities (19-38.1%) and HIV-Associated Dementia (HAD, 11.1-38.9%).

DISCUSSION

In a large sample size we observed that a significant proportion of patients presented with CSF-E: the prevalence of this condition varied largely according to the applied definitions and underlying CNS conditions. We observed that HAD, white matter abnormalities and cerebrovascular disorders were the top three categories identified as having the highest CSF-E prevalence by all the definitions, except for definition D; these categories were also those featured by the highest relative prevalence variability. Interestingly, we observed a CSF-E trend among HAND described by an increasing prevalence along with the worsening of neurocognitive impairment severity according to all definitions, but D. Our sample included few patients with inflammatory conditions and very heterogeneous CNS infections potentially leading to secondary CSF-E, having no possibility to differentiate and better explore this issue. A patient presenting with plasma HIV-RNA below 20 copies/mL and a CSF HIV-RNA of 21 copies/mL would be categorized, by all definitions, as a CSF-E. What may be the clinical and scientific consequences of adopting such a low-threshold definition? A CSF-E diagnosis triggers a remarkable time and resource-consuming clinical workout, a tailored follow-up and primarily it should prompt to reassess and eventually modify the ongoing cART, aiming at a better CNS-targeted combination in terms of penetration and efficacy [7]. According to definition C, we observed a substantially high prevalence of primary asymptomatic CSF-E; current knowledge suggests no relevant worsening over time but a significantly higher immune activation; [8] however, additional biomarkers able to exclude associated CNS damages may be helpful in avoiding unnecessary treatment changes. Still, overestimation may occur and be detrimental. HIV-RNA quantification uncertainty increases at low-level/residual viremia and the estimates precision is limited by biological and analytical variability [9,10]. The lower the

viremia, the higher are the coefficients of variation of modern real-time PCR assays, widely ranging (26.7%-83.1%) [9] and presenting total intra-assay variations of up to 0.26 Log10 RNA cp/mL [10], which would account for 57 (20.7%) of our CSF-E cases by definition C. Furthermore, viral blips have been shown to be analytically reproducible in less than a fifth of the samples and this may potentially be applied also to CSF HIV-RNA measurements [9].

Limitations of the present study are mainly represented by the retrospective design and the lack of data on antiretroviral treatment duration and CSF-E persistence, so that we may have also included slow CSF suppressors and CSF viral blips cases.

In conclusion taking into account current laboratory limits, the very high prevalence and the few prospective studies, we believe that CSF escape definition need to be accurately determined according to meaningful conditions or outcomes.

REFERENCES

1. Anderson AM, Muñoz-Moreno JA, McClernon DR, Ellis RJ, Cookson D, Clifford DB et al. **Prevalence and Correlates of Persistent HIV-1 RNA in Cerebrospinal Fluid During Antiretroviral Therapy.** *J Infect Dis* 2017; **215**(1):105-113.
2. Peluso MJ, Ferretti F, Peterson J, Lee E, Fuchs D, Boschini A et al. **Cerebrospinal fluid HIV escape associated with progressive neurologic dysfunction in patients on antiretroviral therapy with well controlled plasma viral load.** *AIDS* 2012; **26**(14):1765-74.
3. Joseph J, Cinque P, Colosi D, Dravid A, Ene L, Fox H et al. **Highlights of the Global HIV-1 CSF Escape Consortium Meeting, 9 June 2016, Bethesda, MD, USA.** *J Virus Erad* 2016; **2**(4):243-250.
4. Dravid A, Natrajan K, Kulkarni MM, Saraf CK, Mahajan US, Kore SD et al. **Discordant CSF/plasma HIV-1 RNA in individuals on virologically suppressive antiretroviral therapy in Western India.** *Medicine (Baltimore)* 2018; **97**(8):e9969.
5. Di Carlofelice M, Everitt A, Muir D, Winston A. **Cerebrospinal fluid HIV RNA in person living with HIV**. *HIV Med* 2018; **19**(5):365-368.
6. European AIDS Clinical Society. **EACS Guidelines, Version 9.0** [Guidelines]. October 2017.
7. Calcagno A, Barco A, Trunfio M, Bonora S. **CNS-Targeted Antiretroviral Strategies: When Are They Needed and What to Choose**. *Curr HIV/AIDS Rep* 2018; **15**(1):84-91.
8. Edén A, Nilsson S, Hagberg L, Fuchs D, Zetterberg H, Svennerholm B et al.

Asymptomatic Cerebrospinal Fluid HIV-1 Viral Blips and Viral Escape During Antiretroviral Therapy: A Longitudinal Study. *J Infect Dis* 2016; **214**(12):1822-1825.

9. Ruelle J, Debaisieux L, Vancutsem E, De Bel A, Delforge ML, Piérard D et al. **HIV-1 low-level viraemia assessed with 3 commercial real-time PCR assyas show high variability**. *BMC Infect Dis* 2012; **12**:100.
10. Brambilla D, Reichelderfer PS, Bremer JW, Shapiro DE, Hershow RC, Katzenstein DA et al. **The contribution of assay variation and biological variation to the total variability of plasma HIV-1 RNA measurements. The Women Infant Transmission Study Clinics. Virology Quality Assurance Program**. *AIDS* 1999; **13**(16):2269-79.