Discordant CSF/plasma HIV-1 RNA in patients with unexplained low-level viraemia

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Abstract The central nervous system has been proposed as a sanctuary site where HIV can escape antiretroviral control and develop drug resistance. HIV-1 RNA can be at higher levels in CSF than plasma, termed CSF/plasma discordance. We aimed to examine whether discordance in CSF is associated with low level viraemia (LLV) in blood. In this MRC-funded multicentre study, we prospectively recruited patients with LLV, defined as one or more episode of unexplained plasma HIV-1 RNA within 12 months, and underwent CSF examination. Separately, we prospectively collected CSF from patients undergoing lumbar puncture for a clinical indication. Patients with durable suppression of viraemia and no evidence of CNS infection were identified as controls from this group. Factors associated with CSF/plasma HIV-1 discordance overall were examined. One hundred fifty-three patients were recruited across 13 sites; 40 with LLV and 113 undergoing clinical lumbar puncture. Seven of the 40 (18 %) patients with LLV had CSF/plasma discordance, which was significantly more than 0/43 (0 %) with durable suppression in blood from the clinical group (p = 0.005). Resistance associated mutations were shown in six CSF samples from discordant patients with LLV (one had insufficient sample for testing), which affected antiretroviral therapy at sampling in five. Overall discordance was present in 20/153 (13 %) and was associated with nadir CD4 but not antiretroviral concentrations in plasma or CSF. CSF/plasma discordance is observed in patients with LLV and is associated with antiretroviral resistance associated mutations in CSF. The implications for clinical practice require further investigation.

Keywords HIV · Cerebrospinal fluid · Antiretroviral agents · Drug resistance · Viral · Central nervous system

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Introduction

The central nervous system (CNS) has been proposed as a sanctuary site where HIV-1 replication may continue during stable antiretroviral therapy (ART). Concentrations of many antiretrovirals (ARVs) in cerebrospinal fluid (CSF) fall below the minimum inhibitory concentration for wild type virus, and there is large inter-individual variability in CSF penetration (Letendre et al. 2010b; Vissers et al. 2010; Best et al. 2012; Peluso et al. 2012; Ciccarelli et al. 2013; Cusini et al. 2013). ARVs may also have reduced activity in some of the cell populations that are susceptible to HIV infection in the CNS (Aquaro et al. 2002; Gray et al. 2013). HIV-1 RNA can be found at higher concentrations in CSF than in plasma in some patients (Eden et al. 2010; Rawson et al. 2012), termed CSF/plasma discordance, which may result in the compartmentalised detection of drug-resistant variants in CSF (Cunningham et al. 2000; Venturi et al. 2000; Strain et al. 2005).

Unexplained intermittent or persistent low-level viraemia (LLV) in plasma is a common clinical problem, occurring in up to a quarter of ART-treated patients (Doyle and Geretti 2012). The source of this virus is not clear and the clinical significance of LLV is debated (Doyle and Geretti 2012; Doyle et al. 2012). Whether the CNS can act as a sanctuary site for HIV-1 infection in these patients is not known. In this MRC-funded multicentre prospective study, we aimed to investigate the occurrence of CSF/plasma discordance in patients with LLV compared to patients with durable suppression of HIV-1 RNA in blood. We also aimed to investigate factors associated with discordance in the two groups combined, including patients in the clinical group that were not durably suppressed or had evidence of CNS infection.

Methods

Study population

We prospectively recruited HIV-1 positive adults (≥18 years) receiving ART from 13 centres in the UK. Written informed consent was obtained from all participants. The study was approved by the North Wales Research Ethics Committee (Central and East).

Patients were recruited in two groups. In the first group, patients with LLV underwent lumbar puncture (LP) for research purposes. LLV was defined as at least one episode of unexplained plasma HIV-1 RNA viraemia above detection threshold (40 copies/ml) within the previous 12 months. No upper limit was specified. We included patients with blips and persistent LLV. We excluded those with an alternative explanation for virological non-suppression such as adherence <95 % on self-report or recent commencement of ART. Recent modification of ART was not an exclusion.

