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Cerebrospinal fluid HIV-1 escape in patients with neurocognitive symptoms: pooled data from a neuro-HIV platform and the NAMACO study

Running head: CSF HIV-1 escape in cognitive symptoms

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ETHICAL STATEMENTS

The ethics committees of each cantonal hospital centre approved the NAMACO study protocol and the ethics committee of the Canton of Vaud approved the neuro-HIV platform protocol (Study protocol number 44/11, 7th February 2011). All participants signed informed consent prior to inclusion in the respective studies.

MEETINGS

This work was presented in the form of oral communication at the virtual Conference on Retroviruses and Opportunistic Infections (vCROI) on March 6-10, 2021, and at the Joint Annual Meeting of the Swiss Society for Infectious Diseases (SSI) at Montreux on September 2-3, 2021.

ABSTRACT / KEY WORD PAGE

Background

Despite modern antiretroviral therapy, HIV-1 RNA escape into the cerebrospinal fluid (CSF) may occur. We examined the prevalence of and factors associated with CSF HIV-1 escape among people living with HIV (PLWH) in Switzerland.

Setting

The Neurocognitive Assessment in the Metabolic and Aging Cohort (NAMACO) study is an ongoing, prospective, longitudinal, multicenter study within the Swiss HIV Cohort Study. The neuro-HIV platform is a multi-disciplinary, single-day outpatient consultation at Lausanne University Hospital.

Methods

We pooled data from the NAMACO study and the neuro-HIV platform participants who underwent lumbar puncture (LP) between 2011 and 2019. Both patient groups had neurocognitive symptoms. CSF HIV-1 escape was defined as the presence of quantifiable CSF HIV-1 RNA when plasma HIV-1 RNA was suppressed or CSF HIV-1 RNA greater than plasma HIV-1 RNA when the latter was detectable.

Results

Of 1166 PLWH assessed, 288 underwent LP. CSF HIV-1 escape was observed in 25 PLWH (8.7%) of whom 19 (76%) had supressed plasma HIV-1 RNA. Characteristics of PLWH were comparable whether they had CSF HIV-1 escape or not, including comorbidities, time since HIV diagnosis (15 vs 16 years, p=0.9), median CD4 nadir (158.5/mm^3 vs 171/mm^3, p=0.6), antiretroviral CSF-Penetration-Effectiveness score (7 vs 7 points, p=0.8), neurocognitive diagnosis based on Frascati criteria and radiological findings.

Conclusions

In this large pooled sample of PLWH with neurocognitive symptoms, CSF HIV-1 escape occurred in 8.7% of PLWH. PLWH with CSF HIV-1 escape presented no distinctive clinical or paraclinical characteristics. We conclude that LP is unavoidable in confirming CSF HIV-1 escape.

Keywords: neuro-HIV, CSF escape, neurocognitive, compartmentalised infection, reservoir

TEXT

INTRODUCTION

Early after acute infection, human immunodeficiency virus-1 (HIV-1) invades the brain tissue where it establishes latency, thus contributing to the formation of a central nervous system (CNS) reservoir [1]. Despite the high efficacy of modern antiretroviral therapy (ART) in reducing both plasma and cerebrospinal fluid (CSF) HIV-1 ribonucleic acid (RNA) [2], higher HIV-1 RNA levels in CSF than plasma are sometimes observed in ART-treated individuals. This discordance is referred to as CSF HIV-1 escape [3, 4]. Concern has been raised that viral persistence in the CSF may result in chronic immune activation and inflammation [5, 6] and, more rarely, to compartmentalised drug resistance leading to ongoing neuroinflammatory damage [7-10]. Whilst the exact origin of detectable CSF HIV-1 remains unclear, hypotheses include ongoing viral replication within the brain itself and transitory CSF compartment exposure to virus transported via migrating blood cells [5, 11]. Management of CSF HIV-1 escape rests mainly on ART optimisation, including genotypic viral resistance testing in plasma and CSF, avoiding atazanavir-based and dual ART therapies, and considering double-dose dolutegravir in cases with documented or suspected integrase strand transfer inhibitor (INSTI) resistance [12], although no universally accepted practice currently exists due to insufficient scientific evidence.

Among people living with HIV (PLWH) on ART, the reported prevalence of CSF HIV-1 escape is highly variable between studies, ranging from 3% to 28% [10, 13-23] (**Table 1**). Predictive factors for CSF HIV-1 escape include low ART CNS penetration [16], protease inhibitor (PI)-based ART regimens [16, 20, 21], persistent low-level viremia [19, 24, 25], length of time on ART [17, 26], drug resistant virus in the CSF [8, 25, 26], low CD4 count nadir [3, 20, 25, 26] and CSF pleiocytosis [14, 20, 21]. However, many of these studies reporting on predictive factors were case series and/or included PLWH

from different ART eras in whom lumbar puncture (LP) was obtained for highly disparate reasons. While asymptomatic CSF HIV-1 escape is described[17, 27], a wide spectrum of neurocognitive symptoms and signs associated with CSF HIV-1 escape has been described, ranging from mild memory impairment to HIV-associated dementia [28]. However, given that CSF HIV-1 RNA levels do not always correlate with neurocognitive symptoms and signs [17, 25], the relationship between CSF HIV-1 escape and clinical characteristics is yet to be elucidated.

