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Blood Brain Barrier function, ART neuroactivity and risk of CSF HIV escape

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Background. It is debated whether HIV cerebrospinal fluid (CSF) escape (HIVE) may be linked to patients' features or to ineffective antiretroviral (ARV) CSF penetration. Aim was to assess CSF penetration and activity of ARVs and correlation with blood brain barrier (BBB) dysfunction and to estimate risk and predictors of HIVE.
Methodology. Retrospective study on CSF/plasma paired samples from HIV+ patients taking cART undergoing lumbar puncture (LP) because neurologically symptomatic or for CNS staging. HIVE was: a) detectable CSF HIV-RNA with concurrent plasma <50 cp/mL; b) CSF HIV-RNA >1.0 $\log_{10}$ higher than in concomitant plasma. ARVs concentrations in CSF and plasma were measured by mass spectrometry methods. CSF/plasma concentration ratio was calculated and 95% CSF inhibitory quotients (IQ_{95}) derived, and considered optimal if >1. Impaired BBB was defined by CSF/plasma albumin quotient x 10^3 (Q_{alb}), according to age-normalized reference ranges (Reiber, Clin Chem, 1995). Adjusted ORs of HIVE were estimated by fitting a logistic multivariable regression model.
Results. 133 CSF/plasma pairs from 120 patients (2000-2014). Male 79.7%; median age 47y (IQR 40-51); CD4 count <200 cells/mm^3 47.4%. At LP, 58.7% was on TDF/FTC, 3% ABC/3TC, 10.5% ZDV/3TC, 36.8% EFV, 27.1% LPV/r, 13.5% ATV/r, 11.3% DRV/r. Proportion of samples with IQ_{95} >1 was lower for LPV/r and ATV/r (p=0.014). After stratification by Q_{alb}, the proportion of CSF with IQ_{95} >1 in the two strata was still similar for EFV, ATV/r and DRV/r, but not for LPV/r (p=0.05 at interaction test). A positive correlation between Q_{alb} and CSF/plasma ARV concentration ratio (R=0.686, p<0.001) and ARV IQ_{95} (R=0.658, p<0.001) was found.
HIVE was detected in 19/133 samples (14.4%). Only in a subset of 73 asymptomatic pts, duration of current cART [AOR 1.98 per year more (95%CI 1.27-3.12)] and CSF $\log_{10}$ HIV-RNA [AOR 17.91 (95%CI 1.04-307.55)] was associated to an increased HIVE risk by multivariable logistic regression. IQ_{95} of third drug was not associated with HIVE [AOR 0.84 95%CI 0.20-3.50], whereas Q_{alb} was associated to increased HIVE risk [AOR 5.75; 95%CI 1.10-30.1].
Conclusions. BBB dysfunction may influence neuropenetration (CSF/plasma drug concentration ratio) and efficacy (IQ_{95}) of ARVs and play a role on asymptomatic HIVE mechanism. Despite the fact that action of ARVs in the CNS, except for DRV/r, was far below, the role of drug exposure and activity in CSF on HIVE pathogenesis remains controversial.

Background

Blood Brain Barrier (BBB) plays a crucial role in the regulation of drug passage in the CNS. Chronic immune activation due to HIV replication in the CNS could be associated with an increased BBB permeability as well as several inter-individual factors (including age, gender and comorbidities), but also intrinsic characteristics of the specific molecules, such as legacy effects.

There are limited data on the association between viral replication and CSF antiretroviral concentrations. Thus, a BBB dysfunction affects the penetration of different antiretrovirals and the efficacy on viral replication in the intrathecal compartment.

Previously published data showed that the albumin ratio (Q_{alb}) is considered the best proxy of the BBB function and that CSF 95% inhibitory quotient (IQ_{95}) is a useful tool of integration between drug exposure and viral susceptibility.

Objectives

Aims of our study were:
- to assess CSF penetration and activity of ARVs in the CNS compartment and correlation with BBB dysfunction
- to determine factors associated with IQ_{95} >1
- to estimate risk and predictors of HIVE, overall and in a subgroup of samples from asymptomatic HIV positive patients

Methods

Cross-sectional study of 133 CSF/plasma paired samples from 120 cART receiving HIV+ patients undergoing lumbar puncture (LP) because neurologically symptomatic or for lymphoma staging.

HIVE was defined as: a) detectable CSF HIV-RNA with concurrent plasma <50 cp/mL; b) CSF HIV-RNA >1.0 $\log_{10}$ higher than in concomitant plasma.

ARVs concentrations in CSF and plasma were measured by mass spectrometry methods.

CSF/plasma ARVs concentration ratio and 95% CSF inhibitory quotient (IQ_{95}=CSF drug concentration/IC95) were calculated, and IQ_{95} was considered optimal if >1. Impaired BBB was defined by CSF/plasma albumin quotient x 10^3 (Q_{alb}) > age-normalized reference threshold .