It was not considered ethically justifiable to perform research LP on aviraemic controls without neurological symptoms; therefore, we prospectively recruited patients undergoing LP for a clinical indication and collected extra CSF at the time of LP. From this group, we identified patients with durable suppression of HIV-1 RNA detection limits (40 copies/ml) over the past year and no evidence of CNS infection to act as controls for those with LLV.

In a separate analysis, we examined factors associated with discordance in the two groups combined, including patients in the clinical group that were not durably suppressed or had evidence of CNS infection.

Virology testing

HIV-1 RNA was measured centrally in simultaneous plasma and CSF samples by the Abbott RealTime HIV-1 assay (Maidenhead, UK) with a lower limit of quantification of 40 copies/ml, as previously described (Garcia-Diaz et al. 2013). CSF/plasma discordance was defined as CSF HIV-1 RNA levels >0.5 log10 higher than those in plasma based on criteria from other studies (Rawson et al. 2012), and our own work showing raised CSF inflammatory cytokines at this degree of discordance (Nightingale et al. 2016). Discordant samples underwent Sanger (population) sequencing of reverse transcriptase (RT, amino acids 1 to 335), protease (amino acids 1 to 99) and integrase (amino acid 1 to 288) to detect RAMs, as previously described (Stockdale et al. 2015).

Drug concentrations

ARV concentrations were determined in CSF and plasma by liquid chromatography-tandem mass spectrometry (LC-MS/MS). 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 external quality assurance standards.

The revised CNS penetration effectiveness (CPE) score (2010) was calculated by assigning a predefined number of
points to each component of the ART regimen, as proposed by Letendre et al. (Letendre et al. 2008, 2010a). Medication adherence was assessed using the modified medication adherence self-report inventory (MASRI) questionnaire at the time of LP.

Cognitive screening tools

In order to screen for issues related to cognitive impairment, patients with LLV underwent assessment with the following tools. Symptoms of cognitive difficulties were assessed by asking patients to answer whether they had problems with memory, reasoning and attention, as described by Simioni et al. (Simioni et al. 2010), and used in international guidelines (European AIDS Clinical Society 2014) [21] [21] [20] 20. Patients were considered to have cognitive symptoms when answering “yes, definitely” on at least one of the three questions. Patients were also screened for cognitive impairment using the International HIV Dementia scale (Zipursky et al. 2013). This scale uses three brief tests to assess motor speed, psychomotor speed and memory-recall. A score of less than ten has been considered potentially abnormal (Sacktor et al. 2005). Functional impairment was measured using the Instrumental Activities of Daily Living scale (Heaton et al. 2004; Mind exchange 2013). The maximum score of eight means no impairment in the following activities: telephoning, shopping, preparing food, housekeeping, laundry, travel, medications and finances. A score of seven or less indicates potential functional impairment. Mood disorders were assessed using the Hospital Anxiety and Depression scale, addressing depressive (HAD-D) and anxious (HAD-A) symptoms separately. Patients were considered depressed or anxious if the HAD-D or HAD-A subscale score was at least ten out of 21 (Savard et al. 1998, 1999). Cognitive testing was not performed in the clinical group as this could be confounded by intercurrent illness.

Statistical analysis

Mann-Whitney U test was used to compare continuous non-parametric variables. Fisher’s exact and chi-squared tests were used for categorical data. The geometric mean of log_{10} ARV concentrations in plasma and CSF was compared between discordant and non-discordant patients with Student’s t test and also combined with time post dose in a multivariate logistic regression analysis using the forced entry method. A p value of 0.05 was used to determine statistical significance. When correlating CSF and plasma HIV-1 RNA, levels below detection were assigned an arbitrary mid-point value between zero and the 95% detection rate. All analyses were performed using SPSS v22.

Results

Study population

A total of 153 ART-treated patients were recruited; 40 patients with LLV undergoing LP for research purposes and 113 with clinically indicated LP.

In the 40 patients with LLV, plasma HIV-1 RNA had been measured a median of five times (IQR 4, 6) in the previous 12 months. HIV-1 RNA had been detected >50 copies/ml on 117 of 198 occasions; median HIV-1 RNA levels during viraemic episodes was 92 copies/ml (IQR 59, 179). Eight patients (20 %) reported cognitive symptoms on questionnaire. One patient (3 %) had a score below ten on the International HIV Dementia scale and one other patient had a score below eight on the Instrumental Activities of Daily Living scale. Ten patients (25 %) were anxious and two patients (6 %) were depressed according to the Hospital Anxiety and Depression scale.

