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**Cerebrospinal fluid HIV-1 escape according to different thresholds and underlying comorbidities:  
is it time to assess the definitions?**

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

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## 1 **Summary:**

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

9

## 10 **Manuscript:**

11

## 12 **BACKGROUND**

13 In the combination antiretroviral treatment (cART) era, HIV-related comorbidities and  
14 eradication of the virus from reservoirs represent major challenges. In view of this, the study  
15 of selective HIV replication observed in the cerebrospinal fluid (CSF) of treated and suppressed  
16 patients plays an eminent role. This phenomenon, called CSF viral escape (CSF-E), has in fact  
17 been associated erratically with central nervous system (CNS) complications [1-4] and  
18 represents a rare opportunity to understand the establishment and persistence of the virus in  
19 compartmentalised reservoirs [3]. CSF-E prevalence varies widely from 0,7% to 42,3%, as  
20 recently reviewed [3,5]; this difference may be explained by variability in methods and clinical  
21 features of patients' samples and by the surprisingly large range of adopted CSF-E definitions  
22 [3-5]. An International Consortium has been recently founded: [3] one of its aims is to find  
23 consensus on CSF-E through the identification of clinically meaningful CSF HIV-RNA  
24 thresholds and level of discordance with plasma HIV-RNA [3]. To date, without a shared CSF-  
25 E definition both clinical management and research opportunities are debatable. Aiming at  
26 unravelling these issues, we described and compared the prevalence of CSF-E in treated  
27 patients according to different escape definitions and several underlying CNS conditions.

28

## 29 **METHODS**

30 We conducted a retrospective (1993-2018) multi-centric observational study (Rome, Milan,  
31 Brescia, Turin). We included data on HIV-positive adult patients on cART with available  
32 paired plasma and CSF HIV-RNA measurements on samples collected maximum 14 days  
33 apart. Asymptomatic patients/participants enrolled in research studies (A/R) and those with  
34 lymphomas without CNS involvement (LYM) were included for comparisons. The ethics

1 approval was obtained at each Institutions in the context of other ongoing studies. The used  
2 CSF-E definitions included:

- 3 A. Detectable CSF HIV-RNA with undetectable plasma HIV-RNA or CSF HIV-RNA 1  
4  $\text{Log}_{10}$  higher than plasma if the latter was detectable;
- 5 B. Detectable CSF HIV-RNA with undetectable plasma HIV-RNA or CSF HIV-RNA 0.5  
6  $\text{Log}_{10}$  higher than plasma if the latter was detectable;
- 7 C. Detectable CSF HIV-RNA with undetectable plasma HIV-RNA or CSF HIV-RNA  
8 higher than plasma if the latter was detectable;
- 9 D. Detectable CSF HIV-RNA with undetectable plasma HIV-RNA

## 11 RESULTS

12 We included 1095 paired measurements of CSF and plasma HIV-RNA from 774 patients.  
13 Median age was 47 years (40-53); 601 (77.6%) were male; 523 (47.7%) and 556 (50.8%)  
14 plasma and CSF HIV-RNA measurement were below the lower limit of quantification. CSF-E  
15 cases overall were: 164 (15.0%; A), 200 (18.3%; B), 275 (25.1%; C) and 108 (9.9%; D). The  
16 median (IQR) distance between plasma and CSF collection for HIV-RNA measurement was 0  
17 (0-14) days, while CSF-plasma HIV-RNA difference among CSF-E cases were: 1.18 (0.37-  
18 1.68; A), 0.91 (0.48-1.59; B), 0.61 (0.30-1.35; C) and 0.55 (0.099-1.55; D)  $\text{Log}_{10}$  cp/mL.  
19 Similar CSF-E prevalence was observed according to calendar years. CSF-E prevalence  
20 according to the four definitions among A/R, LYM and those with HIV-associated  
21 neurocognitive disorders (HAND) is depicted in Fig.1, while according to all the different  
22 underlying clinical conditions is shown in Supplementary Figure.

23 CSF-E prevalence in cART-treated patients significantly varied according to escape definitions  
24 and CNS affections. Definition C, recently adopted by EACS guidelines [6], was associated  
25 with the highest rate of escape in any considered category (Fig.1 and Supplementary Figure).  
26 The lowest prevalence was described by definition D in any group. Asymptomatic CSF-E  
27 prevalence among A/R and LYM ranged significantly from 9.7 to 20.0% and from 9.0% to  
28 25.1% (definition D-C), as well as for all other included clinical categories. The highest  
29 prevalence was observed in patients with cerebrovascular diseases (9.1-36.4%), white matter  
30 abnormalities (19-38.1%) and HIV-Associated Dementia (HAD, 11.1-38.9%).

## 32 DISCUSSION

33 In a large sample size we observed that a significant proportion of patients presented with CSF-  
34 E: its prevalence varied largely according to the applied definitions and underlying CNS

1 conditions. We observed that HAD, white matter abnormalities and cerebrovascular disorders  
2 were the top three categories identified as having the highest CSF-E prevalence by all the  
3 definitions, except for definition D; these categories were also those featured by the highest  
4 relative prevalence variability. Interestingly, we observed a CSF-E trend among HAND  
5 described by an increasing prevalence along with the worsening of neurocognitive impairment  
6 severity according to all definitions, but D. Our sample included few patients with  
7 inflammatory conditions and very heterogeneous CNS infections potentially representing  
8 secondary CSF-E, having no possibility to differentiate and better explore this issue.

