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MAJOR ARTICLE

Distribution of Human Immunodeficiency Virus (HIV) Ribonucleic Acid in Cerebrospinal Fluid and Blood Is Linked to CD4/CD8 Ratio During Acute HIV

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Background. Human immunodeficiency virus (HIV) ribonucleic acid (RNA) levels in the plasma and cerebrospinal fluid (CSF) are correlated in chronic HIV infection, but their dynamics have not been characterized during acute infection.

Methods. This study analyzed predictors of CSF HIV RNA and relative degree of CNS viral transmigration expressed as plasma minus CSF HIV $\log_{10}$ RNA (PC_{ratio}) during untreated acute HIV infection. Cerebrospinal fluid immune markers were compared between groups with different PC_{ratio}.

Results. One hundred seventeen mostly male (97%) participants in the RV254 cohort in Bangkok, Thailand, had a median age of 28 years and an estimated median 18 days duration of infection; 43 (37%) were Fiebig stages I/II. Twenty-seven (23%) had CSF HIV RNA <80 copies/mL. Those with quantifiable levels (n = 90) had median CSF HIV RNA and PC_{ratio} of 3.76 and 2.36 $\log_{10}$ copies/mL, respectively. Human immunodeficiency virus RNA peaked at Fiebig III in plasma and Fiebig IV in CSF. In multivariable analyses, plasma HIV RNA and CD4/CD8 ratio independently correlated with CSF HIV RNA ($P < .001$), whereas CD4/CD8 ratio predicted PC_{ratio} ($P = .018$). Participants with PC_{ratio} <1 had higher CSF neopterin, soluble (s)CD163, interleukin-6, and sCD14 levels (all $P < .05$).

Conclusions. CD4/CD8 ratio independently correlated with CSF HIV RNA and PC_{ratio}, suggesting that immune responses modulate central nervous system viral entry at early infection.

Keywords. acute HIV infection; CD4/CD8 ratio; CSF HIV viral RNA level; HIV CNS invasion; RV254.

Our understanding of central nervous system (CNS) involvement in human immunodeficiency virus (HIV) infection has evolved over the last 2 decades. The transmigration of HIV into the CNS from the systemic circulation is thought to initiate neuropathogenic processes, including forms of HIV encephalitis (HIVE) and HIV-associated neurocognitive disorder. These processes can occur across all stages of HIV infection and treatment and are not solely determined by markers of HIV disease severity such as current $CD4^+$ T-lymphocyte count [1, 2]. In particular, it has become clear that the CNS is affected early in the course of infection, evidenced by the detection of HIV ribonucleic acid (RNA) in the cerebrospinal fluid (CSF) as early as 8 days from transmission [3], frequent neurological signs and symptoms during acute HIV infection [4], and abnormalities in neuroimaging and CSF markers of inflammation during acute and primary HIV infection [5, 6].

The entrance of HIV into the CNS ultimately leads to infection of resident CNS cells including microglia and astrocytes, setting the stage for the potential of HIV persistence even after antiretroviral therapy is initiated. The potential of the CNS as reservoir for HIV is supported by symptomatic CSF viral escape [7, 8]—a measurable CSF HIV RNA level with an undetectable plasma level in treated individuals presenting with neurological disease—and the isolation of CNS-specific viral strains through sequencing and phylogenetic studies [9–11]. Understanding the viral dynamics between the systemic circulation and the CNS compartment is crucial to elucidate the pathogenesis of HIV-related neurological injury and the development of CNS reservoirs. However, the literature to date is primarily based on samples of treatment-naive, chronically HIV-infected individuals, in whom CSF HIV RNA level is closely associated with that in plasma and the severity of immunodeficiency [12–15]. Reports from primary infection cohorts (within months of initial infection) suggest a similar

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relationship between CSF and plasma HIV RNA levels but with a larger difference between the 2 compartments [5, 16]. The relationship between plasma and CSF HIV RNA levels during the earliest weeks of HIV infection is unknown. Understanding these factors may allow development of strategies to effectively prevent establishment of reservoirs in the CNS and HIV related injury. We examined paired blood and CSF HIV RNA data from the SEARCH010/RV254 cohort, a prospective longitudinal study in Bangkok, Thailand, aiming to define the viral dynamics between CSF and plasma across various Fiebig stages and to identify factors associated with CSF HIV RNA level.

