HIV-1 RNA detection in cerebrospinal fluid in those on antiretroviral therapy experiencing incomplete viral suppression in plasma

Sam Nightingale[1], Anna Maria Geretti[1], Martin Fisher[2], Alan Winston[3], Mark Nelson[4], Steve Taylor[5], Andrew Ustianowski, Jonathan Ainsworth, Richard Gilson, Edmund Ong, Clifford Lean, Jane Minton, Frank Post, Laura Else[1], Victoria Watson[1], Stacey King[1], Munir Pirmohamed[1], Tom Solomon[1,13], Saye Khoo[1], on behalf of the PARTITION study team

[1] University of Liverpool, [2] Royal Sussex Hospital, [3] Imperial College London, [4] Chelsea and Westminster Hospital, [5] Birmingham Heartlands Hospital, [6] North Manchester Hospital, [7] North Middlesex Hospital, [8] UCH/Mortimer Market Centre London, [9] Newcastle Royal Victoria Infirmary, [10] Edinburgh Western General Hospital, [11] Leeds General Infirmary, [12] Kings College Hospital, [13] Walton Centre for Neurology and Neurosurgery.

INTRODUCTION

The central nervous system (CNS) has been proposed as a sanctuary site where HIV-1 replication may continue during seemingly suppressive antiretroviral therapy (ART). Concentrations of most antiretrovirals (ARVs) in cerebrospinal fluid (CSF) are low, often falling below the minimum inhibitory concentration for wild type virus, and there is a large inter-individual variability. [1-6]

HIV-1 RNA can be at higher levels in CSF than in plasma.[7,8] Several studies have shown mutations conferring resistance to several ARV classes in the CSF when not present in plasma.[9-11] Altering ART based on CSF resistance data has led to clinical improvement and decrease in CSF HIV-1 RNA in case series.[2,12]

The goal of ART is sustained suppression of HIV-1 RNA in plasma, commonly interpreted as a viral load <50 copies/ml.[13] Some treated individuals do not maintain viral suppression below this level and it is not clear whether this is related to ongoing viral replication in sanctuary sites such as the brain.

Sensitive assays can now detect HIV-1 RNA below the 50 copies/ml threshold in a proportion of stably treated patients. However the source and significance of residual plasma HIV-1 RNA detection at this level remains controversial.[14]

The aim of this study was to investigate HIV-1 RNA levels and ART concentrations in the CSF in those with a) intermittent or persistent low-level plasma HIV-1 RNA detection above 50 copies/ml, and b) residual plasma HIV-1 RNA detection below 50 copies/ml.

METHODS

Penetration of AntiRetroviral Therapy InTO the Nervous system (PARTITION) was a multicentre UK study recruiting subjects from two groups: Group A – HIV-1 infected adults (≥18 years) undergoing investigation for possible neurological disease. Group B – Unexplained intermittent or persistent HIV-1 RNA detection ≥50 copies/ml within the previous 12 months, in the absence of neurological disease.

HIV-1 RNA levels were measured by the Abbott RealTime HIV-1 assay. A modified assay was used for sensitive HIV-1 RNA testing in subjects with unquantifiable HIV-1 RNA levels on standard testing. The assay 50% and 95% detection thresholds were 1 and 3 HIV-1 RNA copies/mL, respectively for 8 ml plasma input.

CSF/plasma discordance was defined as CSF HIV-1 RNA levels >0.5 $\log_{10}$ copies/ml higher than measured in the matched plasma sample or the upper range of the limit limit of sensitivity in plasma (40 copies/ml). Residual plasma HIV-1 RNA detection was defined as a level between 1 and 49 copies/ml.

