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Publication date
2021

Document Version
Final published version

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ISBN 978-94-6421-470-3

The research described in this thesis was supported by the National Institutes of Health (R01MH095613, R01NS084911, R01MH106466) as well as a cooperative agreement (W81XWH-18-2-0040) between the Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc., and the U.S. Department of Defense (DOD), with added support from the U.S. National Institute of Allergy and Infectious Disease and the National Institute of Mental Health.

Characterizing the Neurological Impact of Acute HIV Infection and Its Outcomes After Immediate Initiation of Antiretroviral Therapy

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Universiteit van Amsterdam
op gezag van de Rector Magnificus
prof. dr. ir. K.I.J. Maex
ten overstaan van een door het College voor Promoties ingestelde commissie,
in het openbaar te verdedigen
op dinsdag 12 oktober 2021, te 15.00 uur

door Yin Cheung Phillip Chan
geboren te Hong Kong

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CONTENTS

Chapter 1:
General Introduction

The development and distribution of antiretroviral therapy (ART) has converted Human Immunodeficiency Virus type 1 (HIV-1) infection from an incurable disease to a manageable chronic illness. The 95-95-95 mission from the Joint United Nations Programme on HIV/AIDS (UNAIDS) aims to achieve not only early HIV-1 diagnosis and prompt ART initiation but also HIV-1 viral suppression among people living with HIV (PLWH). To date, PLWH on suppressive ART enjoy a comparable life expectancy to HIV-negative populations [1, 2]. However, ART can only suppress HIV-1 replication but not eradicate the virus because of the persistent viral reservoir that harbours integrated, replication-competent provirus within host cellular DNA [3]. PLWH therefore have to persevere with lifelong ART, without which plasma HIV-1 RNA will rebound within weeks [4]. Despite suppressive ART, PLWH still suffer from immune dysfunction that may account for their higher risk of non-communicable diseases than the HIV-negative populations [2, 5, 6], leading to less years of morbidity-free survival [1].

HIV-1 is a multi-system disease that involves the central nervous system (CNS). Apart from the risk of developing CNS opportunistic infections (OI) due to immunodeficiency, many untreated PLWH eventually develop cognitive and motor impairment known as AIDS dementia complex (ADC) [7, 8], now re-labelled as HIV-associated dementia (HAD). HAD is characterized by a combination of cognitive, motor and behavioral abnormalities. The risk of HAD increases as immunodeficiency worsens [8]. Similar to incomplete systemic immune recovery, the benefit of ART is incomplete in the CNS. The neurocognitive deficits in HAD are not completely reversed by suppressive ART. Further, neurocognitive deficits due to HIV-1 infection, now collectively labelled as HIV-associated neurocognitive disorder (HAND) in ART era, remains prevalent. Although HAD, the most severe form of HAND, has become rare (2-4%), milder forms of HAND remain common (20-30%) among treated PLWH, suggesting the persistence of HAND [9]. Although cognitive decline in HAND appears to occur years after HIV-1 transmission, contemporary evidence suggests that HIV-1 neuroinvasion takes place during acute infection. Intrathecal and cerebral inflammation and neurologic (including cognitive) dysfunction are readily detected in infected individuals during acute HIV infection (AHI) [10, 11]. The overall picture suggests that the neuropathogenesis of HIV-1 begins at the time of acute infection. Biological events at AHI and their impact on the trajectory of CNS outcomes have not been a major focus of systematic research studies until recently. Further, it is unclear whether initiating ART during AHI might fully resolve CNS inflammation and reverse the risk for future cognitive dysfunction. A crucial issue is whether PLWH who initiate ART during AHI maintain healthy cognitive function without the eventual development of HAND.

This PhD thesis consists of four sections. **Section 1 (Chapter 1 & 2)** describes the general outlook of neurocognitive issues among PLWH who initiate ART during chronic HIV-1 infection. **Sections 2 (Chapter 3-6) and 3 (Chapter 7-9)** include a collection of CNS-focused reports based on the RV254 acute HIV-1 infection cohort conducted in Bangkok, Thailand. **Section 4 (Chapter 10 & 11)** describes how the RV254 cohort contributes to CNS-relevant HIV-1 cure research through its structured design in collecting CNS data.

Chapter 1 is a study conducted in a sample of PLWH initially presenting for care during the chronic phase of HIV-1 infection in Hong Kong. These ART-naïve clinic attendees underwent cognitive screening prior to and 6 months after ART. It revealed high frequencies of impaired cognitive test performance among study participants before ART. Further, poor screening test performance persisted despite ART introduction and plasma HIV-1 suppression at follow-up, suggesting an incomplete reversal of cognitive impairment after ART. **Chapter 2** reviews the epidemiology and causes of the persistence of HIV-related cognitive impairment in the ART era. It highlights the need to understand neuropathogenesis at the early phase of infection.

Examining Acute HIV-1 Infection in the RV254 Study, Bangkok, Thailand

Thailand has among the highest HIV-1 prevalence in Asia and the Pacific, accounting for 9% of the region's total population of PLWH. With efforts from the government, community and non-government organizations, Thailand has achieved enormous progress in controlling the HIV-1 epidemic. Although the figures of official testing, treatment and viral suppression to achieve UNAIDS' 90-90-90 targets are incomplete, latest report estimates that around 80% of PLWH in Thailand are on ART (2019). Among them, more than 95% have achieved viral suppression. Mother-to-child transmission has been eliminated in Thailand, with a transmission rate below 2%. In 2018, Thailand began to scale up pre-exposure prophylaxis (PrEP), making it nationally available for people at high risk of HIV-1 transmission. Apart from the achievements in limiting HIV-1 transmission, Thailand has pioneered HIV research in the region. The RV254 acute HIV-1 infection cohort conducted at the South East Asia Research Collaboration on HIV (SEARCH), Thai Red Cross AIDS Research Centre (TRCARC) is amongst the largest AHI cohorts in the world. It has enrolled more than 600 participants since 2009. Through the enrollment of individuals with AHI, the RV254 study

SEARCH Office & Thai Red Cross AIDS Research Centre

offers a unique research opportunity to investigate the interactions between HIV-1 and the CNS starting within days after HIV-1 transmission. The study characterizes the participants' neurological profiles through neurological examination, screening for mood disorders, neurocognitive assessment and optional procedures including lumbar puncture (LP) and magnetic resonance imaging (MRI) of the brain. These investigations are conducted immediately after the AHI diagnosis before ART is initiated and are repeated regularly during longitudinal follow-up. As almost all RV254 participants initiate ART within days after the AHI diagnosis, the study also informs the clinical benefits of treating HIV-1 during acute infection.

Characterizing Neurological Parameters during Acute HIV Infection

Section 2 of this thesis focuses on data generated from the baseline study interval (AHI) of the RV254 cohort. **Chapter 3-5** are studies based on LP and CSF analyses in RV254. RV254 participants undergo optional LP at baseline and regularly every 48 weeks after treatment initiation. Similar to all CSF studies, one of the biggest concerns of LP is the development of post-LP headache (PLPH). **Chapter 3** retrospectively examines the incidence of PLPH in RV254,focusing on its relationship with the design of LP needles (traditional cutting-edge needle vs. atraumatic needle) and the volume of CSF collection (10ml vs. 20ml).

In the initial CSF study of RV254 [11], HIV-1 RNA was detected in CSF as early as at Fiebig stage I or 8 days after estimated transmission. **Chapter 4** is the follow-up CSF report which focuses on the relationship between HIV-1 neuroinvasion and the corresponding blood parameters.

The optional LPs performed in RV254 occasionally reveal unexpected CSF abnormalities. Recently, there has been a global resurgence of syphilis infection, which is particularly common among PLWH. Thailand is no exception and the incidence of syphilis increased from 2-3 cases per 100,000 in 2009 to 12 cases per 100,000 in 2018. It is indeed relatively common to uncover untreated syphilis at AHI during RV254 enrollment. **Chapter 5** reports a RV254 participant who had concomitant asymptomatic neurosyphilis during AHI. Syphilis is known to worsen HIV-1 infection. Incident syphilis is linked to elevated plasma HIV-1 RNA and reduced CD4+ T-cell level in untreated PLWH [12-14]. **Chapter 6** investigates the immunological effect of untreated syphilis on HIV-1 during acute infection.

Characterizing Longitudinal Neurological Outcomes in RV254

Following an immediate initiation of ART during AHI, RV254 participants are longitudinally followed at the SEARCH clinic. With low rates of treatment failure (1%) and drop-out (6.8%), RV254 provides a unique opportunity to comprehensively examine

the immunological and neurological outcomes of treating HIV-1 at the earliest possible stage of infection, which has been previously under-explored. One of the initial reports of RV254 described persistent cognitive dysfunction among a quarter of RV254 participants after 6 months of ART [10]. **Chapter 7** follows up on the stability of cognitive test performance in a larger group of RV254 participants who sustained stable plasma viral suppression for 6 years after ART initiation during AHI.

Several factors may negatively impact the long-term stability of cognitive function in PLWH on ART. One of them is persistent cerebral immune activation, evidenced by the elevation of CSF immune activation markers [15, 16] and heightened signals of microglial activation in positron emission tomography (PET) studies [17, 18]. Another factor that closely associates with cerebral immune activation is the phenomenon of CSF HIV-1 escape. CSF HIV-1 escape, defined by a measurable level of CSF HIV-1 RNA in the presence of plasma viral suppression, occurs in up to 10% of PLWH on stable ART [19]. CSF HIV-1 escape is mostly asymptomatic. However, some individuals with CSF viral escape develop neurological complications including headache, cognitive complaints, epilepsy and an overt encephalitis syndrome [20-23]. Further, CSF inflammatory markers are elevated in individuals with asymptomatic escape [24, 25], suggesting its potential role to fuel persistent cerebral inflammation. The incidence of CSF HIV-1 escape in the RV254 cohort is reported in **Chapter 8**, suggesting a benetfit of treatment in acute HIV-1 infection on this clinical and biological outcome.

Apart from cerebral immune activation, ART-related neurotoxicity has long been considered as a contributor to HIV-related neurocognitive impairment in the ART era. Efavirenz (EFV), a non-nucleoside reverse transcriptase inhibitor (NNRTI), was previously used as a key component in the first-line ART for treatment-naïve PLWH. However, multiple studies have reported the adverse neurological effects of EFV [26]. Starting in 2017, the recommendation for EFV as first line therapy for HIV-1 treatment was replaced by Dolutegravir (DTG), a new integrase inhibitor. Although DTG has a superior safety profile as compared to EFV, early post-marketing reports cast concerns about its adverse effects on sleep and mood [27, 28]. Based on the Thai and international guidelines, RV254 participants received EFV-based ART since 2009. Upon DTG availability at the SEARCH clinic in 2017, existing RV254 participants were systematically switched to DTG-based regimens. **Chapter 9** examines mood and cognitive performance in the cohort before and after this switch.

RV254 in Connection with HIV-1 Cure and Analytical Treatment Interruption (ATI) Studies

The establishment of the HIV-1 viral reservoir during early infection marks one of the major obstacles to cure the disease. To date, long-lived CD4+ memory T-cells are believed to be the major source of the HIV-1 reservoir. Interruption of ART invariably

leads to reactivation of replication-competent virus harbored in these cells which fuel a new round of cell infection including the CNS. Given the highly-challenging requirement for lifelong ART adherence and the incomplete restoration of immunological function by ART, HIV-1 cure remains essential for PLWH. However, the deleterious CNS complications after ART interruption reported in prior human and animal studies [29-32] raise concern for the design of HIV-1 cure studies, as ATI is an inevitable component of interventional studies that aim to assess impact on the HIV reservoir and the goal of HIV remission. The potential impact of the CNS on HIV-1 eradication is reviewed in **Chapter 10** of **section 4**.

By the end of 2020, SEARCH has completed four ATI studies [32-35] among which three involved interventional agents for HIV-1 cure. All participants were co-enrolled from the RV254 cohort. Apart from close monitoring of plasma HIV-1 RNA level, a subset of participants underwent optional LP, brain MRI, and neurological assessment prior to and after ATI upon the rebound of plasma HIV-1 RNA. The neurological data collected in these studies were analyzed and presented in **Chapter 11**.

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SECTION 1
Current Understanding of Neurocognitive Impact in Chronic HIV Infection

Chapter 2:
Cognitive screening in treatment-naïve HIV-infected individuals in Hong Kong - a single center study

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doi: 10.1186/s12879-019-3784-y

ABSTRACT

Background: HIV-associated neurocognitive disorder (HAND) remains prevalent in the era of combination antiretroviral therapy (cART). The prevalence of HAND in Hong Kong is not known.

Methods: Between 2013 and 2015, 98 treatment-naïve HIV-1-infected individuals were referred to and screened by the AIDS Clinical Service, Queen Elizabeth Hospital with (1) the International HIV Dementia Scale (IHDS), a screening tool that targets moderate to severe HAND, (2) the Montreal Cognitive Assessment (MoCA), a frequently used cognitive screening test and (3) the Patient Health Questionnare-9 (PHQ-9), a 9-item questionnaire that evaluates depression symptoms. Within the study period, 57 of them completed the second set of IHDS and MoCA at 6 months after baseline assessment.

Results: Most participants were male (94%), with a median age of 31 years. At baseline, 38 (39%) and 25 (26%) of them scored below the IHDS (≤10) and MoCA (25/26) cut-offs respectively. Poor IHDS performers also scored lower on MoCA ($p=0.039$) but the correlation between IHDS and MoCA performance was weak ($r=0.29$, $p=0.004$). Up to a third of poor IHDS performers (13/38) showed moderate depression (PHQ-9>9). In the multivariable analysis, a lower education level ($p=0.088$), a history of prior psychiatric illness ($p=0.091$) and the presence of moderate depression ($p=0.079$) tended to be significantly associated with poor IHDS performance.

At follow-up, 54 out of 57 were on cART, of which 46 (85%) had achieved viral suppression. Their blood CD4+ T-lymphocytes and IHDS scores were higher at follow-up compared to baseline values (both $p<0.001$) but their MoCA performance was similar at both assessments. Of note, 17 participants in this subgroup scored below the IHDS cut-off at both assessments.

Conclusions: Poor IHDS performance, and likely cognitive impairment, was frequently observed in treatment-naïve HIV-infected individuals in our locality. A considerable proportion continued to score below the IHDS cut-off at 6 months after cART. Depression was frequently observed in this vulnerable population and was associated with poor IHDS performance.

BACKGROUND

The introduction of combination antiretroviral therapy (cART) has changed HIV-1 infection from a life-threatening disease to a manageable chronic condition, and life expectancy of people living with HIV (PLWH) is approaching that of non-infected individuals [1]. However, the impact of cART is less significant in the central nervous system (CNS). While CNS opportunistic infections are rare, cognitive impairment remains common in PLWH on cART [2-4]. It is estimated that the frequency of HIV associated dementia (HAD), the most severe form of HIV associated cognitive disorder (HAND), has dropped from 20% to 2% among PLWH in the cART era. However, the rate of milder forms of HAND still ranges from 15% to 55% in different cohorts [2-4].

Apart from the impact on work performance and quality of life, cognitive impairment in PLWH could affect drug adherence and hence virological control [5]. In Hong Kong, the population of PLWH exceeded 8000 in 2016 with around 700 new cases reported each year and the prevalence of cognitive impairment in this vulnerable population is largely unknown. In a multi-center cross-sectional study of 10 Asia-Pacific regions in 2008, cognitive impairment was reported in 12% of 647 clinic-followed PLWH, but this percentage was 23% among the 61 Hong Kong participants [6]. Estimating the local prevalence of cognitive impairment is essential for health funding, resource allocation and patient support.

This study aimed to estimate the frequency of possible cognitive impairment in cART-naïve HIV-infected individuals referred to HIV-clinic service in Hong Kong. We compared cognitive screening test performance before and after cART and identified potential risk factors that were associated with poor test performance.

METHODS

Study Design and Participants Recruitment

Participants included all consecutive treatment-naïve referrals to the AIDS Clinical Service, Queen Elizabeth Hospital, Hong Kong, between October 1, 2013 and October 31, 2015. Only ethnic Chinese were included to ensure standardization of population and the cognitive screening procedures. Individuals with a known history of major medical or neurological disorders were excluded. At enrollment, the participants underwent clinical assessment, cognitive and depression screening, and laboratory tests. They were reassessed approximately six months later. All participants received written information sheets for the study and provided verbal informed consent. This study was approved by the Kowloon Central / Kowloon East Research Ethics Committee of Hospital Authority, Hong Kong (Reference number: KC/KE-14-0065/FR-1).

Clinical and Laboratory Data Acquisition

At baseline, demographic data, social history and past medical history, including route

of HIV-1 transmission, were collected. Laboratory tests included blood CD4+ T-lymphocytes level, HIV-1 subtyping, HIV-1 ribonucleic acid (RNA) quantification, and serology of syphilis and viral hepatitis (B and C). Blood CD4+ T-lymphocytes and HIV-1 RNA levels were tested again in parallel with cognitive screening at follow-up. Individual cART regimens were recorded based on the CNS penetration-effectiveness (CPE) index [7]: a high CNS penetrating regime was defined as CPE > 7.

Cognitive Function and Depression Assessment

A formal diagnosis of HAND relies on detailed neuropsychiatric and functional status assessment conducted by a clinical psychologist (CP) [3] but such assessments require a significant amount of time and resources. The International HIV dementia scale (IHDS) is a validated brief 3-minute screening test that is ideal for this pilot study which aims to estimate the frequency of cognitive impairment in newly referred patients. IHDS examines motor speed, psychomotor speed and memory-recall function [8] and a score of ≤ 10 (out of 12) shows a reasonable sensitivity (64-74%) and specificity (55-66%) in identifying moderate to severe HAND [9]. The Hong Kong version of the Montreal Cognitive Assessment (MoCA) was added to supplement the breadth of cognitive screening (visuospatial / executive, naming, memory, attention, language, abstraction, delayed recall, and orientation). In our locality, a cut-off of 21/22 (out of 30) has been validated for evaluating mild cognitive impairment in elderly and stroke patients [10, 11], while a cut-off of 25/26 is used in the original English version and previous HAND-related studies in Asian societies including Singapore [12] and Korea [13, 14]. Both cut-off values were included in the results for reference.

At both baseline and follow-up assessments, participants were screened with IHDS and MoCA by CP or trained research assistants. At baseline, participants also completed the Patient Health Questionnare-9 (PHQ-9), a 9-item questionnaire which scores each of the 9 DSM-IV criteria from "0" (not at all) to "3" (nearly every day) over the previous 2 weeks [15]. The Chinese version of PHQ-9 has been validated in Hong Kong [16], with a cut-off score > 9 for moderate depression [15].

Statistical Analysis

Continuous variables were presented as median and interquartile ranges (IQR). Pearson's Chi-squared test and Fisher's exact test were used for categorical variables. Non-parametric Mann-Whitney U test was used for continuous variables. Multivariable logistic regression models were fitted to identify baseline factors associated with poor IHDS performance (cut-off: <10). McNemar and Wilcoxon signed-rank test were used to compare variables before and after cART accordingly. A p-value < 0.05 was considered significant. Statistical analyses were performed using SPSS Version 20.0 (International Business Machines Corporation, New York, USA).

