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Blood-Brain Barrier Disruption Is Initiated During Primary HIV Infection and Not Rapidly Altered by Antiretroviral Therapy

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Background. We explored the establishment of abnormal blood-brain barrier (BBB) permeability and its relationship to neuropathogenesis during primary human immunodeficiency virus (HIV) infection by evaluating the cerebrospinal fluid (CSF) to serum albumin quotient (Q_{Alb}) in patients with primary HIV infection. We also analyzed effects of initiating combination antiretroviral therapy (cART).

Methods. The Q_{Alb} was measured in longitudinal observational studies of primary HIV infection. We analyzed trajectories of the Q_{Alb} before and after cART initiation, using mixed-effects models, and associations between the Q_{Alb} and the CSF level of neurofilament light chain (NFL), the ratio of N-acetylaspartate to creatinine levels (a magnetic resonance spectroscopy neuronal integrity biomarker), and neuropsychological performance.

Results. The baseline age-adjusted Q_{Alb} was elevated in 106 patients with primary HIV infection (median time of measurement, 91 days after infection), compared with that in 64 controls ($P = .02$). Before cART initiation, the Q_{Alb} increased over time in 84 participants with a normal baseline Q_{Alb} ($P = .006$) and decreased in 22 with a high baseline Q_{Alb} ($P = .011$). The Q_{Alb} did not change after a median cART duration of 398 days, initiated at a median interval of 225 days after infection ($P = .174$). The Q_{Alb} correlated with the NFL level at baseline ($r = 0.497$ and $P < .001$) and longitudinally ($r = 0.555$ and $P < .001$) and with the ratio of N-acetylaspartate to creatinine levels in parietal gray matter ($r = -0.352$ and $P < .001$ at baseline and $r = -0.387$ and $P = .008$ longitudinally) but not with neuropsychological performance.

Conclusion. The Q_{Alb} rises during primary HIV infection, associates with neuronal injury, and does not significantly improve over a year of treatment. BBB-associated neuropathogenesis in HIV-infected patients may initiate during primary infection.

Keywords. HIV/AIDS; PHI; primary HIV infection; BBB; Blood brain barrier; neuropathogenesis.

Chronic exposure to human immunodeficiency virus (HIV) can lead to neurological complications, with one third of untreated individuals with advanced AIDS developing HIV-associated dementia [1, 2], a syndrome of severe cognitive, motor, and behavioral disturbances primarily associated with subcortical atrophy [3]. Although the incidence of HIV-associated dementia has significantly decreased with the advent of combination antiretroviral therapy (cART), a milder spectrum of neurocognitive deficits persists despite treatment [1, 2, 4]. As this persisting impairment may at least in part represent irreversible alterations of central nervous system (CNS) integrity accrued before the initiation of cART, investigative efforts have been drawn toward elucidating neuropathogenesis during the early stages of HIV neuroinvasion and examining the effects of early cART on these processes.

Primary HIV infection (PHI) refers to the first phase of infection, from the time of transmission up to 12 months after transmission [5]. HIV infiltrates the CNS during PHI [6–8], as indicated by the presence of HIV RNA in the cerebrospinal fluid (CSF) compartment, even in the absence of neurological symptoms [1, 8–11]. CNS immune activation accompanies this viral invasion, as reflected by elevations in the CSF white blood cell count and the soluble CSF biomarkers neopterin (reflecting macrophage activation) and CXCL-10/IP-10 (a lymphocyte chemokine) and by T-lymphocyte activation in CSF [1, 12–15]. Furthermore, markers of immune activation may reflect the degree of viral load and neurocognitive impairment [16]. Magnetic resonance spectroscopy (MRS), a noninvasive quantitative MR technique that measures alterations in cerebral

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metabolite levels, demonstrates that inflammatory cerebral metabolites are elevated in acute HIV infection (ie, the period before antibody seroconversion) and longitudinally increased over time in PHI before cART initiation [1, 17, 18]. Thus, crucial processes during the primary phase of viral infection may underlie the initiation of HIV-associated CNS injury.

It is speculated that increased blood-brain barrier (BBB) permeability is a critical contributor to HIV neuropathogenesis because disruption of this regulatory interface facilitates CNS infiltration of potentially harmful substances from the periphery, resulting in compounding viral entry and susceptibility to the inflammatory assault of immune cells [19, 20]. To exert its neurological effects, HIV-1 and/or its viral products must first traverse the BBB. Although data suggest that, at this initial stage, HIV is transported to the CNS via trafficking of infected immune cells across a largely intact BBB, increased permeability of the BBB has been implicated in the progression of HIV neurological dysfunction [19, 21–24]. The ratio of the albumin level in the CSF to that in serum (also known as the CSF to serum albumin concentration quotient [Q_{Alb}]) is the best established fluid marker for BBB permeability [25]. Albumin is synthesized exclusively in the liver and is largely excluded from the CSF. Upon deregulation of the neurovascular unit and sequential loss of tight junctions, BBB permeability to albumin increases, resulting in an increased Q_{Alb}.

