Antiretroviral naïve and treated patients can harbor viruses in CSF more resistant than in plasma.

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ABSTRACT

Background: The neurological disorders in HIV-1 infected patients remained prevalent. The issue of whether HIV-1 in cerebrospinal fluid (CSF) originates from viral replication sources in central nervous system or from peripheral blood and the consequences on antiretroviral (ARV) resistance is still a subject of debate. In this study, the resistance of HIV-1 in plasma and CSF was compared in a multicenter study supported by the "Agence Nationale de Recherche sur le Sida et les Hépatites Virales" (ANRS).

Methodology: Blood and CSF samples were collected at time of neurological disorders for 244 patients in 22 centers in France and 1 center in Switzerland. Demographic data and ARV treatment were collected. The viral loads (VL) were > 50 copies/mL in both compartments. Bulk genotypic resistance tests were realized on both plasma and CSF. The genotypic susceptibility score (GSS) of current treatment was calculated according to the 2013 ANRS AC-11 genotype interpretation algorithm: 0 (resistant), 0.5 (intermediate) or 1 (susceptible) to the drugs. Comparisons between groups were performed by using non-parametric tests.

Results: On 244 patients, 89 and 155 were ARV-naïve and ARV-treated, respectively. The variables were in median: age 42.79 and 44.61 years; CSF VL 4.64 and 4.09 log_{10} copies/mL; plasma VL 5.10 and 3.70 log_{10} copies/mL; nadir CD4 119 and 59 cells/mm^3; CD4 131 and 230 cells/mm^3 for ARV-naïve and ARV-treated respectively. In ARV-naïve patients, detection of mutations in CSF and not in plasma viruses were reported for the reverse transcriptase (RT) gene in 2 of 89 patients (2.2%) (K101E, Y181C, M184I, T215Y) and for the protease gene in 11 of 89 patients (12.36%) (L10I/V, V11I, L33F/V, M36I, I62V, L63P, V77I, V82A, L89M). In ARV-treated patients, 19 of 155 (12.3%) patients had HIV-1 mutations only in the CSF for the RT gene and 30 of 155 (19.4%) patients for the protease gene. Three mutations appeared statistically more prevalent in the CSF in comparison with the plasma: M41L (p= 0.0455), T69N (p= 0.0455) and T215Y (p=0.0455). Furthermore, 79.7% of ARV-treated patients had viruses with a similar GSS of current treatment in the two compartments, 11.5% with a GSS in CSF < GSS in plasma, and 8.8% with a GSS in CSF > GSS in plasma.

Conclusions: For ARV-naïve and ARV-treated patients, in most of cases, resistance mutations were present in both studied compartments. However, in some cases, the virus was more resistant in CSF than in plasma reducing the number of future therapeutic options. Then, in case of neurological troubles with a positive HIV-1 VL in CSF, the genotype should be recommended both in plasma and CSF to adjust the ARV handling.

BACKGROUND

- Infection of the central nervous system (CNS) with human immunodeficiency virus 1 (HIV-1) can lead to the development of HIV-1-associated dementia, HIV-1 encephalitis or neurological disorders.
- Although, treatment with fully active antiretrovirals (ARV) has reduced the frequency of HIV-1 infections of the CNS, neurological disorder symptoms are still observed in HIV-1 ARV-naïve patients and ARV-treated patients [1-6].
- HAART has an effect on HIV-1 RNA levels in cerebrospinal fluid (CSF) [7].
- Low therapeutic concentrations of the ARV could contribute to viral resistance, and consequently, lead to virological failure [8]. Such differences of ARV concentrations in several compartments could result in discordant HIV-1 RNA levels between plasma and CSF and/or ARV drug resistance mutations [9-12].

OBJECTIVES

- Currently, little information on the resistance patterns in the CNS is actually available in clinical practice in a large cohort of patients having a viral load (VL) > 50 copies/mL in plasma and in CSF.
- In this study, the resistance of HIV-1 in plasma and CSF was studied and compared in a multicenter study supported by the ANRS.

PATIENTS AND METHODS

- **Patients**
  - 244 patients who experienced VL > 50 copies/mL in plasma and CSF at time of neurological disorders : 89 ARV-naïve and 155 ARV-treated.
  - 22 centers in France and 1 center in Switzerland.
  - Participating laboratories belong to Agence Nationale de Recherches sur le Sida et les hepatites virales (ANRS) and participate in the ANRS quality control assessment of HIV-1 drug resistance sequencing [13].
  - The study was approved by the scientific committee of Action Coordonnée 11-AC11 ANRS.