The objectives of this study were to evaluate the prevalence of CSF HIV-1 escape among PLWH in Switzerland and to identify potential predictive factors and clinical characteristics associated with this phenomenon.

METHODS

Study design

In a cross-sectional observational analysis, we retrospectively identified and pooled data from PLWH from two different patient groups in Switzerland who had undergone LP. Patients from each group were included in the current study regardless of plasma HIV-1 RNA level and ART regimen.

The first patient group was a subset of the Neurocognitive Assessment in the Metabolic and Aging Cohort (NAMACO) study, an ongoing, prospective, longitudinal, multicentre and multilingual study which includes people with HIV aged ≥ 45 years, enrolled in the Swiss HIV Cohort Study (SHCS) [29, 30]. While the NAMACO study recruits asymptomatic and symptomatic patients, all participants diagnosed with neurocognitive

impairment (NCI) as part of the NAMACO study evaluation are offered formal neurological examination, brain magnetic resonance imaging (MRI) and LP (targeted testing approach, opt-out consent), as recommended by the European AIDS Clinical Society [12]. NAMACO participants do not undergo formal psychiatric evaluation but all complete the Center for Epidemiologic Studies Depression (CES-D) scale, a questionnaire rating depressive symptoms [29]. For the current study, we included NAMACO study participants diagnosed with NCI who had undergone LP (**Figure 1**). In the NAMACO study database, it was possible to identify ART changes but not possible to determine whether the change occurred in response to CSF escape.

The second patient group comprised patients assessed at a multi-disciplinary, single-day outpatient neuro-HIV platform, conducted at Lausanne University Hospital. The neuro-HIV platform receives two to three PLWH with symptomatic or suspected NCI per month [31]. Platform patients can be of any age, from all over Switzerland, and enrolled in the SHCS or not. All are offered a full diagnostic work-up with specialist neurological, neuropsychological, psychiatric and HIV evaluation, brain MRI and LP (universal testing approach, opt-out consent) [32]. Depression is diagnosed using the Montgomery-Asberg Depression Rating Scale (MADRS) scale and following formal psychiatric assessment. Medical records and all clinic letters were available for all neuro-HIV platform patients seen at our centre.

Standard Protocol Approvals, Registrations, and Patient Consents

The ethics committees of each cantonal hospital centre approved the NAMACO study protocol and the ethics committee of the Canton of Vaud approved the neuro-HIV platform protocol (Study protocol number 44/11, 7th February 2011). All participants signed informed consent prior to inclusion in the respective studies.

Definitions

Patients were classified as symptomatic when reporting neurocognitive symptoms or when presenting objective neuropsychological abnormalities. NCI was diagnosed and classified upon clinical neuropsychological evaluation according to Frascati criteria [33]. Based on this classification, non-HIV associated NCI refers to NCI considered to be due to untreated or suboptimally treated conditions not directly linked to HIV, such as psychiatric disorders (including depression), substance use, ART toxicity and structural damage associated with neurodegenerative disorders, previous opportunistic CNS infection, stroke, or trauma. In cases for which both HIV- and non-HIV-associated factors were identified in the same patient, the most probable cause was defined after discussion between different specialists in the neuro-HIV platform and based on neuropsychological evaluation in the NAMACO study. No patient in our study had a CNS co-infection upon assessment for CSF HIV-1 escape. All patients had plasma HIV-1 RNA measured at the time of CSF HIV-1 RNA measurements. In our study, CSF HIV-1 escape was defined as either the presence of quantifiable HIV-1 RNA in the CSF at any level when plasma HIV-1 RNA was undetectable or as CSF HIV-1 RNA greater than plasma HIV-1 RNA when the latter was detectable, as previously described and currently recommended by the European AIDS Clinical Society [12, 34]. Detectable HIV-1 RNA was defined as any HIV-1 RNA detected above the local laboratory's PCR detection limit, which was at 20 copies per ml in our study. Measurement of CSF white blood cell count, total protein and oligoclonal bands, and peripheral blood CD4 count was performed using routine methods. Genotypic viral resistance tests were performed in CSF or plasma samples when clinically indicated and technically feasible. ART penetration in the CNS was estimated using the CNS penetration-effectiveness (CPE) score, as proposed by Letendre *et al* in the CNS HIV Antiretroviral Therapy Effects Research (CHARTER) Study [35]. Finally, standard clinical 3-Tesla MRI protocols

were employed, encompassing high resolution T1-weighted magnetisation, as well as T2* mapping, MP2RAGE, diffusion tensor imaging and Flair 3D sequences. MRI examination in NCI is performed to exclude structural brain abnormalities which may explain neurocognitive symptoms, rather than to 'diagnose' HIV-associated NCI. As no specific radiological pattern has been recognized to date, MRI examinations performed in the two patient groups were considered abnormal in the presence of any pathological finding reported by the radiologist, regardless of the finding's clinical relevance, including grey or white matter lesion (of any degree) with or without contrast-enhancement, brain atrophy or mass effect.