In this study, we aimed to elucidate the natural history of BBB permeability during PHI and to determine whether changes in permeability, if present, were associated with biomarkers of neuropathogenesis. Additionally, we sought to determine whether BBB permeability was responsive to cART initiated during PHI. These results provide novel understanding of the changes to the brain microenvironment that begin during initial HIV infection and the persistence of these alterations in the setting of early, virologically suppressive cART.

MATERIALS AND METHODS

Study Design

Individuals with PHI were recruited into prospective longitudinal studies of CNS HIV in Gothenburg, Sweden, and San Francisco, California, between 1986 and 2014, as previously described [9]. Participants were enrolled within the first year after HIV transmission, as confirmed by the standard serologic testing algorithm for recent HIV seroconversion (STAHRS) [26], and all but 3 were ART naive. A subset began cART at variable times during follow-up for reasons outside of the study. None of the participants had a prior neurological disease history. A history of substance abuse was not an exclusion criterion, but no participants reported same-day substance abuse, which would have led to censoring of data. The date of HIV transmission was approximated as 14 days before the onset of seroconversion symptoms, when present [27]; otherwise, it was approximated as midway between the dates of the last negative and first positive result of an enzyme immunoassay [28]. HIV-uninfected volunteers were recruited from the San Francisco community and had no history of neurological conditions or active systemic diseases.

Ethics

The study protocol was approved by the institutional review board of each institution involved. All study participants gave written consent.

Data Collection and Laboratory Analysis

CSF and blood specimens were obtained and neuropsychological testing and MRS were performed at each visit as previously described [23, 29]. Study visits were scheduled at baseline, 6 weeks, and every 6 months thereafter, although there was participant variation in the timing and duration of follow-up.

CSF neurofilament light chain (NFL) levels were measured with the NF-light enzyme-linked immunosorbent assay kit (UmanDiagnostics, Umeå, Sweden), a sensitive immunoassay [21], with reference values for the upper limit of normal (ULN) of 380 ng/L (for individuals aged 18–29 years), 560 ng/L (for those aged 30–39 years), 890 ng/L (for those aged 40–59 years), and 1850 ng/L (for those aged >59 years) [21]. CSF and plasma albumin levels were measured by nephelometry (Behring Nephelometer Analyzer; Behringwerke, Marburg, Germany). Q_{Alb} was calculated as the ratio of the CSF level of albumin, in milligrams/liter, to the plasma level of albumin, in grams/liter [22]. Upper limits of normal were based on previously established values of <6.8 for ages <45 years and <10.2 for ages >45 years [30]. CSF WBCs, lymphocytes, total protein, and HIV RNA were measured as previously described [9]. Viral loads of <50 copies/mL were assigned a value of 49 copies/mL (1.69 on $\log_{10}$ scale).

Neuropsychological performance was determined through the appraisal of gross and fine motor skills, processing speed, executive function, learning, and verbal memory, using a battery of 11 tests. Performance was summarized as an aggregate total *z* score and a brief NPZ-4 score (including grooved pegboard, digit symbol, finger tapping, and timed gait).

Brain magnetic resonance imaging (MRI)/MRS were performed at the San Francisco site only. MRS data were processed and analyzed with the spectral fitting software siTools, which uses a parametric model of known (metabolites) and modeled (macromolecules) spectral components to fit all resonances and nonparametric parameters to the baseline value. Metabolite disturbances can indicate neuropathology, including inflammation and injury. The ratio of the peak area under the curve for the metabolite N-acetylaspartate to the peak area under the curve for creatine-containing metabolites (NAA:Cr) is a putative marker of neuronal viability and number. We focused spectral acquisition on the parietal gray matter, as we have previously identified metabolite abnormalities in this region during PHI [17, 23].

Statistical Analysis

Baseline characteristics were summarized as frequencies for categorical variables and median values and interquartile ranges for continuous variables. Nonparametric, χ^2, and Fisher exact tests were used for group comparisons. The mixed-effects model was used to analyze longitudinal change of Q_{Alb} after transmission. This model includes both fixed and random effects in the same analysis, allowing for variation in the number and interval of participant follow-up visits. Baseline age was included as a fixed-effect covariate in the model. To account for a possible nonlinear trajectory of Q_{Alb} over time, a quadratic term (t^2) was included as a fixed-effect covariate. The model included a personal intercept for each participant as a random effect, allowing the baseline Q_{Alb} to vary for each participant. As log-transformed results were comparable to non–log-transformed analysis, the latter results are reported. The plot of fitted group mean Q_{Alb} trend was superposed on observed individual data. Between-subject and within-subject correlation were evaluated using the Bland and Altman method [31, 32].

Statistical analyses were performed using the SPSS 23.0 statistical package (IBM, Armonk, NY). The significance level was set as a P value of < .05 (2 sided).