- **Genotyping resistance testing**
  - Reverse transcriptase (RT) and protease resistance mutations were determined in each laboratory using the ANRS consensus technique (http://www.hivfrenchresistance.org), a Bayer TrueGene Kit, an Abbott ViroSeq Kit or an in-house method.
  - In ARV-naïve patients, protease and RT mutations were identified from the consensus statement of the list for genotypic surveillance of transmitted HIV-1 drug resistance [14].
  - In ARV-treated patients, RT and protease mutations were identified from the last International AIDS Society-USA (IAS) resistance testing panel (http://www.iasusa.org) and were interpreted with the last ANRS genotypic algorithm (http://www.hivfrenchresistance.org).
  - According to the ANRS algorithm, the global genotypic susceptibility score (GSS) was calculated on ARV currently available (n=18) as follows: 1 for a sensitive drug and 0 for a resistant or possible resistant drug.

- **Statistical methods**
  - Quantitative variables were summarized by means of median and Inter Quartil Range (IQR) and qualitative variables by percent.
  - Comparisons between independent groups were performed using Kruskal-Wallis non-parametric test. The McNemar test was applied to compare the prevalence of mutations in both plasma or CSF in the same group of patients. No correction for multiple testing was made and the analysis was done with SAS (version 9.4).

RESULTS

Characteristics	ARV-naive Patients (n=89)	ARV-treated Patients (n=155)
Age, years (IQR)	42 (37-50)	44 (38-51)
Male (%)	68.8	61.0
B subtype in CSF (%)	55.8	51.0
B subtype in plasma (%)	54.8	51.1
CSF HIV-1 RNA, log_{10} copies/mL, [median (IQR)]	4.64 (3.78-5.23)	4.09 (3.45-4.71)
Plasma HIV-1 RNA, log_{10} copies/mL, [median (IQR)]	5.10 (4.54-5.72)	3.70 (2.73-4.69)
Nadir CD4 cell count/mm^3, [median (IQR)]	119 (27-332)	59 (18-156)
CD4 cell count/mm^3 [median (IQR)]	131 (38-343)	230 (102-413)
Current treatment (%)		
NRTIs+PIs	na	62.6
NRTIs+PIs+IIs+other	na	11.0
NRTIs+NNRTIs	na	5.2
PIs	na	5.2
Other	na	16.0

Table 1: Characteristics of the studied population (n=244)
IQR, interquartile range; CSF, cerebrospinal fluid; na, non-applicable; NRTIs, nucleoside reverse transcriptase inhibitors; NNRTIs, non-nucleoside reverse transcriptase inhibitors; PIs, protease inhibitors; IIs, integrase inhibitors. The other combinations were present at less than 5%: NRTIs; NRTIs+PIs+entry inhibitors; PIs+entry inhibitors; NRTIs+PIs+IIs; NRTIs+PIs+IIs+entry inhibitors; NRTIs+NNRTIs+PIs+entry inhibitors; NRTIs+IIs; NRTIs+IIs+other; PIs+IIs; NNRTIs+PIs; NRTIs+NNRTIs+PIs; NRTIs+NNRTIs+PIs+IIs+other.
In the regimen of ARV-treated patients, NRTIs: FTC/3TC (47.5%), ABC (25.8%) and TDF (25.0%); PIs: LPV (20.5%), DRV (14.7%) and ATV (13.1%). NNRTIs: (13.1%).

A

Plasma \ CSF	9	10	11	12	14	15	16	17	18	Total
9	1									1
11			1							1
12				1						1
14		1			2					3
15						3				3
16							2			2
17								1		1
18								2	75	77
Total	1	1	1	1	2	3	2	3	75	89

B

Plasma \ CSF	2	3	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Total
2	1																1
3		1															1
4			1														1
5			2														2
6				2				1*									3
7					2												2
8						2											2
9							1										1
10							1	1			1						3
11									4								4
12								1		4	1	1			1		8
13							1				7						8
14			1	1							1	14				1	18
15													9	1		1	11
16										1		1		13	1	1	17
17												1	2		23	2	28
18													1		5	32	38
Total	1	1	4	3	2	2	3	2	5	5	10	17	12	14	30	37	148

Table 3: Comparison of global genotypic susceptibility score of HIV-1 viruses in plasma and CSF compartments.
A: in naïve patients; B: in treated patients

The results are expressed in the table as the number of patients having a global genotypic susceptibility score in plasma and in CSF.
In green, HIV-1 had a higher global GSS in plasma than in CSF. In red, HIV-1 had a similar global GSS in plasma and CSF. In blue, HIV-1 had a higher global GSS in CSF than in plasma.
- This patient had a global GSS at 11 in CSF and 6 in plasma.