Statistical analysis

Statistical analyses were performed using Stata SE 17 (StataCorp, College Station, TX). Descriptive results were expressed as proportion (percentage) or as median with interquartile range (IQR). The Pearson χ^2 or the Fischer's exact test (for categorical variables), or the Wilcoxon Rank-Sum test (for continuous variables) were used to evaluate differences in characteristics between groups.

Data Availability

Data not provided in the article because of space limitations may be shared (anonymised) at the request of any qualified investigator for purposes of replicating procedures and results.

RESULTS

From March 2011 to April 2019, we assessed a total of 1166 patients across the two patient groups (NAMACO and neuro-HIV platform) of whom we excluded 878 patients who did not undergo LP. Among the remaining 288 patients, 25 (8.7%) were diagnosed with CSF HIV-1 escape (**Figure 1**). Of these, only three patients of 25 (12%) had CSF HIV-1 RNA <1.0 $\log_{10}$ greater than plasma HIV-1 RNA.

We observed no significant difference between patients with and without CSF HIV-1 escape in terms of their demographic profile, comorbidities or brain MRI findings (**Table 2**). Among patients with CSF HIV-1 escape, 17 (68%) had an abnormal MRI of whom seven (41.1%) had cortical or subcortical atrophy, four (23.5%) had white matter abnormalities, one (5.8%) had contrast-enhancing lesions and five (29.4%) had unknown radiological abnormalities (missing data). No significant difference among different MRI patterns was observed between patients with and without CSF HIV-1 escape. We found that detectable plasma HIV-1 RNA was more frequent in patients with CSF HIV-1 escape (six patients of 25, 24%) compared to patients with no CSF escape (28 patients of 263, 10.7%, p=0.04; Cohen's d=0.49, 95% CI: 0.08-0.90). We found no significant difference in neurocognitive performance based on Frascati criteria (p=0.9). Among patients with CSF HIV-1 escape, neuropsychological evaluation revealed normal neurocognitive function in four patients (16%), HIV-associated NCI in ten patients (40%) and non-HIV-associated NCI in 11 patients (44%). Among the 11 patients with non-HIV-associated NCI, nine (81.8%) had depression and one had mental retardation (9%); the specific cause was not available for the last patient. Symptoms of depression were present in 18.3% (28/153) of NAMACO study participants, based on a CES-D score >16 (moderate to severe depressive symptoms) and depression was diagnosed in 82.8% (111/132) of neuro-HIV platform patients, based on MADRS and psychiatric evaluation. Overall, there was no significant association between depression and CSF HIV-1 escape (p=0.5). Regarding ART, we found no difference in CPE score (p=0.8) nor in ART regimens, although we

observed a trend towards more patients on dual therapy (including dolutegravir plus lamivudine or rilpivirine) in the CSF HIV-1 group (p=0.07) (**Table 2**). Regarding CSF analysis, abnormal CSF white blood cells were more prevalent in the CSF HIV-1 escape group (p<0.001). We observed no difference in CSF proteins between the two groups (p=0.5). Among patients evaluated in the neuro-HIV platform, the presence of CSF-specific oligoclonal bands as a sign of intrathecal IgG synthesis did not differ between patients with or without CSF HIV-1 escape (**Table 2**).

Of the 25 patients with CSF HIV-1 escape, three (12%) had low levels of detectable plasma HIV-1, defined as plasma HIV-1 RNA >50 copies/mL and <200 copies/ml, but this proportion was not significantly different to that among patients with no CSF escape (p=0.08). When comparing other plasma HIV-1 cut-offs, namely plasma HIV-1 RNA >50 copies/ml and <400, <500 or <1000 copies/ml, a significant difference was observed between patients with and without CSF escape (p=0.02, p=0.02 and p=0.03, respectively). However, we could not examine persistent low-level viremia (requiring at least two consecutive plasma HIV-1 RNA measurements after 12 months of ART [36]) due to the lack of longitudinal data. Furthermore, isolated HIV-1 resistance in CSF (with ART-sensitive HIV-1 in plasma) was observed in three patients with CSF HIV-1 escape (12%). However, viral genotyping was not routinely performed in all patients and was unsuccessful in patients with low CSF HIV-1 RNA. Among patients with CSF HIV-1 escape, ART switch was proposed in six patients (of 25, 24%), all from the neuro-HIV platform. The switch indication was isolated CSF viral resistance in three patients and ART optimisation in terms of burden pill, potential toxicity, pharmacokinetics and/or CPE score in the other three patients. Characteristics of patients diagnosed with CSF HIV-1 escape are shown in **Table 3**.