RESULTS

Study Participant Characteristics

A total of 106 participants with PHI fulfilled the inclusion criteria and had available Q_{Alb} values. Nine participants experienced clinically overt neurological disorders during seroconversion: there were 2 cases of meningitis, 5 of headache with photophobia, 2 of brachial neuritis, and 1 each of Guillain-Barre syndrome, facial palsy, and encephalitis. Total visits ranged from 1 to 13, with a median of 2, and the maximum follow-up duration was 3572 days, with a median of 50 days. The majority of participants were infected with HIV subtype B [9].

The baseline characteristics of participants with PHI and uninfected controls are presented in Table 1. The median duration of HIV infection in participants with PHI was 91 days; the plasma viral load during PHI was 1.8 $\log_{10}$ greater relative to that in the CSF compartment. As compared to the HIV-uninfected participants, the PHI cohort had a higher percentage of males and was younger. As expected, participants with PHI had a lower $CD4^+$ T-cell count, an elevated $CD8^+$ T-cell count, and a decreased ratio of $CD4^+$ T cells to $CD8^+$ T cells. As previously reported, CSF white blood cell counts were elevated in the PHI group, as well as CSF levels of neopterin, a marker of macrophage activation. Despite the younger age, participants with PHI had levels of NFL and equivalent CSF total protein—2 parameters that increase in level with normal aging—that were greater than those for the uninfected group, [21, 33, 34].

BBB Permeability at Baseline

At baseline, the mean age-adjusted Q_{Alb} was elevated in the PHI cohort (5.9; 95% confidence interval [CI], 5.5–6.3) as

Table 1. Baseline Demographic and Clinical Characteristics of Study Participants, by Human Immunodeficiency Virus (HIV) Status

Characteristic	Primary HIV Infection (n = 106)	No HIV Infection (n = 64)	*P*
Male sex, % (no.)	94 (100)	82 (52)	.001
Age, y	36 (29–46)	43 (34–50)	.003
Study site			
San Francisco, CA	82	64	
Gothenburg, Sweden	24	0	
Time after HIV transmission, d	91 (53–149)	…	
$CD4^+$ T-cell count, cells/μL	567 (402–709)	808 (678–1009)	<.001
$CD8^+$ T-cell count, cells/μL	954 (714–1358)	487 (343–733)	<.001
$CD4^+$ to $CD8^+$ T-cell ratio	0.528 (0.391–0.791)	1.76 (1.32–2.18)	<.001
HIV RNA load, $\log_{10}$ copies/mL			
In plasma, $\log_{10}$ copies/mL	4.69 (4.08–5.34)	…	
In CSF, $\log_{10}$ copies/mL	2.83 (2.14–3.51)	…	
Plasma to CSF ratio	1.81 (1.33–2.28)	…	
CSF WBC count, cells/mm^3	6 (2–11)	2 (0–3)	<.001
CSF total protein level, mg/dL	41 (31–51)	41 (31–54)	.611
NFL level, pg/mL	518 (391–819)	411 (320–550)	<.001
CSF neopterin level, nmol/L	9.6 (6.8–20.4)	5.0 (4.1–6.8)	<.001
Neurosymptomatic ARS, % (no.)	8.5 (9)	…	
Study visits, no.	2 (1–3)	…	
Follow-up duration, d	50 (0–450)	…	

Data are median value (interquartile range), unless otherwise indicated.

Abbreviations: ARS, acute retroviral syndrome; CSF, cerebrospinal fluid; NFL, neurofilament light chain; WBC, white blood cell.

compared to that for controls (5.0; 95% CI, 4.4–5.6; P = .02). On the basis of previously published reference values [30], the baseline Q_{Alb} was above the age-specific ULN in 22 participants with PHI (21%), hereafter referred to as the "high baseline Q_{Alb} subgroup." The remaining 84 participants with PHI with baseline Q_{Alb} values below the ULN are referred to hereafter as the "normal baseline Q_{Alb} subgroup." The baseline clinical characteristics of these 2 subgroups are summarized in Table 2. Four of seventeen participants (24%) in the high baseline Q_{Alb} subgroup had neurosymptomatic seroconversion, compared with 8 of 64 (13%) in the normal baseline Q_{Alb} subgroup, although the difference was statistically insignificant. An elevated NFL level, CSF total protein level, CSF neopterin (but not blood neopterin) level, and $CD8^+$ T-cell count and a decreased ratio of plasma to CSF levels of HIV RNA were found in the high baseline Q_{Alb} subgroup as compared to the normal baseline Q_{Alb} subgroup.