METHODS

Study Design and Participants

We selected participants enrolled into the SEARCH010/RV254 cohort between April 2009 and December 2016 with available CSF data. Methodology and primary goals of this cohort study have been described elsewhere [3, 4]. In brief, these individuals were enrolled through voluntary HIV counseling and testing services offered in Bangkok and Pattaya, Thailand, where specimens were systematically screened for acute HIV infection. Enrolled participants were classified into Fiebig stages I to V defined by a hierarchical algorithm from pooled nucleic acid testing, sequential immunoassay, p24 antigen, and Western blot testing [17]: Fiebig I: RNA+, p24 antigen−; Fiebig II: p24 antigen+, IgM−; Fiebig III: IgM+, Western blot−; Fiebig IV: Western blot indeterminate; Fiebig V: Western blot+ without p31 protein band [17]. All participants were assessed at acute infection, immediately initiated combination antiretroviral therapy (cART), and were then followed longitudinally for up to 10 years. The current analysis focused on baseline, cross-sectional pre-cART data. Optional lumbar punctures (LP) were typically performed within 48 hours of acute HIV infection confirmation. All participants provided written informed consent to participate in the study. The study protocol was approved by the institutional review boards of Chulalongkorn University (Bangkok, Thailand), Walter Reed Army Institute of Research (Silver Spring, MD), University of California, San Francisco (San Francisco, CA), and Yale University (New Haven, CT).

Clinical Data and Laboratory Measures

Demographic and clinical parameters of LP participants were collected at baseline, including $CD4^+$ and $CD8^+$ T-lymphocyte count, plasma HIV RNA level, Fiebig stage (I to V), and the presence of acute retroviral syndrome, defined as ≥3 qualifying symptoms using a standardized checklist. Cerebrospinal fluid and plasma HIV viral RNA quantification were completed using the Roche Amplicor HIV-1 Monitor Test V1.5 or Roche COBAS TaqMan HIV-1 V2.0. The lower limit of quantification of plasma HIV RNA was 50 and 20 copies/mL, respectively, depending on the platform used. The lower limit of quantification of CSF HIV RNA was 80 copies/mL. In the following data analyses, a level of 80 copies/mL was assigned to the CSF samples with HIV RNA below the lower limit of quantification.

Cerebrospinal Fluid Soluble Marker Analysis

Cerebrospinal fluid inflammatory markers reflecting T-lymphocyte recruitment (CXCL10) and monocyte/macrophage (interleukin [IL]-6, CCL2, soluble [s]CD163, sCD14, and neopterin) recruitment and activation were evaluated in parallel to HIV RNA level in a subgroup of LP participants. Cerebrospinal fluid IL-6, sCD163, CXCL10, and CCL2 were quantified using a Luminex multiplex enzyme-linked immunosorbent assay (ELISA) array (EMD Millipore, Billerica, MA) per manufacturer's instructions. Data were collected on a FlexMap 3D system. A subset of samples assessed early during the course of the trial were quantified for CXCL10 and CCL2 by custom ELISA array (Quansys Biosciences, Logan, UT). Data generated from this platform were normalized to the Luminex platform by testing of replicate samples assessed in paired assays. Neopterin (GenWay Biotech, San Diego, CA) and sCD14 (R&D Systems, Minneapolis, MN) were measured by standard chemiluminescent detection ELISA per manufacturer's instructions and read on a VersaMax reader (Molecular Devices, Sunnyvale, CA). All data were analyzed in Prism version 6.0 for Mac OS X (GraphPad, La Jolla, CA) using a 4-parameter fit standard curve.

Statistical Analysis

Human immunodeficiency virus-1 RNA levels were transformed to $\log_{10}$ for analysis. The viral dynamic between plasma and CSF compartments was represented by the plasma to CSF viral load ratio (PC_{ratio}), calculated as $\log_{10}$ (plasma HIV RNA level/CSF HIV RNA level), and mathematically equivalent to $\log_{10}$ plasma HIV RNA minus $\log_{10}$ CSF HIV RNA. A PC_{ratio} less than 1 indicates the CSF HIV RNA level is greater than one tenth of that in the plasma, a frequently cited reference in untreated chronic infection. Results were reported as median and interquartile range (IQR) or frequency and percentage, as appropriate. Fisher's exact test and the non-parametric Mann-Whitney *U* test were used to compare categorical and continuous variables, respectively. Factors associated with CSF HIV RNA level and PC_{ratio} were examined using a linear regression model. Factors with a *P* value <.10 in univariate analysis were included in the subsequent multivariable analysis using the backward stepwise method. Statistical analyses were performed using SPSS version 21.0 (IBM Corp., Armonk, NY).

RESULTS

Characteristics of Study Participants

During the sampling period, 430 individuals were enrolled into SEARCH010/RV254. Among these, 117 (27%) underwent baseline LP. The LP participants had similar demographic (age, sex) and HIV infection parameters (plasma HIV RNA level, Fiebig stages, and percentage of acute retroviral syndrome

presentation) with the rest of the cohort ($P > .05$). They were predominantly male (97%) with a median age of 28 years. Nineteen (16%) participants were identified in Fiebig I, 24 (21%) in Fiebig II, 52 (44%) in Fiebig III, 15 (13%) in Fiebig IV, and 7 (6%) in Fiebig V. Median estimated days from HIV transmission to study enrollment was 18 (IQR, 14–23). Most of them were infected with CRF01_AE subtype (83%), followed by CRF01_AE/B (7%), B (4%), CRF01_AE/B/C (1%), and nontypable (7%). All participants initiated cART after the LP, except for 10 who started treatment before the procedure (median 2 days, range 1–6 days). One participant was on pre-exposure prophylaxis before the acute HIV infection diagnosis. All of these participants were included in the following analyses because sensitivity analyses excluding these participants yielded similar findings.