Discordant samples underwent sequencing of reverse transcriptase (RT, amino acids 1 to 335) and protease (amino acids 1 to 99) to detect drug resistance mutations, as previously described (Doyle 2012). Mutations were evaluated according to the Stanford HIV Drug Resistance Database.[15]

ARV concentrations were determined in CSF and plasma by tandem liquid chromatography-mass spectrometry. Drugs were extracted by protein precipitation (plasma) or directly injected (CSF). Standard curves were made up in artificial CSF (Harvard Apparatus, Holliston, MA, USA), and matrix effects assessed by post column injection. Assays were validated against repeated runs of quality controls (inter and intra-assay variability) and EQA standards.

The revised CPE score (2010) was calculated for each ART regimen.[16]

Statistical analysis

Mann Whitney U test was used to compare continuous variables. Fishers exact and Chi squared tests were used for categorical data. Log_{10} CSF and plasma HIV-1 RNA were compared with Wilcoxon matched-pairs signed rank test. Variables demonstrated to be significant ($p<0.1$) in univariate analysis went forward for multivariate logistic regression using SPSS version 20. This was done using a forward stepping process, adding one variable at a time starting with the smallest p values. A p value of 0.05 was used to determine statistical significance and a backward stepping approach was used to test the consistency of the models.

Ethics

Informed consent was obtained from all participants. The study was approved by the North Wales Research Ethics Committee (Central and East).

RESULTS

A total of 145 subjects were recruited, 105 (72%) from group A and 40 (28%) from group B. Overall CSF and plasma HIV-1 RNA levels were directly correlated, (Spearman r=0.5855; p<0.0001). HIV-1 RNA was lower in CSF than plasma, p=0.0362. Median HIV-1 RNA levels were 20 copies/ml (IQR 3,50) in CSF vs. 20 copies/ml (IQR 10,44) in plasma; 106 (73%) plasma HIV-1 RNA measurements and 108 (74%) CSF HIV-1 RNA measurements were below 50 copies/ml. The CSF/plasma discordance rate was 19 (13.1%) and in these subjects median HIV-1 RNA levels were 579 copies/ml (IQR 195,2028) in CSF vs. 14 copies/ml (IQR 10,78) in plasma (figure 1).

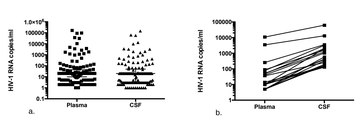


Figure 1.
a. HIV-1 RNA in plasma and CSF, n=145.
b. Plasma and CSF HIV-1 RNA in 19 subjects with CSF/plasma discordance

Factors associated with CSF/plasma discordance

Differences in measured variables among subjects with and without CSF/plasma discordance are shown in table 1. A logistic regression model including nadir CD4 and white-MSM was significantly associated with CSF/plasma discordance (p=0.008). Only white-MSM made a significant contribution to the model (p=0.031) suggesting the 2 variables explain the same variation in the outcome. Nadir CD4 was higher in white-MSM, median 174 cells/mm^3 (IQR 48,302) vs. median 102 cells/mm^3 (IQR 32,241), p=0.0369). Adjusted odds ratio for white-MSM was 0.176 (95% confidence interval 0.036,0.851) suggesting this group was associated with an 82.4% decreased risk of CSF/plasma discordance.