DISCUSSION

In this cross-sectional analysis of a large case series of PLWH with suspected or confirmed NCI, the prevalence of CSF HIV-1 escape was 9% (25 patients), based on the latest definition [12, 34]. Among the 19 patients with CSF HIV-1 escape and undetectable plasma HIV-1 RNA, no parameter (or group of parameters) was predictive of CSF viral escape, demonstrating that LP is the only means of confirming this phenomenon.

An important consideration when estimating CSF HIV-1 escape prevalence is whether the study population is symptomatic or exclusively asymptomatic. Asymptomatic CSF HIV-1 escape refers to patients who are free of symptoms but who have detectable CSF HIV-1 RNA. This should be distinguished from asymptomatic NCI, which implies the presence of mild NCI without functional impairment (unimpaired activities of daily living) independent of CSF viral status [33]. While most of patients in our study reported neurocognitive symptoms, formal neuropsychological evaluation did not confirm NCI in 16% of patients with and 15% of patients without CSF HIV-1 escape. In comparison, among studies of exclusively symptomatic patients, CSF HIV-1 escape prevalence ranged from 3-6% [10, 15, 18] to 28% [16]. Similarly, studies including both symptomatic and asymptomatic patients showed a prevalence varying between 4% and 28% [13, 14, 19-23]. Among asymptomatic patients in one case series by Eden *et al*, CSF HIV-1 escape prevalence was estimated at 10% [17] (**Table 1**).

Consistent with previous studies [15-17, 20], it was not possible in our study population to differentiate between patients with or without CSF HIV-1 escape on the basis of demographic profile, comorbidities, brain MRI findings or neurocognitive diagnosis based on Frascati criteria (**Table 1**).

These results underline the lack of sensitivity and specificity of these diagnostic tools, which is partly due to the non-specific clinical features associated with CSF escape. In other words, it still remains unknown why some CSF HIV-1 escape events are associated with cognitive impairment while others are not. Regarding CSF analysis, we found elevated CSF white blood cells to be a strong predictive factor for CSF HIV-1 escape, as previously described [14, 20, 21], but this was not the case for CSF proteins.

As for HIV-related characteristics, detectable plasma HIV-1 RNA was associated with CSF HIV-1 escape (p=0.04). However, although 90% of patients without CSF HIV-1 escape had undetectable HIV-1 RNA in plasma, three quarters of patients with CSF HIV-1 escape also had well-controlled infection. This is similar to previous reports [15, 20, 22]. We did observe an association between plasma HIV-1 viral loads up to 400 copies/ml and CSF HIV-1 escape (p=0.02). However, this association was not observed when using a cut-off of 200 copies/ml (p=0.08), probably due to low sample size. Of note, the interpretation of this result is limited by the cross-sectional nature of our study and the lack of consecutive plasma viral load measurements. A similar association between CSF HIV-1 escape and viremia was reported by Nightingale *et al* in a prospective multicentre study of 153 patients in the United Kingdom, where CSF HIV-1 escape was found in seven out of 40 patients (18%) with low-level viremia compared to none of the 43 patients who had complete (plasma) viral suppression (p=0.005) [25]. Another cross-sectional study of 69 asymptomatic patients with undetectable viremia in two clinical centres in Sweden and California reported that the number of plasma viral blips was strongly associated with CSF HIV-1 escape, with a median of 2.5 blips (IQR 1-4) compared to zero blips (IQR 0-1) in the group without CSF HIV-1 escape (p=0.001) [17]. Recurrent CSF HIV-1 escape in patients with historical evidence of low-level viremia has been also described [19].

Regarding ART, the CPE score was not predictive of CSF viral escape in our study. Several other studies have failed to demonstrate an association between CPE and CSF viral escape [17, 20, 21]. Protease inhibitor-based regimens have been shown to be associated with more frequent CSF HIV-1 escape [16, 20, 21], although this was not confirmed in our study. Similarly, we found no difference in dolutegravir use between patients with and without CSF HIV-1 escape although patients on dolutegravir were under-represented in our series. Finally, we observed a trend towards more CSF HIV-1 escape among PLWH on dual therapy, as previously described [23]. This remains to be confirmed by studies with longer patient follow-up.

Two small retrospective case series have described patients with undetectable plasma HIV-1 RNA on ART who developed new neurological and/or neurocognitive symptoms without alternative diagnoses and who had detectable CSF HIV-1 RNA [3, 26]. In our study of 25 patients with CSF HIV-1 escape, almost half were diagnosed, paradoxically, as having non-HIV associated NCI. These patients were mostly from the neuro-HIV platform group and so symptomatic by definition. The diagnosis of non-HIV-associated NCI could have been based on concomitant depressive symptoms or diagnosed depression. Equally, NAMACO study factors not directly linked to HIV, such as toxic substance use or concomitant medicines with neurological toxicity, were present in some patients, which may have deferred or masked HIV-associated NCI according to current definitions [33, 37]. Some of these patients may still have been classified correctly as having non-HIV associated NCI, with CSF HIV-1 escape representing a CSF viral blip without clinical consequence rather than persistent CSF viral replication. In keeping with this, four patients (16%) diagnosed with CSF HIV-1 escape had no proven NCI.

