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Cerebrospinal Fluid Viral Load Across the Spectrum of Untreated Human Immunodeficiency Virus Type 1 (HIV-1) Infection: A Cross-Sectional Multicenter Study

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Background. The aim of this large multicenter study was to determine variations in cerebrospinal fluid (CSF) HIV-RNA in different phases of untreated human immunodeficiency virus type 1 (HIV-1) infection and its associations with plasma HIV-RNA and other biomarkers.

Methods. Treatment naive adults with available CSF HIV-RNA quantification were included and divided into groups representing significant disease phases. Plasma HIV-RNA, CSF white blood cell count (WBC), neopterin, and albumin ratio were included when available.

Results. In total, 1018 patients were included. CSF HIV-RNA was in median (interquartile range [IQR]) 1.03 $\log_{10}$ (0.37–1.86) copies/mL lower than in plasma, and correlated with plasma HIV-RNA (r = 0.44, $P < .01$), neopterin concentration in CSF (r = 0.49, $P < .01$) and in serum (r = 0.29, $P < .01$), CSF WBC (r = 0.34, $P < .01$) and albumin ratio (r = 0.25, $P < .01$). CSF HIV-RNA paralleled plasma HIV-RNA in all groups except neuroasymptomatic patients with advanced immunodeficiency (CD4 < 200) and patients with HIV-associated dementia (HAD) or opportunistic central nervous system (CNS) infections. Patients with HAD had the highest CSF HIV-RNA (in median [IQR] 4.73 (3.84–5.35) $\log_{10}$ copies/mL). CSF > plasma discordance was found in 126 of 972 individuals (13%) and varied between groups, from 1% in primary HIV, 11% in neuroasymptomatic groups, up to 30% of patients with HAD.

Conclusions. Our study confirms previous smaller observations of variations in CSF HIV-RNA in different stages of HIV disease. Overall, CSF HIV-RNA was approximately 1 $\log_{10}$ copies/mL lower in CSF than in plasma, but CSF discordance was found in a substantial minority of subjects, most commonly in patients with HAD, indicating increasing CNS compartmentalization paralleling disease progression.

Keywords. HIV-1; cerebrospinal fluid; HIV-RNA.

It has previously been observed that asymptomatic, untreated individuals living with human immunodeficiency virus (HIV) with advanced immune dysfunction usually have relatively low cerebrospinal fluid (CSF) HIV RNA, in contrast to plasma HIV RNA, which normally increases as HIV disease progresses [1–6]. In a large multicenter study, we aimed to (a) investigate whether these observations could be confirmed, (b) compare the CSF HIV RNA in various stages of the course of infection and during opportunistic complications of HIV disease, and (c) hypothesize possible explanations.

HIV invades the central nervous system (CNS) shortly after acquisition of infection. HIV-RNA can be detected almost simultaneously in CSF and plasma during primary infection and remains detectable in the CSF throughout the untreated disease course [1–3]. The dynamics of CSF HIV-RNA during the chronic asymptomatic phase of infection generally parallel that of plasma, where CSF HIV RNA gradually increases over time, albeit at a lower level than in plasma. In patients with severe immunodeficiency but no neurological complications, surprisingly low levels of CSF HIV-RNA have been reported [1, 4, 7].

In contrast, in patients with HIV-associated dementia (HAD), or CNS opportunistic infections and malignancies, CSF viral load often exceeds that in plasma [1, 8–10].

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By establishing a uniquely large multicenter cohort we were able to study variations in CSF HIV-RNA at different phases of untreated HIV infection and observe how these variations correspond to plasma. In addition, we examined the relationship between CSF HIV-RNA and markers of CSF inflammation and blood brain barrier integrity, which may assist our understanding of the biological processes involved.

METHODS

Study Design and Participants

By collecting retrospective data from eight different centers in the cities of Gothenburg, Rome, Sydney, Turin, Milan, Bucharest, San Francisco, and London between 1982 and 2017, we established a cohort of 1018 participants. All were treatment naive and had undergone CSF examination for CSF HIV-1 RNA quantification, either due to CNS symptoms or in the course of clinical studies. Other analyses, described in detail below, were included when available. The participants were divided into pre-defined groups similar to those used in previous studies [11, 12], each group representing a significant phase of HIV disease: primary infection (PHI, defined as within the first 12 months of an initial human immunodeficiency virus type 1 (HIV-1) infection [13, 14]); 5 groups of neuroasymptomatic subjects defined by $CD4^+$ T-cell count thresholds (> 500 cells/μL; 350–499 cells/μL, 200–349 cells/μL, 50–199 cells/μL, and < 50 cells/μL); HAD; opportunistic CNS infections and malignancies (CNS OI); and "elite" controllers (HIV positive ≥ 1 year, with ≥ 2 plasma HIV-RNA < 50 copies/mL despite no antiretroviral therapy [ART] [15]). The CNS OI group included patients with cytomegalovirus (CMV) encephalitis, Epstein-Barr virus-associated primary CNS lymphoma (PCNSL), cryptococcal meningitis, tuberculous meningitis, progressive multifocal leukoencephalopathy (PML), and CNS toxoplasmosis.

The diagnoses of HAD and each of the opportunistic neurological CNS complications were based on the Centers for Disease Control and Prevention (CDC) and American Academy of Neurology AIDS Task force criteria, using standard clinical and laboratory evaluations [16–18].

Our study was approved by Institutional Review Boards at each study site and informed consent was obtained from all participants.

CSF and Blood Measurements

CSF and blood samples were collected and analyzed according to current clinical routines.

Blood CD4+ T-cell counts, blood and CSF albumin levels, and CSF white blood cell counts (WBC) were performed using routine methods. Pleocytosis was defined as CSF WBC ≥ 5 cells/μL. Albumin ratios were calculated as CSF albumin (mg/L)/plasma albumin (g/L) and used to evaluate BBB function. Reference values were < 6.8 for individuals aged 45, and < 10.2 for those ≥ 45 years old [19].