RESULTS

During the study period, 102 patients were referred, of whom 4 individuals were excluded from participation: two were cART-experienced, one had a CNS opportunistic infection, and one had intellectual disability. None were lost to follow-up but one participant succumbed before follow-up due to a non-HIV-related illness. Fifty-seven completed the follow-up assessment within the study period.

Of the 98 enrolled individuals (Table 1), more than a third (n=37, 38%) were referred from our in-patient service for continuation of care. Most were male (n=92, 94%) with a median age of 31 years (IQR 26–43). Sexual contact among men who have sex with men (MSM) was the predominant route of HIV acquisition (77%). HIV-1 subtypes CRF01_AE (42%) and B (37%) were the predominant strains. Eighteen (18%) had a previous AIDS-defining illness. The median pre-cART blood HIV-1 RNA level was 5.07 (IQR 4.68-5.47) log10 copies/mL and the median nadir CD4+ T-lymphocyte level was 270 (IQR 106-376) cells/μL.

Risk Factors of Poor IHDS Performance at Baseline

At baseline, 38 participants (39%) scored ≤ 10 on IHDS. Their demographic and clinical characteristics were compared to those who scored above this cut-off (Table 2). In the univariable analyses, poor IHDS performers had higher rates of prior psychiatric illness (24% vs. 8%, p=0.034) and moderate depression (PHQ-9 > 9) (34% vs. 17%, p=0.048). They tended to have lower education level (40% vs. 57% tertiary education, p=0.097) and blood CD4+ T-lymphocyte counts (241 (IQR 69-320) vs. 298 (IQR 160-400), p=0.078). Age, sex, smoking, alcohol and substance use, blood HIV-1 RNA level, syphilis, and hepatitis C co-infection did not associate with poor IHDS performance (all p>0.1). In the multivariable analysis, prior psychiatric illness (adjusted odds ratio (aOR) 2.99; 95%CI 0.84-10.66, p=0.091), moderate depression (aOR 2.52; 95%CI 0.90-7.06, p=0.079) and lower education level (tertiary education: aOR 0.47; 95%CI 0.20-1.17, p=0.088) tended to be significantly associated with poor IHDS performance, while CD4+ T-lymphocyte level was no longer significant.

Correlation between IHDS and MoCA Performance at Baseline

Poor IHDS performers also performed worse on the MoCA compared to those who scored above the IHDS cut-off (27 (IQR 24-28) vs. 28 (IQR 26-29), p=0.039). Participants' IHDS scores were only weakly correlated with their MoCA performance (Bivariate correlation (Spearman's): r=0.29, p=0.004). In particular, nearly one third (n=24) of the 73 participants who scored above the MoCA cut-off (>25) scored below the IHDS cut-off. The participants' IHDS and MoCA scores are illustrated in Figure 1.

Clinical and Cognitive Screening Outcomes at 6 months Follow-up

Within the study period, 57 participants completed their second assessment, approximately 6 months after the baseline assessment. Compared to those who did not

yet complete the second assessment, this subgroup of participants had a lower baseline IHDS score (10 (IQR 10-11) vs. 11 (IQR 11-12), p<0.001). They also had a high proportion of poor IHDS performers (58% vs 12%, p<0.001). However, both subgroups were statistically similar in terms of demographic and clinical parameters except that the subgroup with follow-up had a higher blood HIV-1 RNA at baseline (5.11 vs 4.91 log10 copies/ml, p=0.010) (Supplementary Table 1).

Fifty-four of the 57 participants who completed both assessments were on cART (Table 3) and 46 (85%) had achieved viral suppression at follow-up (blood HIV-1 RNA < 20 copies/ml). Their blood CD4+ T-lymphocyte level and IHDS performance both improved compared to baseline (468 (IQR 261-673) vs. 248 (IQR46-355) cells/mm3, p<0.001 and 11 (IQR 10-12) vs. 10 (IQR 10-11), p<0.001 respectively). None of them converted from above to below the IHDS cut-off at follow-up and their MoCA test performances were statistically similar between two assessments (p>0.1). Seventeen out of thirty-one participants who scored below the IHDS cut-off at baseline again scored below the cut-off at follow-up.

Linear regression was employed to determine factors that were associated with the change in IHDS scores (i.e. follow-up IHDS score minus baseline IHDS score) (Supplementary table 2). In the univariable analysis, older age was associated with IHDS improvement (Mean difference 0.03, 95% CI (0.01 to 0.06), p=0.013). Tertiary education and history of prior psychiatric illness, which tended to be significantly associated with IHDS performance at baseline, were not associated with IHDS change (p>0.1). HIV-related factors, including plasma CD4+ T-lymphocytes nadir, viral suppression status, and CPE index of cART were not associated with change of IHDS scores (p>0.1).

DISCUSSION

This study estimated the frequency of possible cognitive impairment based on an IHDS cut-off (≤10) that targets more severe forms of HAND. Recent studies suggest a higher cut-off of ≤ 11 to improve the IHDS sensitivity towards milder forms of HAND [17, 18]. At baseline, up to 40% of this group of relatively young, male predominant, cART-naïve individuals scored below the IHDS cut-off. In the MoCA test that examined a different set of cognitive domains, 26% and 8% scored below the original English version (25/26) and locally validated (21/22) cut-offs, respectively. Of note, six participants scored below both IHDS and MoCA 21/22 cut-offs, suggesting multi-domain cognitive impairment. Poor IHDS performers also scored lower on the MoCA but the correlation was weak. Up to a third of participants with normal MoCA scores (>25) scored below the IHDS cut-off, suggesting a discrete impairment in motor, psychomotor and memory-recall functions without a major involvement in cognitive domains examined by MoCA. Thus, a stand-alone MoCA test would be insufficient for cognitive screening in

this group of individuals.

The subgroup of participants who completed the second cognitive screening within the study period had poorer IHDS performance and higher blood HIV-1 RNA levels than the remaining participants while their MoCA performance and clinical parameters were similar. The higher rate of poorer IHDS performers in this subgroup seems random, rather than driven by HIV-1 RNA level, as HIV-1 RNA was not associated with IHDS performance in the multivariable analysis at baseline.

At follow-up, this subset of participants showed an improvement in IHDS performance after cART, but the increment in score was modest. Moreover, a considerable proportion of these participants again scored below the IHDS cut-off at follow-up. Improvement in MoCA test performance was also absent. The overall lack of improvement in both tests could be due to irreversible neurocognitive impairment, driven by HIV-1 infection and/or other etiologies. Other possibilities include the insensitivity of IHDS and MoCA to detect cognitive improvement or a delayed onset of cognitive improvement up to 9 months after cART [19, 20].

At baseline, education, prior psychiatric illness and co-existing moderate depression (PHQ-9>9), but not HIV-specific parameters (HIV-1 RNA level and CD4+ T-lymphocytes nadir) [2, 4, 21] or co-infections (syphilis and HCV) [22, 23], tended to be independently associated with poor IHDS performance. The association between IHDS performance and education, particularly years of education, was previously highlighted in another study [17]. Better education is generally considered as a protective factor against cognitive impairment, likely due to a better cognitive reserve that contributes to resilience against neuropathological insults [24].

The association between mood disorders and cognitive impairment is frequently reported. In particular, major psychiatric illnesses including depression are linked to long term structural brain changes [25] and cognitive decline [26]. Depression is frequently observed in HIV-infected populations [27] and is also a risk factor for HAND [21]. Differentiating the effect of depression from HAND in neurocognitive assessment is challenging and requires detailed neuropsychiatric assessment [28]. However, none of the factors that were associated with baseline IHDS performance nor HIV-related parameters or CPE index of cART were associated with the change of IHDS performance after cART. This general lack of association could be related to the aforementioned irreversible cognitive impairment, insensitivity of the IHDS, or brief interval between assessments for observing the benefit of cART on neurocognitive functioning.

We observed poor IHDS performance in 39% of treatment-naïve HIV-infected study participants, a frequency based on a cut-off that targets for more severe forms of HAND. Despite the usefulness of MoCA in degenerative neurocognitive diseases, MoCA alone

may not be an ideal screening tool in HIV-infected populations because of the limited correlation with IHDS outcomes. A considerable proportion of our participants had concomitant moderate depression symptoms (PHQ-9>9), which tended to be independently associated with poor IHDS performance. Despite cART resulting in virological control in the majority of the group with follow-up, poor IHDS performance persisted in a sizable proportion of them. The findings highlight the need for comprehensive allied health support in contemporary HIV care, including cognitive and mood assessment, and cognitive rehabilitation may be needed. Hong Kong has a relatively young HIV-infected population with a median age under 40. As this population of PLWH ages due to the improved survival and an as yet stable number of newly reported cases, the service demand for cognitive and mood disorders is expected to increase.

Our study has its limitations. First, although the co-dominant HIV-1 B and CRF_01AE subtypes in our participants is compatible with the local HIV-1 strains pattern [29], they had a relatively high rate of recent hospitalization and might not fully match the new local HIV-1 cases in terms of disease severity. The frequency of AIDS in this study was 18%, compared to 14% (218/1558) among total newly reported cases for the corresponding period in Hong Kong (HIV Surveillance Report 2014 and 2015, Centre for Health Protection, Department of Health). Second, repeating cognitive tests at 6 months after cART could be too early to observe cognitive improvement [19, 20]. Third, the higher rate of poorer IHDS performers at baseline among the subgroup with follow-up assessment may overestimate the frequency of persistent cognitive impairment after cART. Lastly, cognitive outcomes in this study were estimated by IHDS and MoCA, which are designed for screening purposes. The lack of HIV-negative controls limits the tests' validity in estimating the frequency of cognitive impairment of the study participants.

CONCLUSION

Our study suggests that moderate to severe cognitive impairment exists in a considerable proportion of treatment-naïve PLWH in Hong Kong. The population of PLWH in Hong Kong is relatively young and is going to expand further. Our findings support the need for implementing cognitive and mood disorder assessments in routine HIV clinical care. This approach may reduce longer term neurocognitive impairment and alleviate its socioeconomic consequences.

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This is a pre-copy editing, author-produced PDF of an article accepted for publication in *BMC Infectious Diseases* follwing peer review. The definitive published article is available online at: https://doi.org/10.1186/s12879-019-3784-y.

Table 1. Demographic and Clinical Characteristics of Participants at Baseline (N=98)	
Male, n (%) **Female, n (%)**	92 (94) 6 (6)
Age, year	31 (26-43)
Tertiary education, n (%)	49 (50)
Current Smoker, n (%)	31 (32)
Current or ex-drinker, n (%)	22 (22)
History of substance Use, n (%)	41 (42)
Prior psychiatric illness, n (%)	14 (14)
Route of transmission, n (%) **MSM** **Other**	 75 (77) 23 (23)
HIV-1 subtype, n (%) **CRF01_AE** **B** **Other**	 41 (42) 36 (37) 21 (21)
AIDS[#], n (%)	18 (18)
Blood CD4+ T-cells nadir^ (cells/µL)	270 (106-376)
Blood HIV-1 RNA, log10 copies/ml	5.07 (4.68-5.47)
Hepatitis B virus co-infection, n (%)	6 (6)
Hepatitis C virus co-infection, n (%)	7 (7)
Syphilis co-infection, n (%)	40 (41)
Recent in-patient care, n (%)	37 (38)
IHDS ≤ 10, n (%)	38 (39)
MoCA ≤ 25, n (%)	25 (26)
MoCA ≤ 21, n (%)	8 (8)
MoCA score	27 (25-28)
Moderate Depression (PHQ-9 >9)^, n (%)	23 (24)

Median (IQR) is presented unless specified otherwise.
[#] Defined by the presence of AIDS-defining illness regardless of CD4+ T-lymphocyte levels; ^ n = 96.
Abbreviations: IHDS = International HIV Dementia Scale, MoCA = Montreal Cognitive Assessment, PHQ-9 = Patient Health Questionnare-9, MSM = men who have sex with men

Table 2. Factor Associated with Cognitive Impairment Status According to IHDS Score

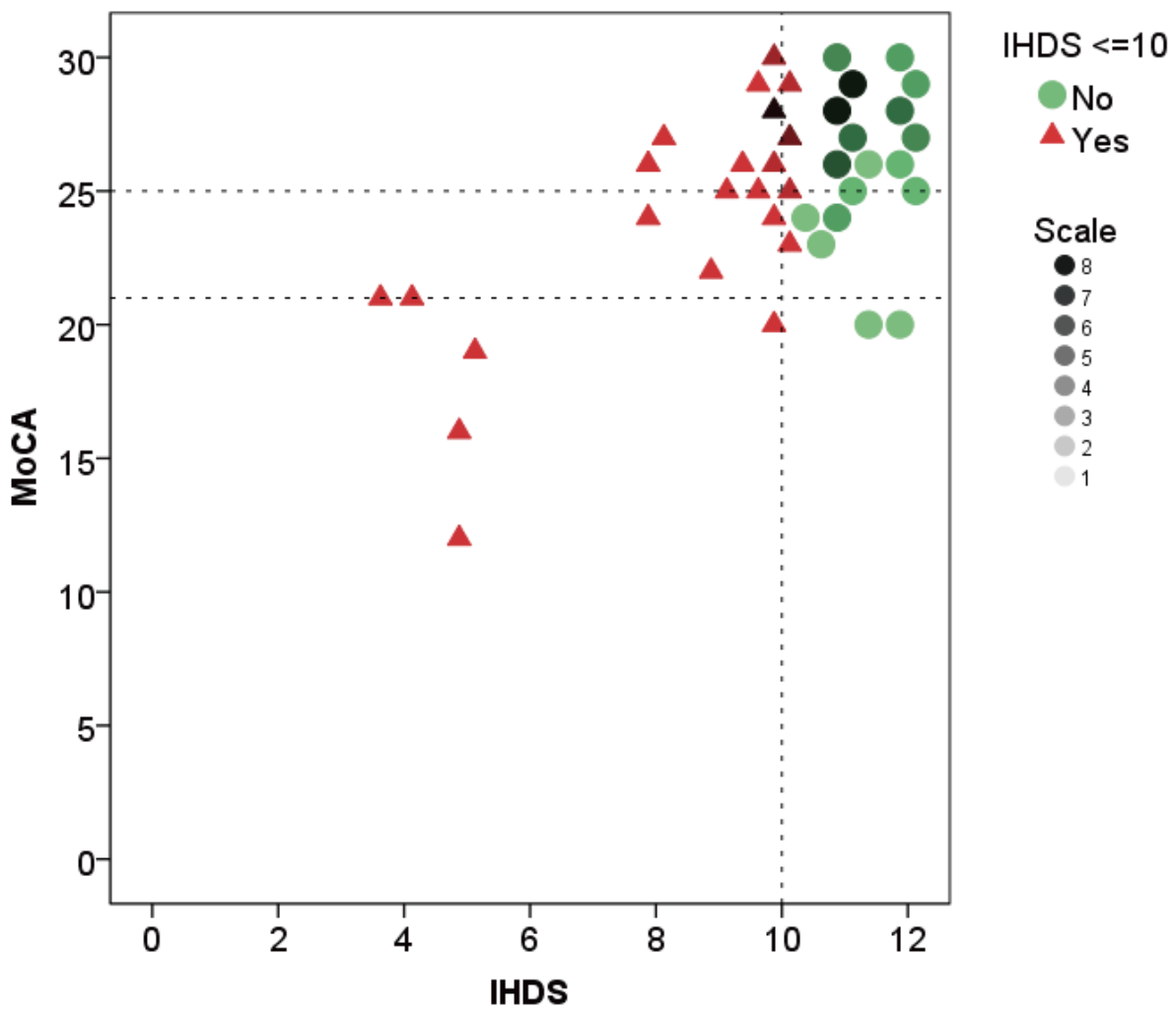
			Univariable Analysis		Multivariable Analysis	
	Non-impaired (IHDS > 10) (n=60)	**Impaired (IHDS ≤ 10) (n=38)**	**Odds Ratio (95% CI)**	**p-value**	**Adjusted Odds Ratio (95% CI)**	**p-value**
***Female sex, n (%)**	3 (5)	3 (8)	1.63 (0.31-8.52)	0.674		
Age, year	31 (25-40)	32 (26-45)		0.358		
#Tertiary education, n (%)	34(57)	15 (40)	0.50 (0.22-1.14)	0.097	0.47 (0.20-1.17)	0.088
#Current smoker, n (%)	19 (32)	12 (32)	1.00 (0.44-2.25)	1.000		
#Current or ex-drinker, n (%)	14 (23)	8 (21)	0.88 (0.33-2.34)	0.792		
#History of substance use, n (%)	23 (38)	18 (47)	1.45 (0.64-3.30)	0.377		
#Prior psychiatric illness, n (%)	5 (8)	9 (24)	3.41 (1.05-11.13)	0.034	2.99 (0.84-10.66)	0.091
Blood HIV-1 RNA, log_{10} copies/ml	5.01 (4.61-5.39)	5.11 (4.81-5.75)		0.139		
CD4+ T-cells nadir (cells/µL)	298 (160-400)	241 (69-320)		0.078		NS
#CD4+ T-cells nadir < 200 cells/µL, n (%)	18 (30)	13 (34)	1.21 (0.51-2.89)	0.662		
***Hepatitis C virus co-infection, n (%)**	5 (8)	2 (5)	0.61 (0.11-3.32)	0.703		
#& Syphilis co-infection, n (%)	22 (37)	18 (47)	1.56 (0.68-3.55)	0.294		
^PHQ-9 score	5 (2-8)	8 (4-12)		0.127		
#^Moderate depression (PHQ-9>9), n (%)	10 (17)	13 (34)	2.67 (0.99-7.17)	0.048	2.52 (0.90-7.06)	0.079

Median (IQR) is presented unless specified otherwise.
* Fisher's Exact test; # Pearson Chi-square test.
& History of infection (enzyme immunoassay (EIA)-Treponemal pallidum assay (TPA) positivity); ^ n = 96.
Abbreviations: IHDS = International HIV Dementia Scale; MoCA = Montreal Cognitive Assessment; PHQ-9 = Patient Health Questionnare-9; NS = Not significant.