	CSF/plasma discordance		
	Yes (n=19)	No (n=126)	p value
Age, years (IQR)	46 (43,50)	46 (41,54)	0.9568
Gender male, n (%)	14 (73.6)	110 (87.3)	0.1552
Risk group, n (%)			0.094
MSM*	7 (36.8)	84 (66.7)	
Heterosexual	9 (47.3)	30 (23.8)	
IDU	0 (0.0)	3 (2.4)	
Vertical tx	1 (5.3)	2 (1.6)	
Not disclosed	2 (10.5)	7 (5.6)	
Ethnicity, n (%)			0.0737
White*	10 (52.6)	92 (73.0)	
Black	8 (42.1)	22 (17.5)	
Asian	1 (5.3)	5 (4.0)	
Other/mixed	0 (0.0)	7 (5.6)	
Years since HIV diagnosis, median (IQR)	10 (6.25,15.75)	9 (5,16)	>0.9999
CD4, median cells/mm^3 (IQR)			
Nadir	31 (21,196)	150 (47,280)	0.0174
Current	374 (189,472)	466 (307,700)	0.0919
ART at sampling, n (%) P1/r	14 (73.7)	75 (59.5)	0.3617
NNRTI	4 (21.1)	49 (38.9)	0.2006
NRTI	17 (89.5)	108 (85.7)	1.000
RAL	5 (26.3)	13 (10.3)	0.0631
MVC	3 (15.8)	7 (5.6)	0.1262
CPE, median (IQR)	7 (6,8)	7 (6,8)	0.6425
CSF WCC** >5 cells/mm^3 (%)	5 (45.5)	4 (4.1)	0.0004
CSF WCC**, median cells/mm^3 (IQR)	3 (0,21)	0 (0,2)	0.0388

Table 1.
Factors associated with CSF/plasma discordance.*White-MSM vs. other associated with CSF/plasma discordance, n=6 (7.5%) and 13 (20.0%) respectively (p=0.0455). **Excluding 17 subjects in group A with CNS infection, and 19 subjects with no CSF WCC measurement.

Within group A, 12 (11.4%) subjects had CSF/plasma discordance. The most frequent indications for LP in group A were to investigate cognitive symptoms, (n=43, 41.0%), and headache (n=20, 19.0%). Indication for LP, adherence and diagnosis of CNS infection did not differ significantly between those with and without CSF/plasma discordance (table 2).

	CSF/plasma discordance		
	Yes (n=12)	No (n=93)	p value
Indication for LP, n (%)			0.3449
Inv. cog symptoms	5 (41.6)	38 (40.9)	
Headache	2 (16.7)	18 (19.4)	
Abnormal neurological examination	1 (8.3)	6 (6.5)	
Follow up CNS infection [1]	3 (25.0)	5 (5.4)	
Seizure	0 (0.0)	5 (5.4)	
Measure CSF HIV viral load	0 (0.0)	2 (2.2)	
In clinical trial [2]	0 (0.0)	3 (3.2)	
Peripheral neuropathy	0 (0.0)	2 (2.2)	
Acute confusion	1 (8.3)	0 (0.0)	
Sepsis	0 (0.0)	1 (1.1)	
Staging for CNS lymphoma	0 (0.0)	1 (1.1)	
HTLV-1	0 (0.0)	1 (1.1)	
Possible neurosyphilis	0 (0.0)	7 (7.5)	
Possible encephalitis	0 (0.0)	2 (2.2)	
Possible multiple sclerosis	0 (0.0)	1 (1.1)	
Possible CNS malignancy	0 (0.0)	1 (1.1)	
CNS infection confirmed/not excluded, n (%)	3 (25.0)	14 (15.1)	0.4067
Self reported adherence ≥95%, n (%)	6 (75.0)	68 (87.2)	0.309

Table 2.
Details of group A: indications for lumbar puncture, outcome and adherence.

CSF HIV-1 RNA detection in subjects with and without consistent HIV-1 RNA suppression <50 copies/ml in plasma.

In group B the median plasma HIV-1 RNA over the preceding 12 months was 66 copies/ml (IQR 23,132). Thirty-three (83%) subjects had 1 or more plasma HIV-1 RNA measurement below 50 copies/ml within 12 months, the remaining 7 (17%) has persistent low-level viraemia, median 144 copies/ml (90,279).

Group B was compared to a subset of 39 subjects from group A with consistent HIV-1 RNA suppression below 50 copies/ml over the preceding 12 months, and no CNS infection (figure 2). Differences in measured variables between the two groups are shown in table 3. CSF/plasma discordance was not analysed in a logistic regression model as there were no subjects with discordance in the consistent suppression group.