HIV-RNA levels were quantified by real-time polymerase chain reaction (PCR) using the assay available at the respective site and time.

Neopterin was analyzed in CSF and serum using a commercially available immunoassay (NEOPT-SCR.EIA 384 Det., Thermo Fisher Scientific – BRAHMS GmbH, Henningsdorf, Germany) with an upper normal reference value of 8.8 nmol/L in plasma and 5.8 nmol/L in CSF [6].

Samples collected before PCR methods were introduced in clinical routine were retrospectively analyzed using stored aliquots of CSF and blood (frozen at –70°C after centrifugation) as part of clinical studies or clinical procedures independent of this study.

Statistical Methods

Descriptive statistics were performed using SPSS (IBM SPSS version 24 software, Armonk, New York, USA) or Prism 9.0 (GraphPad Software, San Diego, California, USA). Continuous variables are reported as median (interquartile range) and were $\log_{10}$ transformed where appropriate for the tests used. Comparisons between groups were done using either Mann-Whitney test or 1-way ANOVA with Dunnett's T3 post hoc test. Correlations were explored using Spearman's rank correlation.

RESULTS

Background clinical and laboratory characteristics for each subject group are summarized in Table 1.

Study Population

The cohort of 1018 participants (76% male) had a median age of 38 (32–47) years.

Table 2 shows the number of participants in each group and the center from which they were recruited.

HIV-RNA

CSF HIV-RNA was characteristically lower than plasma HIV-RNA, with a median (IQR) difference of 1.03 (0.37–1.86) $\log_{10}$ copies/mL (Table 1 and Figure 1). CSF HIV-RNA discordance, defined as CSF≥ plasma HIV-RNA in untreated individuals [20] (ie, a plasma:CSF ratio ≤ 0) was found in 126 of 972 patients (13%) with a wide variation between the various groups. Figure 2 shows the proportion of patients with CSF HIV-RNA > plasma HIV-RNA by groups.

CSF HIV-RNA was significantly higher in patients with HAD compared to all other subgroups (4.73 $\log_{10}$ copies/mL [3.84–5.31], Table 3). The HAD group also had the smallest plasma:CSF ratio: 0.54 (–0.18 to 1.34); 30% (n = 38) exhibited CSF discordance. Neuroasymptomatic patients with CD4 < 50 had the highest plasma HIV-RNA levels (5.35 $\log_{10}$ copies/

Table 1. Subject Characteristics Across the Nine Groups

	Participants	Age	Gender	CD4	CD8	HIV-RNA			CSF WBC	CSF Neopterin	Serum Neopterin	Albumin Ratio
						Plasma	CSF	ΔPlasma–CSF				
	N	Years (IQR)	Male: Female	cells/μL (IQR)	cells/μL (IQR)	log10 copies/mL (IQR)	log10copies/mL (IQR)	log10 copies/mL (IQR)	cells/μL (IQR) (% abnormal)[a]	nmol/L (IQR) (% abnormal)[a]	nmol/L (IQR) (% abnormal)[a]	(IQR) (% abnormal)[a]
PHI	136	34 (28–43) ND 3	122:8 ND 6	533 (383–699) ND 7	955 (689–1360) ND 13	4.90 (4.26–5.59) ND 1	2.99 (2.30–4.01)	1.77 (1.22–2.30) ND 1	6 (2–12) (56%) ND 7	14.3 (8.60–24.5) (95%) ND 93	15.3 (9.48–21.8) (79%) ND 40	4.92 (3.76–6.64) (21%) ND 30
NA > 500	63	39 (30–46)	41:19 ND 3	630 (543–706)	1072 (880–1314) ND 1	4.16 (3.52–4.66)	3.17 (2.17–3.74)	0.87 (0.49–1.59)	5 (2–12) (52%)	11.1 (7.03–20.9) (88%) ND 3	10.8 (8.3–16.7) (66%) ND 6	5.10 (3.70–6.52) (8%) ND 4
NA 350–499	79	40 (32–47)	58:17 ND 4	410 (389–456)	948 (694–1287) ND 1	4.45 (3.92–4.86)	3.64 (2.69–4.30)	0.72 (0.26–1.18)	6 (3–10) (58%) ND 2	14.3 (8.8–22.8) (90%) ND 7	12.0 (8.6–19.8) (73%) ND 10	4.95 (3.66–6.26) (19%) ND 1
NA 200–349	129	37 (31–43) ND 10	78:41 ND 10	260 (231–300)	920 (612–1239) ND 2	4.79 (4.28–5.17)	4.06 (3.47–4.47)	0.66 (0.24–1.26)	6 (2–14) (61%) ND 1	17.1 (11.7–25.1) (97%) ND 14	18.9 (11.1–23.6) (91%) ND 17	4.88 (3.70–6.87) (18%) ND 11
NA 50–199	140	42 (35–49)	88:39 ND 13	110 (82–150)	737 (543–1049) ND 4	5.16 (4.66–5.70) ND 1	4.07 (3.41–4.80)	0.97 (0.31–1.68) ND 1	3 (0–8) (40%)	21.1 (11.7–34.5) (94%) ND 22	22.4 (15.9–34.3) (95%) ND 44	5.10 (3.81–7.15) (15%) ND 16
NA < 50	134	42 (35–49)	88:35 ND 11	20 (10–35)	340 (194–476) ND 1	5.35 (4.91–5.76) ND 1	3.19 (2.42–3.87)	2.13 (1.40–2.76) ND 1	0 (0–1) (9%)	16.5 (9.3–34.7) (87%) ND 15	25.0 (18.2–43.3) (98%) ND 49	5.00 (3.80–6.41) (13%) ND 8
HAD	144	40 (33–46) ND 1	85:17 ND 42	60 (16–172) ND 4	476 (259–825) ND 43	5.18 (4.66–5.58) ND 19	4.73 (3.84–5.35)	0.54 (–0.18 to 1.34) ND 19	3 (1–10) (41%) ND 15	63.5 (33.0–120) (99%) ND 36	27.4 (19.5–51) (95%) ND 50	9.80 (5.69–13.25) (63%).ND 76
CNS OI	174	36 (32–44) ND 2	126:36 ND 12	30 (10–80) ND 15	412 (248–729) ND 101	5.09 (4.57–5.60) ND 24	4.11 (3.05–4.83)	0.81 (0.26–1.81) ND 24	2 (1–11) (34%) ND 17	44.5 (18.9–101) (94%) ND 138	32.6 (16.5–212.1) (96%) ND 148	7.50 (5.15–9.45) (48%) ND 141
ECs	19	51 (37–60) ND 3	7:7 ND 5	1100 (738–1362)	653 (538–1037) ND 1	1.40 (1.40–1.40)	1.40 (1.40–1.40)	0.00 (0.00–0.00)	1 (0–2) (6%) ND 1	5.90 (4.7–8.2) (53%) ND 2	8.0 (6.03–10.8) (50%) ND 3	4.10 (3.52–5.03) (0%) ND 1
Total	1018	38 (32–47) ND 19	693:219 ND 106	164 (37–390) ND 26	715 (450–1095) ND 167	4.92 (4.33–5.54) ND 46	3.80 (2.86–4.59)	1.03 (0.37–1.86) ND 46	3 (1–9) (42%) ND 43	19.7 (10.8–39.0) (92%) ND 330	19.3 (11.0–29.6) (87%) ND 367	5.20 (3.90–7.31) (22%) ND 293