In the clinical group, 113 patients underwent LP to investigate cognitive symptoms (n = 50), headache (n = 20), suspected CNS infection or malignancy (n = 18), follow-up of a previous CNS infection (n = 8), abnormal neurological examination (n = 7), seizure (n = 5), neuropathy/radiculopathy (n = 2) and others (n = 3). On the basis of clinical findings and results of LP CNS infection was excluded in 92/113 patients (81 %). Where CNS infection was proven or probable, the diagnoses were neurosyphilis (n = 4), cryptococcal meningitis (n = 2), cryptococcal immune reconstitution inflammatory syndrome (n = 2), herpes simplex virus type-2 meningitis (n = 1), viral radiculopathy (n = 1), Ebstein-Barr virus in CSF of uncertain significance (n = 4) and no diagnosis (n = 7). Details of alternative diagnoses in those without CNS infection were not recorded; symptoms were cognitive (n = 43), headache (n = 16), suspected CNS infection or malignancy (n = 12), follow-up of a previous CNS infection (n = 4), abnormal neurological examination (n = 7), seizure (n = 5), neuropathy/radiculopathy (n = 2) and others (n = 3). In this group, 43/113 (38 %) patients had durable HIV-1 RNA suppression for 12 months and no CNS infection. The remaining 70 either had CNS infection (n = 21), were not suppressed (n = 26) or were not durably suppressed (n = 23).

CSF/plasma HIV-1 RNA discordance

CSF/plasma discordance was found in 7/40 (18 %) patients with LLV. None of the 43 patients in the clinical group with durable suppression had discordance. The difference in rate of discordance between patients with LLV (18 %) and durable suppression (0 %) was statistically significant, p = 0.005 (Fig. 1). In patients with LLV, there was no difference in magnitude or frequency of HIV-1 RNA detection over the previous 12 months between those with (n = 7) and those without
(n=33) discordance. There were no significant differences in the rate cognitive symptoms, International HIV Dementia Scale results, functional impairment on the Instrumental Activities of Daily Living scale, or tests of anxiety and depression. There was a trend to more frequent raltegravir use in patients with LLV compared to durable suppression (11/40 versus 19/133, p = 0.060), which likely reflects use of integrase inhibitors to intensify ART in patients with viral persistence.

In the clinical group, discordance was found in 13/113 (12 %) patients. All discordant patients in this group were either not suppressed in plasma, not durably suppressed in plasma or had CNS infection. When combined with the LLV group, the rate of discordance overall was 20/153 (13 %). Overall HIV-1 RNA levels in plasma and CSF were directly correlated (Spearman r = 0.51; p < 0.001). HIV-1 RNA levels were <50 copies/ml in 113/153 (74 %) plasma samples and 115/153 (75 %) CSF samples. In discordant patients median HIV-1 RNA levels were 501 copies/ml (<210, <2016) in CSF versus <40 copies/ml (<40, <40) in plasma. Differences in measured variables among patients with and without CSF/plasma discordance are shown in Table 1. Patients with discordance showed a lower nadir (but not current) CD4 cell count and a higher CSF white cell count. Lower rates of discordance were observed in white MSM compared with black heterosexuals, which may reflect the higher nadir CD4 cell count in this group (median 173 versus 110 cells/mm³, p = 0.029).

![Fig. 1](image.png) CSF HIV-1 RNA in 40 patients with intermittent or persistent low-level plasma HIV-1 RNA detection in the last year versus 43 patients from a clinical cohort with durable HIV-1 RNA suppression in the last year. Filled circles represent discordant samples.