Table 3. Clinical and Cognitive Outcomes of the Subset of Participants with Follow-up (n=54)*			
	Baseline	**Follow-up**	**p-value**
CD4+ T-lymphocyte (cells/ µL)	248 (46-355)	468 (261-673)	<0.001
On cART with CPE > 7, n (%)	-	7 (13)	-
HIV-1 RNA suppression#, n (%)	-	46 (85)	-
IHDS score	10 (10-11)	11 (10-12)	<0.001
IHDS ≤ 10, n (%)	31 (57)	17 (32)	<0.001
MoCA score	27 (26-29)	27 (26-28)	0.818
MoCA ≤ 25, n (%)	13 (24)	12 (22)	1.000
MoCA ≤ 21, n (%)	4 (7)	4 (7)	1.000

Median (IQR) is presented unless specified otherwise.
* Only included participants who were on cART
# Blood HIV-1 RNA level < 20 copies/mL
Abbreviations: IHDS = International HIV Dementia Scale; MoCA = Montreal Cognitive Assessment; CPE = CNS penetration-effectiveness

Figure 1. Participants' Performance in IHDS and MoCA at Baseline



Supplementary Table 1. Demographic and Clinical Characteristics of Participants with and without Second Cognitive Screening			
	Participants with Second Cognitive Screening (n=57)	**Participants without Second Cognitive Screening (n=41)**	**p-value**
***Female Sex, n (%)**	2 (4)	4 (10)	0.233
Age, year	31 (26-45)	29 (24-40)	0.219
#Tertiary education, n (%)	26 (46)	23 (56)	0.306
#Current smoker, n (%)	19 (33)	12 (29)	0.669
#Current or ex-drinker, n (%)	10 (18)	12 (29)	0.260
#History of substance Use, n (%)	26 (46)	15 (37)	0.371
#Prior psychiatric illness, n (%)	9 (16)	5 (12)	0.834
Blood HIV-1 RNA, log_{10} copies/ml	5.11 (4.90-5.63)	4.91 (4.52-5.35)	0.010
CD4+ T-cells nadir (cells/µL)	267 (60-361)	272 (178-437)	0.130
CD4+ T-cells nadir < 200 cells/µL	20 (35)	11 (27)	0.386
***Hepatitis C virus co-infection, n (%)**	3 (5)	4 (10)	0.447
#&Syphilis co-infection, n (%)	24 (42)	16 (39)	0.760
IHDS score	10 (10-11)	11 (11-12)	<0.001
IHDS ≤ 10, n (%)	33 (58)	5 (12)	<0.001
MoCA ≤ 25, n (%)	13 (23)	12 (29)	0.469
MoCA ≤ 21, n (%)	4 (7)	4 (10)	0.716
MoCA score	27 (26-29)	27 (25-28)	0.290
#^Moderate depression (PHQ-9>9), n (%)	15 (26)	8 (20)	0.588

Median (IQR) is presented unless specified otherwise.
^ n = 96; # Pearson Chi-square test; * Fisher's Exact test.
Abbreviations: IHDS = International HIV Dementia Scale, MoCA = Montreal Cognitive Assessment; PHQ-9 = Patient Health Questionnare-9

Supplementary Table 2: Factor Correlation with IHDS Changes*		
	IHDS mean difference (95% CI)	**p-value**
Female sex	0.78 (-087 to 2.45)	0.345
Age, year	0.03 (0.01 to 0.06)	0.014
Tertiary education	-0.17 (-0.81 to 0.46)	0.592
Current smoker	-0.04 (-0.40 to 0.31)	0.811
Current or ex-drinker	0.24 (-0.60 to 1.09)	0.565
History of substance use	-0.17 (-0.81 to 0.46)	0.592
Prior psychiatric illness	-0.62 (-1.45 to 0.21)	0.139
HIV-1 RNA suppression	-0.08 (-0.98 to 0.81)	0.850
CPE index ≥ 8	0.13 (-0.81 to 1.08)	0.777
CD4+ T-cells nadir, per 100 cells/µL	-0.1 (-0.3 to 0.05)	0.135
CD4+ T-cells nadir < 200 cells/µL	0.45 (-0.19 to 1.10)	0.165
Hepatitis C co-infection	-0.26 (-1.63 to 1.13)	0.712
Syphilis co-infection	-0.25 (-0.89 to 0.39)	0.432

*IHDS change = Follow-up IHDS score minus Baseline IHDS score
Statistical method: Linear regression with IHDS change as dependent variable
Abbreviation: CPE = CNS Penetration-Effectiveness

Chapter 3:
Cognitive Impairment and Persistent CNS Injury in Treated HIV

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Current HIV/AIDS Reports volume 13, pages209–217(2016)
doi:10.1007/s11904-016-0319-7

ABSTRACT

The implementation of combination antiretroviral therapy (cART) has changed HIV infection into a chronic illness, conveying extensive benefits, including greater longevity and advantages for the central nervous system (CNS). However, studies increasingly confirm that the CNS gains are incomplete, with reports of persistent immune activation affecting the CNS despite suppression of plasma HIV RNA. The rate of cognitive impairment is unchanged, although severity is generally milder than in the pre-cART era. In this review, we discuss cognitive outcomes from recently published clinical HIV studies, review observations on HIV biomarkers for cognitive change, and emphasize longitudinal imaging findings. Additionally, we summarize recent studies on CNS viral invasion, CD8 encephalitis, and how CNS involvement during the earliest stages of infection may set the stage for later cognitive manifestations.

Introduction

Combination antiretroviral therapy (cART) has transformed the HIV epidemic into a chronic yet complex illness that is largely manageable for people with access to life-saving therapies [1]. Combination therapies are now deliverable in as few as one to two pills daily; with adherence and longitudinal follow-up, they are highly successful in suppressing plasma HIV RNA to levels undetectable by standard assays. Yet, cognitive impairment remains frequent, has been quantified in study participants despite plasma HIV RNA suppression, and is associated with markers of ongoing immune activation [2, 3].

Multiple lines of evidence confirm that cART is inadequate to ameliorate central nervous system (CNS) injury for all patients. In research studies, among participants with suppression of plasma HIV RNA, CNS microglial activation is apparent by positron emission tomography (PET, [11C]-PK11195 tracer) and the extent of microglial activation is associated with worse cognitive performance on executive functioning tests [4•]. Diffusion tensor imaging (DTI), a method that evaluates the integrity of deep brain fibers, uncovers CNS injury among suppressed participants, and the degree of compromised CNS fiber integrity correlates to worse cognitive performance [5–7]. Persistent blood monocyte activation measured by the monocyte/macrophage scavenger receptor, CD163, is apparent in participants with suppressed plasma HIV RNA who have HIV-associated neurocognitive disorder (HAND) [3]. Even markers of brain injury have been identified in some individuals with chronic infection, as measured by cerebrospinal fluid (CSF) neurofilament (NFL) elevations in 8 % of asymptomatic suppressed HIV-infected participants compared to only 2 % of controls [8].

While many studies indicate HIV-specific mechanisms, particularly that related to inflammation, there is a similar bulk of data to emphasize the role of comorbidities in current era cognitive impairment. The CNS Anti-Retroviral Therapy Effects Research

(CHARTER) study conducted at five academic centers in the USA and published in 2010 noted the greatest probability of cognitive impairment among individuals with comorbidities, including cerebrovascular disease, head injuries, substance use, and depression. More recently, in a brain autopsy series of 144 HIV-infected individuals, about 50 % had moderate or severe cerebral small vessel disease (CVD), with the extent of CVD associating with previous exposure to protease inhibitors [9•]. This study also noted correlations between HAND and mild CVD [OR (confidence intervals) 4.8 (1.1–21.2)]. Although this important study provides unequivocal evidence of high rates of CVD in HIV, the enrollment period (1999–2011) may expose cohort biases related to the antiretroviral medications used during that era that are no longer broadly employed.

A recent review itemized the likely increased risk of clinical stroke in the HIV-infected population, a finding that may be particularly important for women [10]. HIV may also contribute to young age of stroke, not only among treatment naïve individuals but also those during early phase of treatment [11]. Vasculopathies have been associated with HIV, including vessel remodeling by atherosclerosis, elongation and distension of vessels [12, 13], and alterations in cerebral vasoreactivity [14].

More recently, cognitive impairment in the setting of HIV has been linked to multimorbidity, defined as the presence of two or more independent disease processes that can augment each other and achieve worse outcomes. The Veterans Aging Cohort Study (VACS) index of multimorbidity combines age, traditional HIV clinical measures (e.g., plasma HIV RNA and current CD4+ count), and non-HIV disease markers from more than one organ system (e.g., renal and liver function, anemia, and hepatitis C co-infection) [15]. The possible multisystem contributions to HAND is suggested in a recent Women's Interagency HIV Study (WIHS) publication linking liver fibrosis to cognitive performance in models that were adjusted for both hepatitis C and HIV [16]. Both studies, however, raise concern that the relationship is not mechanistic but rather an indication of common underlying pathophysiological processes, most probably chronic inflammation.

The diagnostic challenge associated with risk for other neurodegenerative disorders, such as Alzheimer's disease (AD), as etiology to cognitive impairment in older HIV-infected patients is an emerging issue. A recent publication employed the more general criteria of mild cognitive impairment (MCI), nosology typically used in HIV-uninfected studies and often prodromal of AD. Participants were virally suppressed and over age 50 years and had a seven-times higher risk of meeting MCI criteria compared to seronegative control (n = 75) [17]. With treatment earlier in the course of HIV, HAND is increasingly found to be independent of clinical markers of immune compromise (e.g., CD4+ counts). Among young treatment-naïve participants (age 27–42 years, n = 608) with CD4+ counts >500 cells/μL, 20 % had at least mild impairment in neuropsychological testing and 3 % had moderate-to-severe impairment

[18•]. While worse performance was associated with duration of HIV, it was also linked to diagnosis of diabetes and the presence of cardiovascular risk factors that compromise the Framingham risk score.

Longitudinal studies support the risk for persistent cognitive impairment, and large cohort studies in the USA uncover challenges with broad uptake of therapy. The Multicenter AIDS Cohort Study (MACS) completed a longitudinal substudy of 197 HIV-infected men who have sex with men (MSM) sampled to be free of major cognitive confounding factors [19]. Across a 3-year period, the frequency of HAND increased from 25 to 31 % ($p = 0.048$), and during this period, 77 % remained stable, 13 % deteriorated, and 10 % improved in their diagnostic HAND staging. In this sample, 75 % of participants were on cART at baseline and the mean CD4+ count was 589 cells/mm3. Rates of persistent impairment, deterioration, and improvement among successfully suppressed participants were not reported, although progression was linked to poorer treatment outcome variables (e.g., viral load). Similarly, the CHARTER study reported the 3-year incidence and predictors of cognitive change in 436 enrollees of whom 46 % were impaired at baseline and noted that 61 % remained stable, 17 % improved, and 23 % declined [20]. Predictors of cognitive change included treatment-related variables, disease severity indicators, baseline demographics, and premorbid intelligence quotient.

These two major US cohort studies highlight an urgent gap in implementing treatment. Although rates of cognitive impairment exceeded 50 % in the CHARTER cohort, less than half had suppressed plasma HIV RNA and 44 % of those on cART were not suppressed. In the WIHS (cross-sectional) conducted in the US and the MACS (longitudinal) reports, only 53 and 70 % of participants achieved undetectable HIV RNA during study visits, respectively. Given that suppression of plasma virus is a universally accepted early step in treating cognitive symptoms and that treatment-related factors were related to cognition, these studies uncover a crucial opportunity for treatment of persistent impairment.

Substantial controversy remains regarding the research categorization of asymptomatic neurocognitive impairment (ANI) within the HAND nosology [21]. The ANI designation may be used in as many as 70 % of research participants who are impaired on cognitive testing, but the absence of functional symptoms is often determined only by self-report [22]. Several studies buttress the likelihood that the "asymptomatic" terminology is misleading and that these individuals remain at risk for outcomes associated with impairment. Compared to unimpaired HIV-infected participants, ANI individuals more frequently convert to symptomatic disease [23]. In a study of mixed asymptomatic and symptomatic participants with HAND, researchers uncovered impairment in the objective skills needed to manage health care literacy [24]. In a separate study, individuals with ANI were found to perform similarly to participants with mild

symptomatic impairment (mild neurocognitive disorder (MND)) on both neuropsychological tests and objective tests of everyday function, yet ANI participants discrepantly reported normal or above normal perceived performance [25]. Symptoms, such as irritability and communication difficulty, are more readily identified when collateral information can be acquired, but proxy input is not commonly included in research studies [26]. These studies highlight important contributions to the asymptomatic designation driven by impaired insight and less proximal objective informants than are typical in non-HIV neurodegenerative disorder studies.

Some researchers have questioned whether HAND terminology appropriately attributes cognitive deficits to HIV alone. In the Cognitive Impairment in People with HIV in the European Region (CIPHER) study of 248 healthy HIV-infected participants (mean CD4+ count of 550 cells, 88 % on cART), investigators found no difference in the frequency of cognitive impairment compared to demographically similar HIV-uninfected controls [27]. These data should be interpreted with caution given such a limited sample of controls (n = 45) of which nearly one third met criteria for cognitive impairment and the sample selection from regular clinic attendees. The authors noted important differences in rates of moderate to severe depression in this study, affecting 29 % of HIV-infected participants compared to only 8 % of HIV-uninfected controls ($p < 0.001$). Given knowledge that HIV is linked to motor, behavioral, and cognitive manifestations, such a high discrepancy in rates of depression highlights continued concern for HIV-related CNS morbidity.

Biomarkers for HAND

To date, markers of immune activation and neuronal injury show greatest promise as potential biomarkers of HAND, although no marker has emerged to have sufficient performance characteristics for clinical use [3]. CSF monocyte/macrophage inflammatory markers MCP-1 and sCD14 correlate to MR spectroscopy (MRS) neuronal damage and inflammatory abnormalities among treated participants, 70 % of which achieved CSF and plasma viral suppression [28]. CSF sCD14 was also found to associate with CSF NFL, a marker of neuronal injury, in another cross-sectional analysis studying a mixed group of participants that were either treatment-naïve or off treatment (n = 48) [29]. These findings demonstrate the importance of understanding the role of monocytes/macrophages in HAND, particularly noting the associations of these markers to HAND despite virologic suppression. This is also bolstered by a recent study using a myeloid only mouse model, demonstrating that macrophages from humans can sustain and transmit infection [30].

Some data have shown that T-lymphocyte activation is linked to HAND. In a study of 86 HIV-infected participants with about 70 % achieving viral suppression, the upregulation of HLA-DR, a marker for T-lymphocyte activation, was detected on CD4+ and CD8+

T-lymphocytes in both blood and CSF samples compared with healthy controls [31]. Decreasing CD4/CD8 ratios in the CSF and the increased frequencies of HLA-DR expressing CD4+ and CD8+ T cells in the CSF also were found to associate with HAND severity and T2 MRI signal abnormalities in the periventricular white matter and basal ganglia [31].

Potential relationships between persistent intracellular HIV DNA and cognitive impairment were explored in a retrospective study of 44 HIV-infected participants with plasma and CSF viral suppression. Here, higher HIV DNA levels were associated with cognitive impairment severity in the subset of older participants (age 50–71, n = 26) [32]. These associations were not detected cross-sectionally in a separate but similar study (n = 80, CSF and blood viral suppression = 97 %); however, change in HIV DNA did link to change in cognitive performance in some domains [33].

CSF NFL is a structural component of myelinated axons and can be detected with active neuronal damage. NFL was shown to be sensitive for detecting HIV-associated dementia (HAD), the most severe form of HAND, and was superior to other CSF markers, including total and phosphorylated tau (t- and p-tau), soluble amyloid precursor protein (sAPP), and amyloid beta fragments [34]. Regardless of CD4+ counts, individuals with HAD also displayed the most distorted overall CSF biomarker profiles (e.g., CSF NFL, sAPP, t-tau), white blood cell count (WBC), and blood-brain barrier (BBB) integrity by albumin ratio. These findings suggest a more extensive inflammatory response and presence of active neuronal injury in HAD compared to neuroasymptomatic disease. In a longitudinal study of treatment effect (n = 78), CSF NFL levels decreased in 63 % of participants after cART initiation, although levels remained higher than those in healthy controls after cART [8]. Recently, elevated NFL levels in plasma, independent of CSF, have been associated with the presence of HAD in participants not taking cART, implying that NFL in blood could be useful as a minimally invasive biomarker for active neuronal injury in demented patients [35]. Markers of ongoing injury are still needed for patients with suppressed plasma HIV RNA, and blood markers would be optimal.

CNS Imaging in HIV

Imaging studies that investigate mixed treatment populations (e.g., both with and without plasma viral suppression) continue to limit our understanding of disease severity among optimally adherent and suppressed patients. A longitudinal volumetric study of 51 asymptomatic HIV-infected participants (mean plasma HIV RNA = 9608 copies/ml, about 20 % not on cART) demonstrated faster atrophy rates in HIV in regions that included the neocortex (from frontal lobes to parietal lobes) and the thalamus compared to 65 HIV-uninfected controls [36•]. The authors did not report the proportion of HIV-infected participants suppressed on therapy. Those with higher CD4+ counts had slower expansion of Sylvian fissure and slower atrophy rates in the insulae, hippocampi,

and frontal and temporoparietal cortices, a finding that could be interpreted as a window of neuroprotection linked to early treatment initiation [36•]. Another preliminary longitudinal study reports that, despite 24 months of cART that was started within days of infection and with demonstrated plasma viral suppression, volumetric decreases of 2 % were found in caudate ($p = 0.002$), putamen ($p<0.001$), and pallidum ($p = 0.034$), with a 1 % decrease in total subcortical gray matter ($p = 0.002$) (n = 38, median age 29 years, CD4+ count 386 cells/mm3). These preliminary data lacked an HIV-uninfected comparison group to assure that these rates exceeded that of healthy controls; however, given the age of these participants, such atrophy would be unusual [37].

Magnetic resonance spectroscopy (MRS) measures the concentration of key metabolites in the brain and can provide insight into neuronal health, cellular metabolism, and inflammation. Several longitudinal studies suggest that virologic suppression does not achieve complete normalization of MRS abnormalities, such as normalization of N-acetyl aspartate (NAA) reflecting neuronal integrity, or choline (CHO), a marker of inflammation [38, 39]. These persistent MRS abnormalities were seen in HIV regardless of cognitive status. Nadir CD4+ count, duration of HIV infection, and older age correlate with persistent abnormalities in markers of neuronal health (NAA/Cr, n = 260 on stable cART, 75 % with plasma HIV RNA suppression) [40]. In a study highlighted earlier in this review, the ability to detect potential microglial activation linked to cognitive performance among suppressed patients using PET imaging may represent one of the greatest breakthroughs in understanding ongoing brain injury despite effective cART from a study published recently; however, larger studies are needed [4•].

A study of white matter hyperintensities (WMHs) used a combination of DTI and fluid-attenuated inversion recovery (FLAIR) images in over 80 HIV-infected participants with a broad age range. Authors found that older age was associated with an increased frequency of WMH in HIV, but not in controls [41]. Investigations of DTI also uncovered impaired microstructural integrity in the internal capsule, cerebral peduncle, and corona radiata associated with age and HCV co-infection. Alterations in white matter integrity, as measured by DTI, correlated with cognitive performance in older HIV-infected individuals (mean age = 64, 90 % with plasma HIV RNA < 400 copies/ml) compared to age-matched controls [6]. Diffuse white matter alterations were detected in another DTI study comparing HIV-infected participants on suppressive therapy (n = 100) with matched controls (n = 70); however, the contributions from residual pre-cART damage cannot be excluded in any of these studies and, in the later study, the DTI abnormalities correlated with the number of years spent with a CD4+ cell count below 500 cells/μl [7].

The neuroanatomic structures involved in HIV infection are largely consistent across the various imaging modalities, with the most common affected brain regions being the frontal white matter (FWM) [38, 39], basal ganglia (BG) [38, 39, 42, 43], and the

thalami [42]. The effect of HIV infection on frontostriatal circuitry was similarly reported by a meta-analysis combining six task-based functional MRI (fMRI) studies (n = 105 HIV-infected and n = 102 HIV-uninfected controls) where frontostriatal dysfunction correlated to degree of cognitive impairment, disease progression, and treatment effect [44].