Abbreviations: CNS, central nervous system; CNS OI, opportunistic CNS infections and malignancies; CSF, cerebrospinal fluid; EC, "elite" controller; HAD, HIV-associated dementia; HIV, human immunodeficiency virus; IQR, interquartile range; NA, neuroasymptomatic; ND, number of subjects with no available data; PHI, primary HIV infection.

[a]Percentage of subjects with biomarker concentrations above normal upper reference value.

Table 2. Study Population Centers in Cohort

Site	PHI	NA > 500	NA 350–499	NA 200–349	NA 50–199	NA < 50	HAD	CNS OI	ECs
GOT	17	36	46	80	75	59	16	9	4
ROM	3	4	4	7	15	5	14	16	…
SF	85	17	20	25	10	10	11	…	12
MIL	17	…	…	…	…	…	58	96	…
BUCH	2	…	…	…	…	…	…	25	…
TOR	6	3	4	6	27	49	3	18	…
LON	…	…	1	1	…	…	…	…	1
SYD	6	3	4	10	13	11	42	10	2
Total	136	63	79	129	140	134	144	174	19

Abbreviations: BUCH, Bucharest; CNS, central nervous system; CNS OI, opportunistic CNS infections and malignancies; EC, "elite" controller; GOT, Gothenburg; HAD, HIV-associated dementia; HIV, human immunodeficiency virus; LON, London; MIL, Milan; NA, neuroasymptomatic; PHI, primary HIV infection; ROM, Rome; SF, San Francisco; SYD, Sydney; TOR, Turin.

mL [4.91–5.76]), and the largest plasma:CSF ratio (2.13 [1.40–2.76]). The plasma:CSF ratio was also high in the PHI group: 1.77 (1.22–2.30), where only 1% (n = 2) had CSF discordance. These comparisons support the visual impression that viral load increases in parallel in both compartments in the chronic neuroasymptomatic stage (Figure 1), that CSF HIV-RNA decreases in the most immunocompromised stage (CD4 < 50) but not in the case of neurosymptomatic patients (HAD and some CNS OI), where despite low CD4 cell counts, CSF HIV-RNA often exceeds that in plasma.

The heterogenous group with CNS OI had an overall median plasma:CSF ratio of 0.81 (0.26–1.81), with 17% (n = 26) CSF discordance. Viral loads in both compartments did not vary significantly between the different CNS OIs, except in patients with PML, who had significantly lower CSF HIV-RNA levels than patients with cryptococcal meningitis ($P < .01$) and CMV encephalitis ($P < .05$) (Figure 3).

CSF Biomarkers

Pleocytosis was a common finding in all groups (total 42%, n = 404), except for neuroasymptomatic patients with CD4 < 50 and elite controllers (Table 1 and Figure 4).

The vast majority of subjects had elevated neopterin levels in both compartments: 92% in CSF and 87% in plasma (Table 1 and Figure 4C–4D). The highest CSF neopterin was found in HAD: 63.5 (33.0–120) nmol/L, whereas the highest serum neopterin was found among patients with CNS OI: 32.6 (16.5–212.1) nmol/L. Approximately half of elite controllers had normal neopterin levels in both compartments. CSF neopterin was significantly ($P < .001$) higher in subjects with CSF discordance compared to subjects with non-discordance: 33.4 (23.0–83.1) and 17.8 (9.6–32.4), respectively (Supplementary Figure 3).