Cerebrospinal Fluid Human Immunodeficiency Virus (HIV) Ribonucleic Acid Levels in Acute HIV Infection

Among the 117 CSF samples, 90 (77%) had quantifiable HIV RNA, 18 (15%) had undetectable HIV RNA, and 9 (8%) had detectable HIV RNA but an unquantifiable level. Cerebrospinal fluid and plasma viral loads with respect to days after estimated HIV exposure were shown in Figure 1. Overall, the CSF HIV RNA measurable rate was 51% (22 of 43) among early enrollees (Fiebig I and II) and 92% (68 of 74) for participants enrolling during later Fiebig stages (Fiebig III to V). Table 1 shows the demographic and laboratory data of participants grouped according to the CSF HIV RNA quantification status. The group with quantifiable CSF HIV RNA levels presented more often at Fiebig III or later ($P < .001$) and had lower $CD4^+$ lymphocyte count ($P = .002$), higher $CD8^+$ lymphocyte count ($P = .009$), higher plasma HIV RNA level ($P < .001$), and more frequent acute retroviral syndrome presentation ($P = .001$). Among those with quantifiable CSF HIV RNA levels, the median PC_{ratio} was 2.36 $\log_{10}$ copies/mL and ranged from 0.10 to 4.40 $\log_{10}$ copies/mL.

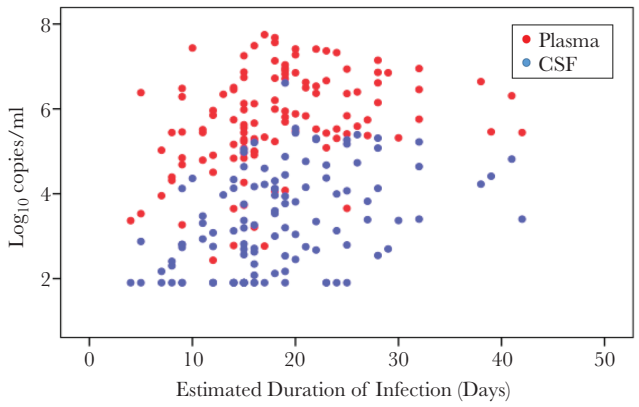

Figure 1. Cerebrospinal fluid (CSF) and plasma human immunodeficiency virus (HIV) ribonucleic acid (RNA) levels with respect to days after estimated HIV exposure. A level of 80 copies/mL was assigned to those who had a CSF HIV RNA level below quantification.

Plasma and Cerebrospinal Fluid Human Immunodeficiency Virus Ribonucleic Acid Level Across Fiebig Stages

Participants' plasma and CSF HIV RNA levels stratified by Fiebig stages are shown in Figure 2. In our samples, the HIV RNA level is always higher than that in CSF. Viewed cross-sectionally, both plasma and CSF HIV RNA level increased sequentially in the early Fiebig stages. The plasma HIV RNA level peaked at Fiebig III (Figure 2a), whereas CSF HIV RNA level peaked at Fiebig IV (Figure 2b).

Determinants of Cerebrospinal Fluid Human Immunodeficiency Virus (HIV) Ribonucleic Acid Level at Acute HIV Infection

Univariate and multivariable linear regression models were applied to identify factors associated with baseline CSF HIV RNA level. Factors that had a P value below .10 in univariate analysis were included in the multivariable analysis using backward stepwise calculation (Table 2). In the univariate analysis, higher plasma HIV RNA level, higher $CD8^+$ T-lymphocytes level, lower $CD4^+$ T-lymphocytes level, lower CD4/CD8 ratio, later Fiebig stage classification (III and later), and presentation of acute retroviral syndrome were associated with a higher CSF HIV RNA level (all $P < .05$). In the multivariable model, only plasma HIV RNA level and CD4/CD8 ratio independently correlated with CSF HIV RNA level (both $P < .001$) with adjusted β coefficients of 0.604 and −0.616 , respectively.

Variability of Plasma-Cerebrospinal Fluid Human Immunodeficiency Virus Ribonucleic Acid Level Difference and Its Determinant

The CSF vs plasma HIV RNA level distribution color-coded by Fiebig stage is shown in Figure 3. Dotted lines of 1–3 log PC_{ratio} were added for reference. Cerebrospinal fluid HIV RNA level, as shown in the regression model, positively correlates with the plasma HIV RNA level. Among the 27 individuals who had a CSF HIV RNA level below the level of detection, half (n = 14) presented at Fiebig stage I, 7 at Fiebig stage II, and the remainder from Fiebig stage III to V. Moreover, one quarter (n = 7) had a relatively high plasma HIV RNA level above 5 $\log_{10}$ despite the unmeasurable CSF HIV RNA level.