It is unclear whether CSF HIV-1 escape represents an active CNS infection despite effective ART or whether, similar to plasma blips, it reflects low-level variations in release of virus into the CSF compartment. The clinical significance of these phenomena is yet to be elucidated. A retrospective longitudinal study by Eden *et al* showed that at least one CSF HIV-1 blip may occur in more than one third of asymptomatic PLWH, with only 3% having repeatedly detectable CSF virus and thus clinically relevant CSF HIV-1 escape [27]. At the other extreme, compartmentalisation of resistant HIV-1 in the CSF may occur with potentially devastating neurological consequences [7, 9], and this phenomenon should always be excluded if technically feasible [24]. In our study, we observed isolated HIV-1 resistance in CSF (with undetectable HIV-1 RNA in plasma) in 12% of patients with CSF HIV-1 escape, a similar figure to that reported in a series of 86 symptomatic patients [10]. Of note, the diagnosis of CSF HIV-1 escape resulted in ART modification in a quarter of our patients, illustrating the benefit of early diagnosis of CSF HIV-1 escape via LP. The choice of the new ART regimen was individualised and based on the treatment (pill burden, toxicity profile, drug-drug interactions), genotypic viral resistance testing, pharmacokinetics, and scientific evidence for CSF HIV-1 escape treatment available *at the time of patient assessment*. While this included review and/or optimisation of CPE score (study period 2011-2019), data from the NAMACO study subsequently demonstrated a non-association between CPE score and NCI [37].

Our study has limitations. First, a potential selection bias exists for NAMACO study participants who could be symptomatic or asymptomatic prior to neurocognitive assessment. Neuro-HIV platform patients were all symptomatic or suspected by clinicians of having NCI. Second, the cross-sectional design did not allow us to determine the persistence of CSF HIV-1 escape or the possibility of late-onset NCI development in initially asymptomatic PLWH with CSF HIV-1 escape, nor the clinical and virological outcome after ART modification. Furthermore, because

we lacked longitudinal analysis, we described patients as having low levels of viremia at the time of plasma sampling which is not the same as 'low-level viremia', a clinical entity which requires at least two consecutive plasma samples showing detectable HIV-1 RNA. Third, our analyses did not account for other neuropsychiatric conditions that can affect neurocognitive performance since these were assessed differently in the two study groups (depressive symptoms in the NAMACO study and formal psychiatric evaluation in the neuro-HIV platform). Fourth, the older age of patients included (mainly due to the NAMACO study inclusion criteria) may limit the generalisability of our findings in younger populations. Finally, while ART adherence is a critical factor in maintaining durable viral suppression [38] and low adherence has been shown to be associated with NCI [39], this could not be fully assessed in our study due to incomplete quantitative data.

Despite these limitations, this study adds to the body of literature reporting CSF HIV-1 escape, being, to date, the largest European case series and the largest case series worldwide studying exclusively symptomatic patients, and one of the largest using the latest CSF HIV-1 escape definition [12, 34]. In addition, our results are pragmatic and reflect real-life situations, while assessing CSF HIV-1 escape in a systematic way. Finally, the median age in our study was 53 years, partly due to the NAMACO age-related inclusion criterion, which is the oldest median age of PLWH in a CSF HIV-1 escape series. This is of particular importance given the ageing of PLWH which is associated with an increasing incidence of non-HIV-related comorbidities, including NCI of non-viral aetiology.

In conclusion, we observed CSF HIV-1 escape in 8.7% of patients with suspected or confirmed NCI in a large case series. Importantly, three quarters of our patients with CSF HIV-1 escape had undetectable plasma viral loads. With the exception of detectable plasma HIV-1 RNA, we found no reliable demographic, clinical, immunological, neurocognitive or radiological predictive factor for CSF HIV-1 escape. Hence, we

conclude that LP is the only means to detect CSF HIV-1 escape, and should be considered in all patients presenting NCI, especially in the absence of other factors not directly linked to HIV, as the identification of CSF HIV-1 escape through LP may prompt ART modification. However, in the exception of documented compartmentalized CSF resistance, it is still unknown whether and to what extent CSF HIV-1 escape might correlate with clinical outcome. Longitudinal studies will improve our understanding of the association between CSF HIV-1 escape and long-term CNS clinical outcomes by exploring the prevalence of viral persistence in the CSF and evaluating the clinical impact of ART modification in patients presenting CSF HIV-1 escape.

