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Cd4+ T Cell Recovery And Cerebrospinal Fluid Escape After Antiretroviral Therapy Initiation In Acute Hiv-1 Infection

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CD4+ T Cell Recovery and Cerebrospinal Fluid Escape After Antiretroviral Therapy Initiation in Acute HIV-1 Infection

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Abstract

Introduction: Up to 30% of individuals treated with antiretroviral therapy (ART) during chronic HIV fail to recover CD4 counts to >500 cells/mm^3 and up to 10% have quantifiable HIV RNA in cerebrospinal fluid (CSF), termed CSF escape, despite plasma viral suppression < 50 copies/mL. Previous studies have shown that ART initiation in the earliest stage of identifiable infection, acute HIV infection (prior to antibody seroconversion), may limit viral reservoir establishment and systemic immune activation and may improve clinical outcomes. We investigated the frequency, associations, and outcomes of suboptimal CD4 recovery (**Project 1**) and CSF escape (**Project 2**) after ART started during acute HIV infection (AHI).

Methods: Thai participants with laboratory-confirmed diagnosis of AHI (Fiebig stages I to V) were started immediately on ART and followed longitudinally with blood sampling, neuropsychological and neurobehavioral testing, and optional lumbar puncture. For **Project 1**, participants with ≥48 weeks of documented HIV RNA <50 copies/mL were stratified by CD4 count at latest study visit to suboptimal recovery (SR; CD4<350 cells/mm^3), intermediate recovery (IR; 350≤CD4<500), and complete recovery (CR; CD4≥500). To assess determinants of CD4 recovery, clinical and laboratory parameters were evaluated at pre-ART baseline and latest study visit. Additional inflammatory and neurobehavioral endpoints were examined at baseline and 96 weeks. For **Project 2**, participants who underwent blood sampling and optional CSF sampling at weeks 24 and 96 were assessed for CSF escape. HIV RNA was quantified using Roche Amplicor and COBAS TaqMan assays with a lower limit of quantitation of 20-50 copies/mL in plasma and 80 copies/mL in CSF. Participants with quantifiable CSF HIV RNA greater than that in plasma during ART were identified as cases of CSF escape.

Results, Project 1: Of 304 participants (96% male, median 26 years old) evaluated after median 144 (range 60-420) weeks of ART initiated at median 19 days (range 1-62) post-exposure, 3.6% (n=11) had SR, 14.5% (n=44) had IR, and 81.9% (n=249) had CR. Degree of CD4 recovery occurred early following ART. Timing of ART initiation by Fiebig stage did not affect CD4 count during treatment. Pre-ART CD4 count in SR compared to CR participants was 265 vs 411 cells/mm^3 (p=0.002). Following ART, the CD8+ T cell count (p=0.001) and CD4/CD8 ratio (p=0.047) were lower in SR compared to CR participants. Compared to the CR group at week 96, the combined SR and IR groups had higher sCD14 (p=0.008) and lower IL-6 (p=0.04) in plasma, without differences in neuropsychological or psychiatric indices. After adjusting for duration of ART, baseline HIV-RNA, and baseline CD4 count, odds of CD4 recovery < 500 cells/mm^3 were higher in those with baseline CD4/CD8 ratio < 1 (odds ratio 3.2, p=0.01), on-ART CD4/CD8 ratio < 1 (odds ratio 2.4, p=0.007), and on-ART CD8 count < 500 cells/mm^3 (odds ratio 3.1, p=0.0005).

Results, Project 2: 204 participants had paired blood and CSF sampling in at least one visit at baseline, week 24, or week 96. The participants were 98% male (199/204) with median age 26 years and baseline Fiebig stage 3 (96/204, 47%), CD4 count 386 cells/mm^3, and plasma HIV RNA 5.87 $\log_{10}$ copies/mL. ART was started at a median of 19 days post estimated infection. At baseline, 126/165 participants (76%) had quantifiable CSF HIV RNA (median 3.13 $\log_{10}$ copies/mL). At week 24 (n=90), two participants (2%) had quantifiable CSF HIV RNA, with one case of CSF escape identified with plasma HIV RNA < 50 copies/mL and CSF HIV RNA 2.50 $\log_{10}$ copies/mL. At week 96 (n=55), one participant (2%) had quantifiable CSF HIV RNA, which did not meet criteria for CSF escape. The two other cases of quantifiable CSF HIV RNA were due to plasma HIV RNA > CSF HIV RNA. The participant with CSF escape was treated

with efavirenz, tenofovir, and lamivudine and had a CD4 count of 840 cells/mm^3 and CSF WBC and CSF protein of 4 cells/mm^3 and 30 mg/dL. His MRI at week 24 showed a small nonspecific T2/FLAIR hyperintense focus in the right high frontal white matter. He did not have a lumbar puncture performed at baseline nor at subsequent visits.

Conclusions: Despite immediate and sustained treatment in AHI, suboptimal CD4 recovery is observed in rare individuals, associated with low pre-ART CD4 count as well as persistent low CD8 count and CD4/CD8 ratio during treatment. While levels of CSF HIV RNA in untreated AHI are high, initiating treatment during AHI results in a very low rate of CSF escape in the first two years of ART. The low rate of CSF escape may also be impacted by high levels of adherence to ART in this cohort or the short duration of ART. Longitudinal monitoring will be required to verify if CSF escape remains rare under long-term ART in early treated individuals.

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Introduction

HIV Infection in the Era of Antiretroviral Therapy

Throughout its storied impact on human health, the human immunodeficiency virus (HIV) epidemic has uniquely intersected with issues of stigma, mental health, sexual identity and expression, and poverty, and once spelled near-inevitable morbidity and mortality for individuals already marginalized by their communities. Now, more than 60% of people living with HIV are receiving antiretroviral therapy (ART), transforming HIV infection into a chronic manageable condition affecting an estimated 37.9 million people worldwide [1]. Substantial progress has been made in reducing HIV transmission, improving screening, and expanding access to ART. However, the number of people living with HIV likely will continue to increase in more than 100 countries, as estimated by incidence-prevalence ratios [1]. Thus, with approximately 23.3 million people on ART and growing, there is considerable interest in long-term outcomes of people on virally suppressive ART. Chronic complications of treated HIV infection include systemic immune dysregulation and inflammation, accelerated cardiovascular disease, insulin resistance, reduced bone mineral density, chronic kidney disease, and HIV-associated neurocognitive disorders (HAND) [2-5]. Notably, this should be distinguished from chronic complications thought to be related to long-term use of certain ART medications [5]. In particular, this section will draw attention to the persistence of immune dysregulation, neurocognitive impairment, and HIV reservoirs, even in the era of ART and in the setting of plasma viral suppression.

Early in the HIV epidemic, even before it was identified as the cause of acquired immunodeficiency syndrome (AIDS), immune activation was described in tandem with very low CD4+ T cell counts in the first case reports of AIDS [6]. Lymphocytes were found to have high expression of CD38, a marker of T cell activation, which were later found to independently predict

more rapid disease progression [7, 8]. Likewise, the markers of innate immune activation neopterin and ß2-microglobulin were found to be elevated in untreated HIV infection, which in particular correspond to monocyte and macrophage activation, respectively [8, 9]. Inflammatory and coagulation indices are also elevated in untreated HIV infection, such as interleukin-6, D-dimer, C-reactive protein, and soluble CD14, and additionally predict morbidity and mortality [10-12]. Even with effective plasma viral suppression on ART, T cell activation persists [13], and inflammatory indices are elevated and predict non-AIDS-defining adverse events independent of pre-ART nadir and recent CD4+ T cell count [14, 15].

Mechanistically, many factors may contribute to persistent immune dysregulation in treated HIV infection. Gut mucosal CD4+ T cells are rapidly depleted in early HIV infection, and the resultant mucosal inflammation damages the epithelial barrier of the gastrointestinal tract leading to intestinal microbial translocation [16]. Both intestinal CD4+ T cell depletion and microbial translocation are thought to contribute to persistent immune activation [17, 18]. Also occurring early in infection, HIV replication in lymphoid tissues results in collagen deposition and fibrosis, likely due to increased T regulatory cell activity and TGF-ß secretion. Lymphatic fibrosis contributes to poor CD4+ T cell reconstitution and immune dysregulation [19, 20]. Co-infection with pathogens such as cytomegalovirus may also be implicated in residual inflammation in treated HIV infection [21, 22]. Beyond T cell activation, other findings of T cell dysregulation have been described in HIV infection, which are only partially ameliorated with ART. T cell exhaustion is marked by upregulation of inhibitory receptor programmed death 1 (PD-1), results in impairment of T cell activity, including reduced cytokine secretion, proliferative capacity, and cytotoxic effector function [23], and occurs in HIV-specific CD8+ T cells in treatment-naïve participants [24]. Other markers such as Tim-3 may also be upregulated in exhausted and impaired T cells in

HIV infection [23, 25]. Commencement of ART only partially downregulates these markers of T cell exhaustion [26]. CD8+ T cells are not only activated and exhausted in untreated and treated HIV infection, their population is also markedly expanded [27]. The coalescence of CD4+ T cell depletion and CD8+ T cell expansion is captured by the metric of the CD4/CD8 ratio, which is increasingly recognized as a measure of immune activation that predicts worse clinical outcomes [28]. Ultimately, inflammation, coagulopathy, and immune activation in ART-suppressed HIV infection are multifactorial processes that contribute to an elevated risk of non-AIDS-related morbidity and mortality. Importantly, immune dysregulation has been linked to poor CD4+ T cell recovery even while on long-term ART [13, 29], a main subject of this thesis.

HIV-associated dementia (HAD) occurred in up to 30% of patients with AIDS-defining illnesses prior to the advent of ART. However, with the widespread use of ART, the burden of HAD has substantially decreased while the burdens of milder forms of HAND have increased, including asymptomatic neurocognitive impairment (ANI) and mild neurocognitive disorder (MND) [2, 30]. This occurs despite the widespread availability of ART in resource-rich settings and despite effective virological suppression systemically and in the central nervous system (CNS). Interestingly, neurocognitive impairment seems to be primarily in the motor, cognitive, and verbal domains in the pre-ART era, and in memory and executive function in the ART era [2]. The increased prevalence of HAND even in the era of ART raises the question of whether ART is effective at suppressing viral replication in the central nervous system (CNS) or modulating neuroinflammation, or whether there are other mechanisms driving persistent neurocognitive impairment including irreversible CNS injury prior to ART initiation, ß-amyloid deposition in the brain, neurovascular injury, or antiretroviral neurotoxicity [2]. Generally, most studies appear to point to a net neurocognitive benefit of ART, though the neurotoxicity of efavirenz is well-

described and recognized [31]. It is still unknown whether ART is sufficient to prevent HAND, though very early treatment initiation may prevent it in a majority of individuals [4, 32]. Because milder HAND remains prevalent in people living with HIV and on ART, specific treatment for HAND can be considered in these individuals including antiretroviral switch, treatment intensification, and potentially newer drugs under investigation such as cenicriviroc and natalizumab [4, 33].

Chronic complications of treated HIV infection, including immune dysregulation and HAND, may stem from persistence of HIV reservoirs, which are not eradicated by long-term ART. HIV reservoirs are established early in infection, may be maintained chronically by ongoing HIV replication, and are thought to predominantly come from resting memory CD4+ T cells that harbor latent proviral DNA [34, 35]. Other important anatomic reservoirs include the genital tract, the gastrointestinal tract, lymphoid tissues (including thymus, gut-associated lymphoid tissue, and lymph nodes), and the CNS. ART may reduce, but does not completely eliminate, reservoirs as measured by total HIV DNA load [36]. Lamers et al demonstrated measurable HIV DNA is present in a majority of autopsy tissues, including brain, sampled in various anatomical sites of individuals on ART with low or undetectable viral load prior to death [37]. There may be some effect of timing of ART initiation upon HIV reservoir size. Earlier treatment in perinatal HIV infection is associated with smaller peripheral blood proviral reservoir size in adolescence [38]. Likewise, treatment during acute HIV infection reduces systemic HIV reservoirs compared to individuals treated for chronic infection. Conversely, progression throughout the stages of acute HIV infection associates with increased HIV reservoir seeding [39]. Specifically within the CNS compartment, early treatment during acute infection has not yet proven to reduce CNS reservoir size, though other benefits have been demonstrated in terms of decreased neuroinflammation and HIV-specific

immune responses in CSF [40]. Cerebrospinal fluid (CSF) escape of HIV, another main subject of this thesis, describes a discordance of detectable CSF HIV RNA alongside undetectable plasma HIV RNA, may be an indicator of active viral replication from CNS reservoirs, and is described later in further detail [41]. Altogether, despite ART, HIV infection remains persistent in the form of difficult-to-eradicate reservoirs in various anatomical compartments. Though HIV remission has been achieved to date in two individuals via hematopoietic stem cell transplant [42, 43], reservoirs represent a chronic disease burden for the vast majority of people living with HIV.