Another 7 individuals had a high level of HIV RNA in CSF with respect to the level in plasma, resulting in a PC_{ratio} below 1 log. This group of outliers (PC_{ratio} <1) was not dominated by late enrollees (3 at Fiebig II; 2 at Fiebig III; 1 at Fiebig IV; and 1 at Fiebig V), and they were infected by a similar HIV subtype as others (6 by CRF01_AE and 1 by recombinant subtype CRF01_AE/B). There were no differences between the outliers, and the rest of group with quantifiable CSF HIV RNA in plasma HIV RNA level ($P = .224$), rate of acute retroviral syndrome presentation ($P = .889$), and duration of infection according to Fiebig staging ($P = .377$).

Linear regression modeling was performed to evaluate the potential factors associated with PC_{ratio} across the entire group (Table 3). In univariate analyses, only CD8 lymphocyte count

Table 1. Demographic and Clinical Parameters According to CSF HIV RNA Detection Status[a]

Characteristics	<LOQ (n = 27)	≥LOQ (n = 90)	*P* Value
Male, n (%)	26 (96)	87 (97)	1.00
Age (year)	26.0 (23–31)	27.5 (23–32)	.902
CD4 T lymphocytes (cells/μL)	555 (354–699)	384 (280–506)	.002
CD8 T lymphocytes (cells/μL)	442 (238–586)	624 (366–1013)	.009
CD4/CD8 ratio	1.12 (0.59–1.57)	0.68 (0.38–0.95)	<.001
Fiebig Stage			
I and II, n (%)	21 (78)	22 (24)	
III to V, n (%)	6 (22)	68 (76)	<.001
Plasma HIV RNA (log_{10} copies/mL)	4.23 (0.92)	6.14 (5.48–6.83)	<.001
CSF HIV RNA (log_{10} copies/mL)	1.90 (0.0) [b]	3.76 (2.81–4.82)	<.001
Plasma-CSF HIV RNA Ratio (PC_{ratio}) (range, log_{10} copies/mL)[c]	-	2.36 (0.10–4.40)	
Presence of ARS, n (%)	14 (52)	77 (86)	.001
Active syphilis, n (%)[d]	2 (7)	6 (7)	1.00

Abbreviations: ARS, acute retroviral syndrome; CSF, cerebrospinal fluid; HIV, human immunodeficiency virus; LOQ, lower limit of quantification (80 copies/mL); RNA, ribonucleic acid; RPR, Rapid Plasma Reagin; VDRL, Venereal Disease Research Laboratory.

[a]Results shown are presented as median (interquartile range) unless specified.

[b]A level of 80 copies/mL was assigned to those who had a CSF HIV RNA level below quantification.

[c]Plasma-CSF HIV RNA Ratio (PC_{ratio}) = log_{10} plasma HIV RNA minus log_{10} CSF HIV RNA.

[d]Defined as positive *Treponema pallidum* hemagglutination and RPR/VDRL, with no treatment 3 months before the acute HIV infection diagnosis.

and CD4/CD8 ratio achieved a *P* value <.05. In the multivariable analysis, CD4/CD8 ratio was the only significant factor, showing a positive correlation (*P* = .018, adjusted β coefficient = 0.380).

Comparison of Cerebrospinal Fluid Immune Activation Markers According to Cerebrospinal Fluid Human Immunodeficiency Virus Detection and PC_{ratio} Status

We compared the levels of the 6 CSF biomarkers of immune activation (neopterin, sCD14, IL-6, CXCL10, CCL2, sCD163) in a subgroup of LP participants categorized by CSF HIV RNA and PC_{ratio}: CSF HIV RNA <80 copies/mL (lower limit of quantification) vs CSF HIV RNA level ≥80 copies/mL with PC_{ratio} >1 vs CSF HIV RNA level >80 copies/mL with PC_{rario} ≤1 (outliers) (Figure 4). In general, all CSF immune activation markers increased with quantifiable CSF HIV RNA, and they were highest in the outlier group. In particular, the group of participants with CSF HIV RNA below quantification had statistically lower levels of all CSF cytokines except sCD14 (all *P* < .05). The outlier group, despite having similar Fiebig staging composition and plasma HIV RNA level to the group with PC_{ratio} >1, showed a significantly higher CSF neopterin, sCD163, IL-6, and sCD14 (all *P* < .05).