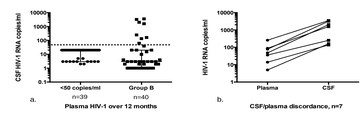


Figure 2.
a. CSF HIV-1 RNA in 40 subjects with intermittent or persistent HIV-1 RNA ≥50 copies/ml versus 39 subjects with consistent HIV-1 RNA suppression <50 copies/ml. Dashed line is at 50 copies/ml.
b. Plasma and CSF HIV-1 RNA in 7 subjects with CSF/plasma discordance

	Plasma HIV-1 RNA detection ≥50 copies/ml in past 12 months		
	Yes (n=40)*	No (n=39)**	p value
CSF/plasma discordance, n (%)	7 (17.5)	0 (0.0)	0.0117
Age, median years (IQR)	45.5 (40.25,51.75)	48.0 (41.0,57.0)	0.3369
Male gender, n (%)	35 (87.5)	35 (89.7)	1.000
Risk group, n (%)			0.5568
MSM	28 (71.8)	27 (67.5)	
Heterosexual	9 (23.1)	9 (22.5)	
IDU	0 (0.0)	1 (2.6)	
Vertical	1 (2.5)	0 (0.0)	
Not disclosed	1 (2.5)	3 (7.7)	
Ethnicity, n (%)			0.2138
White	34 (85.0)	27 (67.5)	
Black	5 (12.5)	8 (20.0)	
Asian	1 (2.5)	1 (2.5)	
Mixed/other	0 (0.0)	3 (7.5)	
Years since HIV diagnosis, median (IQR)	9.6 (4.7,17.4)	12.1 (9.1,20.3)	0.0393
CD4, median cells/mm3 (IQR)			
Nadir	132.0 (43.0,324.5)	195.5 (87.5,289.3)	0.3143
Current	520.0 (395.0,728.5)	523.0 (354.0,770.0)	0.9359
ART, n (%)			
PI	29 (72.5)	22 (56.4)	0.1624
NNRTI	12 (30.0)	17 (43.6)	0.2481
NRTI	33 (82.5)	34 (87.1)	0.7555
RAL	10 (25.0)	1 (2.6)	0.0069
MVC	1 (2.5)	2 (5.1)	0.6153
CPE, median (IQR)	7.0 (6.25,7.0)	7.0 (6.0,8.0)	0.9226

Table 3.
Factors associated with plasma HIV-1 RNA detection ≥50 copies/ml within 12 months. *Group B. **Subset from group A, excluding those with CNS infection, with persistent HIV-1 RNA suppression below 50 copies/ml for 12 months.

Residual HIV-1 RNA detection below 50 copies/ml

49 (33.8%) subjects (24 group A, 25 group B) underwent sensitive HIV-1 RNA quantification. Median volume of plasma tested was 4.5ml (IQR 3,6); median volume of CSF tested was 4ml (IQR 3,7). There was no correlation between volume of sample tested and virus detection (r=-0.1193, p=0.2422). Overall significantly more CSF samples had no HIV-1 RNA detected (n=39) than plasma (p<0.0001).

32 (65.3%) subjects had plasma HIV-1 RNA detection between 1-49 copies/ml, median 13 copies/ml (IQR 6,24). 17 (34.7%) subjects had no HIV-1 RNA detected in plasma; in these subjects the median plasma volume tested was 5ml (IQR 3.5,5.75) giving a median sensitivity of detection of 7 copies/ml (IQR 5,7).

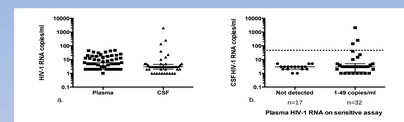


Figure 3.
a. CSF and plasma HIV-1 RNA in 49 subjects undergoing HIV-1 RNA quantification with sensitive assay
b. CSF HIV-1 RNA in 17 subjects with no residual plasma HIV-1 RNA detected versus 32 subjects with plasma HIV-1 RNA detection between 1 and 49 copies/ml. Dashed line is at 50 copies/ml.