Acute HIV Infection: The Earliest Events

Acute HIV infection (AHI) describes the earliest detectable stages of HIV infection, when HIV serology remains non-reactive or inconclusive yet viral replication is detectable in tissue and blood [44]. After exposure and transmission, HIV replicates in the mucosa, submucosa, and lymphoid tissues and is not yet detectable in plasma, which comprises the eclipse phase. Thereafter, HIV RNA becomes detectable in plasma, followed by a predictable sequence of detectable markers of HIV infection [45].

Based on this sequence, AHI is classified by Fiebig stages using laboratory criteria. In AHI, there are sequential assays that test positive: detection of HIV RNA, detection of p24 antigen, anti-HIV-1 (groups M and O) recombinant gp41 IgM enzyme immunoassay, and Western blot (to HIV-1 gp160, gp120, p65, p55, gp41, p40, p31, p24, and p18) (Table 1). Fiebig stage V is estimated to last up to approximately 90 days post-infection [44]. Fourth-generation antibody-antigen testing is standard in the United States and captures Fiebig stages II-VI of AHI, but still misses the pre-p24 eclipse period of AHI. Given this as well as the fact that newly HIV-infected individuals may present later due to the nonspecific symptoms of acute antiretroviral syndrome, diagnosis of AHI may still be delayed or missed [46].

Table 1. Fiebig stages of acute HIV infection.

Stage designation	Laboratory criteria (additive to prior Fiebig stage)
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
Fiebig VI	Western blot+

Data from [44].

Though several routes of HIV transmission are possible (e.g. cervicovaginal, intravenous, penile, rectal), CD4+ T cells and Langerhans cells are likely the first targets of HIV infection. Soon thereafter, viral replication occurs substantially in the gut-associated lymphoid tissues (GALT) within CD4+ T cells expressing high levels of CCR5 coreceptor and then spreads systemically, concurrent with a rapid increase in plasma HIV RNA and a steep decline in peripheral CD4+ T cells. Indeed, the vast majority of transmitted and founder viruses are shown to be R5-tropic [45]. If AHI is left untreated, the CD4 count transiently increases, and later progressively declines, while plasma viral load decreases to a steady state set point [45, 47, 48].

The immune system rapidly responds to AHI. The frequent occurrence of acute antiretroviral syndrome suggests a systemic inflammatory response to AHI. Both soluble and cellular markers of immune activation appear within the first few days of HIV infection, including acute phase reactants, cytokines, and CD8+ T cell activation [49-51]. Early CD8+ T cell activation leads to proliferation that has been shown to improve virological control; however, T cell activation after the first few weeks of infection seem to contribute to immune dysfunction [52]. From the RV254 cohort of Thai participants with AHI, Fiebig I AHI is associated with lower immune activation compared to later Fiebig stages, as measured by soluble CD14 (sCD14), CD4/CD8 ratio, and CD8+ T cell activation [53]. Thus, the immune response appears to be beneficial very early within AHI but may contribute to dysfunction at later stages [54].

Additionally, a substantial proportion of CD4+ T cells in the GALT that are depleted during AHI are Th17 cells that secrete/respond to IL-17, which are integral to maintaining the integrity of the GI tract mucosal barrier. Depletion of Th17 cells in AHI compromises the mucosal barrier, resulting in translocation of GI tract microbes and microbial products that contributes to systemic inflammation seen in AHI [54]. Finally, immune activation markers in CSF are elevated in a subset of individuals with AHI; they include neopterin (marker of macrophage activation) and chemokines CXCL10 and CCL2 (immune cell trafficking) [55].

Given that immune dysfunction may arise even within early HIV infection, ART initiation in AHI continues to be investigated for potential therapeutic benefit. Within the RV254 cohort, ART in AHI reduced markers of inflammation (C-reactive protein, sCD14, hyaluronic acid) and even normalized some when compared to HIV-negative controls (tumor necrosis factor, soluble IL-6 receptor, D-dimer) [56]. Indeed, treatment during the earlier stages of Fiebig I and II AHI was found to have a unique benefit compared to that in Fiebig III, in that it restored mucosal Th17 numbers and polyfunctionality and reversed peripheral and mucosal CD8+ T cell activation [57]. However, initiation of ART in Fiebig I does not appear to confer a unique benefit for restoring CD4/CD8 ratio when compared to initiation of ART at later Fiebig stages, potentially suggesting some degree of irreversible immune activation conferred by AHI [53]. When comparing CSF versus plasma concentrations of neopterin (marker of macrophage activation) and the chemokines CXCL10 and CCL2 (immune cell trafficking), they remained elevated in plasma but in CSF were normalized to levels seen in HIV-negative controls. This suggests a unique benefit of early ART initiation for reducing and reversing neuroinflammation [58].

Studies of AHI and immediate treatment initiation also allow for an assessment of early reservoir establishment and whether this process is modifiable with early interventions, with the

prospect of achieving ART-free HIV remission. The reservoir, as measured by HIV DNA, is established in early infection [39, 59]. Reservoir size also increases throughout the stages of AHI. Participants identified during Fiebig I had lower total blood and gut HIV DNA compared to those in Fiebig stages II and III [39, 53]. Factors impacting viral reservoir size continue to be an area of active investigation but include an early CD8+ T cell response [60] as well as timing of ART. If early infection is allowed to progress without immediate ART intervention, the HIV reservoir dramatically increases and reaches its set point [59]. Early treatment in AHI can significantly reduce this reservoir size [39, 59], and one study has shown that this reduction is sustainable over three years of ART [61]. Importantly, early treatment alone has not shown to produce ART-free HIV remission, even with undetectable HIV RNA or DNA in multiple blood and tissue samples. Even with treatment started at Fiebig I and maintained more than two years, participants experienced rapid viral rebound just weeks after analytical treatment interruption [62]. One participant was able to remain aviremic for more than seven months after analytical treatment interruption [63]. Indeed, even ART initiation within as short as 30 hours of life was not enough to induce ART-free remission in the Mississippi baby [64, 65]. Looking specifically at the CNS compartment, there is evidence of localized benefit of early ART by way of reduced and delayed immune activation and HIV-specific antibodies in CSF [58, 66], but there also is evidence of a persistent HIV-specific CD8+ T cell response in CSF [67]. It is increasingly thought that immune trafficking, signaling, and activation are linked to establishment and persistence of the HIV reservoir [54, 68]. CNS reservoir establishment and persistence may be a consequence of immune cell trafficking, both early in infection and throughout chronic infection. Because there appear to be delays in immune response (perhaps due to trafficking) as well as virological dynamics in the

CNS, there may be a unique benefit of early ART in reducing HIV reservoirs in the CNS as compared to systemic sites of HIV persistence [40, 69].

CD4+ T Cell Recovery During Antiretroviral Therapy

Though ART suppresses ongoing HIV replication in the periphery to undetectable levels, up to 30% of ART-treated individuals with chronic HIV infection fail to achieve CD4+ T cell counts to a normal level (> 500 cells/mm^3) [70]. This phenomenon has been referred to in the literature as immunologic non-response, CD4+ T cell response, and immuno-virological discordance, and it has been associated with factors including older age, viral hepatitis coinfection, lower nadir CD4+ T cell count, longer duration of untreated HIV infection, and worse morbidity and mortality [70-74]. Hereafter referred to as poor CD4 recovery, this phenomenon is more likely to occur in patients who initiate ART late in the course of infection, often associating with a low CD4 nadir [71, 75]. As mentioned earlier, in the initial stages of HIV infection, there is a rapid and severe depletion of circulating CD4+ T cells, followed by a spontaneous but temporary recovery [48]. Initiation of ART within four months of HIV infection to coincide with this temporary recovery period has been associated with improved CD4 recovery [71, 76].

Although it is uncommon to identify individuals during the narrow window of AHI, the RV254/SEARCH 010 study follows a cohort of individuals with AHI in Thailand who are identified and begin ART soon after HIV infection. As described earlier, initiation of ART during AHI partially resolves systemic inflammation and reduces the viral reservoir [53, 56]. Furthermore, initiation of ART during Fiebig stage I results in improved immunological reconstitution compared to Fiebig stages II-IV [53].

Whether identifying and treating HIV at the earliest stages of infection (within one month) reduces the frequency of clinical immunologic non-response is not known, though improved CD4

recovery has been described in ART initiation within four to six months of infection or seroconversion [71, 76, 77]. Whether HIV-associated neuroinflammation or HIV-associated neurocognitive or affective symptoms are associated with poor CD4 recovery have yet to be examined.

CSF HIV Escape During Antiretroviral Therapy

In early HIV infection, central nervous system (CNS) infection can be detected by the presence of HIV RNA in the cerebrospinal fluid (CSF) as early as eight days after infection [55, 78]. Indeed, more than half of RV254 participants with AHI reported neurological symptoms within 12 weeks after diagnosis, with half of those occurring before diagnosis and ART initiation [79]. Thus, the CNS is affected early in HIV infection, both virologically and clinically. Interestingly, virological dynamics between plasma and CSF show that in AHI, CSF HIV RNA is consistently lower than concurrent plasma HIV RNA [55]. Given this and the proposed mechanism of immune cell trafficking that may introduce HIV into the CNS, it is likely that CSF HIV RNA has a delayed appearance behind that in plasma [40].

As discussed in the prior section “Acute HIV Infection”, markers of immune activation and inflammation appear in the CNS in AHI [55]. Importantly, there are neuroimaging findings, specifically magnetic resonance spectroscopy, that are consistent with early CNS inflammation, including elevated choline/creatinine that likely represents immune cell activation [55]. However, neurofilament light chain (NFL) in CSF, a marker for neuronal injury, was not elevated in AHI [80]. It is thus possible that in AHI, the CNS is infected through trafficking of infected immune cells or perhaps viral seeding across a compromised blood-brain barrier, resulting in localized neuroinflammation but without detectable neuronal injury as long as ART is promptly started.

Rapid initiation of ART suppresses HIV replication in the CNS and reverses neuroinflammation [40, 58].

Importantly, the CNS may continue to be affected uniquely in the phenomenon of CSF escape, in which viral presence is undetectable in the periphery due to ART suppression but is detectable in CSF. Initially thought to be rare, one study has suggested an incidence rate of up to 10% of individuals with CSF escape [81]. An area of active investigation, only recently have there been international consensus guidelines to provide a clearer definition, which are reproduced in Table 2 [82]. CSF escape may vary from asymptomatic to symptomatic, as CSF HIV RNA may be detected only incidentally via unrelated lumbar puncture. However, lumbar puncture may also be indicated in the work-up of new neurological symptoms in an ART-adherent, previously well-controlled patient with HIV [83]. Peluso et al presented a case series of ten patients with symptomatic CSF escape, wherein new neurologic symptoms (sensory, motor, and/or cognitive) prompted lumbar puncture and, for some, neuroimaging and CSF resistance testing. All patients had CSF pleocytosis or elevated CSF protein, and among those who had the studies conducted, most had magnetic resonance imaging (MRI) findings (7 of 8) and CSF resistance mutations (6 of 7). Optimizing the ART regimen clinically improved eight of nine patients [84]. Thus, CSF escape encompasses a clinical phenomenon that can have symptomatic impact in some patients, which may also be resolvable by treatment optimization. In at least some patients with CSF escape, there is evidence of neuroinflammation. Additionally, another potential driver of CSF escape is compartmentalized drug resistance secondary to sub-therapeutic levels of antiretrovirals in the CSF, given the findings of this case series.

Table 2. Recommended guidelines for definitions of cerebrospinal fluid escape, from the Second Global HIV CSF Escape Consortium in 2017

1. When plasma HIV RNA is suppressed, the presence of quantifiable HIV RNA in the cerebrospinal fluid at any level should be considered cerebrospinal fluid HIV RNA escape
2. When plasma HIV RNA is detectable, cerebrospinal fluid HIV RNA greater then plasma HIV RNA, at any level, should be considered cerebrospinal fluid HIV RNA escape
3. Defining symptomatic versus asymptomatic cerebrospinal fluid HIV RNA escape should be based on patient symptomatology
4. Cerebrospinal fluid HIV escape should be actively managed in symptomatic cases

Reproduced from [82].