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AUTHOR CONTRIBUTIONS

Paraskevas Filippidis, Katharine Darling and Matthias Cavassini contributed to the study conception and design. Material preparation, data collection and analysis were performed by Paraskevas Filippidis, José Damas and Benjamin Viala. The first draft of the manuscript was written by Paraskevas Filippidis and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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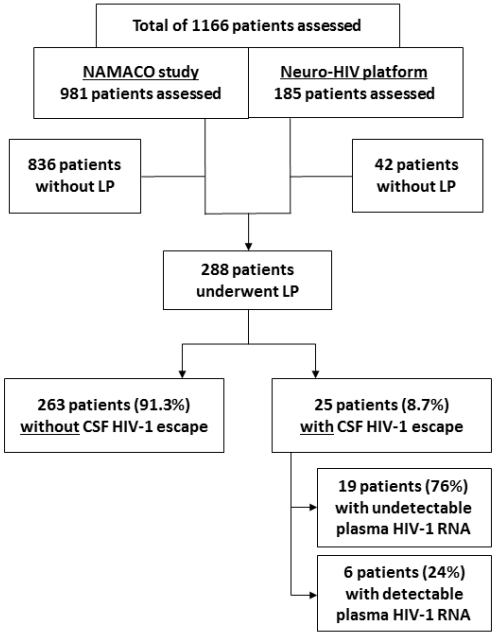
FIGURE CAPTIONS

Figure 1. Flow diagram of included patient



Authors, publication year	Geographical location	Study period	Total participants, n	Age (years)	Definition of CSF HIV-1 escape	Number of CSF HIV-1 escape cases	Estimated prevalence	Asymptomatic† patients with CSF HIV-1 escape, n (%)
Studies including asymptomatic patients								
Eden et al, 2010	Gothenburg, Sweden San Francisco, USA	2002-2010	69	45 (median)	PVL: <50, CVL: >50	7	10%	69 (100%)
Studies including both asymptomatic and neuro-symptomatic patients (sorted by CSF HIV-1 escape definition)								
Calcagno et al, 2015	Torino, Italy	NS	127	46 (median)	PVL: <50, CVL: >50	24	28.6%	NS
Pérez-Valero et al, 2019	USA (HNRC, CHARTER)	2003-2011	1264	46 (mean)	PVL: <50, CVL: >50	55	4.4%	34 (61.8%)
De Almeida et al, 2020	Paraná, Brazil	2018	68	43 (median)	PVL: <50, CVL: >50	7	10.3%	NS
Rawson et al, 2012	London, UK	2008-2010	142	45 (mean)	PVL: <50, CVL: >200 or CVL ≥0.5 $\log_{10}$ PVL	30	21%	NS (mainly symptomatic)
Mukerji et al, 2017	USA (Boston, NNTC)	2005-2016	626	50 (mean)	PVL: <50, CVL: >50 or CVL ≥0.5 $\log_{10}$ PVL	41	6.5%	30 (73.1%)
Trunfio et al, 2020	Torino, Italy	2010-2019	97	NS	PVL: <20, CVL: >20 or CVL ≥0.5 $\log_{10}$ PVL	19	19.6%	NS (mainly symptomatic)
Mukerji et al, 2018	USA (NNTC, CHARTER, HNRC)	2005-2016	1063	46 (mean)	PVL: <50, CVL: >50 or CVL > PVL	77	7.2%	33 (42.9%)
Studies including neuro-symptomatic patients (sorted by CSF HIV-1 escape definition)								
Bavaro et al, 2019	Bari, Italy	2001-2015	75	42.5 (median)	PVL: <25, CVL: >25	2	3%	0 (0%)
Kugathasan et al, 2017	London, UK	2011-2015	146	45 (median)	PVL: <50, CVL: >50	9	6.2%	0 (0%)
Di Carlofelice et al, 2018	London, UK	2015-2017	38	51 (median)	PVL: <20, CVL: >20 or CVL ≥0.5 $\log_{10}$ PVL	2	5.3%	0 (0%)
Dravid et al, 2018	Pune, Western India	2009-2017	71	38 (median)	PVL: <20, CVL: >20 or CVL ≥0.5 $\log_{10}$ PVL‡	20	28.2%	0 (0%)
Filippidis et al, 2022 (current study)	Lausanne, Switzerland	2011-2019	288	53 (median)	PVL: <LOQ, CVL: >LOQ or CVL > PVL	25	8.8%	0 (0%)

Table 1. Prevalence of CSF HIV-1 escape in previous studies (2011-2018)

Studies are grouped based on the presence of symptoms and the definition used for CSF HIV-1 escape. When CSF HIV-1 escape was presented separately from plasma/CSF HIV-1 discordance in a single study, only prevalence of CSF HIV-1 escape is provided in this table.

† Patients were asymptomatic if they reported no neuropsychological symptoms and if they presented no neuropsychological abnormalities.

‡ In this study, the criteria listed are referred to as CSF/plasma HIV-1 discordance.