Insights from Early HIV Infection

HIV RNA has been found in the CSF as early as 8 days following estimated infection [45], and through blood and CSF viral sequencing, independent replication in the CNS has been observed within the first year of HIV infection [46•]. We do not yet understand when brain changes that underlie irreversible, long-term cognitive consequences begin and whether there is a window period for early intervention that can be neuroprotective. The growing body of early HIV infection studies may bridge this gap.

In primary HIV infection (PHI), generally defined as within the first year of infection, a previous report suggested normal performance on neuropsychological testing in most of the participants [47]. Nonetheless, the Chicago Early HIV Infection cohort (CEHI, n = 15, estimated duration of HIV < 100 days, Fiebig III to V) identified worse performance on tasks of psychomotor speed and visual recall [48]. Refining this to the window of acute HIV infection (AHI), a recent study from Thailand (n = 36, 64 % in Fiebig stage I or II) found that as many as 25 % were at least one standard deviation below mean performance on two or more cognitive tests, and this group of cases had higher CSF HIV RNA [49]. At 3 and 6 months post cART, a subset of individuals did not improve.

In cross-sectional DTI studies from the CEHI cohort [48], PHI participants had early impairments in white matter tract integrity at diagnosis. Brain volumetric analyses further revealed reduced parenchyma volumes in PHI compared to controls, suggesting neuronal loss [48]. In contrast, a cross-sectional analysis from the Primary Infection Stage CNS Events Study (PISCES) did not find DTI abnormalities at baseline (median 4 months post infection) compared to controls [50]. A conference proceeding noted earlier in this review identified volumetric reductions over time in deep gray matter structures despite 24 months of suppressive cART [37].

In the PISCES study, BBB integrity was modestly altered during PHI, demonstrated by elevated CSF/plasma albumin ratios, CSF protein levels, and DTI alterations [50, 51]. CSF and plasma concentrations of matrix metalloproteinases (MMPs), a group of extracellular proteases involved in BBB permeability, were compared between 52 PHI and 21 controls in the CEHI study. Here, authors demonstrated reduced plasma MMP-2 in PHI and that elevation of CSF MMP-2 correlated to reduced white matter integrity, basal ganglia volume, and motor speed [52]. Longitudinal studies note that neuroinflammation in PHI can escalate with time in the absence of treatment. A longitudinal MRS study in treatment-naïve PHI reported increases in inflammatory

markers (CHO/Cr and MI/Cr) in the frontal white matter and parietal gray matter over a median of 6 months [53]. Sequential analysis of CSF neopterin concentrations and percentages of activated CD4+ and CD8+ T cells in CSF similarly demonstrated a steady, rising trend in most of these 44 treatment-naïve PISCES participants [54].

Elevated NFL concentration was seen in 44 % of 92 PHI PISCES participants and varied with infection duration [51]. These elevations correlated with reductions in neuronal integrity (NAA/Cr) on MRS, but not with neuropsychological performance, suggesting a pre-clinical brain injury [51]. Treatment led to an attenuation, but not normalization of levels of inflammatory markers in frontal white matter and parietal grey matter. In AHI participants from Thailand, normal CSF NFL levels were seen in all but one participant (n = 32) and remained within normal levels after 24 weeks of cART [55]. Together, these studies highlight CNS HIV infection and immune activation being established very early after initial HIV infection and reasonable support for the conjecture that there may be a window period for protection from neuronal injury after early CNS invasion.

CNS HIV Escape and CD8 Encephalitis

CNS HIV escape is a rare but biologically important phenomenon, defined as either a detectable CSF HIV RNA level despite undetectable plasma HIV RNA level or a CSF HIV RNA level at least one log10 above a fairly well-controlled level in plasma [56, 57]. Patients with symptomatic CNS HIV escape can present with symptoms ranging from mild headache or sensory disturbance to encephalopathy or coma and typically respond to cART adjustments according to CSF HIV genotyping or increasing the CNS penetration effectiveness (CPE) [56, 57]. Drug resistance has been reported in the CSF in case series of symptomatic CNS escape participants treated with long-term suppressive cART, including one after 9 years of a three drug PI-based regimen [58] and another after 6 years of two-drug PI-based regimen [59]. There are currently few published data to guide clinicians on when CNS escape is likely and this is a major gap in the field, since symptomatic CNS escape is likely occurring in the minority of cases of cognitive impairment seen in clinic populations.

A recent cross-sectional study (n = 69) found that 10 % of asymptomatic participants with undetectable plasma HIV RNA had low but detectable levels of CSF HIV RNA [60]. Higher occurrence rates of 15–19 % of participants with detectable CSF HIV RNA despite plasma viral suppression have been reported in cART simplification studies [61, 62]. Among participants with long-term plasma HIV RNA suppression (n = 45, <40 copies/ml), 17 % of CSF samples (12/70) had detectable CSF HIV RNA using a single copy assay with detection limit 0.3 copies/ml [63••]. Despite the exceptionally low level of CSF HIV replication, CSF neopterin was higher in the detectable group compared with the undetectable group, suggesting that macrophage activation may foster low-level HIV persistence or vice versa. This is consistent with other asymptomatic

CSF viral escape reports that employ standard assays for HIV RNA quantitation [60, 64]. As these studies were cross-sectional, it remains less clear if the presence of actively replicating virus in the CNS is transient or persistent and what role asymptomatic CNS escape may plan in CNS compartmentalization or symptomatic escape. Deep sequencing of four symptomatic CNS HIV escape cases revealed the presence of minority variants in CSF, supporting the concept of local CNS replication and differential evolution of HIV in the CNS [65].

CD8 encephalitis, a diagnosis pathologically defined by extensive perivascular and parenchymal infiltration of CD8+ T-lymphocytes [66•], has been reported in treated individuals and further broadens the possible CNS manifestations in treated HIV [66•, 67]. In a case series of CD8 encephalitis, 8 out of 14 individuals had been on stable cART for at least 2 years, with undetectable plasma HIV RNA and either CD4+ count >350 cells/mm3 or CD4/CD8 ratio >0.7 [67]. All presented with unexpected, acute or subacute brain dysfunction (e.g., dizziness, headache, memory disorders, confusion, status epilepticus), and all had elevated CSF lymphocyte counts with a disproportionately elevated CD8/CD4 ratio (CD8+ 65–87 %), in the absence of blood CD8+ lymphocytosis in all but one person. The improvement of CD8 encephalitis after steroid treatment suggests a shared feature with autoimmune conditions; total recovery was reported in 5/14 patients with parallel resolution of MRI brain abnormalities. Both CNS HIV escape and CD8 encephalitis expand our understanding of cognitive impairment in people living with HIV on suppressive cART, as they are proof of concept that CNS viral replication and widespread neuroinflammation can persist and associate with dramatic neurologic symptomatology.

Summary and Recommendations

Conclusions

The persistence of HAND in the era of cART is likely due to multiple etiologies. While cerebrovascular risk factors grow in importance with an aging HIV-infected population, the issue of persistent CNS immune activation despite suppressive cART should not be disregarded, as its presence has been linked with neurocognitive impairment, neuroimaging abnormalities, and neuronal damage markers. The observations from CNS HIV escape offer a potential source of persistent immune activation, and the occurrence of CD8 encephalitis highlights that a partly recovered yet dysfunctional host immune system may be of importance in affecting clinical outcomes.

While there is a persistent risk for developing cognitive impairment despite treatment, hopeful intervention trials are developing. In a recent, small, randomized control trial targeting HIV-infected participants with cognitive symptoms despite suppressive cART in both plasma and CSF (n = 9; HIV RNA < 50 copies/ml), adding a CCR5 inhibitor (maraviroc) to a backbone cART regimen was associated with medium to large effect

sizes, favoring improved global cognitive performance at 6 and 12 months [68]. In another single arm, open-labeled study of maraviroc intensification, cognitive improvement was noted, as was a reduction in intracellular HIV DNA and decreased CD38+ T-lymphocytes, providing mechanistic links to this improvement [69]. With the expanding narrative about usefulness of CCR5 blockade for neuroinflammatory disease [70], CCR5 inhibitors merit larger randomized control trials. Notably, these findings contrast with a study of neuroasymptomatic participants where CCR5 inhibitors were added to first-line therapy (i.e., cART naïve). Authors identified no benefit to augmentation, highlighting the likelihood that the opportunity for augmentation is best guided to those with symptomatic impairment despite cART [71].

Another potential candidate for intervention is paroxetine, which was shown to be beneficial for HIV-infected participants with cognitive impairment in a preliminary conference report [72]. It continues to be reasonable to recommend physical exercise [73] and potentially cognitive rehabilitation [74] in an armamentarium that includes vigilance in management of comorbidities in this complex and multietiology cognitive impairment.

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This is a pre-copy editing, author-produced PDF of an article accepted for publication in *Current HIV/AIDS Reports* follwing peer review. The definitive published article is available online at: https://doi.org/10.1007/s11904-016-0319-7.

SECTION 2
Characterizing Neurological Parameters During Acute HIV Infection

Chapter 4:
Safety of Lumbar Puncture Procedure in an International Research Setting During Acute HIV Infection

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J Virus Erad. 2018 Jan; 4(1): 16–20.
doi: 10.1016/S2055-6640(20)30236-3

ABSTRACT

Background: Cerebrospinal fluid (CSF) sampling at the time of acute HIV infection (AHI) is crucial in understanding the pathogenesis of NeuroAIDS. Here, we report on the safety of performing LP during untreated AHI and follow-up on combination antiretroviral therapy (cART).

Methods: We reviewed records of an AHI protocol from Bangkok, Thailand, including untreated AHI (baseline) and longitudinal visits following immediate initiation of cART to assess rates and risk of PLPH. Cerebrospinal fluid (CSF) volume of 10-20ml was collected using standard cutting-edge needles or atraumatic needles.

Results: From April 2009 to February 2016, 195 LPs were done, 89 (46%) at baseline. LP procedures at baseline were not associated with additional PLPH risk compared to repeat LPs after cART (26/89 (29%) vs. 4/27 (15%), respectively; p=0.134). Higher BMI at baseline (p=0.070) and use of atraumatic needle (p=0.058) had trend level associations with reduced PLPH. Higher volume CSF collection (20ml) was independently associated with lower frequency of PLPH (p=0.024). This association was similar in subgroup analysis using atraumatic needle. CD4+ T lymphocyte count, blood and CSF HIV viral load, Fiebig staging, and the presence of acute retroviral syndrome did not correlate with PLPH (all p>0.05).

Conclusions: The frequency of PLPH during AHI was similar to that seen in the setting of treated HIV and no more frequent with larger CSF volume collection. Our study adds to the existing evidence that atraumatic needles should be used to minimize the risk of PLPH.

INTRODUCTION

Cerebrospinal fluid (CSF) collection by lumbar puncture (LP) is essential for the evaluation of central nervous system (CNS) disorders. In the setting of neurological complications in HIV, CSF examination is critical to exclude CNS viral escape and opportunistic infections. In HIV research studies, CSF provides invaluable information about neuropathogenesis including factors associated with cognitive impairment, which is reported in up to 20% of treatment naive individuals with CD4+ T-lymphocyte count of > 500 cells/mm3 [1] and ranges from 18%-63% of treated patients [2, 3]. Measurement of CSF markers such as neopterin and neurofilament light chain provides insight into CNS inflammation and neuronal injury [4-6], which are incompletely normalized despite systemic virologic suppression, particularly among those with cognitive impairment [7].

Although LP is safe and rarely causes life-threatening complications, post-LP headache (PLPH) is common and develops in up to 40% of individuals undergoing the procedure [8-11]. The reduction in intracranial pressure following LP is believed to cause PLPH and it typically presents with an orthostatic pattern characterized by worsening pain when one moves from supine to erect position. Smaller needle size [12, 13], use of atraumatic (non-cutting edge) spinal needle [14, 15] and replacement of the stylet in the atraumatic needle before needle withdrawal [16] are approaches that tend to reduce PLPH development. In 2005, the American Academy of Neurology (AAN) released a statement supporting the use of atraumatic needles to reduce PLPH [17].

While HIV infection is known to alter coagulation despite treatment [18], a recent LP safety report noted that HIV status did not alter the risk of PLPH [11]. To date, information has not been published on the safety of LP during AHI, nor have studies been performed in a research setting outside of a World Bank classified high income country. This may limit our understanding of the cultural acceptance of LP [19] and LP uptake in the absence of medical expertise required for treating cases of persistent CSF leakage. Intrathecal immune activation is substantial during the time period of AHI [20] and clinically recognized neurological symptoms occur [21] including meningitis [22], leading us to consider whether the frequency and characteristics of adverse events related to LP are altered during AHI. Additionally, although outstanding infrastructure exists for clinical research in an array of settings outside of high income countries, there are no reports of CSF collection safety in low- or middle-income research settings. We examined the frequency and risk factors of PLPH as well as backache and wound infection in AHI from the SEARCH010/RV254 cohort, a prospective longitudinal AHI study underway in Bangkok, Thailand that characterizes participants longitudinally before and after initiation of combination antiretroviral therapy (cART) during AHI, often within weeks of infection.

METHODS

Participants

We included all individuals enrolled into SEARCH010/RV254 between initiation of the study in April 2009 and February 2016. Details of enrollment have been described elsewhere [20]. Briefly, specimens from participants seeking HIV voluntary counseling and testing services at the Anonymous Clinic of the Thai Red Cross AIDS Research Center in Bangkok, Thailand were systematically screened for AHI and enrolled during Fiebig stages I to V, defined by a hierarchical algorithm from pooled nucleic acid and sequential immunoassay (IA) testing [23]. After completion of initial research assessments and the optional LP performed by research physiciansAfter completion of initial research assessments, which include optional LP, nearly all participants initiated cART, typically within 48 hours of AHI confirmation. Optional LP is also performed during post-cART follow-up to determine longitudinal outcome of treating HIV at the earliest stage through CSF markers analyses. All subjects provided written informed consent to participate in the study. The study protocol was approved by the institutional review boards of Chulalongkorn University, Bangkok, Thailand, and the Walter Reed Army Institute of Research, Silver Spring, MD, USA.

Lumbar puncture and clinical data

Research participants underwent optional LP at various time-points in the study, which includes baseline during untreated AHI (n=89), and at 24, 96 and 240 weeks following enrollment. A subset of these participants (n=9) also underwent LP at week 48 as part of co-enrollment in a separate sub-study (No. of all follow-up LP = 106). LP procedure was optional and performed by research physicians during untreated AHI (baseline), and optionally repeated at 24, 96, and 240 weeks following enrollment. A subset of these participants (n=9) also underwent LP at week 48 as part of co-enrollment in a separate sub-study. Demographic and procedure-specific data collected for each LP included age, sex at birth, body mass index (BMI), type of spinal needle used (i.e. cutting edge (Quincke) vs. atraumatic (Sprotte®, 22-gauge, PAJUNK® Medizintechnik)), volume of CSF collected, any same-day invasive study procedure performed (e.g. lymph node biopsy, sigmoid colon biopsy, leukopharesis) and previous exposure to LP. Additional clinical parameters collected at baseline included CD4+ T-lymphocyte cell count, plasma and CSF HIV RNA level, Fiebig stage, and the presence of acute retroviral syndrome (ARS), defined as ≥ 3 qualifying symptoms using a standardized checklist.

Two alterations in the LP standard operating procedure (SOP) took place during the study period. In July 2010 the spinal needle utilized was systematically changed from a cutting edge to atraumatic needle. There was a simultaneous emphasis to encourage vigorous fluid replacement through intravenous or oral route to reduce the chance of dehydration headache. Second, the standard operating procedure for CSF volume collection increased from 10ml to 20ml beginning in April 2015.

Adverse events records and grading

Adverse events including headache, backache and wound infection related to LP were collected by trained physicians and extracted from the research adverse event database. Designation as LP related was considered for symptoms with onset within 3 days after LP. All events were self-reported by participants or collected by research nurses during research follow-up visits. Treatment and hospital admission records, if applicable, were retrieved from the research clinical notes. Adverse events were graded from I to IV according to Division of AIDS (DAIDS) Table for Grading the Severity of Adults and Pediatric Adverse Events (Version 2.0, Nov 2014).

Statistical analysis

In order to minimize the selection bias that participants who experienced PLPH at week 0 were less likely to consent for repeated LP, as suggested by fewer PLPH in subjects with LP experience ($p<0.01$, OR 0.32, 95% CI 0.14-0.75), we decided to restrict our analysis to first ever LP. Results are reported as mean and standard deviation or frequencies and percentage, as appropriate. All the demographic data and clinical factors were linked to the date of procedures. Factors associated with PLPH were examined using logistic regression models that included factors of significance to p-value < 0.05 in individual univariate analyses. Statistical analyses were performed using IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.

RESULTS

During the study period, 347 participants were enrolled into the cohort and 116 participants underwent one or more LPs accounting for 195 LPs. Nearly all (94%) were contributed by male participants who had a mean age of 29 (range 18-50) years. The age and sex of cohort participants with or without LP were similar (Mean age 28.5 vs. 28.1 years, $p=0.294$ and Male 95% vs. 97% $p=0.746$, respectively). Atraumatic needles were used in 88% (169/192) of the procedures and a volume of 20ml CSF was collected in 29% (57/195). Among all enrollees with LP, 33% (64/195) had other invasive procedures on the same day. Almost 46% (n=89/195) of procedures were completed at baseline, with 66% (59/89) and 53% (47/89) taking place in participants with ARS and pre-existing headache, respectively.

Frequency of post-lumbar puncture complications

We noted PLPH in 19% (38/195) of LP with 68% (n=26) of these occurring in untreated AHI (i.e. baseline). The frequency of PLPH was 29% (26/89) at baseline and 11% (12/106) at follow up (week 24: 14% (9/63), week 48: 11% (1/9), week 96: 6% (2/31), and week 240: 0% (0/3)). Among them, 47% (18/38) were grade I (mild), 37% (14/38) were grade II (moderate) and 16% (6/38) were grade III (severe), defined as an inability to perform usual social and functional activities. All PLPH incidents

resolved after conservative management and none required epidural blood patch. There was no grade IV (life-threatening) event. We noted no cases of wound infection, and backache occurred in 8% (15/195) with 12 (80%) grade I and 3 (20%) grade II. All resolved without consequence.

Factors associated with post lumbar puncture headache

Atraumatic needle (OR=0.22, 95% CI 0.07-0.68, p=0.010) and a higher volume (20ml vs 10ml) of CSF collection (OR=0.13, 95% CI 0.03-0.57, p=0.002) was associated with reduced frequency of PLPH (Table 1). We noted no association between PLPH and age, sex or BMI (p>0.05). Neither lumbar puncture during untreated AHI (baseline, p=0.134) nor multiple invasive procedures on the day of LP (p=0.981) was associated with PLPH. In multivariate analysis, atraumatic needle (p=0.058) and a higher CSF volume collection (p=0.024) remained associated with reduced frequency of PLPH.

Analyses limited to untreated AHI

During untreated AHI, use of atraumatic needle (OR 0.25, 95%CI 0.08-0.79, p=0.024), collection of 20ml vs. 10ml of CSF (OR 0.09, 95%CI 0.01-0.68, p=0.005) and a higher BMI (OR 0.85, 95% CI 0.71-1.01, p=0.070) was associated at trend level with a reduced frequency of PLPH (Table 2). In multivariate models that included needle type and volume of CSF collected, atraumatic needle (p=0.094) and higher CSF volume collection (p=0.047) remained associated with reduced frequency of PLPH. Level of blood and CSF HIV RNA, CD4+ T-cell count, diagnosis in Fiebig stage I/II vs. III-V, presence of acute retroviral syndrome (ARS) at enrollment, pre-existing headache (from ARS) and other ARS symptoms (not shown) were not associated with PLPH (p-values > 0.05).