Longitudinal BBB Permeability in PHI Before cART Initiation

The individual Q_{Alb} trajectories for all participants in the PHI group over the duration of the study before cART initiation are plotted in Figure 1. A mixed-model analysis to evaluate the natural history of BBB integrity in the overall PHI group before cART initiation did not reveal a significant change in Q_{Alb} over time (–0.000436/day; P = .092). Figure 2 compares the trajectories of the high and normal baseline Q_{Alb} groups. The high baseline Q_{Alb} subgroup showed a declining trend (–0.00305/day; P = .011), while the Q_{Alb} in the normal baseline Q_{Alb} subgroup initially increased (0.00144/day; P = .006)

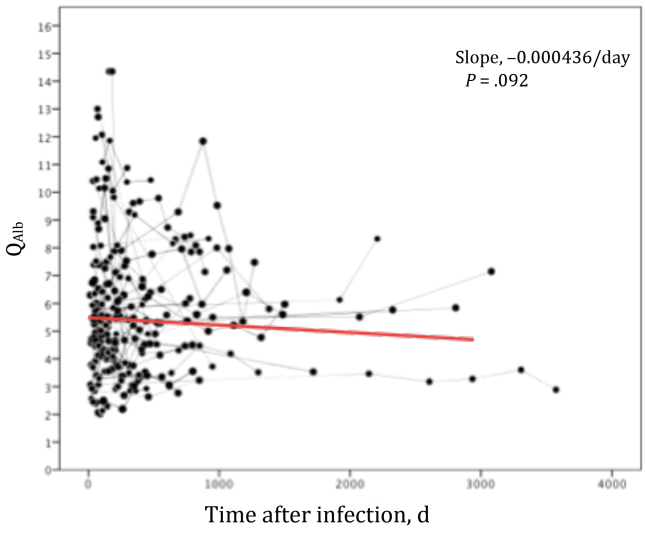

Figure 1. Natural history of blood-brain barrier integrity before combination antiretroviral therapy in the total cohort. Q_{Alb}, cerebrospinal fluid to serum albumin concentration quotient.

Table 2. Baseline Clinical Characteristics of Participants With Primary Human Immunodeficiency Virus (HIV) Infection, by Baseline Cerebrospinal Fluid (CSF) to Serum Albumin Concentration Quotient (Q_{Alb}) Subgroup

Characteristic	High Baseline Q_{Alb} (n = 22)	Normal Baseline Q_{Alb} (n = 84)	P[a]
Age, y	36 (29–45)	37 (28–46)	.797
Time after HIV transmission, d	85 (60–125)	92 (51–150)	.785
$CD4^+$ T-cell count, cells/μL	596 (484–681)	550 (389–730)	.469
$CD8^+$ T-cell count, cells/μL	1294 (792–1620)	915 (706–1200)	.023
$CD4^+$ to $CD8^+$ T-cell ratio	0.463 (0.321–0.791)	0.530 (0.391–0.803)	.376
HIV RNA load, log_{10} copies/mL			
In plasma, log_{10} copies/mL	4.60 (3.91–5.39)	4.69 (4.09–5.32)	.629
In CSF, log_{10} copies/mL	3.23 (1.77–3.87)	2.73 (2.14–3.43)	.340
Plasma to CSF ratio	1.27 (0.464–2.16)	1.84 (1.50–2.29)	.020
CSF WBC count, cells/mm^3	7 (4–13)	5 (2–11)	.087
CSF total protein level, mg/dL	59 (52–74)	37 (28–42)	<.001
NFL level, pg/mL	857 (468–1474)	498 (360–729)	.008
Neopterin level, nmol/L			
In blood	18.0 (10.8–28.9)	14 (9.4–21.3)	.183
In CSF	14.3 (8.4–32.0)	9.0 (6.5–17.4)	.035
Baseline Q_{Alb}	9.18 (7.5–11.3)	4.66 (3.57–5.75)	<.001
Neurosymptomatic ARS, % (no.)	24 (4)	13 (8)	.169[b]
Study visits, no.	2 (1–3)	2 (1–3)	.617
Follow-up duration, d	48 (0–398)	51 (0–455)	.715

Data are median value (interquartile range), unless otherwise indicated.

Abbreviations: ARS, acute retroviral syndrome; CSF, cerebrospinal fluid; NFL, neurofilament light chain; WBC, white blood cell.

[a]Values <.05 were considered statistically significant.

[b]Comparison involved 17 participants in the high baseline Q_{Alb} subgroup and 64 in the normal baseline Q_{Alb} subgroup and was performed using the Fisher exact test.