| Table 1 | Univariate analysis of factors associated with discordant HIV-1 RNA levels in paired CSF and plasma samples |
|----------------|-------------------------------------------------|
| CSF/plasma discordance |  | p value |
| Yes (n=20) | No (n=133) |
| Age, median years (IQR) | 46 (43, 50) | 46 (41, 54) | 0.913 |
| Gender male, n (%) | 15 (75) | 117 (88) | 0.156 |
| Risk group/ethnicity, n (%) | 0.007 |
| White MSM | 7 (35) | 80 (60) |
| Black heterosexual | 8 (40) | 17 (13) |
| Other | 5 (25) | 36 (27) |
| CD4, median cells/mm³ (IQR) |  |  |
| Nadir | 32 (21, 256) | 159 (49, 281) | 0.030 |
| Current | 374 (190, 613) | 464 (310, 707) | 0.163 |
| Years since HIV diagnosis, median (IQR) | 10 (7, 16) | 9 (5, 16) | 0.833 |
| ART at sampling, n (%) | 0.178 |
| PI/r based | 14 (70) | 74 (55) |
| NNRTI based | 3 (15) | 47 (35) |
| Other | 3 (15) | 12 (9) |
| CPE, median (IQR) | 7 (7, 10) | 7 (6, 8) | 0.250 |
| Self reported adherence <95 %, n (%) | 2 (10) | 12 (9) | 1.000 |
| CSF WCC median cells/mm³ (IQR) | 11 (<1, 21) | <1 (<1, 3) | 0.018 |
| CNS infection, n (%) | 3 (15) | 18 (14) | 0.740 |

Risk group and ethnicity were combined due to co-linearity; 89 % of MSM were white. CSF white cell count was tested in 24 (60 %) of LLV patients and all patients in the clinical cohort.

MSM men who have sex with men, IVDU intravenous drug user, IQR interquartile range, PI/r ritonavir-boosted protease inhibitor, NNRTI non-nucleoside reverse transcriptase inhibitor, NRTI nucleoside/tide reverse transcriptase inhibitor, RAL raltegravir, MVC maraviroc, CPE CNS penetration effectiveness score 2010.
CSF resistance

CSF samples from 6/7 discordant patients with LLV underwent sequencing for the detection of HIV-1 RAMs (one patient had insufficient CSF for resistance testing). All six samples tested showed RAMs; this affected one or more of the ARVs taken at the time of sampling in 5/6 patients (Table 2). At least one RAM had been previously identified in plasma in all six patients.

In the clinical group, 12/13 CSF samples from discordant patients underwent sequencing (one patient had insufficient CSF for resistance testing). Five CSF samples did not amplify despite repeated attempts and one sample only amplified for the protease gene. Of the six CSF samples successfully tested, all showed RAMs; this affected one or more of the ARVs taken at the time of sampling in all six patients (Table 2). CSF RAMs had been previously identified in plasma in one patient (patient 18).

Resistance testing of plasma virus was attempted in patients 7, 10 and 11. Plasma virus from patients 7 and 10 did not amplify. Plasma from patient 11 showed no NRTI or NNRTI RAMs; the integrase gene did not amplify.

Plasma and CSF pharmacokinetics

ARV concentrations in plasma and CSF are shown in Table 3. No significant differences were observed between those with or without discordance for any ARVs tested in either CSF or plasma. There were also no significant differences in ARV concentrations between those with LLV versus durable suppression. No associations were observed with CPE score or ART use, either for individual ARVs or drug class of regimen (Table 1).

Table 2  CSF ARV resistance associated mutations (RAMs) in 20 patients with CSF/plasma discordance

