

INTRATHECAL IMMUNE ACTIVATION AND VIRAL CHANGES IN HIV-INFECTED NAÏVE PATIENTS WITH ADVANCED DISEASE AFTER SHORT TERM HAART TREATMENT

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ABSTRACT

Background. Compartmentalization of HIV in central nervous system (CNS) can lead to HIV-associated neurocognitive disorders (HANDs), either for persistent HIV replication in CNS or for HIV-related CNS immune activation. We correlated the change of CNS viral parameters and immune activation in naïve HIV-infected patients (pts) with advanced disease with or without HANDs after ART introduction.

Methods. HIV-infected pts with CD4 <200/ μ l starting ART with ZDV/3TC and LPV/r. Neurocognitive tests were performed at baseline. HIV-RNA, pro-inflammatory cytokines (IL-6, IL-10, INF- γ , TNF- α , TGF- β 1, TGF- β 2) and chemokines (MIP-1 α , MIP-1 β , MCP-1) were measured on plasma and cerebrospinal fluid (CSF) at baseline and T3 by enzyme-linked immunosorbent assay. Wilcoxon log rank sum test was used.

Results. At baseline HANDs was diagnosed in 5 out of 10 pts. Baseline CSF HIV RNA levels were not different between pts with normal and impaired tests (3.26 log₁₀ vs. 3.36 log₁₀ copies/ml). Conversely, when cytokine and chemokine expression was compared between the two groups, 10-fold higher median CSF IL-6 (3.5 vs 0.3 pg/ml) and MIP-1 β levels (2.1 vs 1.1 pg/ml) were found in pts with HANDs as compared to pts with normal cognitive function. All pts receiving ART showed significant decrease in plasma and CSF HIV RNA levels. However all the pts displayed a significant discrepancy in the HIV RNA in plasma and CSF Δ levels between T0 and T3 (1.5 log₁₀ copies/ml vs 0.0 log₁₀ copies/ml, respectively), indicating a more slowly HIV decrease in CSF than in plasma. Despite the significant drop in HIV RNA both in plasma and in CSF no significant changes in cytokine and chemokine expression were observed in CSF from T0 to T3 either in patients with HANDs or in pts without CNS involvement. In particular, despite effective HAART, IL-6 and MIP-1 β remained persistently higher in pts presenting HANDs.

Conclusions. Different decrease rate of HIV RNA between plasma and CSF support the compartmentalization of HIV replication in advanced disease. CSF HIV RNA levels did not seem to be primarily associated with impaired neurocognitive function. Conversely, pro-inflammatory cytokines levels were strictly associated to the development of cognitive disorders. Moreover, irrespective of HIV RNA control in CSF, cytokine and chemokine CSF expression was not modified by HAART. Both findings suggest a major role of pro-inflammatory cytokines in the pathogenetic mechanisms of HANDs development in advanced HIV infected pts.

BACKGROUND

HIV enters the CNS during the initial phases of infection, replicating in different cellular populations in each phase of the disease and leading to compartmentalization of HIV infection.

HIV-associated neurocognitive disorders (HANDs) affects up to 30% of HIV-infected people, and up to 50% of HIV-positive advanced-naïve patients despite the introduction of HAART.

Development of HANDs seems to be due to a double mechanism: viral replication in the CNS by itself and the subsequent intrathecal immune activation, principally sustained by activated glial cells and macrophages.

We analyzed the variation of HIV RNA level and immune activation at baseline and after 12 weeks of antiretroviral treatment in HIV-positive advanced naïve patients, with and without HANDs, with the following objectives:

- to assess the proportion of HIV-positive patients with HANDs and to possibly correlate the extent of HIV replication to the neuropsychological deficit
- to compare the impact of HAART on viral load decay in both the liquor and plasmatic compartments
- to evaluate the role of intrathecal immune activation as a marker of HANDs and to analyze the effect of HAART on immune activation parameters

STUDY DESIGN



- BLOOD SAMPLE**
 - CD4+, HIV-RNA
 - IL-6, IL-10, INF- γ , TNF- α , TGF- β 1, TGF- β 2, MIP-1 α , MIP-1 β , MCP-1

- LUMBAR PUNCTURE**
 - HIV-RNA
 - CMV-DNA, EBV-DNA, JC/BK-DNA
 - chemical-physical parameters
 - cytokine panel IL-6, IL-10, INF- γ , TNF- α , TGF- β 1, TGF- β 2
 - chemokine panel MIP-1 α , MIP-1 β , MCP-1

- NEUROPSYCHOLOGICAL EVALUATION**
- NEUROIMAGING** (cranium RMN)

- BLOOD SAMPLE**
 - CD4+, HIV-RNA
 - IL-6, IL-10, INF- γ , TNF- α , TGF- β 1, TGF- β 2, MIP-1 α , MIP-1 β , MCP-1

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METHODS

Patients: 10 HIV-positive patients with CD4+ cell counts < 200/ μ l, naïve to HAART (advanced naïve) (table 1).