Albumin ratio was significantly ($P < .001$) higher in the HAD group compared to all groups, with the exception of CNS OI. Sixty-three percent of patients with HAD had an elevated

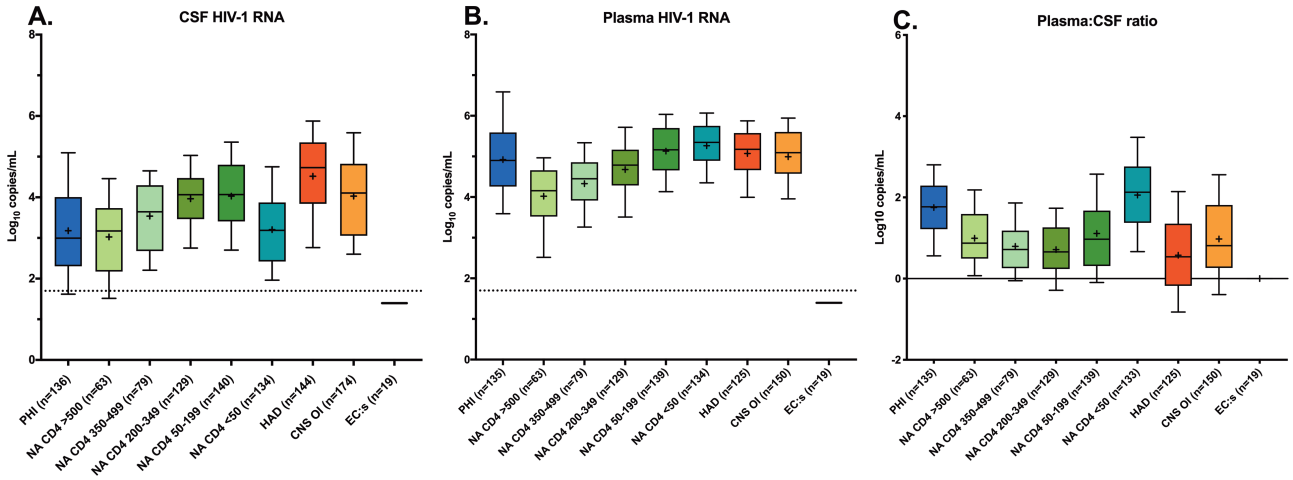

Figure 1. HIV-RNA concentration in CSF and plasma in subject groups. Boxes depict interquartile ranges with median (line) and mean ("+"), whereas whiskers represent 10th to 90th percentiles. HIV-RNA concentrations are displayed as $\log_{10}$ copies/mL, whereas the dotted line in panels (*A*) and (*B*) indicates 50 (1.70 $\log_{10}$) copies/mL. Findings and statistical comparisons between the groups are described in the text and Tables 1 and 3. *C*, Difference in viral load between plasma and CSF ($\log_{10}$ plasma HIV-RNA – $\log_{10}$ CSF HIV-RNA); the grid line represents the difference of zero $\log_{10}$ copies/mL. Number of subjects (n) in each group is showed in brackets following the group name. Abbreviations: CD4, $CD4^+$ T lymphocyte count; CNS, central nervous system; CNS OI, CNS opportunistic infections; CSF, cerebrospinal fluid; EC, elite controller; HAD, HIV dementia complex; HIV, human immunodeficiency virus; HIV-1, human immunodeficiency virus type 1; NA, HIV+ neuroasymptomatic patients; PHI, primary HIV infection; n, number of subjects.

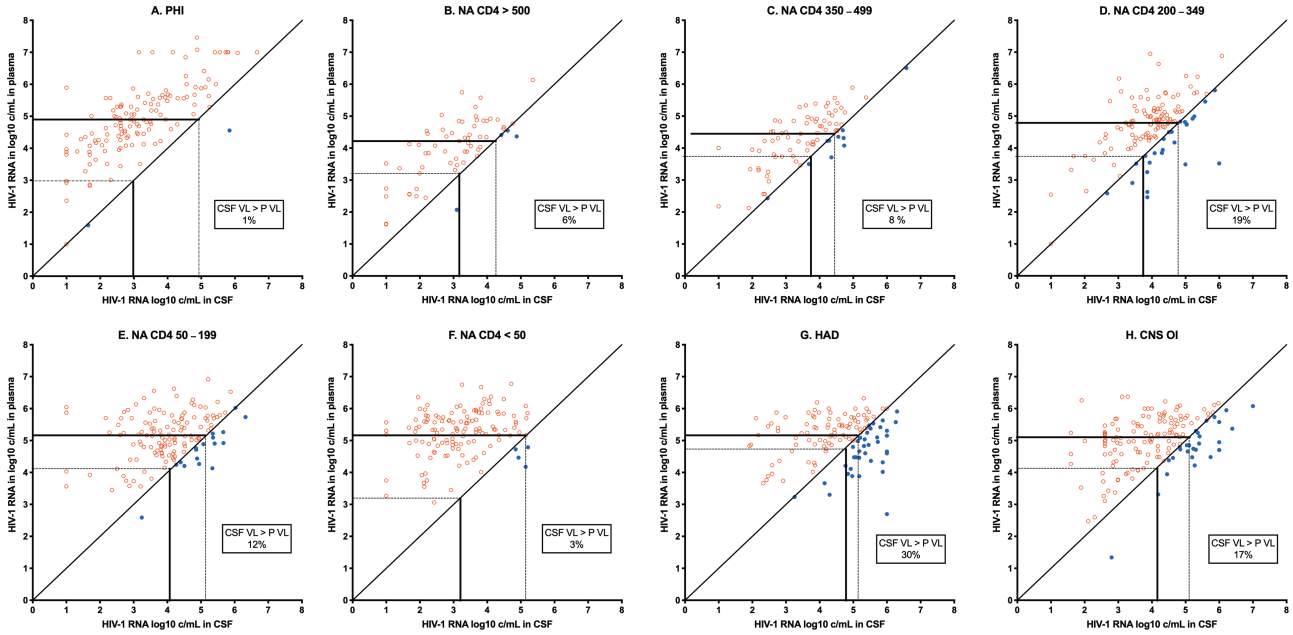

Figure 2. Relationship between CSF HIV-RNA and plasma HIV-RNA among groups. Panels (*A–H*) show the relationship between plasma- and CSF HIV-RNA in 8 predefined groups. Red circles represent patients with plasma HIV-RNA > CSF HIV-RNA; blue circles indicate patients with CSF HIV-RNA > plasma HIV-RNA. Thick black lines represent the median HIV-1 RNA levels in CSF and plasma. Dotted line is an aid to visualize the median difference in each of the 2 body compartments. Abbreviations: CD4, CD4+ T lymphocyte count; CNS, central nervous system; CNS OI, CNS opportunistic infections; CSF, cerebrospinal fluid; Elite, elite controller; HAD, HIV dementia complex; HIV-1, human immunodeficiency virus type 1; NA, HIV+ neuroasymptomatic patients; P, plasma; PHI, primary HIV infection; VL, viral load.