| Patient ID | Plasma RNA copies/ml | CSF RNA copies/ml | ART at sampling | PI | NRTI | NNRTI | INT |
|------------|----------------------|-------------------|----------------|----|------|-------|-----|
| Low-level viremia group |
| 1 | <40 | 138 | TDF/FTC/DRV/r | – | M184I | – | – |
| 2 | <40 | 254 | EFZ/TDF/FTC | Insufficient sample | – | – | – |
| 3 | 48 | 2028 | DRV/r/TFD/RAL | None | D67N, K70R, L74V, M184V, T215Y, K219E | L100K, K103N | T66I, Y143C |
| 4 | 78 | 3234 | DRV/r/MVC/ETR | None | A62V, K65R, M184V | None | – |
| 5 | 88 | 1569 | TDF/FTC/DRV/r | None | M184I | None | – |
| 6 | <40 | 162 | TDF/FTC/DRV/r | None | D67N, M184V, T215Y, K219Q | V108I | – |
| 7 | 258 | 3518 | ABC/3TC/DRV/r | None | L74I, M184V | None | – |
| Clinical cohort |
| 8 | 40 | 1038 | TDF/FTC/DRV/r | None | 1081, E138A |
| 9 | 52 | 1231 | TDF/FTC/RAL/MVC | Did not amplify | – | – |
| 10 | 3443 | 13088 | 3TC/AZT/EFZ | – | None | V108I, E138A |
| 11 | 10817 | 63010 | TDF/FTC/RAL | Did not amplify | – | – |
| 12 | <40 | 400 | ABC/3TC/DRV/r/RAL | Did not amplify | – | – |
| 13 | <40 | 422 | TDF/FTC/AZT/r | – | T69S, M184I | – | – |
| 14 | <40 | 335 | TDF/FTC/DRV/r | Did not amplify | – | – |
| 15 | <40 | 162 | 3TC/AZT/DRV/r/MVC/ETR | Insufficient sample | – | – |
| 16 | <40 | 129 | DRV/r | Did not amplify | – | – |
| 17 | <40 | 579 | ATZ/r/TDF/FTC | V82AV | M184V, T215I | – | – |
| 18 | <40 | 1981 | DRV/r/RAL/MVC/3TC | None | M184V | V108I | N155H |
| 19 | <40 | 195 | EFZ/TDF/FTC | Did not amplify | – | – |
| 20 | <40 | 405 | LPV/r/AZT/RAL | Did not amplify | – | – |

RAMs in bold are to ARVs taken at the time of sampling. RAMs in italics have been previously detected in plasma. Patient 9 showed CCR5 tropic virus in CSF.

**PI** protease inhibitor, **NRTI** nucleoside/tide reverse transcriptase inhibitor, **NNRTI** non-nucleoside reverse transcriptase inhibitor, **INT** integrase inhibitor, **ATZ** atazanavir, **DRV** darunavir, **LPV** lopinavir, **ETV** etravirine, **MVC** maraviroc, **NVP** nevirapine, **RAL** raltegravir, **RPV** rilpivirine, **r** ritonavir, **EFZ** efavirenz, **TDF** tenofovir, **FTC** emtricitabine, **3TC** lamivudine, **AZT** zidovudine
Table 3  Antiretroviral concentrations in CSF and plasma

|            | n  | Drug concentration (ng/ml) | Geo mean | 95 % CI |
|------------|----|---------------------------|----------|---------|
| Plasma     |    |                           |          |         |
| Tenofovir  | 62 | 89.5                      | 73.1–110.7 |         |
| Emtricitabine | 65 | 187.9                     | 136.5–264.9 |         |
| Lamivudine | 24 | 383.7                     | 225.4–688.7 |         |
| Darunavir  | 55 | 2971.7                    | 2393.3–3715.4 |       |
| Atazanavir | 19 | 729.5                     | 418.8–1339.7 |        |
| Lopinavir  | 3  | 8570.4                    | 7798.3–9440.6 |       |
| Ritonavir  | 15 | 116.1                     | 55.7–277.3  |         |
| Efavirenz  | 27 | 1741.8                    | 1349.0–2269.9 |       |
| Nevirapine | 10 | 4226.7                    | 2691.5–6807.7 |       |
| Etravirine | 4  | 269.2                     | 64.4–1836.5  |         |
| Rilpivirine| 4  | 68.1                      | 36.0–144.2   |         |
| Maraviroc | 8  | 172.2                     | 88.9–367.3   |         |
| Raltegravir| 11 | 413.9                     | 174.1–1135.0 |       |
| CSF        |    |                           |          |         |
| Darunavir  | 54 | 35.2                      | 27.8–45.4   |         |
| Atazanavir | 20 | 6.4                       | 4.1–11.5    |         |
| Lopinavir  | 3  | 9.6                       | 4.1–43.7    |         |
| Efavirenz  | 26 | 14.7                      | 8.9–20.4    |         |
| Nevirapine | 12 | 1559.6                    | 1127.2–2197.9 |       |
| Maraviroc | 9  | 6.5                       | 2.7–40.1    |         |
| Raltegravir| 14 | 25.9                      | 18.1–38.7   |         |

Geometric mean and 95 % confidence interval for drug concentrations in plasma and CSF. No significant associations were observed with CSF/plasma discordance.