DISCUSSION

We have previously reported a high rate of CSF HIV RNA detection (15 of 18) in study participants with acute HIV infection, with detectable CSF HIV RNA as early as 8 days after estimated infection [3]. In the current analysis, the rate of HIV RNA above the lower limit of quantification in CSF was 51% at Fiebig I and

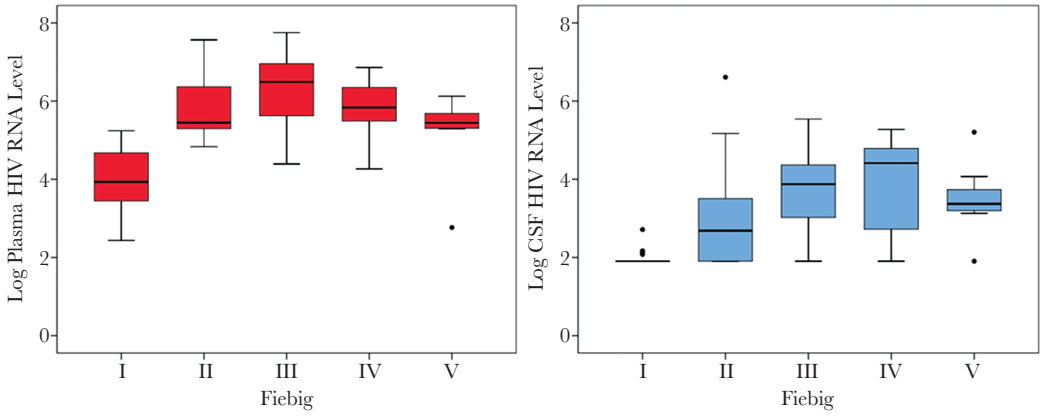

Figure 2. Human immunodeficiency virus (HIV) ribonucleic acid (RNA) level by Fiebig stage (a) plasma and (b) cerebrospinal fluid (CSF). Median (solid line), interquartile range (extent of boxes), 1.5 times of interquartile range (whiskers), and outliers/extreme outliers (stars) are indicated. Number of participants by Fiebig stage (n): I (19); II (24); III (52); IV (15); V (7). A level of 80 copies/mL was assigned to those who had a CSF HIV RNA level below quantification.

Table 2. Linear Regression Analysis on CSF HIV RNA Level (n = 117)[a]

	Univariate Analysis		Multivariable Analysis	
Characteristics	β Coefficient (95% CI)	*P* Value	Adjusted β Coefficient (95% CI)	*P* Value
Age (years)	−0.001 (−0.031 to 0.028)	.93		
Sex				
Male	Ref			
Female	0.922 (−0.266 to 2.111)	.127		
Plasma HIV RNA (log_{10} copies/mL)	0.685 (0.547 to 0.823)	<.001	0.604 (0.470 to 0.738)	<.001
CD4 (per 100 cells/μL)	−0.273 (−0.385 to −0.161)	<.001		NS
CD8 (per 100 cells/μL)	0.040 (0.006 to 0.074)	.022		NS
CD4/CD8	−0.977 (−1.334 to −0.620)	<.001	−0.616 (−0.903 to −0.330)	<.001
Fiebig Stage				
III and later	Ref			
I and II	−1.238 (−1.629 to −0.848)	<.001		NS
ARS				
No	Ref			
Yes	1.047 (0.559 to 1.535)	<.001		NS
Active Syphilis[b]				
No	Ref			
Yes	0.315 (−0.548 to 1.177)	.471		

Abbreviations: ARS, acute retroviral syndrome; CI, confidence interval; CSF, cerebrospinal fluid; HIV, human immunodeficiency virus; NS, not significant; Ref, reference; RNA, ribonucleic acid; RPR, Rapid Plasma Reagin; VDRL, Venereal Disease Research Laboratory.

[a]A level of 80 copies/mL was assigned to those who had a CSF HIV RNA level below quantification. Factors with $P < .1$ in the univariate analysis were included into the multivariate analysis.

[b]Defined as positive *Treponema pallidum* hemagglutination and RPR/VDRL, with no treatment 3 months before the acute HIV infection diagnosis.

II and 92% at Fiebig III to V. Our findings provide additional evidence that HIV can invade the CNS at the earliest phase of infection. In addition, our assessment of immune activation biomarkers indicates that HIV invasion is not a silent event and is associated with an extensive CNS immunologic response.

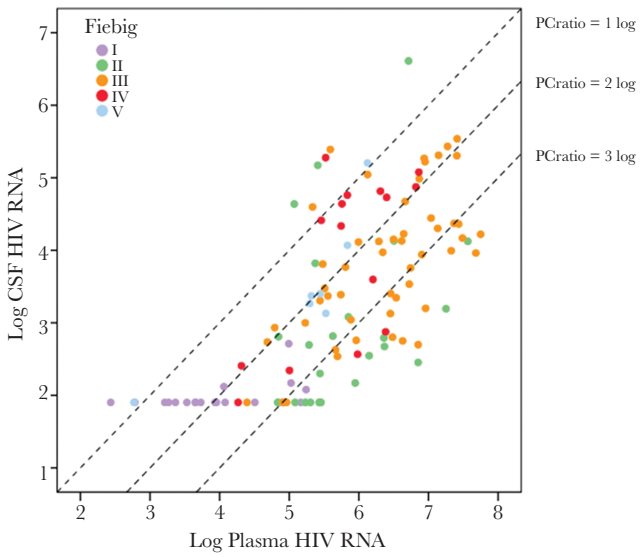

Figure 3. Correlation between cerebrospinal fluid (CSF) and plasma human immunodeficiency virus (HIV) ribonucleic acid (RNA) level by Fiebig staging. A level of 80 copies/mL was assigned to the CSF samples with HIV RNA level below quantification. Correlation coefficient = 0.719; $P < .001$ (Spearman). $PC_{ratio} = log_{10}$ plasma HIV RNA minus log_{10} CSF HIV RNA. Abbreviation: PC_{ratio}, plasma-CSF HIV RNA ratio.