CSF escape may also be secondary to a superimposed neuroinflammatory or neuroinfectious condition [83]. Cases have been described of CSF escape in the context of neurosyphilis, varicella zoster virus meningitis, and neuroborreliosis [85-87]. The possibility of secondary CSF escape from superimposed neuroinflammation makes it difficult to reconcile whether inflammation drives CSF escape via increased immune cell trafficking or blood-brain barrier disruption, or whether CSF escape can drive a localized neuroinflammatory response to localized viral replication.

One concern for CSF HIV escape during ART is that even low-level HIV replication in CSF has been shown to associate with markers of immune activation and CNS injury. Spudich et al characterized a subset of participants in primary HIV infection with CSF HIV RNA < 100 copies/mL who had increased neuroinflammation, as measured by CSF white blood cells (WBC), neopterin, and CXCL10, compared to HIV-negative controls [88]. In a group of patients with < 40 copies/mL of HIV RNA in both plasma and CSF by standard assays, ultrasensitive assays found that those with low-level CSF HIV RNA tended to have higher CSF neopterin [89], even with years of suppressive ART. Similarly, low-level detection of CSF HIV associates with compromised blood-brain barrier integrity and decreased executive function [90]. Thus, while

frank CSF escape (e.g. > 100 copies/mL) may result in new neurological impairment, there is also concern for subclinical or low-level CSF escape that is also pathological.

Finally, one last concern for CSF HIV escape is whether it indicates the presence or long-term persistence of a CNS reservoir [83]. This is especially relevant in the discussion and progress towards achieving ART-free HIV remission, as the CNS reservoir remains an important target. One important point to consider is that persistent CSF escape most likely points to HIV replication from CNS resident cells, i.e. a reservoir. However, not all cases of CSF escape are persistent; indeed some appear to be episodic [91], though this is difficult to know for certain given the paucity of longitudinal CSF data [41]. Episodic CSF escape, otherwise called CSF HIV viral blips, could stem from CNS reservoirs, but could also be explained by transient immune cell trafficking into the CNS that supports local viral replication [41]. Joseph et al investigated cases of CSF escape using deep sequencing, identifying one case of a macrophage-tropic, partially drug-resistant, genetically diverse escape population likely to stem from CNS reservoirs, and two cases of T cell-tropic, genetically homogenous escape populations likely to stem from immune cell trafficking and transient clonal expansion [92].

Given the potential significance of CSF escape for ongoing inflammation and CNS injury in the setting of peripheral ART suppression, as well as for pointing to a CNS HIV reservoir, it remains unknown whether CSF escape can occur after identifying and treating AHI within the first month of infection. Early ART in AHI appears to confer benefit in terms of mitigating neuroinflammation and neuronal injury, as described above, but whether it confers a benefit in terms of reducing events of CSF escape remains uncertain. Investigating this open question can illuminate whether very early ART reduces CNS persistence of HIV infection [40].

Statement of Purpose

This thesis encompasses two projects pertaining to immunological reconstitution and CNS reservoirs in AHI: to investigate if poor CD4 recovery occurs (Project 1) and if CSF escape occurs (Project 2) when ART is started during AHI. We identified pre-ART (baseline) and on-ART clinical and laboratory parameters associated with poor CD4 recovery, including systemic, central nervous system (CNS), and coinfection factors known to associate with CD4 recovery in chronic infection. We also identified clinical and laboratory factors associated with CSF escape in treated AHI.

Methods

Study Participants

Individuals with AHI identified at the Thai Red Cross AIDS Research Centre (TRC-ARC) in Bangkok were enrolled in the ongoing RV254/SEARCH010 study (clinicaltrials.gov NCT00796146) [93]. The TRC-ARC is an anonymous voluntary HIV testing and counseling center in Bangkok, Thailand. Clients are provided a unique identifier at their first visit, which is used for all subsequent visits. Clients also fill out an optional demographic and risk-behavior questionnaire. RV254/SEARCH010 is a study within TRC-ARC that identifies clients with Fiebig stages I through V AHI, using paired 4th-generation HIV antigen/antibody immunoassay and nucleic acid testing (NAT), and offers immediate ART.

Nonreactive 4th-generation immunoassays had their samples pooled for qualitative NAT screening. Pooled samples with reactive NAT testing were deconstructed, and individual samples were re-tested to identify HIV-positive samples. HIV-positive samples were tested by quantitative NAT, 3rd- and 2nd-generation immunoassays, and p24 Western blots; clients were contacted and

asked to enroll in the study. Clients with reactive 4th-generation immunoassay, nonreactive 3rd-generation immunoassay or nonreactive 2nd-generation immunoassay, and p24 Western blot negative or indeterminate were contacted and requested to enroll in the study. All samples were tested within 24 hours to quickly identify AHI [93]. One participant who was enrolled as Fiebig V was subsequently reclassified to Fiebig VI based on new assay thresholds and was retained in the study.

Duration of infection was estimated based on dates of exposure in the past 30 days. Participants voluntarily documented exposure events within the past 30 days. Exposure events were stratified according to no risk (no exchange of bodily fluids, non-insertive sex, or contact of bodily fluids with intact skin), low risk (receptive oral sex without ejaculation, insertive oral sex, or exposure of mucous membranes to bodily fluids), medium risk (anal or vaginal sex with condom use regardless of HIV serostatus, receptive condom-less oral sex with male partner with either unknown HIV serostatus or positive HIV serostatus with suppressed viral load), or high risk (anal or vaginal sex without condom use, receptive condom-less oral sex with male HIV-positive partner with detectable or unknown viral load, or injection drug use). The HIV exposure date was calculated as the mean date of all exposures in the highest risk category reported by the participant. Exposure events within the past 30 to 60 days were considered only if the participant reported no sexual activity within the past 30 days, or if the participant tested in Fiebig stages III-V and reported high risk exposures in the past 30 to 60 days and lower risk exposures in the past 30 days.

Participants were offered immediate initiation of ART via an accompanying protocol (clinicaltrials.gov NCT00796263). Standard first-line ART through 2016 included efavirenz plus two nucleoside reverse transcriptase inhibitors. Efavirenz could be replaced by ritonavir-boosted lopinavir or raltegravir for intolerance or resistance. A subset received a five-drug regimen that

added raltegravir and maraviroc [94]. The majority were switched from efavirenz to dolutegravir starting in 2017.

Participants underwent serial interviews, examinations, and phlebotomy, with optional procedures. Clinical and laboratory assessments were performed at days 0, 2, 3, 5, 7, 10, and at weeks 2, 4, 8, 12, 16, 20, 24, and every 12 weeks thereafter. Laboratory assessments included CD4+ T cell count, CD8+ T cell count, HIV RNA, and complete blood count. Hepatitis B and C serology testing was performed every 48 weeks. Infection with hepatitis B or C was confirmed with plasma viral load testing. Additional laboratory assessments included biomarkers of inflammation and immune activation, as listed below. Optional procedures included lumbar puncture, brain MRI/MRS, sigmoid biopsy, leukapheresis, inguinal lymph node biopsy, and genital secretions.

Optional lumbar punctures were obtained at study entry and at weeks 24, 96, and 240. CSF studies included cell count, protein, glucose, and HIV RNA. The remaining cell-free CSF was stored within six hours of collection at −80°C.

T1-weighted and T2-weighted fluid-attenuated inversion recovery (FLAIR) MRI brain scans were optionally obtained at study entry on the same 1.5T GE scanner, and were independently interpreted by two neuroradiologists.

An optional neuropsychological testing battery was obtained at study entry and weeks 12, 24, and 96. It consisted of the Grooved Pegboard test in the nondominant hand (fine motor function), Color Trails 1 and Trail Making A (psychomotor speed), and Color Trails 2 (executive functioning/set-shifting). Raw neuropsychological testing results were standardized to healthy Thai control participants from equivalent age and education strata to calculate z-scores [95]. A

mean of z-scores was computed (NPZ-4) for an overall metric of neuropsychological testing performance.

Participants completed the Thai version of the Hospital Anxiety and Depression Scale (HADS), a 14-item scale with anxiety and depression subscales of 7 items each. Each item is scored from 0 to 3, with a total score range of 0 to 21 per subscale.

All participants provided written informed consent prior to enrollment in the cohort. The research protocol was approved by institutional review boards at Chulalongkorn University Hospital, Yale School of Medicine, UCSF, and the Armed Forces Research Institute for Medical Sciences.

Project 1 included all participants who initiated ART between April 2009 and April 2016 with at least 48 weeks of documented HIV-RNA < 50 copies/mL, regardless of time to suppression. Eligible participants were stratified by latest CD4+ T cell count to suboptimal recovery (<350 cells/mm^3), intermediate recovery (350-499 cells/mm^3), and complete recovery (≥500 cells/mm^3), in agreement with and for appropriate comparison to prior studies of CD4 recovery in chronic HIV [70]. None of the participants enrolled in analytic treatment interruption or interventional substudies before the latest follow-up visit used in the analysis.

Project 2 included all participants who initiated ART between April 2009 and April 2019 with paired blood and CSF sampling in at least one visit at study enrollment (baseline), week 24, or week 96. CSF escape was defined as paired CSF HIV RNA greater than plasma HIV RNA at week 24 or week 96, as per recent international consensus definitions [82].

Sampling and Laboratory Testing

For Project 1, clinical and laboratory parameters were assessed at baseline and latest study visit. Blood and CSF markers of immune activation, neuropsychological (NP) testing, and mood

assessments were examined at a standardized interval of 96 weeks after starting ART. CD4+ T cell count was assessed at all available study visits to investigate longitudinal trends. For Project 2, clinical and laboratory parameters were assessed at baseline, week 24, and week 96.

CD4+ T cell count was measured by single- and dual-platform flow cytometry (Becton-Dickinson). HIV RNA in plasma was performed using the COBAS AMPLICOR HIV-1 Monitor Test v1.5 or COBAS Taqman HIV-1 Test v2.0 (Roche Molecular Systems). Lower limit of quantification for plasma HIV RNA was 50 and 20 copies/mL, respectively, depending on the platform used. CSF samples were diluted fourfold for volume requirements for detection of HIV RNA, with a lower limit of quantification at 80 copies/mL using the Taqman platform.

Plasma soluble CD14 (sCD14), intestinal fatty acid binding protein (I-FABP) (R&D Systems), and hyaluronic acid (Corgenix) were measured by ELISA. C-reactive protein was measured by electrochemiluminescence assay (Meso Scale Discovery). D-dimer was measured by enzyme-linked fluorescent assay (bioMerieux). Tumor necrosis factor-alpha (TNF-alpha) and high-sensitivity interleukin 6 (IL-6) were measured by the Luminex platform (Millipore). All assays for biomarkers were performed in duplicate on cryopreserved acid citrate dextrose plasma for research purposes only following a single thaw with the exception of I-FABP (two thaws). Anti-hepatitis C antibodies, hepatitis B surface antigen (HBsAg), and anti-HBsAg antibodies were measured by chemiluminescent microparticle immunoassay (Abbott). Syphilis testing was measured by B VDRL Antigen BD Difco (Becton Dickinson), Macro-Vue RPR Card Tests (Becton Dickinson), and Serodia TPPA (Fujirebio Diagnostics). CSF levels of neopterin were measured by ELISA (GenWay Biotech).

Statistical Analysis

Data were reported as median (interquartile range, IQR) values, except when otherwise indicated. Comparisons between CD4 recovery groups were performed using the Mann-Whitney *U* test or Kruskal-Wallis test for continuous variables, χ^2 test for categorical variables, and linear regression for slope analyses. Slope analyses were performed by assessing CD4 change from baseline to week 48 on ART. Multivariate logistic regression analyses were adjusted for baseline CD4 count, baseline HIV-RNA, and duration on ART. Statistical tests were two-sided, and differences were considered significant at $p<0.05$. Differences were considered suggestive of a trend at $p<0.10$. Analyses were performed using SPSS Statistics (version 24; IBM), R (version 3.6.1; R Foundation for Statistical Computing), and Prism (version 7.0; GraphPad) software.