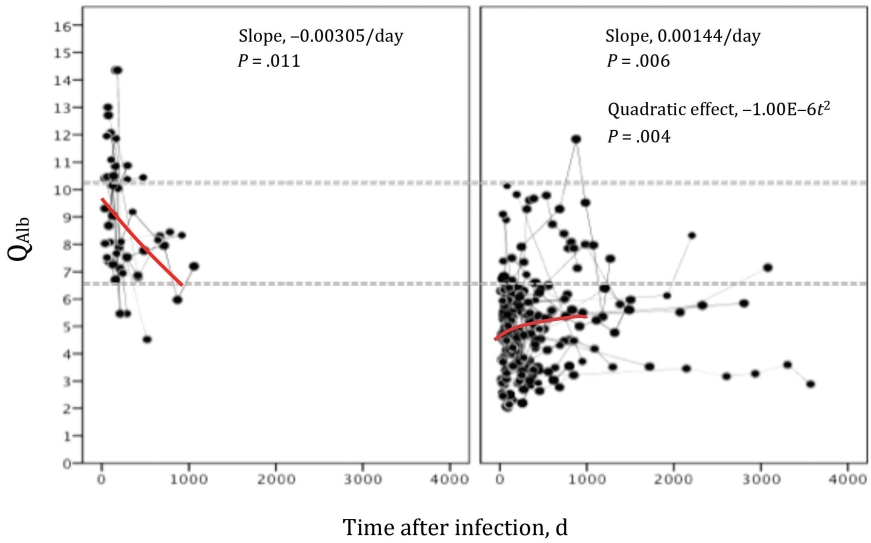

Figure 2. Natural history of blood-brain barrier integrity before combination antiretroviral therapy (cART) upon cohort stratification. Graphs demonstrate individual participant and overall trajectory of the cerebrospinal fluid to serum albumin concentration quotient (Q_{Alb}) in cART-naive participants upon stratification into high and low baseline Q_{Alb} subgroups. Dashed gray lines indicate the upper limits of normal for participants aged <45 years (Q_{Alb}, 6.5) and those aged >45 years (Q_{Alb}, 10.2).

and reached a plateau quickly (quadratic time effect $P = .004$). These results indicated the heterogeneous time effect in the 2 subgroups.

Correlation of BBB Integrity With Markers of Neuropathogenesis

To further evaluate the implications of elevated Q_{Alb}, correlations between Q_{Alb} and markers of neuronal health were evaluated in pre-cART study intervals (Figure 3). Partial correlation coefficients were calculated to correct for the confounding effects of age, as the Q_{Alb} and NFL level both directly correlate with age. The Q_{Alb} demonstrated a strong positive correlation with the NFL level, a marker of active neuronal injury, upon cross-sectional analysis at baseline (r = 0.497; $P < .001$) and longitudinally, with both between-participant (r = 0.555; $P < .001$) and within-participant (r = 0.523; $P = .001$) analysis. Q_{Alb} inversely correlated with NAA:Cr, a cerebral metabolite biomarker of neuronal health, upon cross-sectional analysis at baseline (r = –0.352; $P = .015$) and longitudinally, with between-participant analysis (r = –0.387; $P = .008$) but not within-participant analysis (r = 0.218; $P = .125$). MRS was performed at a median interval of 114 days after infection. Q_{Alb} did not correlate with composite *z* scores (total *z* or NPZ4) of neuropsychological testing at baseline or in longitudinal analysis (data not shown).

Characteristics of cART Recipients

Fifty-eight participants with PHI initiated a cART regimen during study follow-up, although 1 participant was excluded because of virologic failure (defined as 2 consecutive plasma samples with an HIV RNA load of >50 copies/mL after 6 months of ART). Treatment regimens were heterogeneous, consisting of 10 integrase-based, 25 protease-based, and 22 nonnucleoside reverse transcriptase inhibitor–based regimens with 19 distinct combinations. cART was initiated at a median interval of 225 days after infection, with a median duration of follow-up during cART of 402 days. Table 3 compares the cross-sectional laboratory parameters before (ie, during the last visit before treatment initiation) and after (ie, during the last visit of the study) cART initiation in those who initiated cART. There was improvement in most parameters after approximately a year of cART, with suppression of plasma and CSF HIV RNA levels to the lower limit of detection by polymerase chain reaction ($P < .001$), increased $CD4^+$ T-cell counts ($P < .001$), decreased WBC counts ($P < .001$), and decreased blood and CSF neopterin levels ($P < .001$). In this comparison, the NFL level and albumin ratio did not significantly change with cART (640 vs 670 ng/L [$P = .911$] and 5.18 vs 5.09 [$P = .851$], respectively).

Longitudinal History of BBB Integrity Following cART Initiation

A mixed-model analysis was performed to assess the longitudinal trajectory of Q_{Alb} over 13 months of cART (Figure 4). Three participants were recruited into the cohort after already having initiated cART (for 29, 27, and 19 days) and thus were included in the linear mixed model (n = 60) but are excluded from the group described in Table 3. As cART was initiated at a median interval of 225 days after infection ($t = 0$ in Figure 4), this time point corresponded with the linear portion shown in Figure 2, in which the quadratic changes of the normal baseline subgroup are resolving and reaching a set point. Thus, the initial analysis was performed with the total group of cART recipients,