	HIV-1 RNA copies/ml				
Subject	Group	Plasma	CSF	ART at sampling	CSF resistance
1	A	52	1,231	RAL/MVC/FTC/TDF	ns
2	A	3,443	13,088	EFZ/AZT/3TC	ns
4	A	10,817	63,010	RAL/FTC/TDF	ns
5	A	<40	400	DRV/ABC/3TC/RAL	ns
6	A	<40	422	ATZ/TDF/FTC	M184I
7	A	<40	335	DRV/FTC/TDF	ns
8	A	<40	162	DRV/AZT/3TC/MVC/ETR	ns
9	A	<40	129	DRV	ns
10	A	<40	579	ATZ/TDF/FTC	ns
11	A	<40	1,983	DRV/3TC/RAL/MVC	ns
12	A	<40	195	EFV/FTC/TDF	ns
13	B	14	138	DRV/FTC/TDF	M184I
14	B	36	254	EFV/FTC/TDF	ns
15	B	48	2,028	DRVr/TDF/RAL	D67N,K70R,L74V,L100I, K103N,M184V,T215Y, K219E/Y,143C
16	B	78	3,234	DRVr/MVC	ns
17	B	88	1,569	DRVr/FTC/TDF	ns
18	B	<40	162	DRVr/FTC/TDF	D67N,I/M184V,T215Y,I/K219Q,I/V108I,I/82A,I
19	B	258	3,518	DRVr/ABC/3TC	ns

Table 5.
Viral detection and CSF resistance in subjects with CSF/plasma discordance

	CSF HIV-1 RNA >0.5log$_{10}$ plasma		
	Yes (n=19)	No (n=126)	p value
Plasma			
Atazanavir, n	2	17	
Median (IQR)	1460 (305.0,2614)	1038 (366.5,1672)	NS
Darunavir, n	11	44	
Median (IQR)	2168 (1196,2732)	3535 (2148,5915)	0.0229
Etravirine, n	1	3	
Median (IQR)	362.7	362.5 (72.51,724.6)	NS
Lopinavir, n	0	3	
Median (IQR)		8731 (8206, 8807)	NS
Maraviroc, n	3	5	
Median (IQR)	169.2 (117.9,178.5)	232.4,88.06,523.4)	NS
Nevirapine, n	0	10	
Median (IQR)		4351 (2416,5004)	NS
Raltegravir, n	2	9	
Median (IQR)	680.1 (122.8,1237)	736.4 (155.4,1465)	NS
Rilpivarine, n	0	4	
Median (IQR)		79.07 (45.99,95.07)	NS
Ritonavir, n	0	15	
Median (IQR)		193.0 (69.0,282.0)	NS
Efavirenz, n	3	24	
Median (IQR)	3231 (714.8,6103)	1709 (1049,2480)	NS
Tenofovir, n	8	54	
Median (IQR)	72.07 (34.89,106.5)	98.06 (63.19,180.3)	0.1155
Emtricitabine, n	7	58	
Median (IQR)	122.8 (45.54,248.9)	260.8 (93.42,610.5)	0.3669
Lamivudine, n	5	19	
Median (IQR)	240.0 (46.5,632.0)	715.0 (136.0,1596)	0.0591
CSF			
Atazanavir, n	2	18	
Median (IQR)	23.87 (1.950,45.80)	11.17 (3.857,17.18)	NS
Darunavir, n	10	44	
Median (IQR)	26.79 (19.63,93.75)	42.37 (19.69,86.54)	0.7542
Lopinavir, n	0	3	
Median (IQR)		9.871 (6.386,15.02)	NS
Maraviroc, n	3	6	
Median (IQR)	47.58 (16.61,78.03)	7.079 (2.296,9.282)	NS
Nevirapine, n	0	12	
Median (IQR)		1497 (1183,2041)	NS
Raltegravir, n	3	11	
Median (IQR)	19.82 (18.04,170.6)	21.30 (15.27,44.95)	NS
Efavirenz, n	2	24	
Median (IQR)	38.87 (28.06,49.68)	13.45 (9.07,20.97)	NS

Table 4.
Plasma and CSF antiretroviral drug concentrations in those with and without CSF/plasma discordance. NS – not significant.