Author Contributions

This author conceived the study for Project 1 based on an identification of gaps in the literature on CD4+ T cell recovery in the setting of very early ART initiation. The adviser assisted with study design to investigate these questions in the RV254 cohort. The author and adviser conceived the study for Project 2 based on prior work on CSF escape, which had not yet been investigated in acute HIV infection. This author worked on-site at the Thai Red Cross AIDS Research Centre in Bangkok, Thailand, in August 2019, to review clinical records, collect and clean data, and perform statistical analyses. Collaborators participated in study coordination, including study cohort oversight, research procedures including blood draw and lumbar puncture, neuropsychological testing, laboratory testing, and data management. Assays were performed by collaborators either locally in Bangkok or at research laboratories in Maryland. This author performed all statistical analyses, apart from assistance provided by Suteeraporn Pinyakorn for slope analyses in Project 1. A full list of collaborators is available in the Acknowledgments section.

Results: Project 1

Study Participant Characteristics

During the RV254/SEARCH010 study period, 304 participants with AHI immediately started ART and had documented viral load < 50 copies/mL for at least 48 weeks (Figure 1), of whom 79 underwent optional CSF sampling. 96% of enrollees were Thai men, the majority men who have sex with men. Median age was 26 years (range 18-57). ART was started at a median 19 days post-estimated infection (range 1-62). Median latest follow-up visit was at 144 weeks (range 60-420).

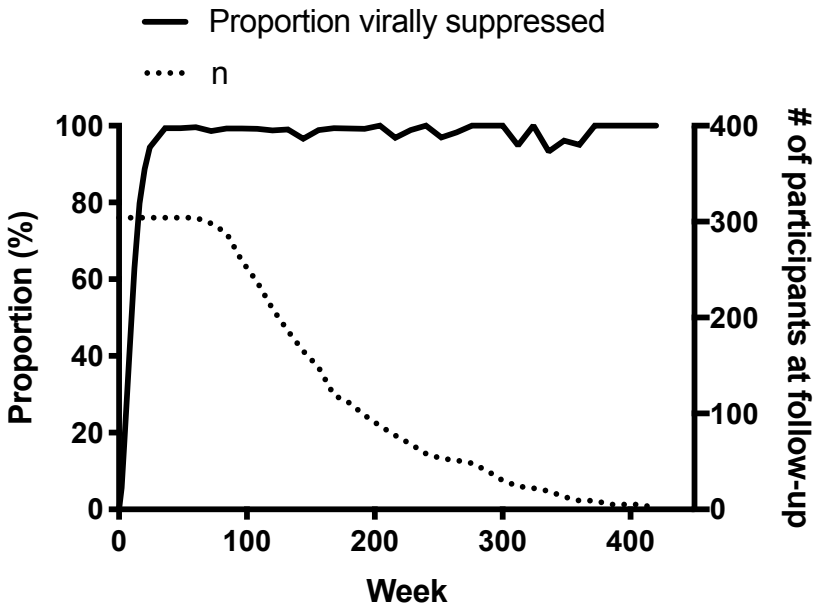

Figure 1. Longitudinal HIV RNA of participants with acute HIV infection. Solid line represents the proportion of participants with HIV RNA < 50 copies/mL at each follow-up study visit week. Dotted line represents the total number of participants at each study visit week.

CD4+ T Cell Recovery After ART in Acute HIV

Of the 304 participants, the most recent CD4 count was <350 cells/mm^3 (suboptimal recovery) in 3.6%, 350-499 cells/mm^3 (intermediate recovery) in 14.5%, and ≥500 cells/mm^3

(complete recovery) in 81.9%. Viral load at enrollment, time from HIV transmission to ART initiation, week of first documented viral suppression, and Fiebig stage at enrollment did not differ between recovery groups (Table 3). Duration of ART was shortest in the intermediate recovery group (median 120 weeks, IQR 84-192, p=0.03) but did not differ between suboptimal (156, 84-180) and complete recovery groups (156, 108-216, p=0.5). Duration of ART was shorter in the combined suboptimal and intermediate recovery group versus complete recovery (p=0.01).

CD4 counts of individuals in the three recovery groups were plotted at each study visit week (Figure 2). Using slope analysis, mean (standard deviation) rates of recovery were 3.1 (3.4), 3.5 (2.1), and 5.5 (4.1) cells/mm^3/week for suboptimal, intermediate, and complete recovery groups, respectively. Recovery rate in intermediate recovery was significantly slower compared to complete recovery (rate difference -2.0 cells/mm^3/week, 95% confidence interval [CI] -3.3 to -0.8, p=0.002). There was a trend of slower rate in suboptimal compared to complete recovery (rate difference -2.4 cells/mm^3/week, 95% CI -4.7 to 0.0, p=0.05). There was no difference between suboptimal and intermediate recovery groups. Individuals were stratified into groups of low (<350 cells/mm^3), medium (350-499 cells/mm^3), and high (≥500 cells/mm^3) baseline CD4 count, and longitudinal CD4 counts within each group were plotted at each study visit week (Figure 3).

CD4 count differed by Fiebig stage at enrollment and was highest for Fiebig stage I at enrollment (p<0.0001, Figure 4). CD4 count at latest study visit was not different by Fiebig stage at enrollment. Change in CD4 count between baseline and latest study visit was different by Fiebig stage at enrollment and was greatest for Fiebig stage II and least for Fiebig stage I at enrollment (p<0.0001).

Table 3. Characteristics of participants treated in acute HIV infection stratified by CD4 recovery group.

Characteristics	Suboptimal Recovery, CD4<350, (*n*=11)	Intermediate Recovery, 350≤CD4<500 (*n*=44)	Complete Recovery, CD4≥500 (*n*=249)	p-value
Age, years (IQR)	23 (20-30)	26 (23-30)	26 (23-33)	0.3
Sex, male:female, *n*	11:0	44:0	237:12	0.3
Risk behavior, *n* (%)				
WSM	0 (0)	0 (0)	12 (5)	
MSW	1 (9)	3 (7)	9 (4)	0.4
MSM	10 (91)	41 (93)	228 (92)	
Thai ethnicity, *n* (%)	11 (100)	43 (98)	244 (98)	0.9
Illicit drug use during HIV exposure, *n* (%)	2 (18)	13 (30)	51 (21)	0.4
HIV-RNA at enrollment (log_{10} copies/mL), median (IQR)	5.9 (5.4-7.3)	6.1 (5.4-6.9)	5.7 (5.2-6.6)	0.3
Time to ART initiation (days), median (IQR)	20 (14-23)	21 (15-28)	19 (15-26)	0.6
1st week of viral suppression, median (IQR)	16 (8-24)	12 (8-24)	12 (8-24)	>0.9
ART duration (weeks), median (IQR)	156 (84-180)	120 (84-192)	156 (108-216)	0.03
Fiebig stage at enrollment, *n* (%)	2 (18)	5 (11)	44 (18)	
Stage I	4 (36)	9 (21)	71 (29)	
Stage II	5 (45)	22 (50)	90 (36)	0.2
Stage III	0 (0)	6 (14)	26 (10)	
Stage IV	0 (0)	2 (5)	18 (7)	
Stage V				

Abbreviations: HIV, human immunodeficiency virus; WSM, women who have sex with men; MSW, men who have sex with women; MSM, men who have sex with men; IQR, interquartile range.

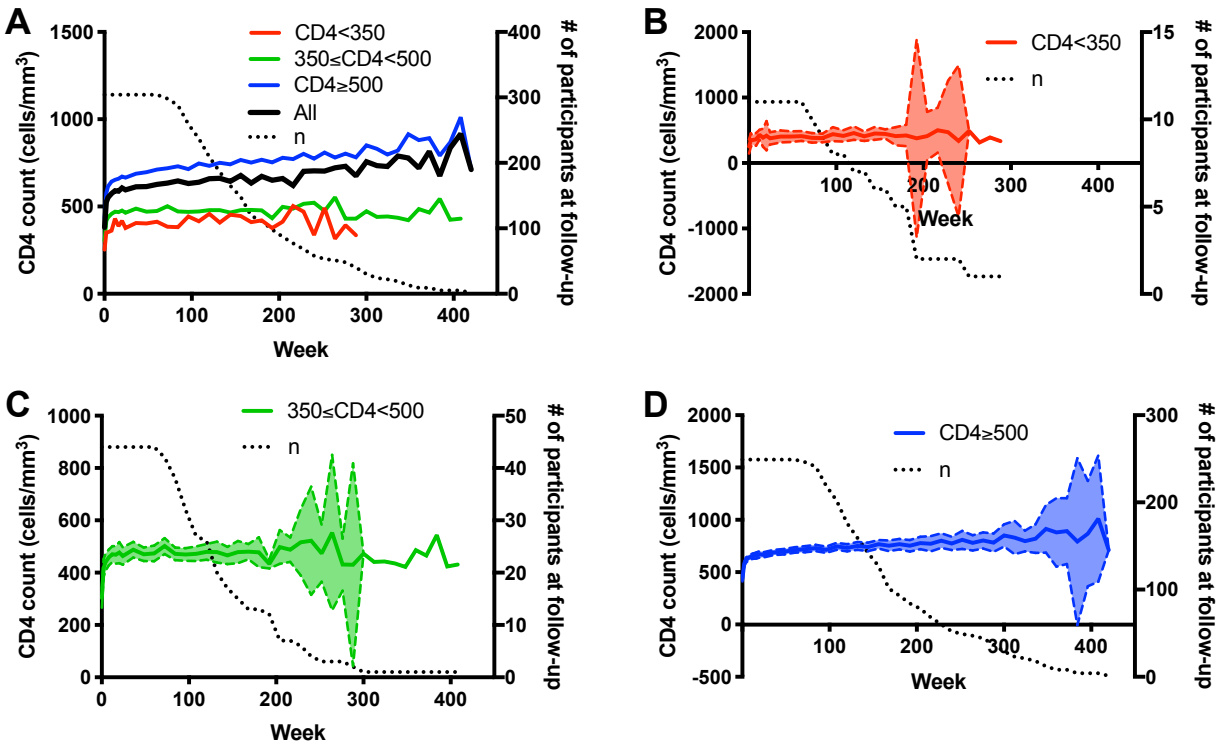

Figure 2. Longitudinal CD4+ T cell counts of participants with acute HIV infection according to suboptimal, intermediate, and complete recovery groups. (A) Mean CD4+ T cell counts of all participants (black) and by suboptimal (red), intermediate (green), and complete recovery (blue) groups. Dotted black line represents the total number of participants across all recovery groups at each study visit week. B-D, CD4+ T cell counts of participants in suboptimal (B), intermediate (C), and complete (D) recovery groups. Solid lines represent the mean CD4+ T cell count at each follow-up study visit week. Dashed lines represent the 95% confidence interval of CD4+ T cell counts at each study visit week. Dotted black lines represent the total number of participants in each respective recovery group at each study visit week. Confidence intervals for panels B-D tend to increase over time due to the declining sample size of participants at each subsequent week of follow-up visit.