Neuropsychological evaluation: trail-making A (speed of processing), trail-making B (executive function), Mauri et al. test (Episodic memory), matrix test (visual and selective attention, speed and accuracy of visual searching), Was-R vocabulary (language), Montgomery-Åsberg Depression Rating Scale (depressive symptoms).

Immunological evaluation: plasma and CSF were collected and used to evaluate:

- IL-6, IL-10, INF- γ , TNF- α , TGF- β 1, TGF- β 2, MIP-1 α , MIP-1 β , MCP-1.

Cytokines and chemokines concentration was assessed by commercial ELISA kit (R&D System, Minneapolis, MN) according to manufacturer's instructions.

Virological evaluation: CSF was used to evaluate HIV RNA by PCR (Taqman)

Statistical analysis: non parametric analyses (Mann-Whitney); comparisons between the different groups were made using a two-tailed T-test. Statistical analysis was performed using the SPSS statistical package (SPSS Inc. Chicago, Illinois, USA).

RESULTS

Table 1

Pt	Age	Sex	Race ^a	CDC	NPS ^b	BASELINE				3 MONTHS			
						CD4+ cell/ μ l	HIV-RNA plasma log ₁₀ cp/ml	HIV-RNA CSF log ₁₀ cp/ml	CSF white cell/ μ l	CD4+ cell/ μ l	HIV-RNA plasma log ₁₀ cp/ml	HIV-RNA CSF log ₁₀ cp/ml	CSF white cell/ μ l
1	27	M	I	C3	+	90	5.55	1.82	<1	116	2.18	1.59	1
2	53	M	C	C3	-	59	5.23	2.78	<1	116	1.59	1.59	1
3	48	M	I	C3	+	59	4.76	4.80	8	190	1.75	2.05	8
4	39	F	C	B3	-	27	5.15	1.87	<1				
5	49	F	C	C3	+	83	5.67	3.37	1				
6	25	F	I	B3	-	106	4.19	3.69	8	474	1.59	1.83	1
7	36	M	C	B3	+	26	5.62	2.62	1	49	1.59	1.59	1
8	26	M	A	C3	-	172	5.16	4.61	18	146	1.84	1.59	<1
9	40	M	C	C3	+	100	4.04	5.4	16	175	1.59	2.6	16
10	41	M	C	C3	-	42	5.87	3.26	<1	234	1.76	1.59	<1

^aI:Hispanic; ^bO:caucasian; ^cA:African.
^bNPS test +: HANDs, test NPS-: controls

Neuropsychological evaluation at baseline.

5/10 patients presented neurocognitive impairment, defined as asymptomatic or mild cognitive impairment. The most altered function was attentive and executive capacity (4/10) and episodic memory (3/10).

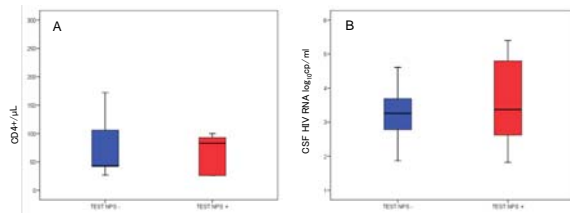


Figure 1. CD4+ T cell count and liquor viral profile at observation.

No significant differences in CSF HIV RNA levels were observed at baseline between patients with HANDs and controls (panel A).

Baseline CD4+ T cell counts were similar in the two groups (panel B).

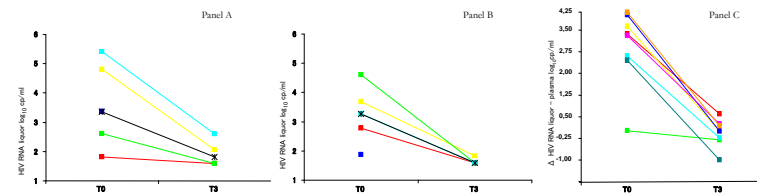


Figure 2. HAART effect on liquor and plasmatic HIV RNA replication.