Our cohort provides a novel parallel comparison between plasma and CSF CSF HIV RNA level across Fiebig stages in acute HIV infection, in which the CSF HIV RNA level peaked at Fiebig IV whereas the plasma HIV RNA peaked at Fiebig III. This is in line with recent studies that report a systemic HIV-specific T-cell expansion and cytokine burst taking place during Fiebig III in both human and primate models [18–20]. Moreover, in a prior report from this cohort, the frequency of activated CD8 T lymphocytes was elevated in CSF samples from later Fiebig stages compared with those from earlier stages [21]. Because the brain is an immune privileged site bound by the blood-brain barrier (BBB), it may delay the entry of infected cells into the CNS, and hence there is a delayed HIV viral expansion in CSF in comparison with plasma [22, 23].

Determinants of Cerebrospinal Fluid Human Immunodeficiency Virus Ribonucleic Acid Level

To date, most CSF HIV RNA level studies have come from chronic HIV infection cohorts and occasionally primary HIV infection cohorts defined as within 1 year of HIV transmission. Plasma HIV RNA has been reported to be predictive of that in CSF [5, 12, 13]. Severity of immunosuppression [24], concomitant CNS opportunistic infection [12, 15], and presence of HIVE [14, 15] may also be independently associated with the CSF HIV RNA level. Our analysis is unique in that we assessed viral dynamics from the bloodstream and CSF at the stage of CNS invasion. By contrast, chronic HIV infection represents a stage of equilibrium in which local viral replication in CNS has already been established and multiple confounding factors may impact CSF HIV RNA level.

Table 3. Linear Regression Analysis on Plasma CSF HIV RNA Ratio (PC_{ratio}) (N = 117)[a]

	Univariate analysis		Multivariable analysis	
Characteristics	β Coefficient (95% CI)	*P* Value	Adjusted β Coefficient (95% CI)	*P* Value
Age	0.012 (−0.012 to 0.035)	.320		
Sex				
Male	Ref			
Female	−0.791 (−1.741 to 0.159)	.102		
CD4 (per 100 cells/μL)	−0.064 (−0.162 to 0.034)	.198		
CD8 (per 100 cells/μL)	−0.029 (−0.056 to −0.001)	.040		NS
CD4/CD8	0.380 (0.068 to 0.692)	.018	0.380 (0.068 to 0.692)	.018
Fiebig Stage				
III and later	Ref			
I and II	0.128 (−0.233 to 0.490)	.483		
ARS Presentation				
No	Ref			
Yes	0.320 (−0.096 to 0.736)	.130		
Active Syphilis[b]				
No	Ref	.627		
Yes	−0.170 (−0.861 to 0.521)			

Abbreviations: ARS, acute retroviral syndrome; CI, confidence interval; CSF, cerebrospinal fluid; HIV, human immunodeficiency virus; NS, not significant; Ref, reference; RNA, ribonucleic acid; RPR, Rapid Plasma Regain; VDRL, Venereal Disease Research Laboratory.

[a]A level of 80 copies/mL was assigned to those who had a CSF HIV RNA level below quantification. Factors with $P < .1$ in the univariate analysis were included into the multivariate analysis.

[b]Defined as positive *Treponema pallidum* hemagglutination and RPR/VDRL, with no treatment 3 months before the acute HIV infection diagnosis.

Although the exact mechanism of HIV CNS entry has not been elucidated, pathways such as free virus entry or a cell-mediated cascade have been hypothesized [25]. The cell-mediated, or so-called "Trojan horse" mechanism, is the most accepted theory, in which HIV is transported across the BBB by infected $CD4^+$ lymphocytes and monocytes. After seeding of