Abbreviations: CHARTER: CNS HIV AntiRetroviral Therapy Effects Research cohort, CSF: cerebrospinal fluid, CVL: CSF viral load, HNRC: HIV Neurobehavioral Research Center (HNRC) cohort, LOQ: Limit of quantification, NNTC: National NeuroAIDS Tissue Consortium, NS: not specified, PVL: plasma viral load

	Total (N=288)	HIV-1 CSF escape (N=25)	No HIV-1 CSF escape (N=263)	*p*
Demographic characteristics				
Female sex	97 (33.5)	9 (36.3)	88 (33.5)	0.8
Age (years)	53 (47 – 59)	53 (48 - 58)	53 (47 - 60)	0.9
Ethnicity				0.8
• White	226 (78.5)	19 (76)	207 (78.7)	
• Black	46 (16)	4 (16)	42 (15.9)	
• Hispano-American	12 (4.2)	2 (8)	10 (3.8)	
• Asian	1 (0.4)	0 (0)	1 (0.4)	
• Other	3 (1.1)	0 (0)	3 (1.1)	
Comorbidities				
Active smoker	101 (35.1)	10 (40)	91 (34.6)	0.8
Metabolic disorder†	26 (9.1)	2 (8)	24 (9.1)	0.8
Cardiovascular disease	36 (12.5)	1 (4)	35 (13.3)	0.1
HIV-related characteristics				
Time from HIV diagnosis (years)	15.5 (9 – 23)	15 (9 - 26)	16 (9 - 23)	0.9
CD4 count nadir (/mm^3)	171 (81 – 237)	158.5 (81 – 236.5)	171 (70 - 278)	0.6
CD4 count current (/mm^3)	600 (446 – 781)	558 (459 - 646)	611 (444 – 790)	0.2
Plasma HIV-1 RNA - detectable	34 (11.8)	6 (24)	28 (10.7)	**0.04***
Low level viremia, plasma HIV RNA > 50 and:				
• < 200 copies/ml	14 (4.9)	3 (12.5)	11 (4.2)	0.08
• < 400 copies/ml	17 (5.9)	4 (16.7)	13 (4.9)	**0.02**
• < 500 copies/ml	17 (5.9)	4 (16.7)	13 (4.9)	**0.02**
ART regimen				
• NNRTI-based therapy	80 (27.8)	5 (20.0)	75 (46.3)	0.6
• PI-based therapy	73 (25.4)	8 (32.0)	65 (24.8)	0.7
• INSTI-based therapy	95 (33.2)	5 (20.8)	90 (34.4)	0.2
• Dual therapy	5 (1.7)	1 (4.0)	4 (1.5)	0.07
CSF Penetration-Effectiveness (CPE) score	7 (7 – 9)	7 (7 - 9)	7 (7 - 9)	0.8
CSF analysis				
CSF HIV-1 RNA	0 (0 - 0)	49 (35 – 190)	0 (0 - 0)	**<0.001**
CSF white blood cell count ≥ 5 cells/mm^3	28 (10.9)	9 (36.0)	19 (8.2)	**<0.001**
CSF proteins >450 mg/l	142 (50.0)	14 (56.0)	128 (49.4)	0.5
CSF-specific oligoclonal bands‡	84/135 (62.9)	7 (63.6)	77 (62.6)	0.9
Neuropsychological classification				
Frascati criteria				0.9
• Absence of NCI	44 (15.3)	4 (16.0)	40 (15.2)	
• Asymptomatic NCI (ANI)	127 (44.1)	9 (36.0)	118 (44.9)	
• Mild neurocognitive disorder (MND)	14 (4.9)	1 (4.0)	13 (4.9)	
• HIV-associated dementia (HAD)	1 (0.4)	0 (0)	1 (0.4)	
• Non-HIV-associated NCI	102 (35.4)	11 (44.0)	91 (34.6)	
Radiology				
Abnormal brain MRI§	206 (71.5)	17 (68.0)	189 (71.9)	0.8

Table 2. Characteristics of patients with and without CSF HIV-1 escape

Data are median (Interquartile range) or number of cases (%).

† Diabetes mellitus or thyroid disorder

‡ Oligoclonal band data available only for patients undergoing lumbar puncture within the neuro-HIV platform

§ Using standard MRI protocols

* Cohen's d = 0.49 (95% CI: 0.08–0.90)

Abbreviations: NNRTI: Non-nucleoside reverse transcriptase inhibitors, PI: Protease inhibitors, INSTI: Integrase strand transfer inhibitor, CSF: cerebrospinal fluid, NCI: neurocognitive impairment, MRI: magnetic resonance imaging