After 3 months of HAART, CSF HIV RNA decreased in both group of patients, with HANDs (panel A) and controls (panel B), without significant differences. Comparison of the HIV viral decay in plasmatic and liquor compartments was analyzed by calculating the difference (Δ) between liquor and plasmatic HIV RNA at baseline and at T3 (panel C). A value > 0.5 log₁₀ copies/ml was observed in 9/10 patients, indicating that the viral load decreased in a slower way into the liquor compartment compared to the plasma.

Table 2. CNS immunoinactivation markers assessment. Values are medians (interquartile range).

Variable	HANDs T0 (liquor)	Controls T0 (liquor)	HANDs T3 (liquor)	Controls T3 (liquor)
MOP-1 pg/ml	638.91 (477.38-1177.9)	447.54 (416.66-501.08)	409.96 (312.76-578.44)	440.33 (405.34-477.36)
IL-6 pg/ml	3.5 (0.8-33.2)	0.2 (0-1.3)	2.3 (0.5-8.7)	3.45 (0-7.2)
IL-10 pg/ml	Undetectable (5/5 pts)	Undetectable (5/5 pts)	Undetectable (5/5 pts)	Undetectable (5/5 pts)
TNF- α pg/ml	Undetectable (5/5 pts)	Undetectable (5/5 pts)	Undetectable (5/5 pts)	Undetectable (5/5 pts)
INF- γ pg/ml	Undetectable (3/5 pts)	Undetectable (5/5 pts)	Undetectable (5/5 pts)	Undetectable (5/5 pts)
MIP-1 α pg/ml	Undetectable (3/5 pts)	Undetectable (4/5 pts)	0.3 (0-1.1)	Undetectable (4/5 pts)
MIP-1 β pg/ml	2.07 (0.9-3.12)	1.73 (0.2-2.3)	1.14 (0.1-3.6)	1.7 (0-2.3)
TGF- β 1 pg/ml	243.16 (35.7-578.44)	405.34 (50-473.26)	10.4 (0-25)	28.45 (0-28.15)
TGF- β 2 pg/ml	96.6 (59.2-155.9)	98.8 (19.7-147.1)	41.7 (32.9-138.3)	79.05 (68.01-94.4)

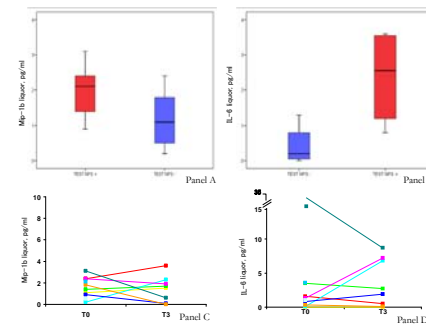


Figure 3. CSF concentration of immune activation markers

Immunoinactivation markers concentrations showed relevant differences between the two groups of patients. MIP-1 β concentration in patients with neurocognitive impairment was twice than that of patients without HANDs (2.1 pg/ml vs. 1.1 pg/ml) (panel A). Moreover, IL-6 concentration was 10 times higher in HANDs patients compared to controls (3.5 pg/ml vs. 0.315 pg/ml) (panel B). No significant alterations in cytokines and chemokines levels were observed after 3 months of HAART treatment in both groups of patients (panel C and D).

CONCLUSIONS

- Neurocognitive impairment is a frequent condition in HIV-positive advanced naïve patients and, in this group of subjects, liquor HIV RNA replication does not seem directly associated to the neurocognitive impairment.

- HAART is effective in reaching liquor viral load decay in patients with and without neurocognitive impairment, but the different reduction speed of HIV RNA in liquor and plasma testify for a persistent replicative activity of HIV within the intrathecal compartment in patients with advanced disease.

- The evaluation of intrathecal immune activation markers displayed higher immune activation in the presence of neurocognitive disorders. HAART was not effective in modifying the liquor concentration of immune activation markers in the brief period in patients with HANDs. These findings support the hypothesis of a critical role of intrathecal immunoinactivation in the pathogenesis of neurocognitive impairment in patients with severe immune depression and suggest the possibility of using these markers in the diagnosis and follow-up of HIV positive patients with HANDs