Additional analyses were performed to examine if the systematic protocol addition of atraumatic needle and volume of CSF collected confounded each other (Table 3). After unification of needle type and CSF volume drawn, both 20ml CSF collection (All first-ever LP, p=0.013; Baseline, p=0.028) and atraumatic needle (All first-ever LP, p=0.068; Baseline, p=0.087) remained significant or trend level associated with reduced PLPH.

DISCUSSION

In our sample of AHI participants, the overall frequency of PLPH was 19%, compared with 2-40% in the literature. None of the PLPH required invasive medical intervention and they all resolved after analgesia and intravenous fluid replacement. No cases of wound infection were observed, and local pain in the back transiently occurred in 8%.

Participant characteristics including low BMI [11, 24], female sex [25-28] and younger age [28-30] have been reported as risk factors of PLPH. This is consistent with our observation of a trend for low BMI associating with PLPH. We did not observe the effect

of sex and age in our cohort, likely owing to the restricted age range with mean below 30 years and a high percentage of male participants (97%).

In a previous report which compared PLPH incidence between chronic HIV infected individuals and healthy participants, the authors reported no increased risk in the HIV-infected group [11]. The overall PLPH frequency was 5.6% in the prior work, compared with 19% in our study. However, our study is quite different from the prior one. In the prior work, all LPs were completed with atraumatic needle, whereas a portion (12%) of our LP procedures were completed with standard cutting edge spinal needle which may contribute to PLPH. By comparing the incidence of PLPH before and after atraumatic needle usage, we showed the beneficial effect of atraumatic needle described in previous reports [9, 29].

Our study is also unique in that it is the first study to report LP safety during AHI, with nearly half of LPs performed at study baseline. The higher frequency of PLPH at baseline in comparison with subsequent time-points after cART initiation (29% vs 11%) may be related to the selection bias as discussed above. After adjusting the bias, neither AHI nor individual clinical factors during AHI correlated with PLPH development. Multiple invasive procedures on the day with LP or pre-existing headache did not increase the risk of PLPH. Nonetheless, headache developed after baseline LP may be secondary to other factors including side effect of immediate initiation of cART that included efavirenz (87/89 of baseline LP participants) and, potentially a heightened awareness of symptoms associated with anxiety in AHI [21]. Last but not least, our experience suggests research LP can be carried out with a good safety standard in a middle income setting.

The paradoxical relationship between PLPH and volume of CSF collection is unexpected. Traditionally, the orthostatic feature of intracranial hypotension headache is thought to be caused by the traction and distortion of pain-sensitive intracranial venous structures [31]. While persistent CSF leakage likely contributes to delayed PLPH, immediate PLPH probably links with an abrupt pressure change related to the CSF volume loss. The study design may have contributed to our finding. All LPs completed with the standard cutting edge withdrew only 10ml, whereas all LP taking 20ml were done with the atraumatic needle. However, we managed to demonstrate the protective effect of atraumatic needle regardless of amount drawn and lack of adverse effects with higher volume draws. To date, research LP procedures seldom collect a volume beyond 20ml [11, 24, 29]. One recent Alzheimer's disease study found that collection of a greater CSF volume was associated with higher rates of immediate post-procedural headache but trended towards lower rate of delayed PLPH at 24 hour follow-up with lower risk of therapeutic blood patch [10]. A volume below 17ml, compared to a volume > 30ml, had a significantly higher rate of follow-up headache and blood patch intervention [10]. The authors suggest the lower intracranial pressure after high volume CSF collection may

facilitate the dural closure and decrease the risk of persistent leakage [10]. As we only collected the PLPH data at 3 days post LP, we could not conclude if the reduction of headache events were mainly of the delayed onset PLPH.

Our study is limited by the fact that orthostatic characteristics of PLPH were not systematically collected during the early phase of our study. This may lead to an over-estimation of PLPH especially at baseline due to the combined effect of ARS and medications. Moreover, there could be selection bias as participants who developed PLPH after the first LP at week 0 may not have undergone further LPs at later time-points. Additionally, we did not have consistent data regarding CSF white cell count and information on CSF/serum albumin ratio were not collected, each of which could clarify the level of CNS inflammation and may correlate with PLPH development. We did not have sufficient information to analyze the number of LP attempts which may increase the risk of PLPH. Lastly, the protective effect of the atraumatic needle may be potentiated by simultaneous introduction of vigorous fluid replacement, although a protective role of fluid replacement on PLPH is controversial [32]. As route and amount of fluid replacement was not specifically captured, we are unable to analyze the effect of fluid replacement on PLPH development.

CONCLUSION

We found no additional risk in development of PLPH or other post-procedural complications during the acute phase of HIV infection as compared to prior studies of chronic HIV. We noted a protective effect from a higher BMI and the use of atraumatic needle, with an assumption of insignificant effect of fluid replacement in reducing PLPH from available literature. Finally, a higher volume of CSF collection (20mL) was not associated with a higher risk of PLPH or required medical interventions. In short, lumbar puncture can be carried out safely in a middle income research setting during acute HIV infection.

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This is a pre-copy editing, author-produced PDF of an article accepted for publication in Journal of Viral Eradication follwing peer review. The definitive published article is available online at: https://doi.org/10.1016/S2055-6640(20)30236-3

Table 1. Participants demographics and procedure related conditions

	LP without PLPH (n=86)	LP with PLPH (n=30)	Univariate OR (95%CI)	p-value	Multivariate OR (95%CI)	p-value
Age (mean (SD)) at LP	28.7 (7.05)	27.9 (7.35)	0.98 (0.93-1.05)	0.600		
Sex, n (%) Male (Ref) Female	 84 (98%) 2 (2%)	 28 (93%) 2 (7%)	 1.003.00 (0.40-22.30)	0.275		
Body mass index (mean (SD))	21.8 (3.02)	20.9 (2.56)	0.89 (0.76-1.05)	0.161		
Vol of CSF collected, n (%) 10ml (Ref) 20ml	 55 (64%) 31 (36%)	 28 (93%) 2 (7%)	 1.00 0.13 (0.03-0.57)	0.002	 0.17 (0.04-0.79)	0.024
Type of needle used[#], n (%) Cutting edge (Ref) Atraumatic	 7 (8%) 79 (92%)	 8 (29%) 20 (71%)	 1.00 0.22 (0.07-0.68)	0.010	 0.33 (0.10-1.04)	0.058
Multi-procedures on the same day, n (%) No (Ref) Yes	 60 (70%) 26 (30%)	 21 (70%) 9 (30%)	 1.00 0.99 (0.40-2.45)	0.981		
LP at baseline, n (%) No (Ref) Yes	 23 (27%) 63 (73%)	 4 (13%) 26 (87%)	 1.00 2.37 (0.75-7.54)	0.134		

[#] 3 LP records (2 at week 0) were excluded due to uncertain type of needle used

Table 2. Subgroup analysis at week 0 (n=89)

	LP without PLPH (n=63)	LP with PLPH (n=26)	Univariate OR (95% CI)	p value	Multivariate OR (95% CI)	p value
Age at LP, mean (SD)	28.9 (6.84)	27.6 (6.92)	0.97 (0.90-1.04)	0.408		
Sex, n (%) Male (Ref) Female	61 (97%) 2 (3%)	24 (92%) 2 (8%)	1.00 2.54 (0.34-19.09)	0.577		
BMI, mean (SD)	22.0 (3.15)	20.7 (2.50)	0.85 (0.71-1.01)	0.070		
CD4, mean (SD)	434 (172)	437 (178)	1.00 (0.997-1.003)	0.944		
Log_{10} blood HIV RNA, mean (SD)*	5.78 (1.08)	5.41 (1.26)	0.75 (0.50-1.12)	0.155		
CSF Log_{10} HIV RNA, mean (SD)	3.50 (1.22)	3.08 (1.19)	0.74 (0.50-1.11)	0.137		
Fiebig stage I/II, n (%) No (Ref) Yes	39 (62%) 24 (38%)	14 (54%) 12 (46%)	1.00 1.39 (0.55-3.51)	0.481		
Type of needle used, n (%) Cutting edge (Ref) Atraumatic	7 (11%) 56 (89%)	8 (33%) 16 (67%)	1.00 0.25 (0.08-0.79)	0.024	1.00 0.36 (0.11-1.19)	0.094
Vol of CSF collected, n (%) 10ml (Ref) 20ml	43 (68%) 20 (32%)	25 (96%) 1 (4%)	1.00 0.09 (0.01-0.68)	0.005	1.00 0.12(0.01-0.98)	0.047
Multi-procedures, same day, n (%) No (Ref) Yes	40 (63%) 23 (37%)	17 (65%) 9 (35%)	1.00 0.92 (0.35-2.40)	0.866		
ARS, n (%) No (ref) Yes	22 (35%) 41 (65%)	8 (31%) 18 (69%)	1.00 1.21 (0.45-3.22)	0.706		
Pre-existing headache, n (%) No (Ref) Yes	28 (44%) 35 (56%)	14 (54%) 12 (46%)	1.00 0.69 (0.27-1.72)	0.419		

[#] 2 LP records were excluded due to unknown needle used (n=87)
* 20/89 of CSF sample showed undetectable HIV RNA VL

Table 3 Non-confounding analysis on LP needle type and volume of CSF drawn on PLPH development					
		All first ever LP		Baseline only	
		OR (95% CI)	p value	OR (95% CI)	p value
LP done with atraumatic needle only	Vol of CSF drawn: 10ml (Ref) 20ml	1.00 0.17 (0.04-0.19)	0.013	1.00 0.12 (0.02-0.98)	0.028
LP with 10ml CSF drawn only	Needle used: Cutting edge (Ref) Atraumatic	1.00 0.33 (0.10-1.04)	0.068	1.00 0.365 (0.11-1.19)	0.087

Chapter 5:
Distribution of Human Immunodeficiency Virus (HIV) Ribonucleic Acid in Cerebrospinal Fluid and Blood Is Linked to CD4/CD8 Ratio During Acute HIV

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J Infect Dis. 2018 Aug 14;218(6):937-945.
doi: 10.1093/infdis/jiy260.

ABSTRACT

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

Methods: This study analyzed predictors of CSF HIV RNA and relative degree of CNS viral transmigration expressed as plasma minus CSF HIV log10 RNA (PC_{ratio}) during untreated acute HIV infection. Cerebrospinal fluid immune markers were compared between groups with different PC_{ratio}.

Results: One hundred seventeen mostly male (97%) participants in the RV254 cohort in Bangkok, Thailand, had a median age of 28 years and an estimated median 18 days duration of infection; 43 (37%) were Fiebig stages I/II. Twenty-seven (23%) had CSF HIV RNA <80 copies/mL. Those with quantifiable levels (n = 90) had median CSF HIV RNA and PCratio of 3.76 and 2.36 log10 copies/mL, respectively. Human immunodeficiency virus RNA peaked at Fiebig III in plasma and Fiebig IV in CSF. In multivariable analyses, plasma HIV RNA and CD4/CD8 ratio independently correlated with CSF HIV RNA ($P < .001$), whereas CD4/CD8 ratio predicted PC_{ratio} ($P = .018$). Participants with PC_{ratio} <1 had higher CSF neopterin, soluble (s)CD163, interleukin-6, and sCD14 levels (all $P < .05$).

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

INTRODUCTION

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

The entrance of HIV into the CNS ultimately leads to infection of resident CNS cells including microglia and astrocytes, setting the stage for the potential of HIV persistence even after antiretroviral therapy is initiated. The potential of the CNS as reservoir for HIV is supported by symptomatic CSF viral escape [7, 8] – a measurable CSF HIV RNA level with an undetectable plasma level in treated individuals presenting with neurological disease – and the isolation of CNS-specific viral strains through sequencing and phylogenetic studies [9-11]. Understanding the viral dynamics between the systemic circulation and the CNS compartment is crucial to elucidate the pathogenesis of HIV-related neurological injury and the development of CNS reservoirs. However, the literature to date is primarily based on samples of treatment-naïve, chronically HIV infected individuals, in whom CSF HIV RNA level is closely associated with that in plasma and the severity of immunodeficiency [12-15]. Reports from primary infection cohorts (within months of initial infection) suggest a similar relationship between CSF and plasma HIV RNA levels but with a larger difference between the two compartments [5, 16]. The relationship between plasma and CSF HIV RNA levels during the earliest weeks of HIV infection is unknown. Understanding these factors may allow development of strategies to effectively prevent establishment of reservoirs in the CNS and HIV related injury. We examined paired blood and CSF HIV RNA data from the SEARCH010/RV254 cohort, a prospective longitudinal study in Bangkok, Thailand, aiming to define the viral dynamics between CSF and plasma across various Fiebig stages and to identify factors associated with CSF HIV RNA level.

METHODS

Study Design and Participants

We selected participants enrolled into the SEARCH010/RV254 cohort between April 2009 and December 2016 with available CSF data. Methodology and primary goals of this cohort study have been described elsewhere [3, 4]. Briefly, these individuals were enrolled through voluntary HIV counseling and testing services offered in Bangkok and

Pattaya, Thailand, where specimens were systematically screened for acute HIV infection. Enrolled participants were classified into Fiebig stages I to V defined by a hierarchical algorithm from pooled nucleic acid testing, sequential immunoassay, p24 antigen, and Western Blot testing [17]: Fiebig I: RNA+, p24 antigen-; Fiebig II: p24 antigen+, IgM-; Fiebig III: IgM+, Western Blot-; Fiebig IV: Western Blot indeterminate; Fiebig V: Western Blot+ without p31 protein band [17]. All participants were assessed at acute infection, immediately initiated combination antiretroviral therapy (cART), and were then followed longitudinally for up to 10 years. The current analysis focused on baseline, cross sectional pre-cART data. Optional lumbar punctures (LP) were typically performed within 48 hours of acute HIV infection confirmation. All participants provided written informed consent. The study protocol was approved by the institutional review boards of all relevant collaborating institutions.

Clinical Data and Laboratory Measures

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

CSF Soluble marker analysis

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

Statistical Analysis

HIV-1 RNA levels were transformed to log10 for analysis. The viral dynamic between plasma and CSF compartments was represented by the plasma to CSF viral load ratio (PC_{ratio}), calculated as log10 (plasma HIV RNA level/CSF HIV RNA level) and mathematically equivalent to log10 plasma HIV RNA - log10 CSF HIV RNA. A PC_{ratio} less than one indicates the CSF HIV RNA level is greater than one tenth of that in the plasma, a frequently cited reference in untreated chronic infection. Results were reported as median and interquartile range (IQR) or frequency and percentage, as appropriate. Fisher's Exact test and the non-parametric Mann-Whitney U test were used to compare categorical and continuous variables, respectively. Factors associated with CSF HIV RNA level and PC_{ratio} were examined using a linear regression model. Factors with a p-value < 0.10 in univariate analysis were included in the subsequent multivariable analysis using the backward stepwise method. Statistical analyses were performed using SPSS Version 21.0 (IBM Corp., Armonk, NY).

RESULTS

Characteristics of Study Participants

During the sampling period, 430 individuals were enrolled into SEARCH010/RV254. Among these, 117 (27%) underwent baseline LP. The LP participants had similar demographic (age, sex) and HIV infection parameters (plasma HIV RNA level, Fiebig stages and percentage of acute retroviral syndrome presentation) with the rest of the cohort (p>0.05). They were predominantly male (97%) with a median age of 28 years. Nineteen (16%) participants were identified in Fiebig I, 24 (21%) in Fiebig II, 52 (44%) in Fiebig III, 15 (13%) in Fiebig IV and 7 (6%) in Fiebig V. Median estimated days from HIV transmission to study enrollment was 18 (IQR 14-23). Most of them were infected with CRF01_AE subtype (83%), followed by CRF01_AE/B (7%), B (4%), CRF01_AE/B/C (1%) and non-typable (7%). All participants initiated cART after the LP except ten who started treatment before the procedure (median 2 days, range 1-6 days). One participant was on pre-exposure prophylaxis before the acute HIV infection diagnosis. All of these participants were included in the following analyses as sensitivity analyses excluding these participants yielded similar findings.

CSF HIV RNA Levels in Acute HIV Infection

Among the 117 CSF samples, 90 (77%) had quantifiable HIV RNA, 18 (15%) had undetectable HIV RNA, and 9 (8%) had detectable HIV RNA but an unquantifiable level. CSF and plasma viral loads with respect to days after estimated HIV exposure were shown in Figure 1. Overall, the CSF HIV RNA measurable rate was 51% (22/43) among early enrollees (Fiebig I & II) and 92% (68/74) for participants enrolling during later Fiebig stages (Fiebig III to V). Table 1 shows the demographic and laboratory data of participants grouped according to the CSF HIV RNA quantification status. The group with quantifiable CSF HIV RNA levels presented more often at Fiebig III or later

(p<0.001) and had lower CD4+ lymphocyte count (p=0.002), higher CD8+ lymphocyte count (p=0.009), higher plasma HIV RNA level (p<0.001), and more frequent acute retroviral syndrome presentation (p=0.001). Among those with quantifiable CSF HIV RNA levels, the median PCratio was 2.36 Log10 copies/ml and ranged from 0.10 to 4.40 Log10 copies/ml.

Plasma & CSF HIV RNA level across Fiebig Stages

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

Determinants of CSF HIV RNA level at Acute HIV Infection

Univariate and multivariable linear regression models were applied to identify factors associated with baseline CSF HIV RNA level. Factors that had a p value below 0.10 in univariate analysis were included in the multivariable analysis using backward stepwise calculation (Table 2). In the univariate analysis, higher plasma HIV RNA level, higher CD8+ T-lymphocytes level, lower CD4+ T-lymphocytes level, lower CD4/CD8 ratio, later Fiebig stage classification (III and later) and presentation of acute retroviral syndrome were associated with a higher CSF HIV RNA level (all p<0.05). In the multivariable model, only plasma HIV RNA level and CD4/CD8 ratio independently correlated with CSF HIV RNA level (both p<0.001) with adjusted β coefficients of 0.604 and -0.616 respectively.

Variability of Plasma-CSF HIV RNA level Difference and Its Determinant

The CSF vs. plasma HIV RNA level distribution color-coded by Fiebig stage is shown in Figure 3. Dotted lines of 1-3 log PCratio were added for reference. CSF HIV RNA level, as shown in the regression model, positively correlates with the plasma HIV RNA level. Among the 27 individuals who had a CSF HIV RNA level below the level of detection, half (n=14) presented at Fiebig stage I, seven at Fiebig stage II and the remainder from Fiebig stage III to V. Moreover, a quarter (n=7) had a relatively high plasma HIV RNA level above 5 log10 despite the unmeasurable CSF HIV RNA level.