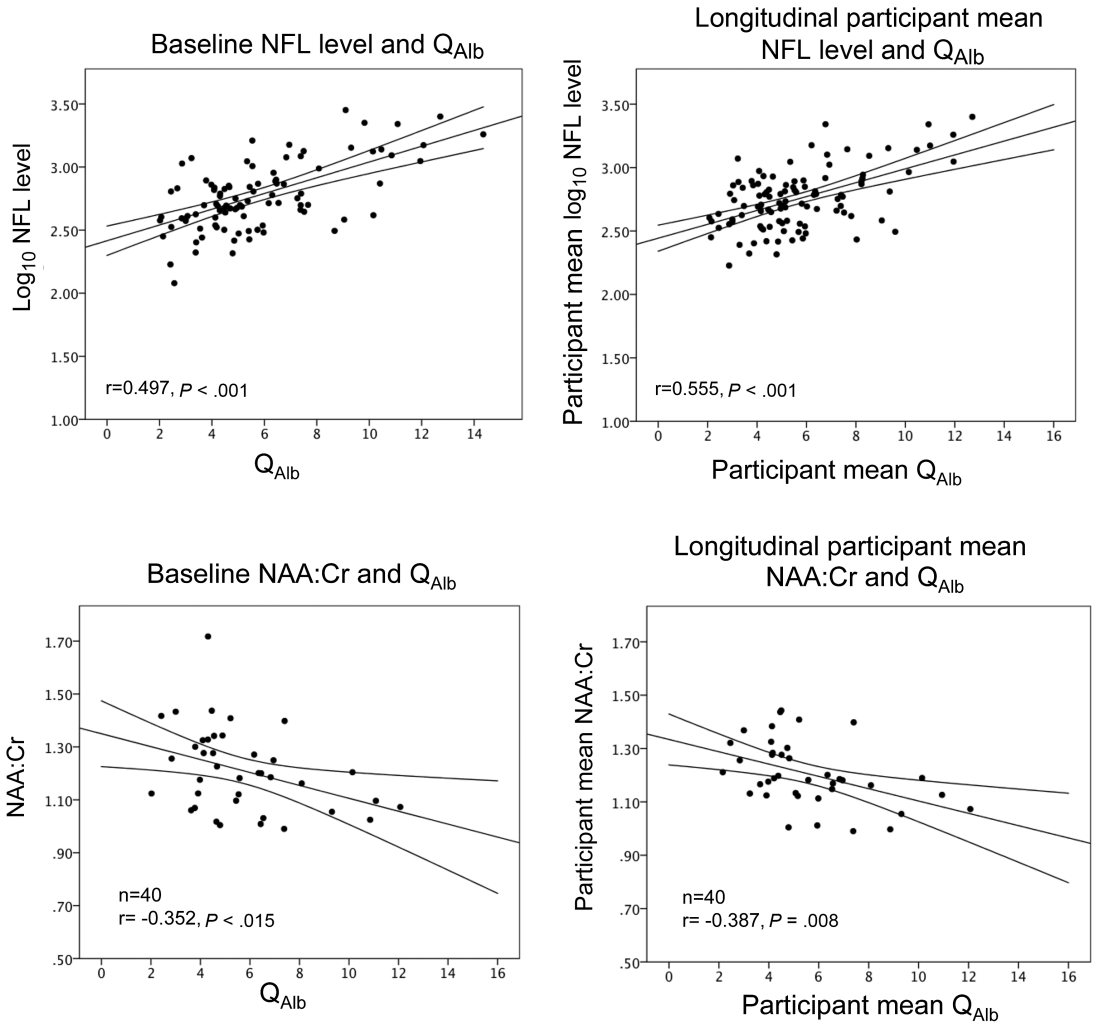

Figure 3. Correlation of blood-brain barrier permeability with clinical and laboratory indicators of neuropathogenesis. Abbreviations: Cr, peak area under the creatinine concentration curve; NAA, peak area under the N-acetylaspartate concentration curve; NFL, neurofilament light chain level; Q_{Alb}, cerebrospinal fluid to serum albumin concentration quotient.

rather than separating cART recipients into the high and normal baseline Q_{Alb} subgroups. There was no significant change detected in Q_{Alb} over the median duration (ie, >1 year) of cART (slope, −0.00369/month; $P = .174$). With group stratification, the high baseline Q_{Alb} subgroup (n = 7) demonstrated no significant change in Q_{Alb} over time ($P = .783$). The low baseline subgroup (n = 53) demonstrated a slope of effectively 0 (slope, 0.00008/month; $P = .004$), similar to the pre-cART plateau.

DISCUSSION

In this study, we analyzed the natural history of BBB permeability and the influence of early cART during primary HIV infection. We show that albumin ratio is mildly elevated in participants with PHI, compared with that for uninfected controls, when correcting for age. This correction is relevant given that BBB permeability increases with normal aging [35] and may explain why previous studies have not reported abnormalities in BBB permeability during PHI when compared to controls, given that most studies of PHI enroll young patients. That being said, we have previously identified moderate elevation of the albumin ratio during PHI [9, 36] and in participants with chronic HIV infection who are cART naive and neuroasymptomatic [9]. Similarly, Li et al have reported a strong association between matrix metalloproteinases—enzymatic surrogate markers of BBB permeability—and neurocognitive status in PHI [37].