albumin ratio, although albumin ratios were normal in all elite controllers (Table 3 and Figure 4).

Correlations Across Groups

Correlations between HIV-RNA, CSF WBC, neopterin, and albumin ratios are shown in a heat map (Supplementary Figure 1). Overall, CSF HIV-RNA was most closely associated with CSF neopterin (r = 0.49, $P < .01$) and plasma HIV-RNA (r = 0.44, $P < .01$). We also explored correlations among the same biomarkers after dividing the patients into four groups: PHI, neuroasymptomatic, HAD and CNS OI (Supplementary Figure 2*A*–2*D*). In the neuroasymptomatic group, CSF HIV RNA was associated with CSF neopterin (r = 0.43, $P < .01$), CSF WBC (r = 0.42, $P <$,01), and plasma HIV-RNA (r = 0.35, $P < .01$), although the plasma:CSF ratios were negatively associated with CSF WBC counts (r = 0.61, $P < .01$). The HAD group showed a somewhat different pattern, with no significant correlation between CSF HIV-RNA and plasma HIV-RNA (r = 0.14, $P = .11$). A weak but still significant association was found between CSF HIV-RNA and CSF neopterin (r = 0.24, $P < .05$) and between CSF HIV-RNA and CSF WBC (r = 0.21, $P < .05$). In the CNS OI group, CSF HIV-RNA was strongest associated with the albumin ratio (r = 0.47, $P < .01$).

DISCUSSION

In this large multicenter study we were able to confirm that overall, HIV-RNA has a parallel dynamic in the plasma and CSF compartments during untreated infection. However, neuroasymptomatic HIV-infected patients with advanced immunodeficiency had low levels of CSF HIV-RNA despite high plasma levels. These low CSF HIV-RNA levels were accompanied by a low CSF WBC count. Moderate CSF pleocytosis is a common finding in early stages of HIV infection [1, 2], implying that CSF HIV-RNA is closely associated with meningeal inflammation and lymphocyte trafficking in neuroasymptomatic HIV. CSF neopterin is usually closely correlated to the presence of viral RNA in CSF in neuroasymptomatic patients [6]. In the present study, we found that CSF neopterin concentrations increased in parallel with declining blood CD4+ cell levels, from in median 11.1 nmol/L in patients with CD4+ counts >500 cells/μL to 17.1 nmol/L in patients with CD4+ cell counts of 200–349 cells/μL. Interestingly however, CSF neopterin concentrations seemed to plateau in individuals with CD4 cell counts < 200 cells/μL, with medians of 21.1 and 16.5, respectively, in patients with CD4 cell counts of 50-199 and <50. Although CSF HIV-RNA and CSF WBC decreased significantly in the most immunosuppressed group of neuroasymptomatic patients (NA CD4 < 50), this was not accompanied by a concomitant decline in CSF neopterin, implying that the level of intrathecal immune activation does not fully correspond to the presence of virions and/or immune cells in CSF in this group of patients. Likely, this residual intrathecal immune activation, despite absence or low levels of CSF viral RNA, reflects at least

Table 3. Comparisons of Investigated Biomarker Concentrations Among Subject Groups