Another seven individuals had a high level of HIV RNA in CSF with respect to the level in plasma, resulting in a PCratio below 1 log. This group of outliers (PCratio <1) was not dominated by late enrollees (three at Fiebig II; two at Fiebig III; one at Fiebig IV; and one at Fiebig V) and they were infected by a similar HIV subtype as others (six by CRF01_AE and one by recombinant subtype CRF01_AE/B). There were no differences between the outliers and the rest of group with quantifiable CSF HIV RNA in plasma HIV RNA level (p=0.224), rate of acute retroviral syndrome presentation (p=0.889) and duration of infection according to Fiebig staging (p=0.377).

Linear regression modeling was performed to evaluate the potential factors associated with PCratio across the entire group (Table 3). In univariate analyses, only CD8 lymphocyte count and CD4/CD8 ratio achieved a p value < 0.05. In the multivariable analysis, CD4/CD8 ratio was the only significant factor, showing a positive correlation (p=0.018, adjusted β coefficient = 0.380).

Comparison of CSF immune activation markers according to CSF HIV detection and PC_{ratio} status

We compared the levels of the six CSF biomarkers of immune activation (neopterin, sCD14, IL-6, CXCL10, CCL2, sCD163) in a subgroup of LP participants categorized by CSF HIV RNA and PCratio: CSF HIV RNA < 80 copies/ml (lower limit of quantification) vs. CSF HIV RNA level ≥ 80 copies/ml with PCratio > 1 vs. CSF HIV RNA level > 80 copies/ml with $PC_{ratio} \leq 1$ (outliers) (Figure 4). In general, all CSF immune activation markers increased with quantifiable CSF HIV RNA and they were highest in the outlier group. In particular, the group of participants with CSF HIV RNA below quantification had statistically lower levels of all CSF cytokines except sCD14 (all p<0.05). The outlier group, despite having similar Fiebig staging composition and plasma HIV RNA level to the group with $PC_{ratio} > 1$, showed a significantly higher CSF neopterin, sCD163, IL-6 and sCD14 (all p<0.05).

Discussion

We have previously reported a high rate of CSF HIV RNA detection (15/18) in study participants with acute HIV infection, with detectable CSF HIV RNA as early as 8 days after estimated infection [3]. In the current analysis, the rate of HIV RNA above the lower limit of quantification in CSF was 51% at Fiebig I and II and 92% at Fiebig III to V. Our findings provide additional evidence that HIV can invade the CNS at the earliest phase of infection. In addition, our assessment of immune activation biomarkers indicates that HIV invasion is not a silent event and is associated with an extensive CNS immunologic response.

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

Determinants of CSF HIV RNA Level

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

While the exact mechanism of HIV CNS entry has not been elucidated, pathways such as free virus entry or a cell-mediated cascade have been hypothesized [25]. The cell-mediated, or so-called "Trojan horse" mechanism, is the most accepted theory, in which HIV is transported across the BBB by infected CD4+ lymphocytes and monocytes. After seeding of the intracerebral perivascular space, local CNS cells including microglia, tissue macrophages, and possibly astrocytes, are infected [26, 27]. The positive correlation between plasma and CSF HIV RNA level in the linear regression model would support either free virus or a cell-mediated cascade hypotheses.

Predictors of Viral Penetration into CNS

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

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

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

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

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

CD4/CD8, PC_{ratio} and the development of cognitive impairment.

Limitations

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

CONCLUSION

We found that the CSF HIV RNA level correlates with that in plasma at acute HIV infection. However, PC_{ratio} is higher in acute than in chronic HIV infection, and is highly variable. The CSF HIV RNA level peaked at Fiebig stage IV, slightly later than the height of activation of HIV-specific blood cytotoxic T cells during Fiebig stage III. We also found that the degree of viral penetration into the CSF as compared to blood could be predicted by CD4/CD8 ratio. Given a similar correlation to cognitive impairment in chronic infection, both PC_{ratio} and CD4/CD8 ratio may be important markers for clinically significant neurologic outcomes in both acute and chronic infection.

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This is a pre-copy editing, author-produced PDF of an article accepted for publication in the *Journal of Infectious Diseases* follwing peer review. The definitive published article is available online at: https://doi.org/10.1093/infdis/jiy260

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

Characteristic	< LOQ (n=27)	≥ LOQ (n=90)	P value
Male, n (%)	26 (96)	87 (97)	1.00
Age (year)	26.0 (23 – 31)	27.5 (23 – 32)	0.902
CD4 T lymphocytes (cells/ul)	555 (354 - 699)	384 (280-506)	0.002
CD8 T lymphocytes (cells/ul)	442 (238 -586)	624 (366-1013)	0.009
CD4/CD8 ratio	1.12 (0.59 – 1.57)	0.68 (0.38 – 0.95)	<0.001
Fiebig Stage **I & II, n (%)** **III to V, n (%)**	 21 (78) 6 (22)	 22 (24) 68 (76)	 <0.001
Plasma HIV RNA (Log_{10} copies/ml)	4.23 (0.92)	6.14 (5.48-6.83)	<0.001
CSF HIV RNA (Log_{10} copies/ml)	1.90 (0.0)[#]	3.76 (2.81-4.82)	<0.001
Plasma-CSF HIV RNA Ratio (PC_{ratio}) (range, Log_{10} copies/ml)[&]	-	2.36 (0.10-4.40)	
Presence of ARS, n (%)	14 (52)	77 (86)	0.001
Active syphilis, n (%)*	2 (7)	6 (7)	1.00

Results shown are presented as median (Interquartile Range) unless specified.
*Defined as positive Treponema pallidum hemagglutination (TPHA) and RPR/VDRL, with no treatment 3 month prior the acute HIV infection diagnosis. [#] A level of 80 copies/ml was assigned to those who had a CSF HIV RNA level below quantification. [&] Plasma-CSF HIV RNA Ratio (PC_{ratio}) = log_{10} plasma HIV RNA minus log_{10} CSF HIV RNA
Abbreviations: LOQ = Lower limit of quantification (80 copies/ml); ARS = Acute retroviral syndrome.

Table 2. Linear Regression Analysis on CSF HIV RNA level (n=117).

	Univariate analysis		Multivariable analysis	
	β coefficient (95% CI)	P value	Adjusted β Coefficient (95%CI)	P value
Age, (years)	-0.001 (-0.031 to 0.028)	0.93		
Sex				
Male	Ref			
Female	0.922 (-0.266 to 2.111)	0.127		
Plasma HIV RNA (Log_{10} copies/ml)	0.685 (0.547 to 0.823)	<0.001	0.604 (0.470 to 0.738)	<0.001
CD4 (per 100cells/µL)	-0.273 (-0.385 to -0.161)	<0.001		NS
CD8 (per 100 cells/µL)	0.040 (0.006 to 0.074)	0.022		NS
CD4/CD8	-0.977 (-1.334 to -0.620)	<0.001	-0.616 (-0.903 to -0.330)	<0.001
Fiebig Stage				
III and later	Ref			
I & II	-1.238 (-1.629 to -0.848)	<0.001		NS
ARS				
No	Ref			
Yes	1.047 (0.559 to 1.535)	<0.001		NS
Active syphilis*				
No	Ref			
Yes	0.315 (-0.548 to 1.177)	0.471		

A level of 80 copies/ml was assigned to those who had a CSF HIV RNA level below quantification. Factors with $p < 0.1$ in the univariate analysis were included into the multivariate analysis.

*Defined as positive Treponema pallidum hemagglutination (TPHA) and RPR/VDRL, with no treatment 3 month prior the acute HIV infection diagnosis. Abbreviation: ARS = Acute retroviral syndrome.

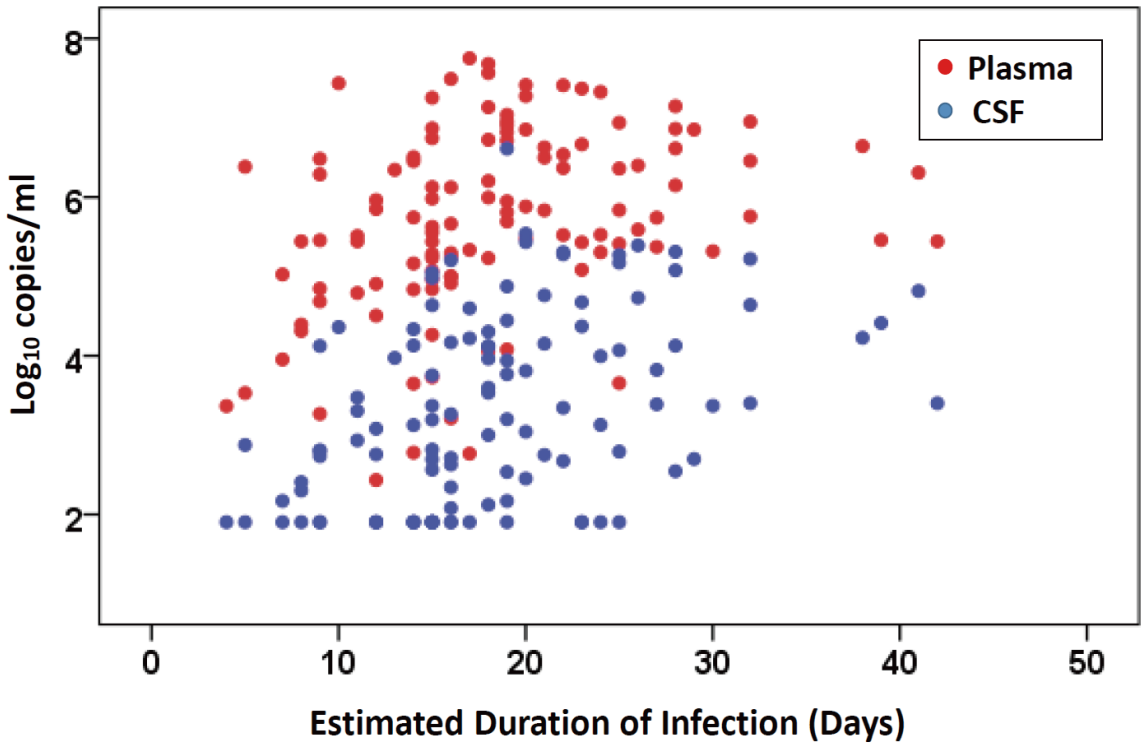
Table 3. Linear Regression Analysis on Plasma CSF HIV RNA Ratio (PC_{ratio}) (N=117)

	Univariate analysis		Multivariable analysis	
	β coefficient (95% CI)	P value	Adjusted β Coefficient (95% CI)	P value
Age	0.012 (-0.012 to 0.035)	0.320		
Sex				
Male	Ref			
Female	-0.791 (-1.741 to 0.159)	0.102		
CD4 (per 100 cells/µL)	-0.064 (-0.162 to 0.034)	0.198		
CD8 (per 100 cells/µL)	-0.029 (-0.056 to -0.001)	0.040		NS
CD4/CD8	0.380 (0.068 to 0.692)	0.018	0.380 (0.068 to 0.692)	0.018
Fiebig Stage				
III and later	Ref			
I & II	0.128 (-0.233 to 0.490)	0.483		
ARS presentation				
No	Ref			
Yes	0.320 (-0.096 to 0.736)	0.130		
Active syphilis*				
No	Ref			
Yes	-0.170 (-0.861 to 0.521)	0.627		

A level of 80 copies/ml was assigned to those who had a CSF HIV RNA level below quantification. Factors with $p < 0.1$ in the univariate analysis were included into the multivariate analysis.

*Defined as positive Treponema pallidum hemagglutination (TPHA) and RPR/VDRL, with no treatment 3 month prior the acute HIV infection diagnosis. Abbreviation: ARS = Acute retroviral syndrome.

Figure 1. CSF and plasma HIV RNA levels with respect to days after estimated HIV exposure.



A level of 80 copies/ml was assigned to those who had a CSF HIV RNA level below quantification.

Figure 2. HIV RNA level by Fiebig stage (a) Plasma & (b) CSF.

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

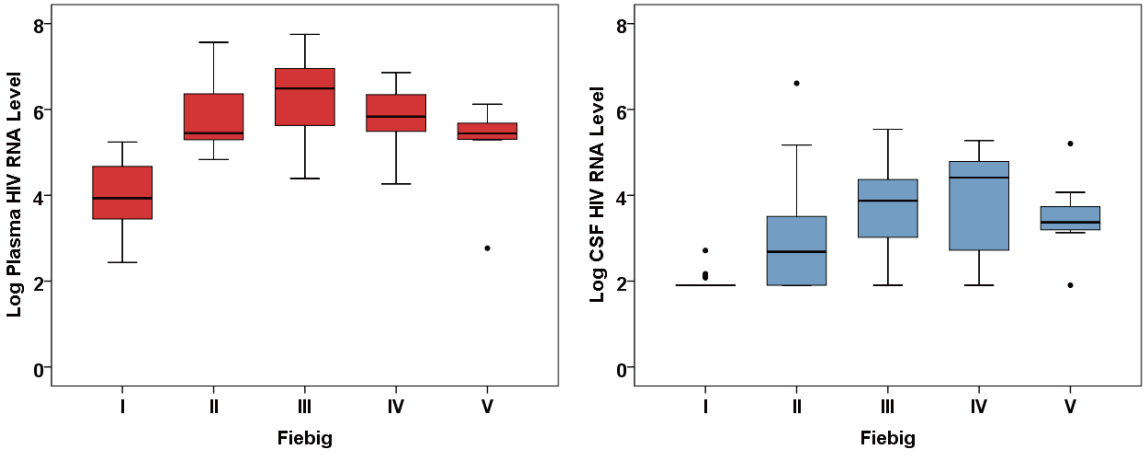


Figure 3. Correlation between CSF and plasma HIV RNA level by Fiebig staging.

A level of 80 copies/ml was assigned to the CSF samples with HIV RNA level below quantification. Correlation coefficient = 0.719; $p < 0.001$ (Spearman).
PC_{ratio} = log10 plasma HIV RNA minus log10 CSF HIV RNA

Abbreviation: PC_{ratio} = Plasma-CSF HIV RNA ratio.

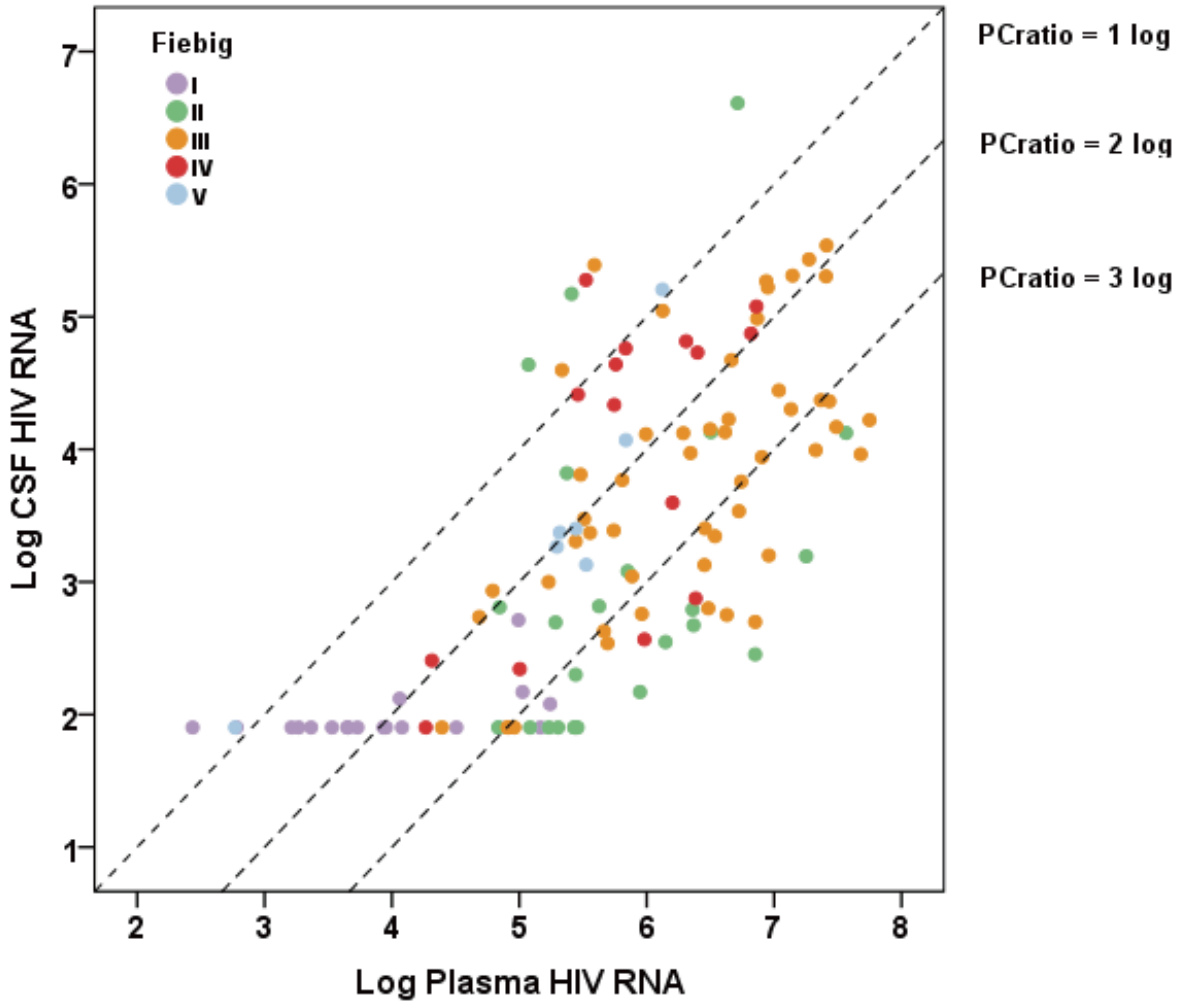
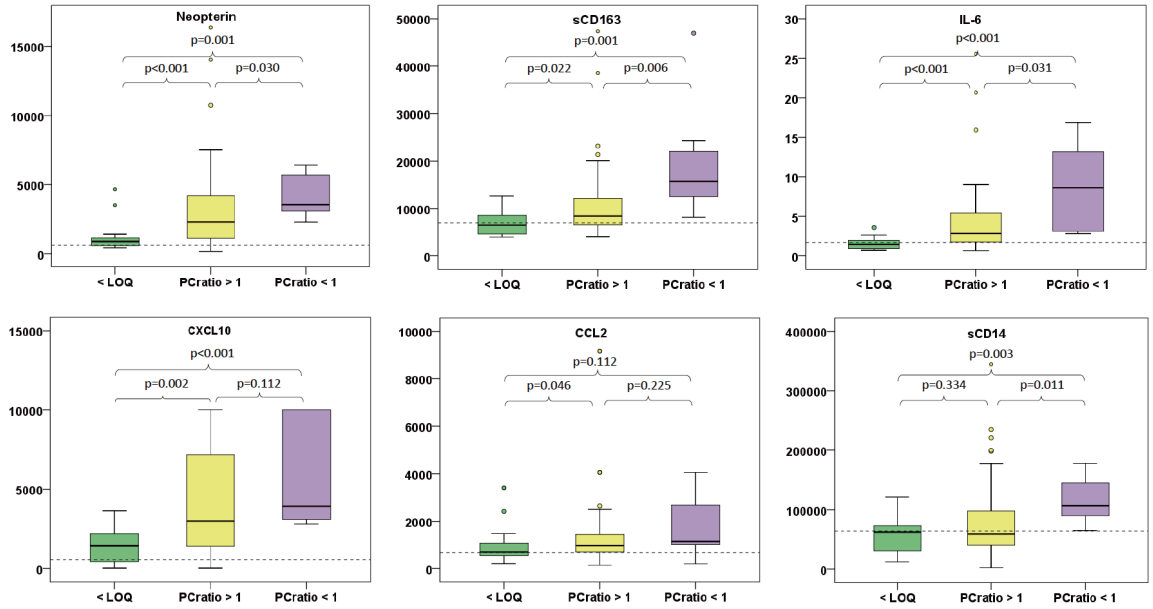


Figure 4. Levels of CSF immune and inflammatory markers in participants grouped by CSF HIV RNA quantification and PCratio status:



CSF HIV RNA below the lower limit of quantification (<LOQ), F quantifiable CSF HIV RNA with PC_{ratio} > 1 (PC_{ratio} > 1), quantifiable CSF HIV RNA with PC_{ratio} < 1 (PC_{ratio} < 1). Median (middle line), interquartile range (extent of boxes), 1.5 times of interquartile range (whiskers) and outliers/extreme outliers (colored dots) are indicated. P value of non-parametric test between groups are shown. Dotted horizontal line: Median level of 22 unmatched HIV negative Thai controls. All markers level are presented in pg/mL. PC_{ratio} = log10 plasma HIV RNA minus log10 CSF HIV RNA

Abbreviation: PC_{ratio} = Plasma-CSF HIV RNA ratio.