The novelty of this study is our finding that BBB permeability undergoes dynamic changes during PHI, even within days of transmission. Two distinct trajectories were noted for the PHI cohort when analysis was stratified by baseline albumin ratio. Those with a normal baseline albumin ratio (ie, a ratio below the ULN) showed a mild initial increase that plateaued within the first 1000 days of infection. Despite the initial rise, the Q_{Alb} remained well below the ULN. As will be discussed below, it may be that there is an element of subclinical injury associated with this mild rise. The subgroup with a

Table 3. Characteristics of Participants with Primary Human Immunodeficiency Virus (HIV) Infection Before and After Initiating Combination Antiretroviral Therapy (cART)

Characteristic	Before Initiation (n = 57)	After Initiation (n = 57)	P[a]
Age, y	41 (29–46)	…	
Time after HIV transmission, d	225 (96–760)	…	
Time before ART initiation, d	19 (3–85)	…	
Follow-up visits, no.	…	2 (1–6)	
cART duration, d	…	402 (192–1060)	
$CD4^+$ T-cell count, cells/μL	431 (282–588)	643 (483–787)	<.001
HIV RNA load, log_{10} copies/mL			
In plasma, log_{10}	4.9 (4.4–5.3)	1.69 (1.69–1.69)	<.001
In CSF, log_{10}	3.4 (2.6–4.0)	1.69 (1.69–1.69)	<.001
Plasma to CSF ratio	1.49 (0.71–2.08)	0.00 (0.00–0.20)	<.001
CSF WBC count, cells/mm^3	4 (6–14)	2 (1–3)	<.001
CSF total protein level, mg/dL	40 (33–50)	35 (29–42)	.001[b]
NFL level, pg/mL	640 (515–965)	670 (453–1072)	.911[c]
Q_{Alb}	5.18 (3.92–6.40)	5.09 (3.87–6.21)	.832
Neopterin level, nmol/L			
In blood	18.4 (8.4–24.9)	7.6 (5.2–12.9)	<.001
In CSF	13.9 (7.8–21.6)	5.2 (4.7–7.7)	<.001

Data are median value (interquartile range). Data before cART initiation were obtained during the last visit before treatment initiation, and data after cART initiation were obtained during the last visit of the study.

Abbreviations: CSF, cerebrospinal fluid; NFL, neurofilament light chain; Q_{Alb}, cerebrospinal fluid to serum albumin concentration quotient; WBC, white blood cell.

[a]Group comparisons were performed using nonparametric analysis for related samples. Values <.05 were considered statistically significant.

[b]Comparison is for 21 paired values.

[c]Comparison is for 42 paired values.

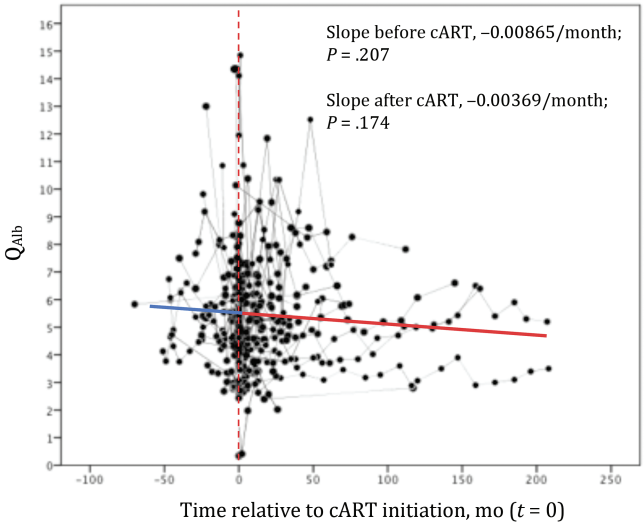

Figure 4. Effects of combination antiretroviral therapy (cART) on the trajectory of blood-brain barrier permeability. Scatterplot shows individual participant trajectories and overall cohort trajectories of the cerebrospinal fluid to serum albumin concentration quotient (Q_{Alb}) before and after cART initiation (indicated by the dashed line at t = 0). Linear mixed model analysis generated the two slopes. Months before cART initiation are negative values.

high baseline albumin ratio demonstrated a marked decline in the albumin ratio within the first 1000 days of infection. Presumably an early rise in albumin ratio occurred immediately following infection, before participant recruitment, and began to resolve during the follow up. Notably, the subgroup with a higher baseline albumin ratio was characterized by a higher percentage of neurosymptomatic seroconversion, elevated levels of CSF markers of axonal injury and immune activation, and a higher ratio of CSF to plasma levels of HIV RNA. These findings suggest that a subgroup of participants with PHI is susceptible to marked BBB disruption, which persists even beyond 1000 days after infection, and is associated with signs of increased CNS involvement. Factors that predispose individuals to one trajectory versus the other warrant further investigation.