Logistic regression analysis was used to determine factors associated with IQ_{95}>1 and HIVE. Variables explored at univariate level were: gender, age, risk factor for HIV, AIDS diagnosis, years of HIV infection, years of ARV exposure, HCV co-infection, CD4 and HIV-RNA at LP, nadir CD4, CPE score at LP, CSF protein, type of NRTI and of third drug used, year of LP, neurological signs and presence of neurological disease at LP, presence of BBB dysfunction. All these variables were retained in a multivariable model if their p-value was <0.1.

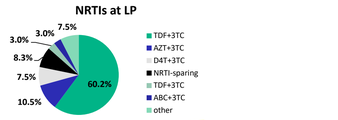
Results-1

Table 1. Characteristics of CSF/plasma pairs

CSF/plasma pairs (n=133) of 120 pts	
Male gender, n. (%)	106 (79.7%)
Age, years, mean (range)	47 (24-79)
Years of HIV infection, median (IQR)	5.4 (0.4-15.7)
Mode of HIV transmission, n. (%)	
Heterosexual	52 (39.1%)
MSM	37 (27.8%)
IVDU	34 (25.6%)
Other/unknown	10 (7.5%)
Clinical characteristics at LP	
Stage C, n. (%)	115 (86.5%)
HCV Ab positivity, n. (%)	28 (21.0%)
Months of cART exposure, median (IQR)	6.5 (2.6-42.3)
Nadir CD4<200 cell/mmc, median (IQR)	80 (30-153)
CD4, T-cell/mmc, median (IQR)	191 (88-337)
CD4 count <200 cell/mmc, n. (%)	64 (52.5%)
Plasma HIV-RNA, $\log_{10}$ cp/ml, median (IQR)	1.7 (1.6-2.5)
Plasma HIV-RNA<50 cp/mL, n. (%)	75 (56.8%)
CSF HIV-RNA, $\log_{10}$ cp/ml, median (IQR)	1.7 (1.6-2.2)
CSF HIV-RNA<50 cp/mL, n. (%)	87 (65.9%)

Neurological symptoms and diseases at LP	
Neurologic symptoms at LP, n. (%)	46 (34.6%)
Neurologic disease at LP, n. (%)	50 (37.6%)
Viral encephalitis	16 (32%)
Bacterial meningo-encephalitis	8 (16%)
HIV-encephalopathy	19 (38%)
CNS opportunistic infections	6 (12%)
Unknown	1 (2%)

CSF: cerebrospinal fluid; IQR: interquartile range; MSM: men who have sex with men; IVDU: intravenous drug users; LP: lumbar puncture; cART: combined antiretroviral therapy.



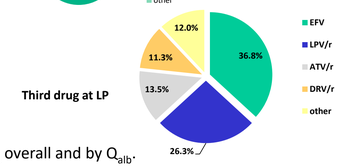
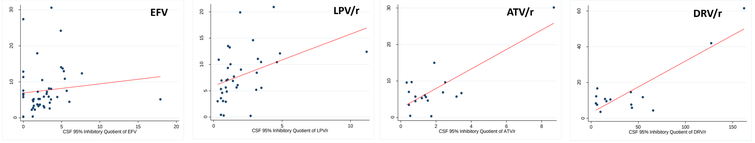


Table 2. CSF parameters on ARVs concentrations and IQ_{95} overall and by Q_{alb}.

Drugs^	Samples n.	HIV escape $	CSF PK, ng/mL, median (IQR)	CSF/plasma ARV drug ratio, median (IQR)	IQ_{95}, median (IQR)	Samples with IQ_{95}>1 *	Samples with IQ_{95}>1	
							Samples with Q_{alb} normal (intact BBB), 79/133 §	Samples with Q_{alb} abnormal (altered BBB), 53/133 #
EFV	49	5 (10.2%)	10.1 (5.9-17.0)	0.7% (0.2%-1.0%)	2.1 (1.2-3.6)	39 (79.6%)	28/35 (80.0%)	11/14 (78.6%)
LPV/r	36	8 (22.2%)	20.5 (13.8-51.0)	0.3% (0.2%-0.6%)	1.2 (0.8-3.0)	22 (61.1%)	7/19 (36.8%)	15/17 (88.2%)
ATV/r	18	4 (22.2%)	9.1 (4.0-15.6)	0.9% (0.5%-1.3%)	1.4 (0.6-2.4)	11 (61.1%)	8/13 (61.5%)	3/5 (60.0%)
DRV/r	15	2 (13.3%)	39 (12.7-104.2)	0.7% (0.5%-1.9%)	20.5 (6.7-54.8)	15 (100.0%)	4/4 (100.0%)	11/11 (100.0%)

^Drugs detected in <5 samples are not included in this table (nevirapine=4, nelfinavir=4, etravirine=2, raltegravir=2, rilpivirine=1, maraviroc=1, tipranavir=1); $ p at Chi square test =0.364; * p at Chi square test =0.014; § p at Chi square test = 0.006; # p at Chi square test =0.171.