All subjects with no HIV-1 RNA detection in plasma, also had no virus detection in CSF. Median CSF volume tested for undetectable samples was 4ml (IQR 3,7) giving a median sensitivity of detection of 7 copies/ml (IQR 3,7).

Ten (31.3%) subjects with residual HIV-1 RNA detection in plasma had HIV-1 RNA detection in CSF, median 19 copies/ml (IQR 4,167). Viral detection in CSF was significantly more frequent in this group than in those without plasma viral detection (p=0.0094). Three (9.4%) of these subjects had CSF/plasma discordance (p=0.5423). One further subject (group B) had lower level CSF/plasma discordance with 40 copies/ml in CSF and 4 copies/ml in plasma.

Subjects with no residual HIV-1 RNA detection in plasma had a longer time since HIV diagnosis, median 17 years (IQR 7.5,27) versus 8.5 years (IQR 3,17), p=0.0445. There were no significant differences observed between the groups in age, sex, ethnicity, risk group, ART treatment, current/nadir CD4 or CPE score.

CSF resistance

HIV-1 RNA from 4 discordant CSF samples underwent SANGER sequencing (table 5). All 4 showed resistance-associated mutations.

For subject 15, the same RT resistance profile observed in the discordant CSF sample had been detected 10 years previously in plasma virus sequenced after virological failure of AZT/3TC/ABC followed by ABC/3TC/EFZ. The patient then started PI/r-based ART and between 2002 and 2008 experienced 5 consecutive HIV-1 RNA measurements between 50 and 542 copies/ml; three of these viraemic samples were sequenced and two, collected while the patient was receiving TDF/ATZ/FOS/r, showed the protease mutation V32I but no RT mutations. In 2008 the patient started TDF/RAL/DRV/r and between 2008 and 2011 experienced two further episodes of viremia (50 and 117 copies/ml respectively); one of the two samples was sequenced with no mutations detected in RT, protease or integrase.

CONCLUSIONS

The CNS may be the source of ongoing HIV-1 replication in a proportion of patients that fail to suppress below 50 copies/mL in plasma, as well as those with residual plasma HIV-1 RNA detection between 1-49 copies/ml. This has implications for the management of low-grade viraemia and 'blips', both in terms of stratifying lumbar punctures and tailoring treatment for effective suppression in the CNS.

Some authors have questioned the significance of CSF/plasma discordance below 200 copies/ml,[7] however these data support that this threshold may be too stringent. Two subjects with CSF HIV-1 RNA 100-200 copies/ml (subjects 13 and 18) had resistance-associated mutations including M184I/V which is associated with impaired replicative capacity.

CSF/plasma discordance, was present in 13.1% of the study population. Some studies in neuroasymptomatic patients undergoing LP for research purposes have demonstrated lower rates of CSF/plasma discordance,[6] however studies on subjects in a clinical setting may more accurately represent those seen in clinical practice.[7]

The association between CSF/plasma discordance and nadir CD4 has been demonstrated in in other studies and is confirmed here.[12] This suggests the presence of HIV-1 RNA in the CNS relates to a longer duration of viral replication and a lower immune status. In this study nadir CD4 was higher in the white-MSM population.

No association between the occurrence of CSF/plasma discordance and CPE score was demonstrated, however this study was not designed to assess such an association. Inter-individual variation in plasma levels of darunavir (and possibly lamivudine) may correspond to effectiveness in the CNS to a greater extent than differences in effectiveness between drugs.

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