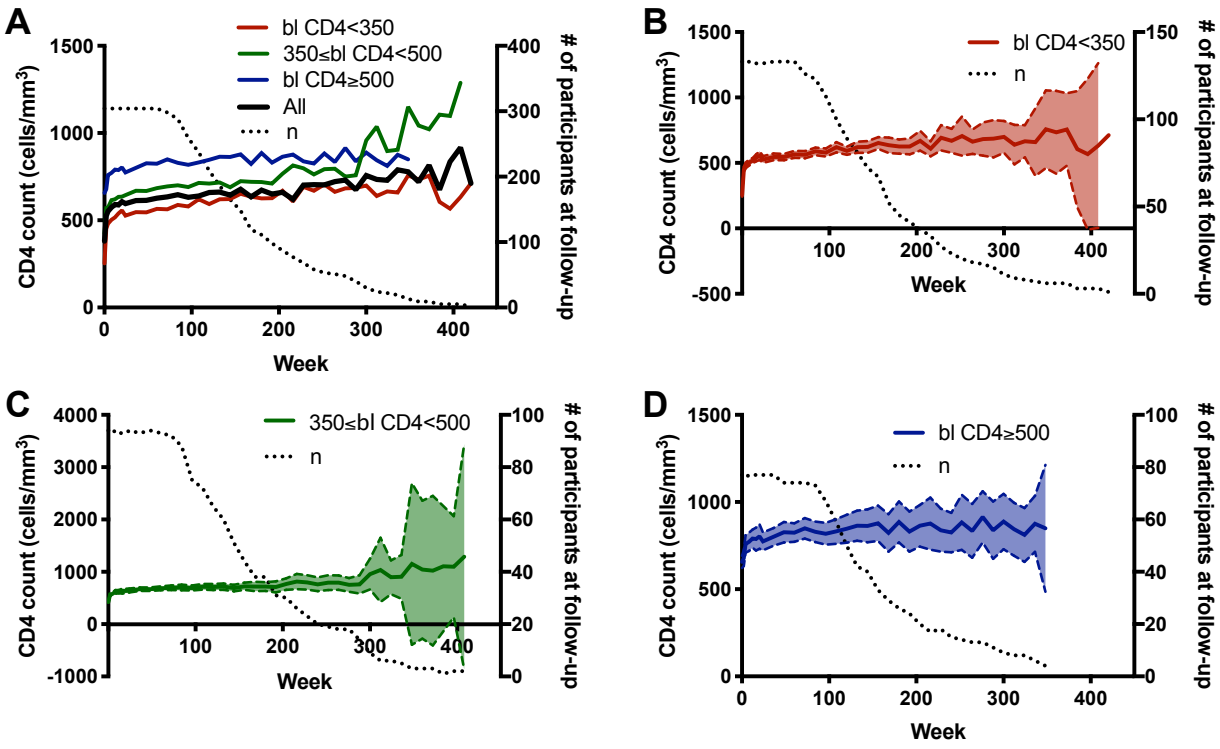

Figure 3. Longitudinal CD4+ T cell counts of participants with acute HIV infection according to low, medium, and high baseline CD4 count. (A) Mean CD4+ T cell counts of all participants (black) and by low (red, baseline CD4 < 350 cells/mm3), medium (green, baseline CD4 350-499 cells/mm3), and high (blue, baseline CD4 ≥ 500 cells/mm3) baseline CD4 count. Dotted black line represents the total number of participants at each study visit week. B-D, CD4+ T cell counts of participants with low (B), medium (C), and high (D) baseline CD4 count. Solid lines represent the mean CD4+ T cell count at each follow-up study visit week. Dashed lines represent the 95% confidence interval of CD4+ T cell counts at each study visit week. Dotted black lines represent the total number of participants in each respective group at each study visit week. Confidence intervals for panels B-D tend to increase over time due to the declining sample size of participants at each subsequent week of follow-up visit.

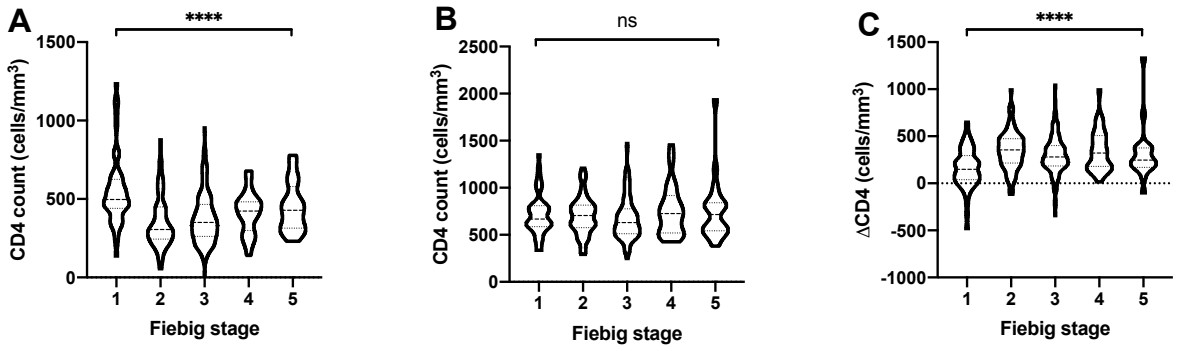

Figure 4. CD4+ T cell count by Fiebig stage at enrollment. Violin plots of CD4 count at baseline (A) and at latest study visit (B) by Fiebig stage at enrollment. (C) Violin plot of change in CD4 count between baseline and latest study visit by Fiebig stage at enrollment. Comparison between groups by Kruskal-Wallis test. **** indicates $p<0.0001$, ns indicates not statistically significant.

Baseline Pre-ART Predictors of CD4 Recovery After ART

At baseline, CD4 and absolute lymphocyte counts were lower in participants with suboptimal compared to complete recovery (CD4 count: median 265 vs 411 cells/mm^3, p=0.002, Table 4). CD8 count, CD4/CD8 ratio, hemoglobin, and platelet count at baseline were not different between suboptimal and complete recovery.

Given the small size of the suboptimal recovery group and the similar CD4 trajectories of the suboptimal and intermediate recovery groups, we combined these two groups and compared baseline predictors with complete recovery. CD4/CD8 ratio and CD4+ T cell, total WBC, absolute lymphocyte, absolute monocyte, and platelet counts were lower in the combined group compared to the complete recovery group at baseline. Intestinal fatty acid binding protein (I-FABP) was lower in the combined group compared to complete recovery. Other markers of inflammation were similar between these groups (Table 4).

Table 4. Pre-ART baseline predictors of CD4 recovery.

Pre-ART Baseline Predictors	Suboptimal Recovery, CD4<350 (*n*=11)	Suboptimal & Intermediate Recovery, CD4<500 (*n*=55)	Complete Recovery, CD4≥500 (*n*=249)	p-value†	p-value‡
CD4 count (cells/mm^3)	265 (91-371)	275 (214-371)	411 (303-533)	0.002	< 0.001
CD8 count (cells/mm^3)	412 (228-575)	448 (298-847)	512 (339-854)	0.09	0.4
CD4/CD8 ratio	0.67 (0.37-0.94)	0.66 (0.35-0.87)	0.81 (0.47-1.2)	0.4	0.003
WBC count (× 10^3 cells/mm^3)	4.5 (3-5.6)	4.7 (4.0-5.6)	5.5 (4.4-7.0)	0.03	0.003
Absolute neutrophil count (× 10^3 cells/mm^3)	2.84 (1.56-3.52)	2.90 (2.24-3.60)	3.13 (2.18-4.22)	0.3	0.2
Absolute lymphocyte count (× 10^3 cells/mm^3)	1.06 (0.69-1.48)	1.18 (0.93-1.91)	1.58 (1.21-2.10)	0.003	< 0.001
Monocyte count (× 10^3 cells/mm^3)	0.38 (0.28-0.63)	0.40 (0.31-0.52)	0.52 (0.42-0.66)	0.1	< 0.001
Eosinophil count (× 10^3 cells/mm^3)	0.045 (0.000-0.308)	0.023 (0.012-0.049)	0.038 (0.018-0.082)	0.9	0.09
Hemoglobin (g/dL)	15 (14-16)	15 (14-16)	15 (14-16)	0.2	0.2
Platelet count (× 10^3/mm^3)	215 (167-233)	215 (180-248)	234 (188-296)	0.1	0.01
sCD14 (μg/L)	-	1.3 (1.1-2.3)[a]	1.6 (1.1-1.9)[b]	-	0.9
IL-6 (pg/mL)	-	0.6 (0.4-1.4)[c]	0.5 (0.3-0.9)[d]	-	0.3
Hyaluronic acid (ng/mL)	-	17.6 (9.7-30.2)[a]	18.0 (9.0-32.4)[b]	-	0.96
Intestinal fatty acid binding protein (pg/mL)	-	738 (343-944)[a]	1044 (637-1559)[b]	-	0.045
C-reactive protein (mg/L)	-	2.1 (0.5-4.2)[a]	1.3 (0.7-3.4)[b]	-	0.96
TNF-alpha (pg/mL)	-	1.3 (0.8-2.1)[c]	1.2 (0.7-2.2)[d]	-	0.9
D-dimer (ng/mL)	-	357 (283-473)[a]	266 (173-435)[b]	-	0.3

Median (IQR).

Abbreviations: CD4, CD4+ T cell; CD8, CD8+ T cell; WBC, white blood cell; sCD14, soluble CD14; IL-6, interleukin-6; TNF-alpha, tumor necrosis factor alpha.

†P-value is for suboptimal recovery vs complete recovery.

‡P-value is for combined suboptimal & intermediate recovery vs complete recovery.

[a]n=8; [b]n=63; [c]n=16; [d]n=118

CSF sample results for suboptimal (CSF n=3) and intermediate (CSF n=7) recovery groups were also combined for comparison to complete recovery (CSF n=69). CSF viral load, WBC count, protein, and glucose were not different between the combined and complete recovery groups. Baseline CSF neopterin trended in elevation in the combined group (n=10) compared to the complete recovery group (n=69, median 2938 vs. 1623 pg/mL, p=0.05, Table 5). Suboptimal versus complete recovery groups did not differ on neuropsychological tests (Color Trails 1, Color Trails 2, Trail Making A, Grooved Pegboard) or psychiatric indices (Patient Health Questionnaire) at baseline (Table 6). Markers for co-infection or exposure to hepatitis B virus, hepatitis C virus, and syphilis at baseline were not associated with CD4 recovery (Table 6).

Table 5. Pre-ART baseline predictors of CD4 recovery, measured in CSF.

Pre-ART Baseline Predictors in CSF	Suboptimal & Intermediate Recovery, CD4<500 (*n*=10)	Complete Recovery, CD4≥500 (*n*=69)	p-value‡
CSF HIV RNA (log_{10} copies/mL)	3.8 (2.9-4.2)	3.0 (2.0-4.1)	0.3
CSF WBC count (cells/mm^3)	0 (0-0)	0 (0-0)	0.7
CSF protein (mg/dL)	38 (26-41)	29 (25-35)	0.3
CSF glucose (mg/dL)	62 (58-66)	61 (57-66)	0.7
CSF neopterin (pg/mL)	2938 (2069-5310)	1623 (958-3140)	0.05

Median (IQR).
Abbreviations: ART, anti-retroviral therapy; CSF, cerebrospinal fluid; WBC, white blood cell.
‡P-value is for combined suboptimal & intermediate recovery vs complete recovery.

Table 6. Additional pre-ART baseline predictors of CD4 recovery.

Pre-ART Baseline Predictors	Suboptimal Recovery, CD4<350	Suboptimal & Intermediate Recovery, CD4<500	Complete Recovery, CD4≥500	p-value[†]	p-value[‡]
Positive anti-HCV, *n* (%)	0/10 (0)	1/54 (2)	2/248 (1)	0.8	0.5
Positive anti-HBs, *n* (%)	5/11 (45)	28/54 (52)	133/249 (53)	0.6	0.8
Positive HBsAg, *n* (%)	1/11 (9)	3/55 (5)	15/249 (6)	0.7	0.9
Reactive VDRL, *n* (%)	2/10 (20)	7/54 (13)	30/249 (12)	0.5	0.9
NPZ-4 score	0.007 (-0.7 – 0.1) *n=11*	-0.002 (-0.5 – 0.4) *n=50*	-0.02 (-0.6 – 0.6) *n=216*	0.4	0.8
Total PHQ score	14 (7 – 16) *n=11*	12 (7 – 15) *n=50*	9 (6 – 14) *n=216*	0.2	0.09

Abbreviations: ART, anti-retroviral therapy; HCV, hepatitis C virus; anti-HBs, hepatitis B surface antibody; HBsAg, hepatitis B surface antigen; VDRL, Venereal Disease Research Laboratory test; NPZ-4, neuropsychological test z-score in four domains (Color Trails 1, Color Trails 2, Trail Making A, Grooved Pegboard); PHQ, Patient Health Questionnaire.
[†]P-value is for suboptimal recovery vs complete recovery.
[‡]P-value is for combined suboptimal & intermediate recovery vs complete recovery.

On-ART Factors Associated with CD4 Recovery

The ART regimen initiated at AHI did not predict CD4 recovery (Table 7). By the time of most recent study visit, 113 of the 304 participants (37%) were switched to a dolutegravir-containing regimen after a median (IQR) duration of ART of 145 (107-212) weeks. There was no association between switch to dolutegravir and CD4 recovery (not shown).

As mentioned earlier, assessment of on-ART factors was performed at the latest study visit week for each participant, which was at median 144 weeks (range 60-420). Some assays and testing, including blood and CSF markers of immune activation, neuropsychological (NP) testing, and mood assessments, were only performed at standardized intervals of 24 and 96 weeks. For these, we focused on assessments at 96 weeks due to our interest in longer term CD4 recovery.

Table 7. Initial ART regimen stratified by CD4 recovery group.