Previous studies have expounded on the association of the albumin ratio with biomarkers of CNS inflammation and injury [20]. We confirm that, during PHI, the albumin ratio correlates strongly with the level of the axonal injury marker, NFL [23], and are the first to demonstrate that it inversely correlates with the metabolic marker of neuronal health, NAA:Cr. NFL is a sensitive marker of active neuronal damage, and its levels correlate with the severity of this damage [38–40]. We have previously shown NFL to be the most sensitive neuronal biomarker for assessing HIV neurodegeneration, as it can detect subclinical injury in neuroasymptomatic individuals, even during PHI [36, 41]. As disease progresses, it is also associated with overt clinical neurological disease, thus reflecting not only structural changes, but also functional changes [38]. Although NFL is not specific for HIV neurodegeneration [36, 39], comorbid neurological conditions were excluded from the study onset. Similar to Q_{Alb}, the NFL level was elevated during PHI, although it was below the ULN (<560 ng/L), possibly indicating subclinical damage, which may explain the lack of correlation with NPZ-4 test results. In line with this conclusion, we have previously shown a lack of correlation between NFL levels and NPZ-4 results during PHI, despite showing moderate elevations when compared to findings for uninfected controls [23, 36]. Similar to the utility of the NFL level as a biomarker of early subclinical injury, MRS has been shown to detect early HIV-associated neuropathogenesis before detection of conventional MRI changes [42]. In a recent study, subjects with chronic HIV infection who have cognitive deficits were shown to have reduced glutamate and NAA levels in several brain regions, most pronounced in the parietal gray matter [43]. Here, we extend that finding to PHI.

Once we demonstrated that BBB permeability was altered in PHI and associated with markers of neuronal pathology, we

assessed whether early cART could remediate these changes. Surprisingly, the effect of cART on BBB permeability has not been intensely evaluated. In an unpublished study, Crozier et al observed the gradual diminishment of the median albumin ratio (from 6.48 to 6.09) in 16 neuroasymptomatic participants with chronic HIV infection after 200 days of cART [44]; thus, although BBB integrity improved over time with cART, a return to baseline or near baseline function may take years. In contrast, Abdulle et al reported no significant reduction in BBB permeability after 2 years of cART in 38 neuroasymptomatic participants [45]. Importantly, the median baseline albumin ratio among participants in the study by Crozier et al was greater than that of participants in the study by Abdulle et al (6.48 vs 4.45), potentially contributing to the discrepancy in the cohort response to cART.

In our study, cART, initiated at a median interval of 225 days after infection, was effective in suppressing CSF and plasma HIV RNA, suggesting medication compliance and effectiveness. Notably, the inflammatory marker neopterin improved to the upper level of normal limits both in the plasma and CSF. Despite this systemic (including CNS) suppression of viral replication and inflammation, the NFL level and albumin ratio were unchanged. The pre-cART measurement of the albumin ratio was comparable to that of the age-matched uninfected controls and thus may indicate that the acute changes of the albumin ratio in the high baseline Q_{Alb} subgroup had largely resolved and reached a level near baseline once cART was initiated at a median interval of 225 days after infection. On the other hand, although the NFL level was below the age-specific ULN (<840 pg/mL), it was significantly elevated as compared to that for uninfected controls and the baseline PHI cohort, given only a marginal age difference. There is a gradual normalization of the NFL level following axonal injury, which is unlikely to persist for over a year [46]. Thus, this persistently elevated level of NFL may reflect continued subclinical injury despite cART and what appears to be a largely normal albumin ratio.

We hypothesize that perhaps (1) the initially altered BBB permeability initiated CNS injury, which persists despite resolution of BBB integrity; (2) the mechanism of injury is independent of BBB integrity; or (3) BBB permeability is mildly elevated and has not fully returned to baseline, resulting in persisting neuronal injury. Alternatively, it is possible that, despite the large sample size, we still have insufficient power to detect a significant change in the NFL level and Q_{Alb} after cART initiation. Further studies are necessary to elucidate the possible explanation. Notably, a previous study showed normalization of the ratio of $CD4^+$ to $CD8^+$ T cells during PHI only when cART was initiated within 6 months after transmission [47]. Furthermore, in a cohort of individuals who started treatment during acute HIV infection, the CSF NFL level was not elevated at baseline nor after 6 and 24 months of cART [48]. The effects of earlier cART intervention on normalization of the albumin ratio should be investigated.

This study has several limitations. Q_{Alb} is affected by many factors not accounted for in this study, including body weight and smoking [35]. Although cholesterol and other cardiovascular risk factors were not routinely screened, none of the participants had a known history of clinically apparent cardiovascular disease, such as coronary artery disease, peripheral vascular disease, or stroke. One participant, in the low baseline group, had a known diagnosis of diabetes mellitus type 2. Abuse of substances such as cocaine has been shown to at least transiently increase BBB permeability [49]; thus, misreporting of ongoing drug use or long-term effects of previous drug use cannot be discounted as confounding factors. Furthermore, given the observational nature of this study, cART regimens were heterogeneous, which may result in distinct effects on the BBB.

BBB permeability undergoes a dynamic process during PHI, demonstrating acute changes within days. We identified 2 subgroups of participants with PHI with different albumin ratio trajectories: one with a presumed acute increase and gradual improvement over the course of infection and a second with a mild initial increase. BBB permeability correlated with markers of neuropathogenesis. Initiation of cART in the first year of infection did not significantly alter BBB permeability in our study. Further investigations should test the effects of earlier cART initiation, especially in individuals with signs of early BBB disruption.

Notes

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