Group Comparisons	HIV-RNA in CSF	HIV-RNA in Plasma	HIV-RNA ΔPlasma–CSF	CSF WBC	CSF Neopterin	Serum Neopterin	Albumin Ratio
Dunnett's T3 Multiple Comparisons	Overall ANOVA *P*						
	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001
PHI vs NA CD4 > 500	NS	< .0001	< .0001	NS	NS	NS	NS
PHI vs NA CD4 350–499	NS	< .001	< .0001	NS	NS	NS	NS
PHI vs NA CD4 200–349	< .0001	NS	< .0001	NS	NS	NS	NS
PHI vs NA CD4 50–199	< .0001	NS	< .0001	NS	NS	< .01	NS
PHI vs NA < 50	NS	NS	NS	< .0001	NS	< .0001	NS
PHI vs HAD	< .0001	NS	< .0001	NS	< .0001	< .0001	< .0001
PHI vs CNS OI	< .0001	NS	< .0001	NS	< .01	< .01	< .05
PHI vs ECs	< .0001	< .0001	< .0001	< .0001	< .0001	NS	NS
NA CD4 > 500 vs NA CD4 350–499	NS	NS	NS	NS	NS	NS	NS
NA CD4 > 500 vs NA CD4 200–349	< .0001	< .0001	NS	NS	NS	NS	NS
NA CD4 > 500 vs NA CD4 50–199	< .0001	< .0001	NS	NS	< .05	< .0001	NS
NA CD4 > 500 vs NA CD4 < 50	NS	< .0001	< .0001	< .0001	NS	< .0001	NS
NA CD4 > 500 vs HAD	< .0001	< .0001	NS	NS	< .0001	< .0001	< .0001
NA CD4 > 500 vs CNS OI	< .0001	< .0001	NS	NS	< .0001	< .01	< .05
NA CD4 > 500 vs ECs	< .0001	< .0001	< .0001	< .0001	<.01	NS	NS
NA CD4 350–499 vs NA CD4 200–349	NS	NS	NS	NS	NS	NS	NS
NA CD4 350–499 vs NA CD4 50–199	< .05	< .0001	NS	NS	NS	< .05	NS
NA CD4 350–499 vs NA CD4 < 50	NS	< .0001	< .0001	< .0001	NS	< .0001	NS
NA CD4 350–499 vs HAD	< .0001	< .0001	NS	NS	< .0001	< .0001	< .0001
NA CD4 350–499 vs CNS OI	< .05	< .0001	NS	NS	< .001	< .01	< .05
NA CD4 350–499 vs ECs	< .0001	< .0001	< .0001	< .0001	< .0001	NS	NS
NA CD4 200–349 vs NA CD4 50–199	NS	< .001	< .05	<.05	NS	NS	NS
NA CD4 200–349 vs NA CD4 < 50	< .0001	< .0001	< .0001	< .0001	NS	< .0001	NS
NA CD4 200–349 vs HAD	< .001	< .01	NS	NS	< .0001	< .0001	< .0001
NA CD4 200–349 vs CNS OI	NS	NS	NS	NS	< .01	< .05	< .05
NA CD4 200–349 vs ECs	< .0001	< .0001	< .0001	< .0001	< .0001	< .01	NS
NA CD4 50–199 vs NA CD4 < 50	< .0001	NS	< .0001	< .0001	NS	NS	NS
NA CD4 50–199 vs HAD	< .01	NS	< .01	NS	< .0001	NS	< .0001
NA CD4 50–199 vs CNS OI	NS	NS	NS	NS	< .05	NS	NS
NA CD4 50–199 vs ECs	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001	NS
NA CD4 < 50 vs HAD	< .0001	NS	< .0001	< .0001	< .0001	NS	< .0001
NA CD4 < 50 vs CNS OI	< .0001	NS	< .0001	< .0001	< .01	NS	< .05
NA CD4 < 50 vs ECs	< .0001	< .0001	< .0001	NS	< .0001	< .0001	NS
HAD vs CNS OI	< .01	NS	NS	NS	NS	NS	NS
HAD vs ECs	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001
CNS OI vs ECs	<.0001	< .0001	< .0001	< .0001	< .0001	< .0001	< .01

Abbreviations: ANOVA, analysis of variance; CNS, central nervous system; CNS OI, opportunistic CNS infections and malignancies; CSF, cerebrospinal fluid; EC, elite controller; HAD, HIV-associated dementia; HIV, human immunodeficiency virus; NA, neuroasymptomatic; NS, not significant; PHI, primary HIV infection; WBC, white blood cell.

in part a more compartmentalized CNS-specific infection that has been demonstrated in advanced disease [3, 21].

CSF neopterin has also been found to correlate with the light subunit of neurofilament protein (NfL) in CSF, indicating an association between immune activation and neuroaxonal injury in untreated neuroasymptomatic patients with HIV [22]. Altogether, the relationships found between CSF HIV RNA, CD4 cell count, CSF WBC, CSF neopterin, and NfL supports the theory that progressive immune activation may increase the risk of subsequent neuroaxonal injury as immunosuppression worsens.

Although there is usually a significant increase in CSF HIV-RNA in patients with HAD, high levels of CSF RNA are also commonly found in neuroasymptomatic patients with HIV, limiting the usefulness of CSF RNA as a single predictor of HIV-related CNS disease [23].

A possible explanation for the shifting of plasma:CSF viral load ratios at various CD4 cell strata during the chronic neuroasymptomatic phase of HIV infection may be that CSF HIV-RNA is mainly a reflection of a continuous re-seeding of immune cells, predominantly CD4+ T lymphocytes. These cells traffic into the CSF from the blood while a patient still