	2NRTI/LPV/r	2NRTI/EFV	2NRTI/RAL	2NRTI/MVC/RAL	2NRTI/EFV/MVC/RAL	**Total**
Suboptimal Recovery, CD4<350	0	8	0	1	2	11
Intermediate Recovery, 350≤CD4<500	0	29	0	0	15	44
Complete Recovery, CD4≥500	1	187	1	0	60	249
Total	1	224	1	1	77	304

Abbreviations: NRTI, nucleoside reverse transcriptase inhibitor; LPV, lopinavir; r, ritonavir; EFV, efavirenz; RAL, raltegravir; MVC, maraviroc.
Fisher's exact test, p=0.1.

On ART, CD8+ T cell count and CD4/CD8 ratio were lower in suboptimal compared to complete recovery (median 318 vs 621 cells/mm^3, IQR 279-628 vs 490-843, p=0.001 and median 1.05 vs 1.18, IQR 0.47-1.20 vs 0.91-1.48, p=0.047, respectively) (Table 8). Additionally, hemoglobin was higher in suboptimal compared to complete recovery. Platelets were diminished in the suboptimal recovery group.

Table 8. On-ART variables associated with CD4 recovery.

On-ART Variables	Suboptimal Recovery, CD4<350 (*n*=11)	Suboptimal & Intermediate Recovery, CD4<500 (*n*=55)	Complete Recovery, CD4≥500 (*n*=249)	p-value[†]	p-value[‡]
CD8 count	318 (279-628)	460 (359-600)	621 (490-843)	0.001	< 0.001
CD4/CD8 ratio	1.05 (0.47-1.20)	0.95 (0.76-1.16)	1.18 (0.91-1.48)	0.047	< 0.001
WBC count ($\times 10^3$ cells/mm^3)	4.88 (4.68-6.25)	4.99 (4.36-5.74)	6.13 (5.34-7.27)	0.02	< 0.001
Absolute neutrophil count ($\times 10^3$ cells/mm^3)	3.37 (2.52-4.45)	2.92 (2.27-3.57)	3.23 (2.48-4.21)	0.9	0.07
Absolute lymphocyte count ($\times 10^3$ cells/mm^3)	0.99 (0.91-1.40)	1.46 (1.24-1.67)	2.19 (1.85-2.48)	2×10^{-7}	< 0.001
Monocyte count ($\times 10^3$ cells/mm^3)	0.41 (0.36-0.64)	0.41 (0.35-0.51)	0.51 (0.40-0.62)	0.3	0.001
Eosinophil count ($\times 10^3$ cells/mm^3)	0.13 (0.09-0.19)	0.13 (0.09-0.20)	0.13 (0.08-0.24)	1.0	0.7
Hemoglobin (g/dL)	15.5 (14.5-16.4)	15.1 (14.4-15.8)	14.7 (14.0-15.6)	0.02	0.04
Platelet count ($\times 10^3$/mm^3)	223 (185-283)	252 (218-277)	273 (237-313)	0.03	0.002
sCD14 at week 96 (µg/L)	-	1.7 (1.6-1.8)[g]	1.1 (1.0-1.2)[h]	-	0.008
IL-6 at week 96 (pg/mL)	-	0.13 (0.11-0.56)[i]	0.56 (0.14-0.97)[j]	-	0.04
Hyaluronic acid at week 96 (ng/mL)	-	15.1 (13.6-21.9)[g]	11.8 (9.0-18.9)[h]	-	0.4
Intestinal fatty acid binding protein at week 96 (pg/mL)	-	1533 (1212-2058)[g]	2953 (1366-3823)[h]	-	0.3
C-reactive protein (mg/L)	-	0.22 (0.19-0.54)[g]	0.49 (0.19-1.08)[h]	-	0.7
TNF-alpha (pg/mL)	-	0.93 (0.40-16.13)[i]	*1.61 (0.25-4.28)*[j]	-	0.6
D-dimer (ng/mL)	-	190 (179-401)[g]	138 (102-256)[h]	-	0.3

Median (IQR). Measurements were performed at latest study visit unless otherwise indicated.
Abbreviations: CD4, CD4+ T cell; CD8, CD8+ T cell; WBC, white blood cell; sCD14, soluble CD14; IL-6, interleukin-6; TNF-alpha, tumor necrosis factor alpha.
[†]P-value is for suboptimal recovery vs complete recovery.
[‡]P-value is for combined suboptimal & intermediate recovery vs complete recovery.
[a]n=23; [b]n=99; [c]n=22; [d]n=23; [e]n=97; [f]n=98; [g]n=3; [h]n=25; [i]n=8; [j]n=66

After combining suboptimal and intermediate recovery groups, we identified on-ART factors that differed between the combined and complete recovery groups (Table 8). Consistent with the differences between suboptimal and complete recovery, CD8 count, CD4/CD8 ratio, and WBC count were lower in the combined group compared to complete recovery. On-ART hemoglobin was higher in the combined group, and platelet count was lower. Absolute lymphocyte and monocyte counts were lower in the combined group. Serum sCD14 at week 96 was elevated and IL-6 was lower in the combined group.

Optional CSF sample size during ART was too small to assess associations between neuroinflammatory markers and CD4 recovery. Suboptimal and complete recovery did not differ in neuropsychological test performance or psychiatric indices at 96 weeks of ART (Table 9). On ART, markers for co-infection or exposure to hepatitis B virus, hepatitis C virus, and syphilis were not associated with CD4 recovery (Table 9).

Table 9. Additional on-ART variables associated with CD4 recovery.

Post-ART Variables	Suboptimal Recovery, CD4<350 (n=11)	Suboptimal & Intermediate Recovery, CD4<500 (n=55)	Complete Recovery, CD4≥500 (n=249)	p-value[†]	p-value[‡]
Positive anti-HCV, *n* (%)	0/2 (0)	1/15 (7)	1/49 (2)	0.8	0.4
Positive anti-HBs, *n* (%)	2/2 (100)	14/16 (88)	42/50 (84)	0.5	0.7
Positive HBsAg, *n* (%)	0/2 (0)	1/16 (6)	8/49 (16)	0.5	0.3
Reactive VDRL, *n* (%)	0/1 (0)	4/9 (44)	16/40 (40)	0.4	0.8
NPZ-4 score at week 96	0.6 (-0.04 – 0.8) *n=7*	0.5 (-0.04 – 0.9) *n=33*	0.5 (0.03 – 1) *n=183*	0.6	0.6
Total PHQ score at week 96	5 (1 – 8) *n=7*	4 (1 – 8) *n=33*	5 (2 – 8) *n=183*	0.9	0.4

Abbreviations: ART, anti-retroviral therapy; HCV, hepatitis C virus; anti-HBs, hepatitis B surface antibody; HBsAg, hepatitis B surface antigen; VDRL, Venereal Disease Research Laboratory test; NPZ-4, neuropsychological test z-score in four domains (Color Trails 1, Color Trails 2, Trail Making A, Grooved Pegboard); PHQ, Patient Health Questionnaire.

[†]P-value is for suboptimal recovery vs complete recovery.

[‡]P-value is for combined suboptimal & intermediate recovery vs complete recovery.

Independent Effect of Pre- and On-ART Factors on CD4 Recovery

We used a multivariate logistic regression model to adjust for duration of ART, baseline HIV-RNA, and baseline CD4 count (Table 10). After adjustment, participants with baseline CD4/CD8 ratio < 1 were more likely to have poor CD4 recovery < 500 cells/mm^3 (odds ratio [OR] 3.2, 95% confidence interval [CI] 1.4-8.4, p=0.01). Participants with baseline I-FABP < 1000 pg/mL were more likely to have poor CD4 recovery (OR 13.4, 95% CI 1.9-276.4, p=0.02). Those with baseline CSF neopterin > 1600 pg/mL had no different odds for poor CD4 recovery after adjustment (OR 2.4, 95% CI 0.2-57.5, p=0.5). Participants with baseline platelet count < 300,000

per mm^3 were more likely to have poor CD4 recovery after adjustment (OR 3.5, 95% CI 1.2-15.3, p=0.04).

After adjustment, odds of poor CD4 recovery were higher in participants with on-ART CD4/CD8 ratio < 1 (OR 2.4, 95% CI 1.3-4.6, p=0.007), CD8 count < 500 cells/mm^3 (OR 3.1, 95% CI 1.6-6.0, p=0.0005), hemoglobin > 14.8 mg/dL (OR 2.0, 95% CI 1.0-4.0, p=0.04), and platelet count < 300,000 per mm^3 (OR 3.6, 95% CI 1.4-11.1, p=0.01). Participants with sCD14 > 1.8 µg/L had no different odds for poor CD4 recovery (p>0.9). Participants with IL-6 < 0.2 pg/mL were more likely to have poor CD4 recovery after adjustment (OR 6.2, 95% CI 1.2-47.4, p=0.04).

Table 10. Factors Associated with CD4 Recovery < 500 cells/mm^3

	Univariate		Multivariate	
Characteristic	OR (95% CI)	p-value	OR (95% CI)	p-value
Baseline CD4 count		< 0.001	-	-
<350 cells/mm^3	7.7 (2.9-26.4)			
350-499 cells/mm^3	2.5 (0.8-9.3)			
≥500 cells/mm^3	1 (ref)			
ART duration		0.02	-	-
48-119 weeks	1.7 (0.9-3.4)			
120-155 weeks	0.9 (0.4-2.0)			
≥156 weeks	1 (ref)			
Baseline HIV-RNA		0.2	-	-
<10^6 copies/mm^3	1 (ref)			
≥10^6 copies/mm^3	1.7 (1.0-3.1)			
Baseline CD4/CD8 ratio		0.002		0.01
<1	3.4 (1.6-8.6)		3.2 (1.4-8.4)	
≥1	1 (ref)		1 (ref)	
Baseline I-FABP		0.07		0.02
<1000 pg/mL	8.8 (1.4-168.7)		13.4 (1.9-276.4)	
≥1000 pg/mL	1 (ref)		1 (ref)	
Baseline CSF neopterin		0.3		0.5
<1600 pg/mL	1 (ref)		1 (ref)	
≥1600 pg/mL	8.7 (1.5-165.7)		2.4 (0.2-57.5)	
Baseline platelet count		0.01		0.04
<300,000 per mm^3	4.7 (1.6-19.8)		3.5 (1.2-15.3)	
≥300,000 per mm^3	1 (ref)		1 (ref)	
On-ART CD4/CD8 ratio		< 0.001		0.007
<1	2.7 (1.5-5.0)		2.4 (1.3-4.6)	
≥1	1 (ref)		1 (ref)	
On-ART CD8 count		< 0.001		< 0.001
<500 cells/mm^3	3.2 (1.8-5.9)		3.1 (1.6-6.0)	
≥500 cells/mm^3	1 (ref)		1 (ref)	
On-ART hemoglobin		0.02		0.04
<14.8 mg/dL	1 (ref)		1 (ref)	
≥14.8 mg/dL	2.0 (1.1-3.9)		2.0 (1.0-4.0)	
On-ART platelet count		0.002		0.01
<300,000/mm^3	3.7 (1.5-10.9)		3.6 (1.4-11.1)	
≥300,000/mm^3	1 (ref)		1 (ref)	
sCD14 at week 96		0.03	-	1.0
<1.8 µg/L	1 (ref)			
≥1.8 µg/L	12 (0.4-406.0)			
IL-6 at week 96		0.1		0.04
<0.2 pg/mL	6.9 (1.4-50.0)		6.2 (1.2-47.4)	
≥0.2 pg/mL	1 (ref)		1 (ref)	

Multivariate models were adjusted for duration of ART, baseline HIV-RNA, and baseline CD4 count.
Abbreviations: CI, confidence interval; OR, odds ratio.

Results: Project 2

Study Participant Characteristics

During the RV254/SEARCH010 study period, 204 participants with AHI immediately started ART and had paired blood and CSF sampling in at least one visit at baseline, week 24, or week 96. 98% of enrollees were Thai men, the majority men who have sex with men. Median age was 26 years (range 18-60). At enrollment, participants were mostly in Fiebig stage III, with median CD4+ T cell count 386 cells/mm^3 (range 91-1302) and median plasma HIV RNA 5.87 log_{10} copies/mL (range 2.43-7.89). ART was started at a median 19 days post-estimated infection (range 3-49).