Figure 1. Scatterplot of CSF 95% Inhibitory Quotient and Albumin Quotient



Pearson correlation R=0.12 p=0.428
Pearson correlation R=0.41 p=0.014
Pearson correlation R=0.79 p<0.001
Pearson correlation R=0.86 p<0.001

Results-2

Table 3. Factors associated with IQ_{95}>1 in all samples

	univariate		multivariate	
	OR (95% CI)	p-value	AOR (95% CI)	p-value
CSF Proteins > 45 mg/L	4.38 (1.52-12.64	0.006	2.44 (0.70-8.47)	0.159
BBB dysfunction	2.99 (1.17-7.61)	0.022	2.64 (0.66-10.53)	0.170
HIV escape	1.35 (0.41-4.43)	0.620	0.88 (0.18-4.21)	0.875
Third drug				
EFV	1.00		1.00	
LPV/r	0.43 (0.16-1.15)	0.094	0.38 (0.12-1.24)	0.111
ATV/r	0.40 (0.12-1.30)	0.129	0.37 (0.05-2.87)	0.344
DRV/r §	-	-	-	-
other	0.26 (0.01-4.47)	0.351	0.88 (0.18-4.21)	0.875

§ risk not calculable because all samples had IQ95>1
In the multivariate model interaction between BBB dysfunction and third drug was tested, p<0.05

Table 4. Factors associated with IQ_{95} >1 in samples stratified by BBB dysfunction

	In 79 (59.4%) samples with Q_{alb} normal		In 53 (39.8%) samples with Q_{alb} abnormal	
	AOR (95% CI)	p-value	AOR (95% CI)	p-value
CSF Proteins > 45 mg/L	1.53 (0.33-7.00)	0.581	13.38 (0.69-260.31)	0.087
HIV escape	1.57(0.12-20.53)	0.729	1.08 (0.07-17.33)	0.958
Third drug				
EFV	1.00		1.00	
LPV/r	0.16 (0.04-0.66)	0.011	10.67 (0.38-298.98)	0.164
ATV/r	0.49 (0.11-2.24)	0.361	0.65 (0.04-11.74)	0.768
DRV/r§	-	-	-	-
Other§	0.28 (0.03-2.44)	0.252	-	-

§ risk not calculable because all samples have IQ95>1

Results-3

Table. 5 HIVE associated factors in all samples

n=133	OR (95%CI)	p-value	AOR (95%CI)	p-value
Type of current regimen				
2 NRTIs+NNRTI	1.00		1.00	
2 NRTIs+PI/r	2.24 (0.79-6.34)	0.130	1.33 (0.33-5.39)	0.687
other	0.32 (0.04-2.83)	0.305	0.43 (0.01-18.17)	0.658
$\log_{10}$ CSF HIV-RNA (1 log more)	3.04 (1.92-4.80)	<0.001	5.25 (2.35-11.70)	<0.001
Q_{alb} abnormal vs normal	2.25 (0.88-5.78)	0.092	1.46 (0.38-5.60)	0.578
Duration of current cART (1 month more)	1.02 (1.00-1.04)	0.113	1.03 (1.00-1.05)	0.019
IQ_{95} of third drug>1	1.30 (0.46-3.65)	0.061	3.01 (0.80-11.33)	0.104

Table 6. HIVE associated factors in n=74 samples from neuro-asymptomatic patients

n=74	OR (95%CI)	p-value	AOR (95%CI)	p-value
Type of current regimen				
2 NRTIs+NNRTI	1.00		1.00	
2 NRTIs+PI/r	3.81(0.91-15.91)	0.067	0.65 (0.13-3.36)	0.608
other	-		-	-
$\log_{10}$ CSF HIV-RNA (1 log more)	5.40 (1.78-16.36)	0.003	17.91 (1.04-307.55)	**0.047**
Q_{alb} abnormal vs normal	3.38 (0.96-11.90)	0.058	5.75 (1.10-30.09)	**0.038**
Duration of current cART (1 month more)	1.04 (1.00-1.07)	0.024	1.98 (1.27-3.12)	**0.003**
IQ_{95} of third drug >1	0.89 (0.23-3.36)	0.861	0.84 (0.20-3.50)	0.816

Conclusions

Our data showed that there is an association between abnormal Q_{alb} levels and HIVE in a subgroup of neurologically asymptomatic patients. However, the role of drug exposure and activity in CSF on HIVE pathogenesis remains controversial.

BBB dysfunction may influence neuropenetration and the efficacy of ARVs. In the absence of BBB dysfunction, neuropenetration of the used drugs does not seem to produce the same effects. DRV/r showed an elevated neuroactivity independently of the presence of BBB dysfunction.

More data are needed to establish the actual impact of ARVs on the CNS both in terms of virological efficacy and neurotoxicity.

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