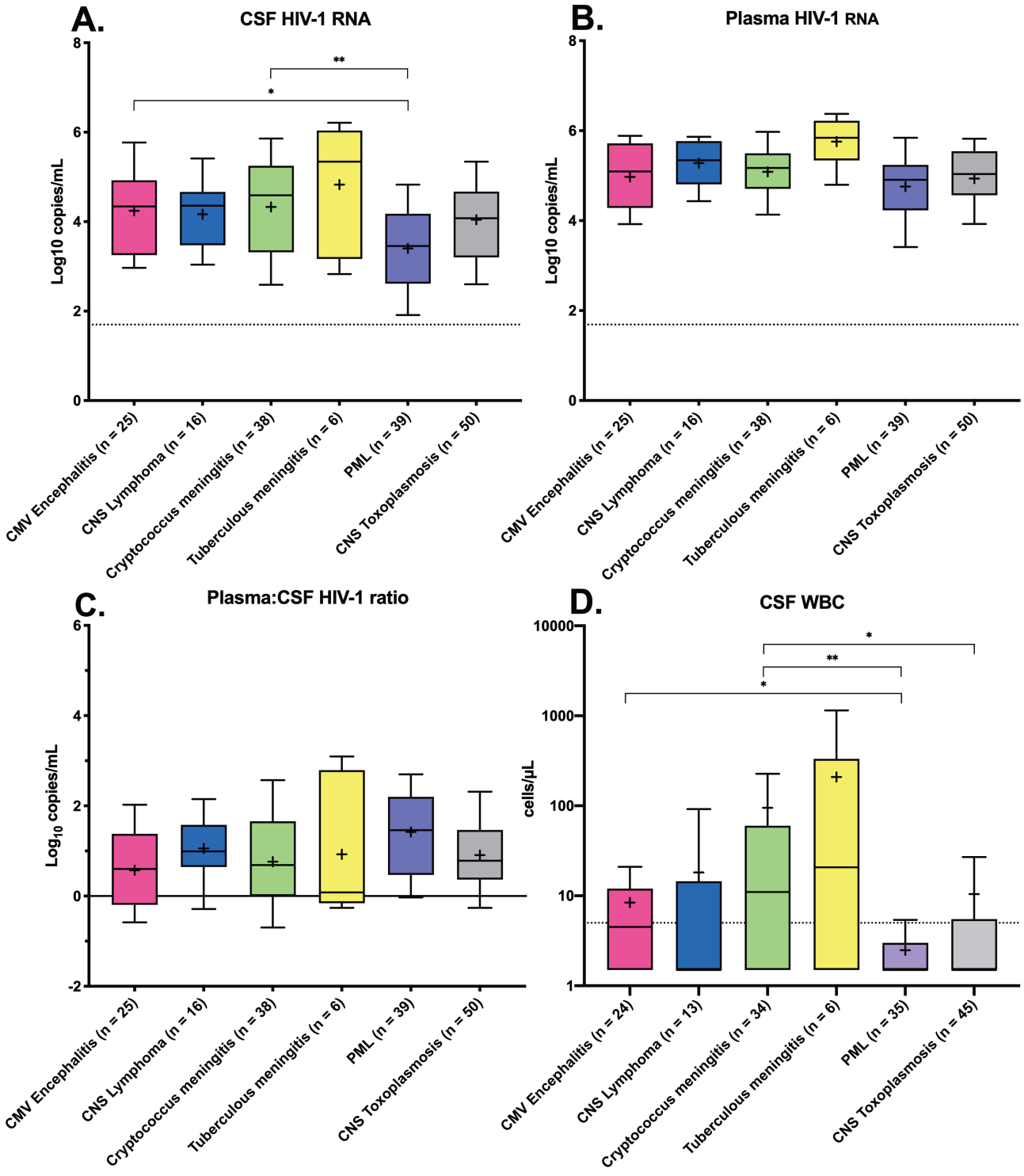

Figure 3. Comparison of viral load among various CNS opportunistic infections. Boxes depict interquartile ranges with median (line) and mean ("+"), whereas whiskers represent 10th to 90th percentiles. Dotted lines represent upper normal reference values and the grid line (*C*) represents the HIV-RNA difference of zero $\log_{10}$ copies/mL. Horizontal brackets show *P* values for significant group differences by Dunnett's T3 post hoc test after ordinary 1-way ANOVA test: * $P < .05$, ** $P < .01$. Abbreviations: ANOVA, analysis of variance; CMV, cytomegalovirus; CNS, central nervous system; CSF, cerebrospinal fluid; HIV-1, human immunodeficiency virus type 1; PML, progressive multifocal leukoencephalopathy; WBC, white blood cell.

has a preserved immune function. However, in late stage disease with advanced T-cell deficiency, this trafficking is impaired and CSF WBC count is low, which likely contributes to the comparatively lower levels of CSF HIV RNA seen in these patients. In the absence of opportunistic infections, the lower CSF HIV RNA levels presumably also contribute to the