At baseline, 126 of 165 participants (76%) had quantifiable CSF HIV RNA with median CSF HIV RNA 3.13 log_{10} copies/mL (range <1.90-6.61). There were no cases where plasma HIV RNA exceeded the paired CSF HIV RNA level. At week 24, of 90 available participants with paired blood and CSF samples, two participants (2%) had quantifiable CSF HIV RNA. At week 96, of 55 available participants with paired blood and CSF samples, one participant (2%) had quantifiable CSF HIV RNA (Figure 5).

At week 24, of the four cases with detectable CSF HIV RNA, one met criteria for CSF escape, with plasma HIV RNA < 50 copies/mL and CSF HIV RNA at 2.50 log_{10} copies/mL. At week 96, the one case of detectable CSF HIV RNA did not meet criteria for CSF escape. All other cases of detectable CSF HIV RNA were associated with plasma virological failure with plasma HIV RNA greater than paired CSF HIV RNA.

Table 11. Characteristics of participants treated in acute HIV infection with paired blood and CSF samples at weeks 0, 24, and 96.

Characteristics	All participants (n=204)	Baseline (n=165)	Week 24 (n=90)	Week 96 (n=55)
Age at enrollment (range)	26 (18-60)	26 (18-60)	27 (18-60)	28 (18-60)
Male, n (%)	199 (98)	160 (97)	86 (96)	53 (96)
Risk behavior, n (%)				
WSM	5 (2)	5 (3)	4 (4)	2 (4)
MSW	7 (3)	6 (4)	1 (1)	2 (4)
MSM	192 (94)	154 (93)	85 (94)	51 (93)
Fiebig stage at enrollment, n (%)	33 (16)	27 (16)	13 (14)	8 (15)
Stage I	47 (23)	37 (22)	20 (22)	11 (20)
Stage II	96 (47)	76 (46)	42 (47)	29 (53)
Stage III	19 (9)	17 (10)	10 (11)	3 (5)
Stage IV	8 (4)	7 (4)	4 (4)	3 (5)
Stage V	1 (0)	1 (1)	1 (1)	1 (2)
Stage VI				
Time to ART initiation (days), median (range)	19 (3-49)	18 (3-49)	19 (7-49)	19 (9-42)
CD4+ T-cells, cells/mm^3 (range)	386 (91-1302)[†]	389 (101-1302)	613 (291-1464)	639 (320-1357)
CD8+ T-cells, cells/mm^3 (range)	517 (81-4556)[†]	515 (102-4556)	575 (178-1352)	628 (260-1575)
Plasma HIV RNA, log_{10} copies/mL, (range)	5.87 (2.43-7.89)[†]	5.83 (2.43-7.89)	<1.30 (<1.30-5.44)	<1.30 (<1.30-4.42)
Proportion of plasma HIV RNA < 50 copies/mL, n (%)	0 (0)[†]	0 (0)	86 (96)	53 (96)
CSF HIV RNA, log_{10} copies/mL, (range)	-	3.13 (<1.90-6.61)	<1.90 (<1.90-3.84)	<1.90 (<1.90-3.14)
Proportion of CSF HIV RNA < 80 copies/mL, n (%)	-	39 (24)	88 (98)	54 (98)
CSF viral escape, n (%)	-	-	1 (1)	0 (0)

Abbreviations: HIV, human immunodeficiency virus; WSM, women who have sex with men; MSW, men who have sex with women; MSM, men who have sex with men; CSF, cerebrospinal fluid.
[†]At time of enrollment.

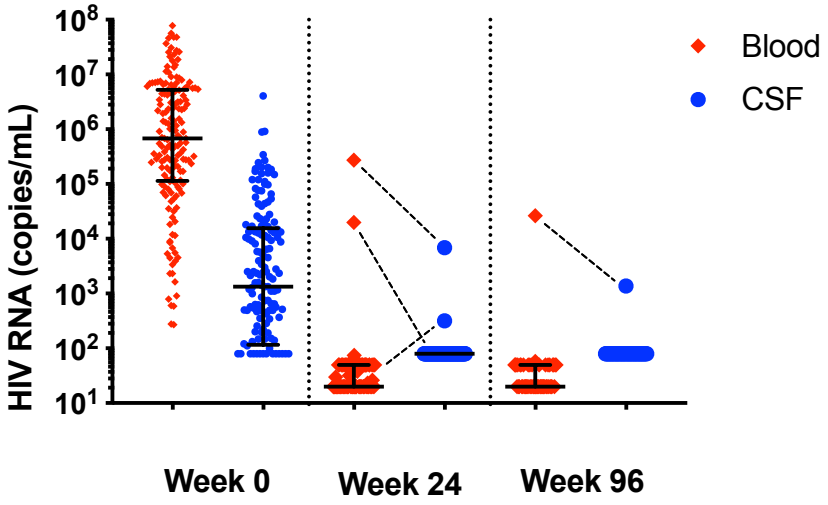

Figure 5. Paired blood and CSF HIV RNA at weeks 0, 24, and 96. Medians and interquartile ranges are indicated by black bars. Dashed lines represent paired blood and CSF HIV RNA from participants who had either blood or CSF HIV RNA greater than the respective limit of quantitation at week 24 or 96.

Clinical Course of CSF Escape During Treatment in Acute HIV Infection

Of 204 eligible participants, one participant at week 24 post-treatment initiation met criteria for CSF escape. This is a 23-year-old male university student who identifies as bisexual, who initially presented with acute retroviral syndrome. His past medical history includes migraines and dengue fever. Three months before his presentation with acute HIV infection, he was treated for chlamydia and gonorrhea infection. He denied alcohol, tobacco, and other drug use. He was diagnosed with acute HIV infection in Fiebig stage IV at estimated 21 days post infection and was immediately started on efavirenz, tenofovir, and emtricitabine. Plasma HIV RNA and CD4+ T cell count responded rapidly to initiation of ART (Figure 6).

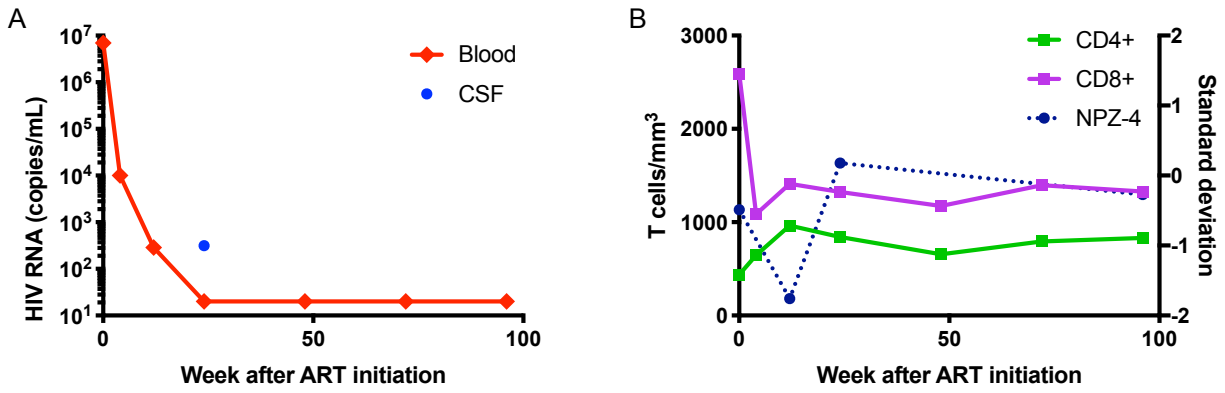

Figure 6. Clinical measurements and neuropsychological testing for the case of CSF escape in AHI. A) Longitudinal blood and CSF HIV RNA for the participant with CSF escape identified at week 24. B) CD4+ and CD8+ T cell counts (left axis) and NPZ-4 score (right axis) for the participant during treatment for acute HIV infection.

This participant had no study lumbar punctures performed other than that at week 24. CSF white blood cell count was 4 cells/mm^3, protein 30 mg/dL, and glucose 62 mg/dL. The participant did not endorse any neurological symptoms at week 24. An MRI was performed at this time, which showed a small nonspecific hyperintense focus in the right high frontal white matter. The participant also underwent neuropsychological testing at weeks 0, 12, 24, and 96 (Figure 6). At week 12, the participant scored an NPZ-4 score of 1.76 standard deviations below the reference population. At all other time points, NPZ-4 scores were unremarkable.

After 76 weeks on ART, this participant was transitioned to dolutegravir, abacavir, and lamivudine per the study protocol, which was unrelated to the CSF findings from week 24.

At time of treatment initiation, the participant had a nonreactive VDRL. No VDRL was performed at week 24. At 96 weeks on ART, the participant had a positive VDRL with a titer of 1:128.

Discussion

Consistent with many other studies emerging from the RV254/SEARCH010 cohort of participants with AHI, prompt treatment initiation in the earliest stages of HIV infection appears to confer unique immunologic and virological benefits that mitigate the immune dysfunction and reservoir establishment seen in treated chronic HIV infection. Not only does early treatment in AHI reduce inflammation [56], it improves immunological reconstitution as measured by CD4+ T cell recovery. Early ART may also reduce the degree of HIV persistence specifically in the CNS compartment, given the very low rate of CSF escape observed in tandem with a prior report of normalization of neuroinflammation [58].

CD4+ T Cell Recovery in Acute HIV Infection

We found that suboptimal CD4 recovery occurs in a small subset of individuals despite treatment in the earliest stages of AHI. Whereas 70-85% of individuals with chronic HIV infection had CD4 recovery to >350 cells/mm^3 after ART, we observed a larger proportion (96.4%) in AHI [70]. A larger proportion of individuals were categorized as intermediate responders (CD4 count 350-499 cells/mm^3) as compared to suboptimal responders (<350 cells/mm^3). Prior work has identified determinants of CD4 recovery in early seroconversion, though in the setting of delayed ART initiation [76, 96]. To our knowledge, our study is the first to examine determinants of CD4 recovery with immediate ART initiation in the earliest stages of HIV infection. Furthermore, few studies have identified determinants of CD4 recovery in chronic HIV infection in an Asian population, and none in early seroconversion or primary HIV infection to our knowledge [97-99].

The degree of lymphatic tissue fibrosis has been shown to correlate with CD4 recovery [19, 100]. As an indicator of a systemic profibrotic state, hyaluronic acid was lower in treated AHI compared to treated chronic HIV infection, yet both remain elevated compared to HIV-uninfected

controls [56]. Taken together, immediate ART initiation in AHI may limit the extent of lymphatic tissue fibrosis compared to that seen in chronic HIV infection, which may explain the lower proportion of suboptimal CD4 recovery in AHI.

When examining the suboptimal and intermediate recovery groups throughout the course of treatment (Figure 2), CD4 count trajectories are similar. Additionally, though not apparent in the aggregate analysis in Figure 2, some individuals with intermediate recovery appear to have transient decreases in CD4 count below 350 cells/mm^3, and may in fact sustain an overall CD4 response around or above the threshold of 350 cells/mm^3. Thus, differences between the combined suboptimal and intermediate recovery group versus complete recovery are more stark and reveal a state of perturbed immunological recovery as compared to differences between suboptimal versus intermediate recovery.

Poor CD4 recovery (< 500 cells/mm^3) is associated with nadir CD4 count, and thus increases from substantially low nadir may not be captured using an absolute threshold as a definition of CD4 recovery. However, our slope analyses show that individuals with complete recovery have a higher recovery rate over a fixed time interval of 48 weeks, indicating greater absolute and relative increases in CD4 count. Other definitions of CD4 recovery have accounted for low nadir, such as an increase of CD4 count ≥ 30% from baseline [101]. We used prior definitions of CD4 recovery as absolute thresholds ≥ 350 or ≥ 500 cells/mm^3 to allow for direct comparison to results in chronic and primary HIV infection. Furthermore, absolute CD4+ T cell count during treated HIV infection is a critical clinical measure for risk of opportunistic infections.

Consistent with a prior description of our cohort, individuals diagnosed and started on ART at Fiebig stage I have a higher baseline CD4 count compared to stages II-IV, with normalization of CD4 count after at least 48 weeks of treatment [53]. Individuals starting on ART at Fiebig stage

I have a lower absolute CD4 increase compared to other stages, suggesting that CD4 decline to nadir occurs at later Fiebig stages but is reversible with immediate ART initiation. Strategically timing earlier ART initiation during an interval of CD4 recovery has been associated with better long-term CD4 recovery [71, 72]. Conversely, ART initiation during an interval of CD4 decline is associated with poor CD4 recovery [102], suggesting that strategic timing of early ART must consider not only nadir CD4 count but also the dynamics of pre-ART CD4 recovery. However, initiation of ART in Fiebig stages II-IV did not affect on-ART CD4 count, suggesting that ART initiation in AHI does not need to be strategically timed and should be prompt.