Chapter 6:
Neurosyphilis during Acute HIV Infection: A CNS Immunologic and Virologic Characterization (Case Report)

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J Acquir Immune Defic Syndr. 2019 Oct 1;82(2):e34-e37.
doi: 10.1097/QAI.0000000000002114.

BACKGROUND

Syphilis is a re-emerging sexually transmitted infection (STI) caused by the spirochete Treponema pallidum. Syphilis and HIV-1 infection are known to impact each other's course, and both invade the central nervous system (CNS) within days after infection [1, 2]. During chronic HIV-1 infection, incident syphilis leads to decline in CD4+ T-lymphocytes and elevated HIV-1 RNA levels in blood [3]. Advanced HIV-1 infection may increase the risk of neurosyphilis [4] and syphilis treatment failure [5]. While a simultaneous diagnosis of HIV-1 and syphilis infection is not uncommon, neurosyphilis diagnosed at the time of acute HIV-1 infection (AHI), and its corresponding features in cerebrospinal fluid (CSF) and brain imaging have not been described. Here, we report an individual who presented with meningeal syphilis at AHI.

Case presentation

A 21-year-old Thai man presented for HIV screening at an anonymous testing center in Bangkok where routine evaluation for AHI is performed [1]. He was diagnosed with Fiebig IV AHI (HIV-1 RNA+, anti-HIV IgM+, Western Blot indeterminate), and consented to enroll into the RV254/SEARCH010 AHI cohort (NCT00796146). He reported multiple sexual encounters with male partners including anal sex without use of condoms within 60 days prior to HIV testing. Acute retroviral syndrome (ARS), with symptoms including fever, headache, malaise, pharyngitis and oral ulcer, started approximately a week before the HIV diagnosis.

His past medical history was unremarkable except for a single episode of childhood seizure of unknown origin. He worked as a sex worker. He was a chronic smoker and daily drinker with occasional use of methamphetamine, but he denied intravenous drug use. Five months before the AHI, his blood rapid plasma reagin (RPR) was 1:32 and he received a single intramuscular injection of penicillin-G.

Cardiovascular, respiratory, abdominal and neurological examinations were unremarkable. There was bilateral tender inguinal lymphadenopathy without penile lesion. Initial laboratory tests showed normal complete blood count, kidney function and clotting profile. Liver enzymes (ALT, AST, GGT) were mildly elevated. The plasma HIV-1 RNA level was 5.04 Log10 copies/ml, CD4+ and CD8+ T-lymphocyte counts were 236 and 370 cells/μl, respectively. STI screening revealed anal chlamydia and gonorrhea, hepatitis C (HCV) infection with HCV RNA 4.47 log10 copies/ml; RPR titer in the local laboratory rose from 1:32 five months ago to 1:128 while plasma venereal disease research laboratory (VDRL) titer was 1:64 (Table 1). Given the possibility of syphilis treatment failure and the fact that late syphilis could not be ruled out based on the available history, 3 once-weekly intramuscular penicillin-G injections (2.4 MIU) were given. Oral azithromycin and intramuscular ceftriaxone were prescribed for chlamydia and gonorrhea infection.

He underwent lumbar puncture (LP) for research purposes, followed by immediate initiation of antiretroviral therapy (ART) consisting of dolutegravir, abacavir and lamivudine. CSF examination revealed a lymphocytic pleocytosis of 105 cells/mm3 with 99% mononucleated cells. CSF protein and glucose levels were normal, and CSF-VDRL was non-reactive. Magnetic resonance imaging (MRI) of the brain (Figure 1 Top) demonstrated hyperintense signals along the sulci of the bilateral occipital and high frontal regions on axial T2-FLAIR (fluid-attenuated inversion recovery) images with fat suppression, suspicious of underlying leptomeningitis. A stored CSF sample was positive for TPHA (Treponema pallidum hemagglutination assay). Consistent with the diagnostic criteria of neurosyphilis in the US CDC guideline for HIV-infected individuals, he was treated with intravenous penicillin G injection, 3 mega-units every 4 hours for 14 days.

His ARS symptoms resolved soon after ART, before penicillin treatment. The plasma HIV-1 RNA was once suppressed (<20copies/ml) at 12 weeks with a CD4+ T-lymphocyte count of 565 cells/μl. Subsequently, he stopped taking ART at 20 weeks for fear of disclosure after moving in with a new partner and presented to us at 24 weeks having an HIV-1 RNA level of 5.44 log10 copies/ml and CD4+ T-lymphocyte count of 443 cells/μl. His blood VDRL titer dropped from 1:64 at baseline to 1:8. Repeat neurological examination was unremarkable. Repeat LP showed incomplete improvement of CSF pleocytosis of 10 cells/mm3 with 100% mononuclear cells. CSF HIV-1 RNA was 3.84 log10 copies/ml. Repeat brain MRI using the same scanner and scanning parameters demonstrated mostly resolved leptomeningeal abnormalities (Figure 1 Bottom) on axial T2-FLAIR images with fat suppression.

Given his unique presentation, his CSF and peripheral blood mononuclear cell (PBMC) samples at baseline and 24 weeks were sent for flow cytometry to examine for neurosyphilis-associated CSF B-lymphocytes pleocytosis [6]. In general, the CSF CD19+ B/CD3+ T lymphocyte ratio (B/T ratio) is less than 0.1 as T-lymphocyte activation is the predominant immune response at AHI [7]. At baseline, 57% of lymphocytes in CSF were CD19+ B-lymphocytes, with a B/T ratio of 1.42. Such B-lymphocyte expansion was compartmentalized in CSF but not in the patient's PBMC sample, in which only 15% of lymphocytes were CD19+ B-lymphocytes and the B/T ratio was 0.25. At 24 weeks, the frequency of CD19+ B-lymphocytes in CSF dropped from 57% to 4%, with a corresponding B/T ratio of 0.06 (Table 1). Together with the improvement in imaging, plasma VDRL and CSF pleocytosis, the findings supported the resolution of neurosyphilis.

DISCUSSION

We describe MRI imaging findings and an unusual pattern of proportions of CSF cellular subsets that we posit are due to a combination of AHI and neurosyphilis. Both HIV-1 and T pallidum can invade the CNS and cause CSF pleocytosis [2], however, AHI mono-infection seldom causes CSF pleocytosis greater than 20 cells/ul (unpublished

observation). Our group has examined CSF samples and brain imaging from more than 100 individuals with untreated AHI, but this is our first encounter of a grossly elevated CSF white cell count (WCC) accompanied by leptomeningeal abnormalities on neuroimaging. Leptomeningitis is a common manifestation of neurosyphilis, presenting as stand-alone meningitis, or in combination with vascular and cranial nerve complications. Our patient lacked overt symptoms but had CSF and neuroimaging abnormalities consistent with meningeal syphilis.

Elevated CSF protein levels and CSF pleocytosis occur in up to 70% of neurosyphilis cases. CSF pleocytosis is more pronounced in HIV-infected individuals who present with neurosyphilis [8]. In particular, CSF pleocytosis in HIV-1 infection is likely linked to CSF HIV-1 RNA levels rather than the severity of the immunocompromised state or to CD4+ T-lymphocyte count [9]. Current recommendations suggests a cut-off of CSF WCC > 5 cells/mm3 to be used as diagnostic criteria for neurosyphilis in HIV-infected individuals on ART with undetectable plasma HIV-1 RNA, whereas a cut-off of CSF WCC > 20 cells/mm3 is recommended for viremic individuals [10].

CSF-VDRL is currently considered the gold standard for diagnosis of neurosyphilis. CSF-VDRL is highly specific, but its sensitivity is reported to be between 30-70% [10]. In contrast, CSF Treponemal specific tests such as CSF-TPHA and CSF-TPPA (Treponemal pallidum particle agglutination) are sensitive but less specific [10]. Recent studies suggest the implementation of a titer cut-off in CSF-TPHA and CSF-TPPA tests to improve their specificity [11, 12]. CSF CXCL-13 (C-X-C Motif Chemokine Ligand 13), a B cell chemoattractant, has also been proposed to improve the diagnostic yield as the CSF usually contains a high concentration of B lymphocytes in neurosyphilis [6].

Our participant's virologic profile was not typical of AHI mono-infection. His CSF HIV-1 RNA level was high with respect to that in plasma. His plasma-CSF HIV-1 RNA ratio (PCratio), mathematically equivalent to log10 plasma HIV-1 RNA minus log10 CSF HIV-1 RNA, was 0.81 log10 copies/ml. In general, the PCratio is around 2 log10 within first year of HIV-1 infection [1, 13], and around 1 log10 in chronic infection. Given the lack of a well-established CNS HIV-1 reservoir at AHI, a reduced PCratio suggests an increased HIV-1 CNS transmigration from the systemic circulation.

We previously reported a reduced PCratio (< 1 log10) in around 7% of individuals at AHI [13]. Reduced PCratio was not associated with systemic syphilis but the effect of neurosyphilis on CSF HIV-1 RNA was unclear. We speculate that the "improvement" of PCratio from 0.81 log10 copies/ml at baseline to 1.60 log10 copies/ml after neurosyphilis treatment was due to eradication of intrathecal T pallidum, which facilitates not only viral replication in chronic HIV-1 infection but also CNS invasion in AHI.

There are growing concerns about the effect of syphilis on HIV-1 infection. While individuals with neurosyphilis showed higher levels of CSF chemokines and systemic monocyte activation than systemic syphilis alone [14], whether neurosyphilis, or prior systemic syphilis, links to a lower cognitive performance is inconclusive [14, 15]. Without clear biomarkers for the duration of syphilis infection, we are unable to determine if our participant acquired the syphilis concurrently with or before the HIV-1 transmission. However, our patient's presentation may indicate a facilitating role of neurosyphilis in accelerating HIV-1 CNS invasion at AHI, which may hasten the establishment of CNS compartmentalization and eventually the development of neurocognitive complications in HIV-1 infection.

CONCLUSIONS

Our case illustrates that neurosyphilis at AHI may closely resemble the radiological and CSF patterns seen in chronic HIV-1 infection. Future research should examine the role of syphilis, and particularly neurosyphilis, in early HIV-1 infection to determine if it alters disease progression.

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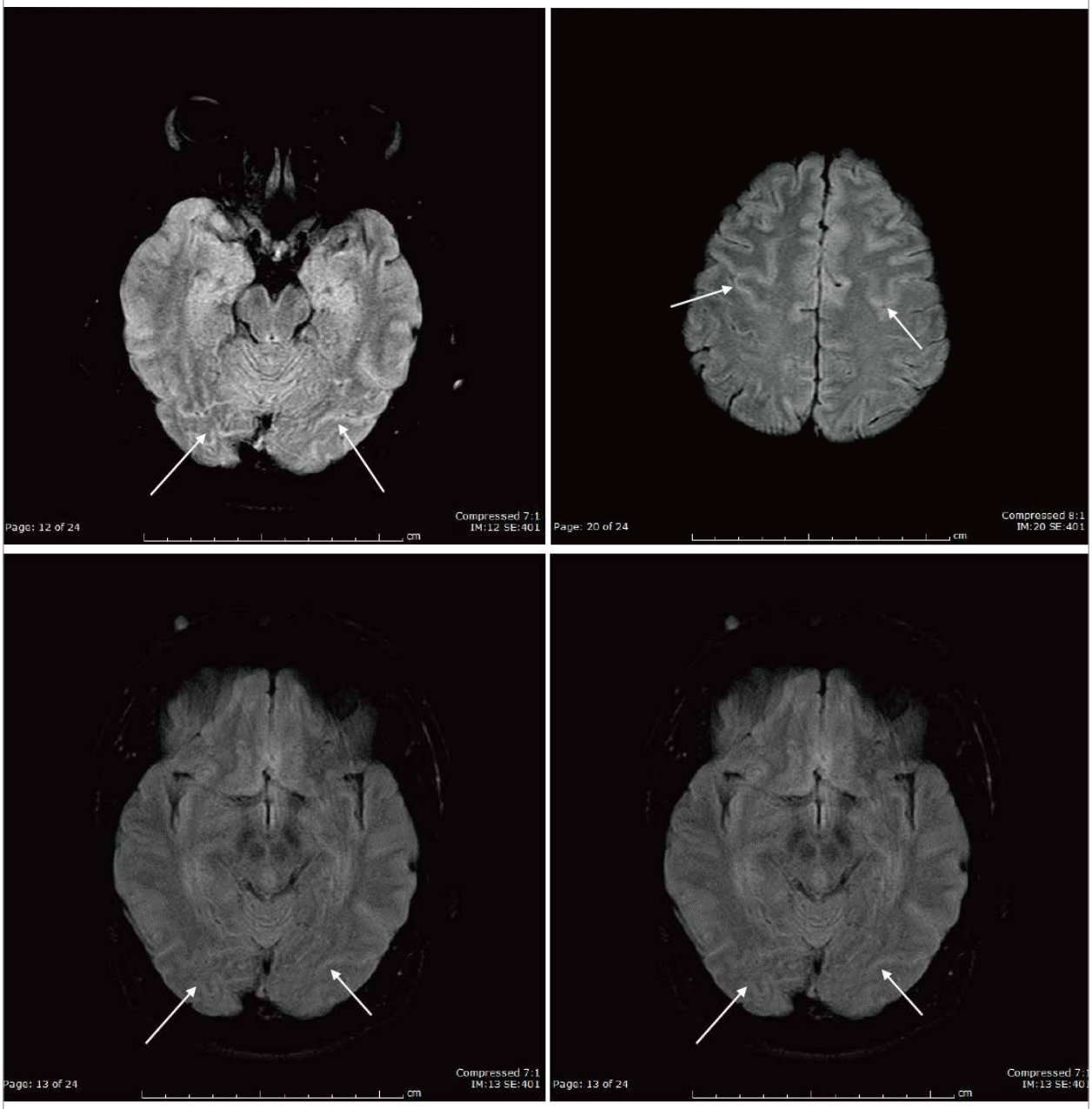
This is a pre-copy editing, author-produced PDF of an article accepted for publication in *Journal of Acquired Immune Deficiency Syndromes* follwing peer review. The definitive published article is available online at:
https://doi: 10.1097/QAI.0000000000002114

Table 1. Laboratory parameters at acute HIV infection and 24 weeks follow-up

	Acute HIV Infection	24-week Follow-up
CD4+ T-lymphocytes, cells/µl	236	443
CD8+ T-lymphocytes, cells/µl	370	1258
CD4/CD8 ratio	0.64	0.352
Plasma HIV-1 RNA, log_{10} copies/ml	5.04	5.44
CSF HIV-1 RNA, log_{10} copies/ml	4.23	3.84
***Plasma to CSF HIV-1 RNA ratio**	0.81	1.60
CSF white cell count, cells/mm^3	105	10
Polymorphs (%)	1	0
Mononuclear cells (%)	99	100
CSF glucose, mg/dL	62.0	64.0
CSF protein, mg/dL	27.9	34.0
Serum VDRL	1:64	1:8
CSF VDRL	Non-reactive	Non-reactive
Flow Cytometry (CSF)		
Frequency of CD19+ B-lymphocytes (%)	56.8	3.6
B/T Ratio	1.42	0.06
Flow Cytometry (PBMC)		
Frequency of CD19+ B-lymphocytes (%)	15.1	11.3
B/T Ratio	0.25	0.15

*Plasma to CSF HIV-1 RNA ratio = Log10 plasma HIV-1 RNA – log10 CSF HIV-1 RNA
Abbreviations: CSF = Cerebrospinal Fluid; B/T ratio = CD19+ B/CD3+ T cell ratio; VDRL = Venereal disease research laboratory

Figure 1.



MRI of the brain (Axial T2-weighted fluid-attenuated inversion recovery (FLAIR) with fat suppression) at acute HIV infection (Top) and 24 weeks (Bottom).
Arrows: Hyperintense signals along the sulci of the bilateral occipital and high frontal regions, suggestive of underlying leptomeningitis.

Chapter 7:
Clinical and Laboratory Impact of Concomitant Syphilis Infection during Acute HIV

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ABSTRACT

Introduction: Cognitive impairment has been reported in people living with HIV-1 (PLWH) with prior syphilis, while PLWH who present with incident syphilis have reduced blood CD4+ T-lymphocyte and elevated HIV-1 RNA levels. However, the clinical, virologic and neurocognitive effects of syphilis during acute HIV-1 (AHI) remain unknown.

Methods: Pre-antiretroviral therapy laboratory outcomes and neurocognitive performance in a 4-test battery in the SEARCH10/RV254 AHI cohort were compared according to syphilis status, determined by serum Treponema pallidum hemagglutination (TPHA), venereal disease research laboratory (VDRL) and syphilis treatment history. Impaired cognitive performance was defined as having z-scores ≤-1 in at least two tests or ≤-2 in at least one test.

Results: Of 595 AHI participants (97% male, median age of 26 years and estimated duration of HIV-1 infection of 19 days), 119 (20%) had history of syphilis (TPHA+), of whom 51 (9%) had untreated syphilis (TPHA+/VDRL+/without prior treatment). Compared to those without syphilis (TPHA-), individuals with untreated syphilis had higher CD8+ T-lymphocyte levels but not higher plasma HIV-1 RNA or lower CD4+ T-lymphocyte levels. Taking into account estimated duration of HIV-1 infection ($p<0.001$), and later Fiebig stages (III-V) ($p<0.001$), those with untreated syphilis had higher CD8+ T-lymphocyte levels ($p=0.049$). Individuals with any syphilis (TPHA+), but not untreated syphilis, had increased odds of impaired cognitive performance than those without ($p=0.002$).

Conclusions: During AHI, individuals with any history of syphilis (TPHA+) had poorer cognitive performance than those without syphilis. However, syphilis did not associate with worsened HIV disease measures as described in chronic infection.