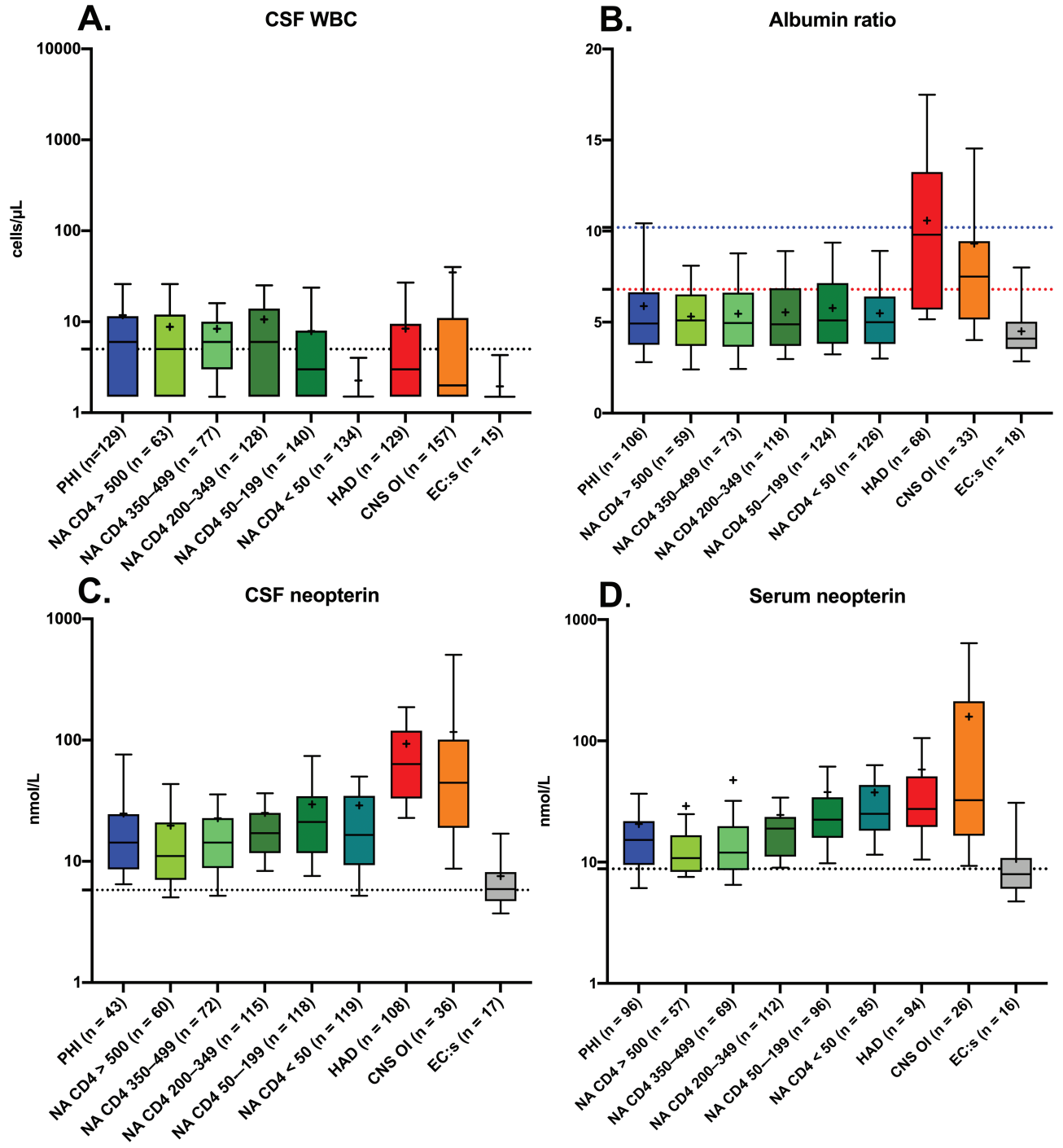

Figure 4. Concentrations of CSF WBC, albumin ratios and neopterin in subject groups. Boxes depict interquartile ranges with median (line) and mean ("+"), whereas whiskers represent 10th to 90th percentiles. Dotted lines represent upper normal reference values in panels (*A*), (*C*), and (*D*). No age adjustment was made according to albumin ratio. Dotted lines in panel (*B*) represent the upper normal reference values for individuals < 45 years (*red*) and those ≥ 45 years of age (*blue*). Findings and statistical comparisons between the group are described in the text and Tables 1 and 3. Number of subjects (n) in each group is showed in brackets following the group name. Abbreviations: CD4, $CD4^+$ T lymphocyte count; CNS, central nervous system; CNS OI, CNS opportunistic infections; CSF, cerebrospinal fluid; EC, elite controller; HAD, HIV dementia complex; NA, HIV+ neuroasymptomatics; HIV-1, human immunodeficiency virus type 1; n, number of subjects; PHI, primary HIV infection; WBC, white blood cell count.

comparatively moderate concentrations of CSF neopterin seen in neuroasymptomatic patients with advanced disease. In contrast, in patients with HAD or CNS OI's, other contributing mechanisms such as compartmentalized viral replication in the CNS or systemic immune cell recruitment result in increased CSF HIV RNA as well as significant increases in CSF neopterin.

By contrast, the majority of patients with HAD have high CSF HIV-RNA levels without CSF pleocytosis, which likely

represents a more compartmentalized infection where the HIV RNA measured in CSF is most likely dominated by virions produced by cells within the CNS compartment itself [1, 24, 25]. In neuroasymptomatic patients, peripheral virus likely has a comparatively greater influence on the CSF viral load. The degree of CNS compartmentalization probably vary in different stages of natural infection [26], a theory further supported by the lack of association between CSF HIV-RNA and plasma HIV-RNA, CSF WBC, and albumin ratio in the HAD group. On the other hand, the degree of HIV CNS compartmentalization is low during PHI [27], where we found CSF discordance to be a rare phenomenon (1%).

Patients with CNS OI's such as CMV encephalitis, tuberculous, or cryptococcal meningitis also more frequently had CSF HIV RNA levels exceeding those of plasma. In these patients, CSF HIV-RNA was also associated with albumin ratio, an association that was not as evident in the other groups (Supplementary Figure 2). This might either be a reflection of facilitated penetration of peripheral virions into the CNS or that blood brain barrier impairment leads to an increased inflammatory response resulting in higher levels of locally replicated virus [28]. CSF HIV-RNA in the CNS OI group was also weakly associated with CSF pleocytosis, a finding previously observed in smaller studies [29, 30] and in other CNS coinfections such as neuroborreliosis and herpes zoster [20, 31]. By contrast, PML, an opportunistic CNS infection associated with only minor CNS inflammation, was not associated with an increased CSF viral burden.

Approximately 10% of all neuroasymptomatic patients in our study had higher CSF than plasma HIV-RNA, a condition which has been designated discordant CSF/plasma HIV-RNA. The frequency of CSF discordance was lower in our investigation compared to previous reports, perhaps as a consequence of the comparatively higher proportion of neuroasymptomatic patients in our cohort [4, 32]. CSF discordance might be accounted for by greater activity of the infection in the CNS compartment than in the peripheral system in the case of neuroasymptomatic disease, a theory supported by the significantly higher CSF neopterin levels found in that subgroup. Whether this is mainly attributable to variation in HIV entry phenotypes and cell tropism, meningeal inflammation, astrocyte involvement, or host factors is still unclear [3, 33–35].

The present study included HIV-infected patients who had not received ART. The widespread availability of effective ART has resulted in a dramatic effect on reducing the risk of HAD and CNS OI. After initiation of ART CSF HIV-RNA usually decays as rapidly as plasma HIV-RNA, and CSF WBC count is normalized [36, 37].

The limitations of our study include the retrospective design, the extensive period of time for patient assessment and sampling, and the different PCR methods used at various locations and times. A major strength is the large number of patients examined by means of lumbar puncture, allowing us to stratify patients into predefined immunodeficiency CD4 groups.

Characterization of CSF findings in different phases of infection can be valuable in the clinical management of patients, and the results from this large study contributes to previous knowledge of CSF characteristics in untreated infection when evaluating neurological symptoms in PLHIV. Our study confirms previous findings that HIV-RNA is detectable in CSF at all stages of HIV infection, and overall parallels that of plasma. However, we found discordant CSF HIV-RNA with higher HIV RNA levels in CSF than in plasma in about 10% of neuroasymptomatic patients without ART and in 30% in patients with HAD.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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