Interestingly, our study showed that lower CD8 count during treatment, but not at baseline, was associated with poor CD4 recovery, consistent with a previous report [103]. This may indicate suppression of thymic output that has been observed to occur in the setting of poor CD4 recovery [104]. However, another study found that higher baseline CD4 count and lower CD8 count were independently associated with improved CD4 recovery [105]. The CD8 count may thus be interpreted as an indicator of thymic output as well as of immune activation.

Poor CD4 recovery was independently associated with lower CD4/CD8 ratio both at baseline and during treatment in AHI. Reduced CD4/CD8 ratio has been found to be a marker for immune dysregulation and activation in the setting of HIV infection that has been associated with an increased risk for non-AIDS related morbidity and mortality, including age-related inflammation and cardiovascular disease [28, 106, 107]. Despite initiating ART at the earliest possible stages of infection, low pre-ART CD4/CD8 ratio remained low on treatment and is a predictor for poor CD4 recovery. For our group of poor responders treated during AHI, CD4/CD8 ratio at baseline was higher than in participants treated during chronic HIV infection, reflecting less immune dysregulation at the earliest stages of infection [101, 105, 107]. Initiating ART during

primary versus chronic HIV infection leads to a higher frequency of CD4/CD8 ratio > 1, with higher CD4/CD8 ratio at baseline associated with a lower risk of poor CD4 recovery [72]. An overall faster time to treatment initiation results in improved CD4/CD8 ratio [108]. Interestingly, despite the rapid initiation of ART in early AHI, other markers of chronic inflammation persist in the RV254/SEARCH010 cohort [56].

We analyzed markers for microbial translocation and systemic inflammation, finding elevated sCD14 in suboptimal CD4 responders after treatment. However, sCD14 did not appear to be independently associated with CD4 recovery after controlling for duration of ART, baseline CD4 count, and baseline HIV-RNA. Some prior studies show increased sCD14, its ligand lipopolysaccharide (LPS) [109], bacterial 16S ribosomal DNA [110], or neutrophil infiltration [111] in poor compared to complete CD4 recovery, while others find no differences [101]. No differences were detected in hyaluronic acid or in systemic inflammatory markers CRP, TNF-alpha, and D-dimer [101]. Surprisingly, in our study IL-6 was elevated in complete recovery. Elevated IL-6 has been associated with increased risk of HIV-associated opportunistic disease [12]. Plasma I-FABP at baseline was higher in complete recovery, suggesting increased enterocyte turnover, contrary to prior findings [111]. However, in our multivariate model, we report an odds ratio for I-FABP with a large margin of error, suggesting this may be a spurious association.

Platelet counts were lower in poor compared to complete recovery both at baseline and after ART, consistent with a prior study [112]. HIV-associated thrombocytopenia has several possible mechanisms, including immune-mediated thrombocytopenia via immune complexes composed of antibodies cross-reactive to HIV and platelet antigens, decreased platelet survival, decreased production, and infection of megakaryocytes [113]. With concurrent thrombocytopenia, the relative monocytopenia observed in poor recovery may be an indicator of suppressed

hematopoiesis. However, there was no association between neutrophil count and CD4 recovery. Given that both T lymphocytes and monocytes are natural hosts for HIV infection and replication, it cannot be excluded that poor CD4 recovery may reflect persistent HIV infection and subclinical replication in both T lymphocytes and monocytes that suppresses their cell counts. A higher hemoglobin was observed in suboptimal versus complete recovery post-ART, contrary to other reports [114].

Poor CD4 recovery trended towards association with neuroinflammation at baseline, as measured by CSF neopterin. However, when controlling for confounders, there was no independent association between CSF neopterin and CD4 recovery. To our knowledge, this is the first study assessing the association between neuroinflammation and impaired CD4 recovery, though other CNS biomarkers have been associated with CD4 recovery [115]. Elevated CSF neopterin has been associated with CSF HIV-RNA and neurological complications of HIV infection [116]. Elevated plasma neopterin has been associated with poor CD4 recovery in treated HIV infection [117]. Suboptimal CD4 recovery may reflect not just a systemic inflammatory state but also a localized neuroinflammatory state, which may have implications for neurological outcomes in poor responders. Further work is needed to investigate associations between CD4 recovery and localized inflammation.

We did not find an association between CD4 recovery and coinfection with syphilis, hepatitis B virus, or hepatitis C virus. Our study may not be sufficiently powered to detect an association between CD4 recovery and HCV coinfection, given the low number of positive anti-HCV samples. Some studies have not shown an association between HBV coinfection and CD4 recovery [118], while others have [119]. Likewise, HCV coinfection has been associated with poor CD4 recovery on ART in some studies [120] but not others [121].

A subset of participants was assessed for CD4+ and CD8+ T cell activation by flow cytometry of CD38+ T cell subsets. However, sample sizes were too low to assess for an association between immunological response and CD38+ expression. CD38+ expression in CD8+ T cells has been associated with a faster rate of CD4+ T cell decline in untreated individuals with HIV [122]. Additionally, higher expression of immune checkpoint receptor and exhaustion marker PD-1 on both CD4+ and CD8+ T cells has been associated with lower CD4 count at baseline and after treatment [24, 123]. This was not assessed in this study of acute HIV infection and is a target for future investigation. Further work is also needed to assess whether expression of IL-7 receptor, regulatory T cell phenotype, and inflammasome upregulation is associated with immunological response in acute HIV infection, as described in treated chronic HIV infection [124-126].

Our study has several limitations. On-ART factors were measured at the week of the latest available study visit, which was lower in the combined suboptimal and intermediate recovery group compared to complete recovery. Thus, the overall longer treatment duration in the complete recovery group possibly confounds the finding of improved CD4+ T cell gains. However, our slope analysis examines rate of recovery over a fixed time interval, showing that the complete recovery group exhibits both higher and faster CD4 recovery. Furthermore, we identified pre- and on-ART factors independently associated with poor CD4 recovery < 500 cells/mm^3 when adjusting for duration of ART, as well as baseline CD4 count and HIV-RNA. Our study did not have sufficient numbers of lumbar punctures after 48 weeks of ART and thus cannot assess the degree of neuroinflammation after ART in poor responders. The participants in this study are young and may have a lower baseline inflammatory state due to young age. Finally, the HIV-uninfected Thai population have a lower CD4+ T cell count reference range compared to predominantly white or European reference populations [127].

CSF Escape in Acute HIV Infection

We found that after immediate ART initiation in acute HIV infection, there is a very low incidence of CSF escape of 1% after 24 weeks and 0% after 96 weeks. Moreover, the singular case of CSF escape identified in our study population was at a low CSF viral load of 316 copies/mL and was not associated with any neurological findings or dysfunction. Prior studies have identified an incidence rate of up to 10% for CSF escape in the setting of treated chronic HIV infection [81], though a follow-up study by the same authors suggested that many of these cases of detectable and quantifiable CSF HIV RNA are likely better described as CSF viral blips since the CSF viral loads mostly are not repeatedly elevated on longitudinal measurements [91]. This episodic CSF viral blip may be mechanistically due to cell trafficking into the CNS, resulting in either HIV release or transient viral replication [41]. Thus, it remains fairly likely that this case of asymptomatic quantifiable CSF HIV RNA paired with undetectable plasma HIV RNA can be considered a CSF viral blip. This may be further corroborated by the lack of CSF pleocytosis. Of note, even with CSF viral blips, there may be an association between low-level CSF HIV RNA and increased neuroinflammation as measured by elevated CSF neopterin levels [91].

An additional consideration is the possibility that this singular case represents secondary CSF escape, given the nonreactive VDRL at baseline and the reactive VDRL at week 96. Although we cannot know when this participant was infected with syphilis, an infection at week 24 cannot be excluded as a possible contributor for CSF escape secondary to neurosyphilis. In secondary CSF escape, elevated CSF HIV RNA is thought to be due to a superimposed infection or inflammatory process causing increased immune cell trafficking into the CNS, which results in localized viral replication detectable in CSF [83]. Again, the lack of CSF pleocytosis speaks against this possibility.

Prior characterizations of this unique AHI cohort have described baseline characteristics of early CNS infection. At baseline, most of our participants had detectable HIV RNA in the CSF, and none had CSF HIV RNA greater than that in plasma [55]. AHI individuals with a higher CSF HIV RNA relative to plasma HIV RNA have a greater degree of systemic inflammation as measured by CD4/CD8 ratio and a greater degree of neuroinflammation as measured by CSF neopterin, soluble CD163, IL-6, and sCD14 [128]. From our results, CSF HIV RNA is almost always suppressed to undetectable levels when initiating ART in AHI with only one case of CSF escape. Further work is needed to characterize whether CSF escape in AHI is associated with neurological or systemic inflammation. Additionally, since the incidence of CSF escape has been associated with longer time on ART in chronic infection [81], long-term follow-up of this cohort is necessary to verify that CSF escape remains rare in treated AHI. The rarity of CSF escape in treated AHI may point to a benefit in very early treatment in terms of reducing CNS persistence and viral reservoirs, though further investigation is needed [40].

This study has several limitations. This singular case of CSF escape was identified in a participant who to date has undergone only one study lumbar puncture, with no CSF samples prior to ART initiation nor at any other study visit. Thus, it is impossible to determine whether the quantifiable CSF HIV RNA at week 24 has been sustained and to distinguish between persistent CSF escape and a CSF viral blip. The level of 316 copies/mL is too low to perform resistance testing using available assays, but further testing could be useful in determining whether selection for drug resistance is driving this case of CSF escape and determining any degree of compartmentalization between blood and the CNS. Further testing could also assess the virological phenotype of this CSF escape population, namely its propensity to infect macrophages (M-tropic) versus CD4+ T cells (T-tropic), which may provide additional clues about whether the population

is produced by CNS reservoirs versus transient immune cell trafficking [41]. We did not measure any other markers in CSF, such as neopterin and sCD14, in this participant. Additionally, with only one case of CSF escape, we cannot draw any statistically significant associations with markers of neuroinflammation or systemic inflammation. In the cohort at large, markers of immune activation in CSF but not plasma normalize after 96 weeks of ART [58]. Finally, due to sample dilution for volume requirements for CSF, the lower limit of quantification of HIV RNA differs between CSF and plasma, and thus our study cannot capture very low-level CSF HIV RNA < 80 copies/mL that may represent cases of CSF escape or CSF viral blips. Recent work has demonstrated that even low-level CSF HIV RNA < 20 copies/mL associates with decreased blood-brain barrier integrity and executive function [90].

Conclusions

In conclusion, suboptimal CD4 recovery following initiation of ART during AHI is low (< 5%) compared to chronic HIV infection and is characterized by a low CD4 count at baseline and persistent low CD8 count during treatment. Poor recovery is also associated with systemic inflammation during treatment. CSF escape is rare (1%) following initiation of ART during AHI. Future work should be directed at determining whether ART should be initiated as early as possible in AHI versus strategic timing of ART within AHI to coincide with spontaneous CD4 recovery in order to optimize long-term CD4 recovery [71]. Future work should also corroborate the low incidence of CSF escape over longer follow-up > 96 weeks and investigate the virological drivers behind CSF escape in treated AHI.

List of Abbreviations

AHI, acute HIV infection; anti-HBs, hepatitis B surface antibody; ART, antiretroviral therapy; CBC, complete blood count; CD4, CD4+ T cell; CD8, CD8+ T cell; CI, confidence interval; CNS, central nervous system; CR, complete recovery; CSF, cerebrospinal fluid; ELISA, enzyme-linked immunosorbent assay; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; HIV, human immunodeficiency virus; I-FABP, intestinal fatty acid binding protein; IL-6, interleukin-6; IQR, interquartile range; IR, intermediate recovery; MSM, men who have sex with men; MSW, men who have sex with women; NPZ-4, neuropsychological test z-score in four domains (Color Trails 1, Color Trails 2, Trail Making A, Grooved Pegboard); OR, odds ratio; PHQ, Patient Health Questionnaire; TNF-alpha, tumor necrosis factor alpha, sCD14, soluble CD14; SR, suboptimal recovery; VDRL, Venereal Disease Research Laboratory test; WBC, white blood cell; WSM, women who have sex with men.

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