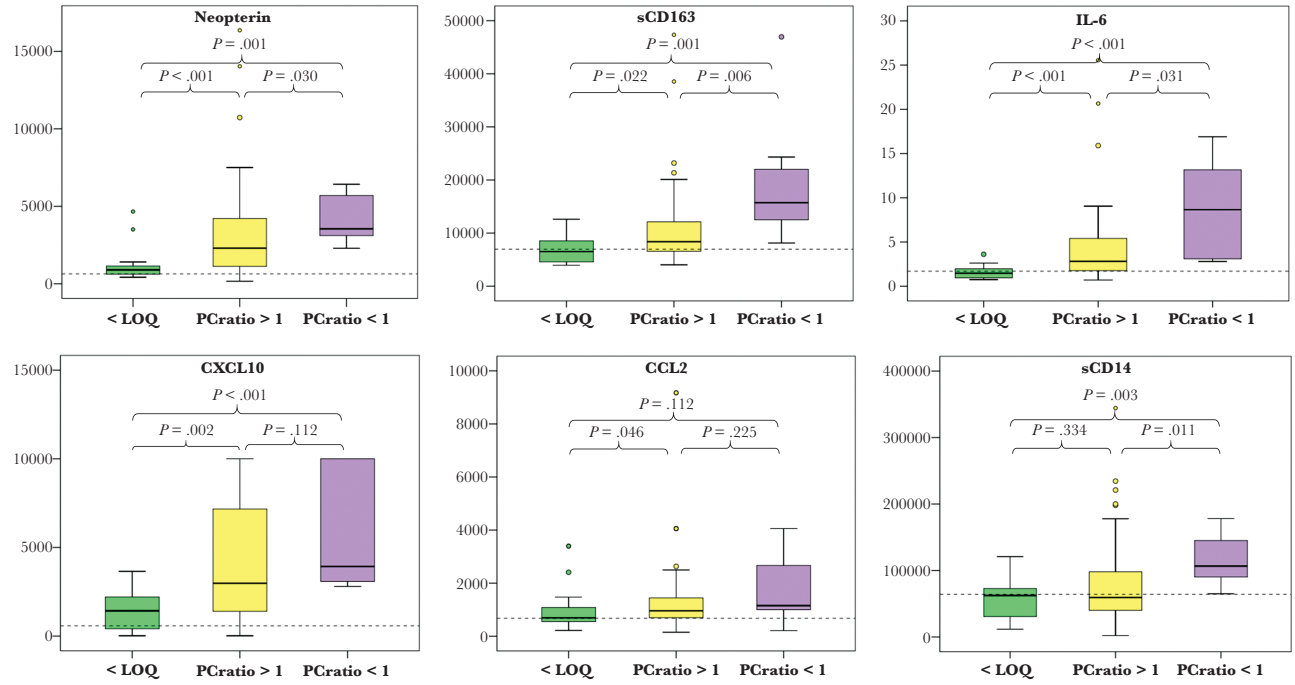

Figure 4. Levels of cerebrospinal fluid (CSF) immune and inflammatory markers in participants grouped by CSF human immunodeficiency virus (HIV) ribonucleic acid (RNA) quantification and PC_{ratio} status: CSF HIV RNA below the lower limit of quantification (<LOQ), F quantifiable CSF HIV RNA with PC_{ratio} >1 (PC_{ratio} >1), quantifiable CSF HIV RNA with PC_{ratio} <1 (PC_{ratio} <1). Median (middle line), interquartile range (extent of boxes), 1.5 times of interquartile range (whiskers), and outliers/extreme outliers (colored dots) are indicated. *P* value of non-parametric test between groups are shown. Dotted horizontal line: median level of 22 unmatched HIV-negative Thai controls. All markers level are presented in pg/mL. $PC_{ratio} = \log_{10}$ plasma HIV RNA minus $\log_{10}$ CSF HIV RNA. Abbreviation: PC_{ratio}, plasma-CSF HIV RNA ratio.

the intracerebral perivascular space, local CNS cells including microglia, tissue macrophages, and possibly astrocytes are infected [26, 27]. The positive correlation between plasma and CSF HIV RNA level in the linear regression model would support either free virus or a cell-mediated cascade hypotheses.

Predictors of Viral Penetration Into Central Nervous System

Previous CSF versus plasma analyses from primary HIV infection cohorts revealed a larger PC_{ratio} than seen in chronic infection [5, 16]. In addition to this observation, we noted a surprisingly large variation of PC_{ratio} in our group of participants. Although some had a high PC_{ratio}, with CSF HIV RNA level below the level of detection in conjunction with high plasma HIV RNA, there was also a group of outliers who had an exceptionally low PC_{ratio} that was similar to that seen in chronic infection [24, 28]. These outliers exhibited heightened CNS immune activation as demonstrated by significantly higher levels of CSF cytokines including neopterin, CXCL10, sCD14, and sCD163.

Our study demonstrates that initial viral invasion into the CNS was only partially governed by the duration of HIV infection, with the lowest rate of measurable CSF HIV RNA of 26% at Fiebig stage I, progressively increasing to over 90% in Fiebig III to V. It is interesting to note that the PC_{ratio} did not correlate with Fiebig stage. Taking Fiebig stage II CSF samples as example, although the rate of measurable CSF HIV RNA level in this group was lower than that of Fiebig stage III to V, 3 of 7 PC_{ratio} outliers came from Fiebig II. This paradoxical finding may suggest that HIV dynamics between the systemic circulation and the CNS, once established, are no longer altered by duration of infection.