A: In ARV-naive patients
96.6% (86/89) **similar** GSS between CSF and plasma;
3.4% (3/89) with a **GSS CSF < GSS plasma;**
0% (0/89) with a **GSS CSF > GSS plasma.**

B: In ARV-treated patients
79.7% (118/148) **similar** GSS between CSF and plasma;
11.5% (17/148) with a **GSS CSF < GSS plasma;**
8.8% (13/148) with a **GSS CSF > GSS plasm**

	ARV-naive patients	ARV-treated patients
HIV-1 drug resistance	• **17.9%** (16/89) sequences with at least one mutation in **plasma** • **11.2%** (10/89) patients with resistance in **plasma.** • **17.9%** (16/89) sequences with at least one mutation in **CSF** • **12.4%** (11/89) patients with resistance in **CSF.**	• **98.0%** (152/155) sequences with at least one mutation in **plasma** • **74.3%** (110/148) patients with resistance in **plasma.** • **98.0%** (152/155) sequences with at least one mutation in **CSF** • **75.0%** (111/148) patients with resistance in **CSF.**
Detection of mutations in CSF and not in plasma viruses	• **2.2%** (2/89) patients for **RT** gene • **12.4%** (11/89) patients for **protease** gene • K101E, Y181C, M184I, T215Y. • L10I/V, V11I, L33F/V, M36I, I62V, L63P, V77I, V82A, L89M.	• **12.5%** (19/152) patients for RT gene • **19.8%** (30/151) patients for protease gene • **M41L**, K65R, D67N, T69D, K70R, L74V, K101E, K103N, V108I, F116Y, Q151M, Y181C, M184V, M184I, G190A, L210W, **T215Y**, T215F, H221Y, M230I. • L10I, L10F, V11I, K20R, K20I, K20T, M36I, M36L, M46I, F53L, I62V, L63P, I64L, I64V, A71V, A71I, A71L, V77I, V82T, V82A, L89V, L89M, L90M, I93L, I93M.
Detection of mutations in plasma and not in CSF viruses	• **2.2%** (2/89) patients for **RT** gene • **9.0%** (8/89) patients for **protease** gene • Y181I , M184I. • L10I, V11I, G16E, M36I, I62V, L63P, I64V, V77I, I93L.	• **10.5%** (16/152) patients for **RT** gene • **13.9%** (21/151) patients for **protease** gene • D67N, **T69N**, K70R, K70E, L74V, V90I, V106I, V106M, V108I, E138Q, Y181C, Y188L, M184I, M184V, L210W, K219E, M230L. • L10I, L10F, G16E, K20I, L24I, V32I, L33I, L33F, M36I, M36L, M46I, I47V, G48V, I50L, I54V, I54L, I54M, Q58E, D60E, I62V, L63P, I64V, I64M, H69K, A71V, A71T, L76V, V77I, V82A, V82I, V82T, I85V, L89M, L90M, I93L.

Table 2: Comparisons of mutations and resistance in CSF and plasma.
In bold, mutations appeared statistically more prevalent: in the CSF: M41L (p= 0.0455); T215Y (p=0.0455). In the plasma: T69N (p= 0.0455).

CONCLUSIONS

- In most cases, HIV-1 resistance is similar between plasma and CSF.
- However, some cases were identified with HIV-1 more resistant in CSF than in plasma, reducing the number of future therapeutic options.
- In ARV-naïve patients, the prevalence of transmitted drug resistance was 11.2 %: similar results to the national sentinel surveillance of transmitted drug resistance in antiretroviral-naïve chronically HIV-infected patients in France over a decade (2001–2011) (9.0%) [15].
- In ARV-treated patients, the prevalence of HIV-1 drug resistance (74.3%) was superior to those previously shown in 2009 in French nationwide study (59%), possibly explained because the present study population is more advanced with higher VL (2.57 vs 3.70 log_{10} copies/mL) and lower CD4 (390 vs 230 cells/mm^3) [16].
- In 3.4% of ARV-naïve and 8.8% of ARV-treated patients, the virus was more resistant in CSF than in plasma.
- Some attention should be paid to patients with similar GSS in both compartments: the resistance mutation set could be different and resulting in different ARV resistance.
- Several factors could explain the discordant presence of HIV-1 mutations and/or ARV resistance between the plasma and the CSF: insufficient drug exposure [9], previous discontinuation of treatment [17], independent replication and evolution of resistance in the CNS [18], CNS infection leading to local immune activation [19, 20], previous virological failure in plasma allowing archiving of ARV-resistance HIV-1 strains in the CNS compartment [10].

- **In conclusion, these results from a large cohort from clinical routine care evidenced some discordances in HIV-1 mutations and/or resistance between plasma and CSF in HIV-1 patients with neurocognitive symptoms.**

- **These results support viral resistance genotypic testing in CSF for patients with neurological disorders and CSF VL > 50 copies/mL to optimize the ARV treatment.**

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