9 A patient presenting with plasma HIV-RNA below 20 copies/mL and a CSF HIV-RNA of 21  
10 copies/mL would be categorized, by all definitions, as a CSF-E. What may be the clinical and  
11 scientific consequences of adopting such a low-threshold definition? A CSF-E diagnosis  
12 triggers a remarkable time and resource-consuming clinical workout, a tailored follow-up and  
13 primarily it should prompt to reassess and eventually modify the ongoing cART, aiming at a  
14 better CNS-targeted combination in terms of penetration and efficacy [7]. According to  
15 definition C, we observed a substantially high prevalence of primary asymptomatic CSF-E;  
16 current knowledge suggests no relevant worsening over time but a significantly higher immune  
17 activation; [8] however, additional biomarkers able to exclude associated CNS damages may  
18 be helpful in avoiding unnecessary treatment changes. Still, overestimation may occur and be  
19 detrimental. HIV-RNA quantification uncertainty increases at low-level/residual viremia and  
20 the estimates precision is limited by biological and analytical variability [9,10]. The lower the  
21 viremia, the higher are the coefficients of variation of modern real-time PCR assays, widely  
22 ranging (26.7%-83.1%) [9] and presenting total intra-assay variations of up to 0.26 Log<sub>10</sub> RNA  
23 cp/mL [10], which would account for 57 (20.7%) of our CSF-E cases by definition C.  
24 Furthermore, viral blips have been shown to be analytically reproducible in less than a fifth of  
25 the samples and this may potentially be applied also to CSF HIV-RNA measurements [9].

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

29 In conclusion taking into account current laboratory limits, the very high prevalence and the  
30 few prospective studies, we believe that CSF escape definitions need to be accurately  
31 determined according to meaningful clinical conditions, viral compartmentalization and  
32 replication capacity or neurological outcomes: in this framework definition B (CSF HIV-RNA  
33 0.5 Log<sub>10</sub> higher than plasma HIV-RNA) might better represent underlying virological process.

## 1 REFERENCES

- 2 1. Anderson AM, Muñoz-Moreno JA, McClernon DR, Ellis RJ, Cookson D, Clifford DB et  
3 al. **Prevalence and Correlates of Persistent HIV-1 RNA in Cerebrospinal Fluid During**  
4 **Antiretroviral Therapy.** *J Infect Dis* 2017; **215**(1):105-113.
- 5 2. Peluso MJ, Ferretti F, Peterson J, Lee E, Fuchs D, Boschini A et al. **Cerebrospinal fluid**  
6 **HIV escape associated with progressive neurologic dysfunction in patients on**  
7 **antiretroviral therapy with well controlled plasma viral load.** *AIDS* 2012; **26**(14):1765-  
8 74.
- 9 3. Joseph J, Cinque P, Colosi D, Dravid A, Ene L, Fox H et al. **Highlights of the Global**  
10 **HIV-1 CSF Escape Consortium Meeting, 9 June 2016, Bethesda, MD, USA.** *J Virus*  
11 *Erad* 2016; **2**(4):243-250.
- 12 4. Dravid A, Natrajan K, Kulkarni MM, Saraf CK, Mahajan US, Kore SD et al. **Discordant**  
13 **CSF/plasma HIV-1 RNA in individuals on virologically suppressive antiretroviral**  
14 **therapy in Western India.** *Medicine (Baltimore)* 2018; **97**(8):e9969.
- 15 5. Di Carlofelice M, Everitt A, Muir D, Winston A. **Cerebrospinal fluid HIV RNA in**  
16 **person living with HIV.** *HIV Med* 2018; **19**(5):365-368.
- 17 6. European AIDS Clinical Society. **EACS Guidelines, Version 9.0** [Guidelines]. October  
18 2017.
- 19 7. Calcagno A, Barco A, Trunfio M, Bonora S. **CNS-Targeted Antiretroviral Strategies:**  
20 **When Are They Needed and What to Choose.** *Curr HIV/AIDS Rep* 2018; **15**(1):84-91.
- 21 8. Edén A, Nilsson S, Hagberg L, Fuchs D, Zetterberg H, Svennerholm B et al.  
22 **Asymptomatic Cerebrospinal Fluid HIV-1 Viral Blips and Viral Escape During**  
23 **Antiretroviral Therapy: A Longitudinal Study.** *J Infect Dis* 2016; **214**(12):1822-1825.
- 24 9. Ruelle J, Debaisieux L, Vancutsem E, De Bel A, Delforge ML, Piérard D et al. **HIV-1 low-**  
25 **level viraemia assessed with 3 commercial real-time PCR assays show high variability.**  
26 *BMC Infect Dis* 2012; **12**:100.
- 27 10. Brambilla D, Reichelderfer PS, Bremer JW, Shapiro DE, Hershow RC, Katzenstein DA et  
28 al. **The contribution of assay variation and biological variation to the total variability**  
29 **of plasma HIV-1 RNA measurements. The Women Infant Transmission Study**  
30 **Clinics. Virology Quality Assurance Program.** *AIDS* 1999; **13**(16):2269-79.

31  
32 **Figures:**

33

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

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

7  
8 **Supplementary Figure. Prevalence of Cerebrospinal Fluid HIV-1 Escape according to**  
9 **different Definitions and Central Nervous System Affections among on cART-treated**  
10 **patients**

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

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