Patient	Patient group	Age (years)	Sex	Time from HIV diagnosis (years)	CD4 count current (cells/mm^3)	CD4 count nadir (cells/mm^3)	ART on diagnosis of CSF HIV-1 escape	Plasma HIV-1 RNA (copies/mL)	CSF HIV-1 RNA (copies/mL)	CSF analysis (Cells, Proteins)	Isolated CSF HIV-1 resistance	ART modification within 6 months
1	NAM	61	F	15	646	110	ABC-3TC-RAL	0	40	2/mm^3, 305 mg/l	No	No
2	NAM	75	M	12	1054	34	ABC-3TC-DTG	0	20	2/mm^3, 478 mg/l		
3	NAM	59	M	22	506	19	TDF-FTC-DRV/r-ETV	0	49	1/mm^3, 430 mg/l		
4	NAM	51	F	9	933	257	TDF-FTC-EFV	0	35	1/mm^3, 270 mg/l		
5	NAM	62	M	19	516	119	RPV-DTG	0	160	5/mm^3, 680 mg/l		
6	NAM	52	F	7	524	170	Unknown (study drug)	0	29	1/mm^3, 240 mg/l		
7	NAM	64	F	2	365	185	TDF-FTC-EFV	0	31	2/mm^3, 700 mg/l		
8	NAM	57	M	28	646	231	ABC-FTC-TDF-FAPV/r	0	55	3/mm^3, 292 mg/l		
9	NAM	56	M	30	323	123	ABC-3TC-ATV/r	0	248	15/mm^3, 596 mg/l		AZT-3TC-RAL-DRV/r†
10	NAM	76	M	12	635	147	TAF-FTC-NVP	0	55	1/mm^3, 605 mg/l		TAF-FTC-NVP-DRV/r†
11	NAM	58	M	30	698	60	TDF-FTC-DRV/r	0	126	25/mm^3, 564 mg/l		TDF-FTC-DRV/r-DTG-ETV†
12	NAM	62	M	30	670	80	TDF-FTC-DRV/r	0	499	18/mm^3, 490 mg/l		TDF-FTC-DTG-NVP†
13	NAM	57	M	26	480	200	ABC-3TC-ATV	0	28	1/mm^3, 380 mg/l		ABC-3TC-DTG†
14	PLAT	53	M	13	601	147	TDF-FTC-ATV/r	34	58	0.5/mm^3, 349 mg/l		No‡
15	PLAT	36	F	5	486	190	TDF-FTC-EFV	0	37	1/mm^3, 267 mg/l		
16	PLAT	43	F	16	223	82	ABC-3TC-DTG	0	35	3/mm^3, 250 mg/l		
17	PLAT	56	M	22	610	203	TDF-FTC-DRV/r	306	2040	5/mm^3, 637 mg/l		Yes§
18	PLAT	57	M	33	578	252	TAF-FTC-DRV/r	60	190	4/mm^3, 518 mg/l		
19	PLAT	43	M	6	558	307	TDF-FTC-RAL	0	43	5/mm^3, 354 mg/l		TDF-FTC-RAL-DRV/r
20	PLAT	47	M	1	171	5	TDF-FTC-RPV	0	27	0.2/mm^3, 590 mg/l		TDF-FTC-RPV-MVC
21	PLAT	64	M	10	406	406	TDF-FTC-ETV-MVC	0	43	4/mm^3, 2081 mg/l		ABC-3TC-ETV-MVC
22	PLAT	43	F	21	559	255	TAF-FTC-NVP	34	40	5/mm^3, 361 mg/l		TAF-FTC-EVG/c-NVP
23	PLAT	48	M	9	349	242	TDF-FTC-DRV/r-RAL	80	301	2/mm^3, 586 mg/l	Yes	TDF-FTC-DRV/r-ETV-MVC
24	PLAT	49	F	12	1322	67	TDF-FTC-DRV/r-MVC	59	930	8/mm^3, 956 mg/l		FTC-DRV/r-MVC-ETV-DTG
25	PLAT	60	F	34	459	NA	ATV/r	41	2720	20/mm^3, 536 mg/l		ABC-3TC-DTG

Table 3. Characteristics of patients diagnosed with CSF HIV-1 escape.

Abbreviations: ART: antiretroviral treatment, CSF: cerebrospinal fluid, F: female, M: male, NAM: NAMACO, PLAT: neuro-HIV platform, ABC: abacavir, 3TC: lamivudine, ATV: atazanavir, /r: ritonavir (booster), RAL: raltegravir, TAF: tenofovir alafenamide fumarate, FTC: emtricitabine, NVP: nevirapine, DRV: darunavir, DTG: dolutegravir, ETV: etravirine, EFV: efavirenz, RPV: rilpivirine, FAPV: fosamprenavir, MVC: maraviroc, NA: not available

† Reason for ART change was not specified (CSF HIV-1 escape, toxicity or other).

‡ Reassessment with a new lumbar puncture was proposed at 6-12 months to exclude CSF viral rebound.

§ ART modification was proposed, but adopted by the patient's treating infectious disease physician only at a later stage (upon receipt of the CSF HIV-1 resistance profile).