Discussion

We observed CSF/plasma discordance in 18 % of patients undergoing a research-based LP for intermittent or continuous LLV. No discordance was observed in patients with durably suppressed plasma viral load undergoing clinically indicated LP. This suggests that a significant proportion of HIV-positive patients with LLV despite ART may have suboptimal control of virus replication within the CNS. CSF examination of discordant patients with LLV revealed RAMs in all samples tested, which related to ART taken at the time of sampling in all but one. Of note, in these patients, plasma viraemia was generally too low to perform resistance testing in blood.

One interpretation of these findings could be that ongoing replication of HIV in a CNS sanctuary site results in seeding to plasma leading to LLV. Alternatively, the observation of LLV and CSF/plasma discordance may represent two coinciding aspects of overall suboptimal virological suppression. It is of interest that in all cases at least part of the resistance detected in CSF virus was consistent with the previous history of failure and older resistance tests in plasma. This, combined with the observed association of discordance with low nadir CD4, suggests that advanced immune suppression may allow the establishment of a viral reservoir in the CNS that may be only partially responsive to ART. This virus may then continue to evolve and develop resistance to some ARVs, for example raltegravir, and thus become progressively less susceptible to suppression than virus in peripheral blood. LLV with a genetically different virus may originate from a similar process of incomplete suppression at other sites such as lymphoid tissue, where drug penetration and activity may also vary (Fletcher et al. 2014). Intermittent reseeding of virus into the CNS during periods of system replication due to another cause (e.g. non-declared poor adherence) may also establish compartmentalised CNS infection (Gisslen et al. 2005; Eden et al. 2010). Regardless of the origin of CSF virus, targeting LP to patients with unexplained blips or persistent LLV in plasma may represent a useful strategy in clinical practice and warrants further investigation. Altering ART based on the resistance profile of CSF variants has led to clinical improvement and decrease in CSF HIV-1 RNA in case series (Canestri et al. 2010; Peluso et al. 2012) and has been adopted by guidelines as best practice (Mind exchange 2013; Williams et al. 2014). In addition, these findings add to the evidence suggesting the CNS compartment must be considered in studies aiming to eradicate HIV from the periphery.

Screening for cognitive impairment in the LLV group with a cognitive symptoms questionnaire and International HIV-Dementia Scale did not reveal associations with CSF/plasma discordance. The numbers are small and the screening methods used are insensitive for milder cognitive disorders (Haddow et al. 2013); nevertheless, these data suggest that discordant patients were relatively asymptomatic and cognitive deficits were not marked. Whether discordance in this group has implications for cognitive dysfunction in the longer term requires clarification in longitudinal studies.

CSF/plasma discordance was present in 12 % of patients undergoing clinically indicated LP. This rate of discordance is similar to other reports from clinical settings (Eden et al. 2010; Rawson et al. 2012). Studies where LP was performed for research purposes in the absence of clinical indications have reported lower rates of discordance (Heaton et al. 2010). CSF/plasma discordance overall was not associated with ART at sampling, CPE score or ARV concentrations in plasma or CSF, but was associated with nadir CD4 cell count. This suggests the presence of HIV in the CNS relates to a longer duration of viral replication and a lower immune status, consistent with the hypothesis of CNS viral persistence discussed above. In cross sectional studies, nadir CD4 has been consistently associated with cognitive impairment (Robertson et al. 2007; Heaton et al. 2010). It has not been clear whether this
sampling and raltegravir RAMs in all four patients on raltegravir
C S Fs a m p l e si n c l u d i n gN R T IR A M si ne i g h to ft e noN R T I sa t
tested showed RAMs. Overall RAMs were demonstrated in 12
pression. In our study, all discordant CSF samples successfully
CNS inflammatory process set up during advanced immunosup-
prior to ART initiation or whether it represents an ongoing
association is due to a legacy effect of CNS damage sustained
in perivascular macrophages, the critical target of HIV
by prescriber bias, whereby patients with CNS problems
are more likely to receive an ART regimen with a
higher CPE score (Garvey et al. 2011).

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interest.

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