The correlation of CD4/CD8 ratio with both CSF HIV RNA level and PC_{ratio} further suggests an independent role of the immune response in modulating viral trafficking across the BBB. Indeed, there is a growing body of literature regarding the relevance of CD4/CD8 ratio as a marker of the immunologic state in both HIV-infected and uninfected populations. In uninflected individuals, a low CD4/CD8 ratio is associated with the immunosenescent state in which T-cell effector reaction and vaccination immunogenicity are hypofunctional [29, 30]. In treated HIV-infected individuals, a persistently low CD4/CD8 ratio represents immunologic dysfunction that is associated with ongoing heightened immune activation [31, 32]. Clinically, a low CD4/CD8 ratio has been linked with impaired cognitive performance [33], vascular complications, malignancy, and mortality in HIV populations [34]. Moreover, a previous cellular composition study found a linked distortion of CD4/CD8 ratio between plasma and CSF samples from HIV-infected individuals [35]. These findings suggested that the CD4/CD8 ratio may serve as an immunologic state marker independent of absolute CD4 or CD8 T-lymphocyte counts.

In light of the recent findings in treatment-naive and treated HIV-infected populations correlating cognitive impairment and high CSF/plasma HIV RNA ratio [28] and low CD4/CD8 ratio [33], respectively, we hypothesize that a lower CD4/CD8 ratio would give rise to early seeding of the CNS reservoir and hence a higher CSF/plasma HIV RNA ratio through enhanced viral penetration. In treated HIV infection, given BBB permeability defects not readily reverted by cART [36] and the association of low CD4/CD8 ratio with increased HIV systemic reservoir size [37, 38] and more frequent viral blips [39], HIV may continue to breach the BBB directly through low-level viremia or indirectly carried by activated immune cells. Both scenarios, pre- and posttreatment, could seed the CNS with a larger pool of infected and immune-activated cells and set the stage for inflammatory-driven injury. In a recent study of HIV-infected participants on suppressive ART using a neurocognitive assessments and a positron emission tomography (PET) radioligand ([11C]PBR28) to measure brain microglial activation, a low CD4/CD8 ratio was associated with heightened CNS immune activation by PET, which, in return, was associated with altered white matter structure in magnetic resonance imaging and poorer cognitive performance [40].

We observed that CD4/CD8 ratio and plasma HIV RNA level are closely associated and surmise that both may link to total body viral load during untreated acute HIV infection. However, (1) the wide spectrum of viral dynamics between plasma and CSF after Fiebig stage I and (2) the positive correlation between PC_{ratio} and CD4/CD8 ratio suggest a modification of viral CNS penetration by the individual's immune response in addition to virologic factors. Without treatment, it is reasonable to anticipate a consequential hastening of CNS infection and injury through the increased viral penetration, which eventually leads to earlier development of neurological complications. Studies focused on treatment-naive early HIV infection would be useful to clarify the linkage among CD4/CD8, PC_{ratio}, and the development of cognitive impairment.

Limitations

This study included a population of predominantly young Thai men who sleep with men with HIV clade CRF AE_01 infection, and the findings may not be applicable to other populations. We may also overestimate the actual CSF HIV RNA level by assigning a lower limit value to those CSF HIV RNA level below the lower limit of quantification, which may affect the intergroup CSF cytokine comparisons. We also acknowledge that although CSF is one measure of CNS exposure to HIV, CSF HIV RNA level does not provide an exact index of degree of brain parenchymal infection. Finally, characterizing the viral dynamic between plasma and CSF compartments by PC_{ratio} is an approach that fulfills linear regression model criteria but may oversimplify nonlinear relationships between the 2 compartments.

CONCLUSIONS

We found that the CSF HIV RNA level correlates with that in plasma at acute HIV infection. However, PC_{ratio} is higher in acute than in chronic HIV infection, and it is highly variable. The CSF HIV RNA level peaked at Fiebig stage IV, slightly later

than the height of activation of HIV-specific blood cytotoxic T cells during Fiebig stage III. We also found that the degree of viral penetration into the CSF compared with blood could be predicted by CD4/CD8 ratio. Given a similar correlation to cognitive impairment in chronic infection, both PC_{ratio} and CD4/CD8 ratio may be important markers for clinically significant neurologic outcomes in both acute and chronic infection.

Notes

Author contributions. All authors have contributed to and approved the manuscript.

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Potential conflicts of interest. J. A. reports other from ViiV Healthcare, other from Merck, other from Roche, and personal fees from Gilead, outside the submitted work. V. V. reports the following: personal fees from ViiV Healthcare and personal fees from Merck, during the conduct of the study; and personal fees from IAS-USA and grants from the National Institutes of Health (NIH), outside the submitted work. S. S. reports grants from NIH, National Institute of Mental Health and National Institute of Neurological Disorders and Stroke, during the conduct of the study, and nonfinancial support from ViiV Healthcare, Inc., outside the submitted work. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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