INTRODUCTION

Chronic HIV-1 infection impacts the course of Treponema pallidum infection (syphilis), and syphilis affects laboratory and clinical parameters in people living with HIV-1 (PLWH). Similar to other sexually transmitted infections (STIs), syphilis is associated with an increased risk of acquisition of HIV-1 infection [1, 2]. Syphilis can potentiate the risk of HIV-1 transmission through mechanisms such as local mucosal disruption and an increase in CCR5 expression in macrophages [3]. Incident syphilis is associated with a decline in CD4+ T-lymphocytes and an elevation of HIV-1 RNA level in PLWH [4-6]. Moreover, PWLH experience a higher rate of syphilis treatment failure compared to individuals without HIV-1 [7-9]. While both pathogens invade the central nervous system (CNS) within days after initial infection [10, 11], neurosyphilis, defined as at least one of the two criteria including reactive cerebrospinal fluid (CSF) - Venereal Disease Research Laboratory (VDRL) test and CSF pleocytosis, may be more common among PLWH [12, 13]. An association between prior syphilis and poorer cognitive performance in chronic HIV-1 infection in the absence of overt neurosyphilis have been inconsistently reported in different studies [14-16]. In a recent study of participants with HIV-1 with reactive blood rapid plasma regain (RPR), neurosyphilis was not directly associated with cognitive impairment. However, those with neurosyphilis had higher levels of CSF inflammatory biomarkers [17], demonstrating its potential role in exacerbating chronic intracerebral immune activation in HIV-1 infection [18].

To date, most HIV-1/syphilis co-infection studies are based in populations with chronic HIV-1 infection and syphilis defined by a positive Treponemal-specific test, in which the durations of both infections are largely unknown. Further, participants in these prior studies have been inconsistently treated with ART with variable degrees of immunodeficiency at incident syphilis. No existing study addresses whether pre-existing or co-acquired syphilis exert negative effects at early stages of HIV-1 infection. Given the global resurgence of syphilis and increasing prevalence of HIV-1/syphilis co-infection [19], understanding whether syphilis could augment early HIV-1 replication and immune abnormalities and thus potentially alter the progress of HIV-1 disease is essential. This study aims to examine the effect of syphilis on acute HIV-1 infection (AHI) by evaluating clinical, virologic and neurocognitive parameters in the SEARCH010/RV254 AHI cohort in Bangkok, Thailand.

METHODS

Study Design and Participants

Participants included all 595 individuals enrolled into the SEARCH010/RV254 cohort (NCT00796146) between April 2009 and March 2019. The methodology and primary goals of the cohort study have been described elsewhere [11]. Briefly, these individuals enrolled at voluntary HIV counseling and testing services offered in Bangkok, Thailand, which systematically screened for AHI. Enrolled participants were classified into Fiebig stages I to V defined by a hierarchical algorithm from pooled nucleic acid testing,

sequential immunoassay, p24 antigen, and Western Blot testing: 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 [20, 21]. Additionally, individuals with major psychiatric disorders and substance dependence were excluded from enrollment (per cohort protocol). All participants were confirmed to have AHI and almost all immediately initiated antiretroviral therapy (ART) within a median of one day of the clinical assessment (IQR 0-1 days), followed by longitudinal evaluations. The current analysis focuses on baseline, cross-sectional pre-ART data. All participants provided written informed consent. The study protocol was approved by the institutional review boards of Chulalongkorn University, Walter Reed Army Institute of Research, The University of California, San Francisco and Yale University.

Clinical Data and Laboratory Measures

Demographic and clinical parameters included CD4+ and CD8+ T-lymphocyte levels, blood HIV-1 RNA level, Fiebig stage (I to V), and the presence of acute retroviral syndrome (ARS), defined as ≥ 3 qualifying symptoms using a standardized checklist [22]. Plasma HIV-1 RNA was quantified by the Roche Amplicor HIV-1 Monitor Test V1.5 or Roche COBAS TaqMan HIV-1 V2.0, with lower limits of quantification of 50 and 20 copies/ml, respectively.

Cognitive Assessment

Most participants (n=561, 94%) underwent a 4-test neuropsychological testing battery at enrollment, including the non-dominant hand Grooved Pegboard test (fine motor function; Lafayette Instrument Company, Lafayette, USA), Color Trails 1 and Trail Making A (Psychomotor speed; PAR, Inc., Lutz, USA) and Color Trails 2 (executive functioning/set shifting; PAR, Inc., Lutz, USA). The raw scores of each test were standardized to control data from 477 HIV-1 seronegative Thais [23]. A composite measure of overall performance (NPZ-4) was calculated based on the mean of individual test z-scores. In addition to the numerical NPZ-4 score, participants were categorized as having impaired cognitive performance if they had a performance with z-scores ≤-1 in at least two tests or ≤-2 in at least one test [24].

Syphilis Status and other Sexually Transmitted Diseases Co-infection

Upon study enrollment, all participants underwent serum hepatitis C (HCV) antibody testing, serum Treponema pallidum hemagglutination test (TPHA), and either serum VDRL or RPR testing (denoted as VDRL in the following analyses). History of any previous syphilis or syphilis treatment were collected through interviews conducted by research physicians at enrollment. We defined syphilis status as follows: 1. No syphilis: negative serum TPHA and VDRL; 2. Any syphilis: positive serum TPHA; 3. Untreated syphilis (at the time of AHI): Positive serum TPHA and VDRL without history of prior syphilis or syphilis treatment. The group with no syphilis (TPHA negative) served as controls in the following analyses examining the effect of syphilis/HIV co-infection at AHI. Additional

concomitant STIs at AHI, including gonorrhea and chlamydia co-infection, were retrieved from the clinical assessment and testing records.

Statistical Analysis

Results were reported as median and interquartile range (IQR) or number and percentage, as appropriate. Effects on categorical and numerical parameters by different syphilis status were compared using Chi-squared test and t-test, respectively. Univariable and multivariable regression analyses were performed to identify factors that were correlated with percentage change of CD8+ T-lymphocytes, in which levels of CD8+ T-lymphocyte were natural log-transformed to better approximate a normal distribution (Shapiro-Wilk W test p-value = 0.04). Factors correlated with impaired cognitive performance were identified using logistic regression. Factors with a p-value $\leq$ 0.20 in univariable analyses were included in backward stepwise multivariable analysis to select the final model with probability to enter = 0.05 and probability to retain = 0.10. The final models only include factors with p-value $\leq$ 0.10. Both models appeared to have good fit. Statistical analyses were performed using SPSS Version 18.0 (IBM Corp., Armonk, NY) and StataCorp. 2017 (Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC).

RESULTS

Of the 595 RV254 participants, 476 (80%) had no serologic evidence of syphilis (TPHA negative); 119 (20%) had any syphilis (TPHA positive) and 51 (9%) had untreated syphilis (15 by VDRL & 36 by RPR). Participants were mostly male (n=579, 97%), with a median age of 26 years and an estimated duration of HIV-1 infection to enrollment of 19 days; 213 (36%) presented at Fiebig stage I or II and 452 (76%) had ARS (Table 1). Of note, 32 out of 119 participants with any syphilis underwent optional lumbar puncture (LP) in the parent protocol, of which 11 of them had untreated syphilis. While headache and general malaise were common among the participants, complaints of overt cognitive dysfunction were absent. In particular, all participants with untreated syphilis were clinically free of neurological complaints that would suggest neurosyphilis. Two LP participants with untreated syphilis were considered to have asymptomatic neurosyphilis due to marked CSF lymphocytic pleocytosis (CSF white blood cell of 105 and 88 cells/mm3) with positive CSF THPA despite negative CSF VDRL. There was no false positive serum VDRL test (i.e. positive VDRL/RPR with negative TPHA). The rate of TPHA positivity rose from less than 5% in 2009 and 2010 to around 20% since 2015, with concomitant untreated syphilis ranging from 10-15% since 2014 except 4% in 2018. (Supplementary Table 1 and Supplementary Figure 2).

Association between Syphilis Status and HIV-1 Disease Parameters

Parameters including Fiebig stage (I/II vs. III-V), presence of ARS, CD4+ and CD8+ T-lymphocyte levels, CD4/CD8 ratio, plasma HIV-1 RNA levels, estimated duration of

HIV-1 infection, and NPZ-4 scores were compared between subgroups with syphilis and controls (TPHA negative) (Table 2). In the group comparisons, participants with untreated syphilis tended to have higher blood CD8+ T-lymphocytes than the controls (541 [IQR 352 to 1170] vs. 508 [IQR 334 to 832], p=0.099). Compared to those without syphilis, those with previous syphilis (TPHA+) had lower NPZ-4 composite score (-0.15 [IQR -0.77 to 0.44] vs. 0.07 [IQR -0.45 to 0.55], p=0.018) and higher rate of impaired cognitive performance (38% vs. 22%, p=0.002). For the rest of the examined parameters, none showed significant association between the control group and individual syphilis group (all p>0.1).

Comparing Clinical Parameters According to Fiebig Staging and Untreated Syphilis Status

Levels of CD4+ and CD8+ T-lymphocyte counts, log10 Plasma HIV-1 RNA and estimated duration of HIV infection were plotted against participants' untreated syphilis status and Fiebig stage to represent the sequential interaction between HIV-1 and immunological responses (Figure 1). Both the control and untreated syphilis subgroup showed a rapid rise of plasma HIV-1 RNA levels from Fiebig I that peaked at Fiebig III. However, there was no distinct pattern, such as consistent elevation of plasma HIV-1 RNA or reduction in CD4+ T-lymphocyte level in the untreated syphilis group. Among the tested parameters, median CD8+ T-lymphocyte level was higher in the untreated syphilis subgroup across Fiebig I to V except Fiebig III.

Syphilis Status as Independent Predictor of CD8 T-lymphocyte Count and NPZ-4 Composite Score

Univariable and multivariable regression models were employed to determine if untreated syphilis and any syphilis were independently associated with higher CD8+ T-lymphocyte level (Table 3) and impaired cognitive performance (Table 4).

Factors with p-values ≤ 0.2 in the univariable analysis on log10 CD8+ T-lymphocyte level included estimated duration of HIV-1 infection (p<0.001), presentation of ARS (p=0.011), AHI presentation at later Fiebig stages (III to V) (p<0.001), untreated syphilis (p=0.164), concomitant chlamydia (p=0.148) and gonorrhea (p=0.187) co-infections but not age, sex, plasma HIV-1 RNA and anti-HCV status. In the multivariable analysis that included factors with p-values ≤0.2, CD8+ T-lymphocyte level increased with higher estimated days after HIV transmission (% change per day = 1.3%, 95%CI [0.7% to 1.9%], p<0.001), later Fiebig stages (% change = 65.5%, 95%CI [46.9% to 86.5%], p<0.001) and untreated syphilis (% change = 20.2%, 95%CI [0.1% to 44.4%], p=0.049) (Table 3).

Factor with p-values ≤ 0.2 in the univariable analysis of impaired cognitive performance included female sex (p=0.171), CD4+ T-lymphocyte level (p=0.183) and any syphilis (p=0.002) but not estimated duration of HIV-1 infection, Fiebig stage, plasma HIV-1 RNA level and CD8+ T-lymphocytes levels. In the multivariable analysis that included

factors with p-values ≤0.2, any syphilis was the only factor independently associated with impaired cognitive performance and the odds of impairment were twice as high among those with any syphilis compared to no syphilis (Adjusted odds ratio = 2.0, 95% CI [1.30 to 3.09], p=0.002 (Table 4).

DISCUSSION

Syphilis is re-emerging globally, particularly among men who have sex with men (MSM) living with HIV-1 [1, 2, 25-27]. Apart from the potential to facilitate HIV-1 transmission [3], in-vitro experiments suggest that syphilis may elicit local and systemic innate and adaptive immune responses [28], as well as increase CCR5 expression in macrophages [29]. Both mechanisms may facilitate HIV-1 infection in susceptible cells and hence might exacerbate HIV-1 viral replication. The observation that incident syphilis contributes to transient plasma HIV-1 RNA elevation and CD4+ T-lymphocytes reduction in chronic HIV-1 infection provides further theoretical rationale to examine the effect of concomitant syphilis on HIV-related parameters during AHI.

Our data originates from an ongoing AHI cohort with predominantly young MSM from one of the major urban centers in Southeast Asia. From 2009 to 2019, there was a general increase in the frequency of any and untreated syphilis in persons with AHI. These findings are consistent with recent epidemiologic reports about syphilis in other societies [2, 13, 25]. We previously reported that untreated syphilis had no measurable effect on CSF HIV-1 RNA levels in a subset of cohort participants who underwent optional LP for research purpose during untreated AHI [11]. The current analysis focused primarily on the effects of clinical parameters by syphilis, which compared individuals with any syphilis and untreated syphilis with those without any prior syphilis at the time of AHI.

In contrast to the linkage between syphilis and lower CD4+ T-lymphocyte and higher HIV-1 RNA levels observed in individuals with chronic HIV-1 infection [4-6], syphilis was not associated with any significant alternation of the two parameters at untreated AHI. Besides, syphilis was not associated with the presence of acute retroviral syndrome or the estimated duration to AHI presentation. Further, standardizing the immunological status by Fiebig stages did not show any perceivable pattern of change in the aforementioned parameters. The overall picture suggested that untreated syphilis did not augment HIV-1 replication, CD4+ T-lymphocytes depletion or accelerate HIV-1 seroconversion across Fiebig stages.

However, untreated syphilis was associated with higher CD8+ T-lymphocyte levels in the multivariable analysis. Unlike later Fiebig stages (III-V) and increased estimated duration after HIV-1 infection which both link directly to the expansion of HIV-1 specific CD8+ T-lymphocytes at AHI [30], untreated syphilis was the only co-infection in our study that

affected CD8+ T lymphocyte level. Previous reports suggested that cytomegalovirus (CMV) and HCV co-infection may contribute to persistently elevated CD8+ T-lymphocyte levels in PLWH [31-33]. Although primary and secondary syphilis may induce peri-lesional infiltration of CD4+ and CD8+ T-lymphocytes [34], its effect on CD8+ T-lymphocytes in the systemic circulation is largely unknown and was not examined in the previous studies regarding incident syphilis in PLWH [5, 6, 35]. In a recent study of 25 AHI individuals of whom 15 had a reactive serum RPR [36], Vδ1/Vδ2 T-lymphocyte ratio in peripheral blood mononuclear cell (PBMC) samples was different between those with reactive vs. non-reactive serum RPR, while blood CD8+ T-lymphocyte levels were similar between the two groups. However, the comparison was based on univariable analysis without adjusting for the effects of HIV-1 related parameters such as Fiebig stage and plasma viral load.

The clinical and biological significance of the mild elevation in the absolute CD8 T-lymphocyte count that we observed in our study is uncertain. Given the fact that HIV-specific T-lymphocytes undergo rapid clonal expansion and robust functionality change during AHI, future phenotyping and functional analysis of T-lymphocytes with measurement of plasma inflammatory markers will be crucial to define more clearly the extent to which syphilis distorts the innate and adaptive immune response towards HIV-1 infection. Another plausible contributor to the CD8+ T-lymphocyte count differences we observed is that syphilis may be a proxy for other unmeasured concomitant STIs which more directly link with elevation of CD8+ T-lymphocyte level. For instance, MSM showed higher CD8+ T-lymphocyte levels and lower CD4/CD8 ratios than men who only have sex with women in a study of 368 HIV-negative participants, in which the differences could be partially explained by the higher CMV seroprevalence among MSM [37].

Instead of the subset of participants with untreated syphilis, participants with any past syphilis had impaired cognitive performance. While the sample size of the untreated syphilis group could be too small to achieve statistical significance, association between prior syphilis and poorer cognitive performance in chronic HIV-1 infection in the absence of overt neurosyphilis has been reported in some studies [14-16]. Conversely, in a study that compared PLWH with HIV-negative controls, PLWH had lower global cognitive scores but history of syphilis was not associated with cognitive impairment after accounting for potential cofounders [16]. In this study, we handled a number of key confounding factors by standardization of neuropsychological test performance for age and educational level, excluding individuals with major psychiatric disorders and substance dependence and including HIV-1 related parameters as well as several concomitant infections in the statistical analysis. Together with the previous reports suggesting an association between syphilis and cognitive impairment, our finding supports a biological impact of syphilis on cognitive function. Neuroinvasion by T. pallidum takes place in at least 30% of infected individuals and in up to 80% of cases, the organism is spontaneously cleared by one's immunity [10]. With the increasing incidence of syphilis reinfection [38] and hence increased risk of repeated T. pallidum

neuroinvasion, the cumulative effect of repeated intracranial immune activation may promote chronic CNS immune activation and neuronal damage, even if a fully established neurosyphilis is absent. Other possibilities include unmeasured sociodemographic risk factors that associate with both syphilis acquisition and poorer neurocognitive performance. For instance, poly-substance use is associated with HIV-1 and syphilis infections in certain populations [39] but local data in Bangkok is not available.

Limitations of our study should be acknowledged. The number of participants with untreated syphilis was limited and they were predominantly male. Many of them did not have regular syphilis screening or were first tested positive for syphilis at the time of AHI. Based on the limited information, we were only able to determine if the participants had untreated syphilis but not stage the untreated syphilis. Staging untreated syphilis at AHI is also challenging due to the overlapping symptoms between new syphilis and ARS including rash, lymphadenopathy and raised liver enzymes. Thus, the actual diagnosis of untreated syphilis could range from acute syphilis co-infected with HIV-1 to latent infection acquired years beforehand. Early vs. late syphilis may impose different biological effects on HIV-1 infection. We did not evaluate the biological effect of VDRL titer due to the inconsistent usage of VDRL and RPR secondary to the availability of laboratory service. Further, participants who received syphilis treatment shortly (days to weeks) before enrollment were considered as treated syphilis but very recently treated syphilis may still pose a biological effect. The period of HIV/syphilis co-infection was also brief, on the order of several weeks, which may have limited the synergistic effects of the two pathogens. Besides, it is important to note that the absolute difference in cognitive performance between TPHA+ and control group in current study (NPZ-4: -0.15 vs. 0.07) and the aforementioned report (Global deficit score: 0.47 vs. 0.31) is relatively limited. Lastly, because few participants underwent lumbar puncture, we were unable to assess the effect of neurosyphilis on neurocognitive test performance.

CONCLUSIONS

Unlike in chronic HIV-1 infection, in our study of 595 participants with AHII, untreated syphilis was not associated with elevated plasma HIV-1 RNA and lower CD4+ T-lymphocyte levels. Instead, it was associated with a higher level of CD8+ T-lymphocytes. Future studies should examine whether syphilis contributes to observed alterations of CD8+ T-lymphocyte levels and CD4/CD8 dysregulation in PLWH on ART. Examination of serial CD8+ T-lymphocyte levels before and after incident syphilis, as well as the level after successful treatment would help clarify such an association. The association between impaired cognitive performance and any syphilis leaves the question of whether uncomplicated syphilis contributes to cognitive impairment in PLWH unanswered. Further effort is necessary to clarify if syphilis, particularly in the setting of recurrent infection, is a stand-alone risk factor of cognitive impairment.

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Database Management:	SP
Cognitive Test Performance Analysis:	RP
Statistical Analysis:	PC, SP, CM, SS
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Contribution of work: We affirm that all authors have contributed to and approved the manuscript.

Table 1. Characteristics of participants at untreated acute HIV-1 infection	
Characteristic	**N=595***
Age, years	26 (22-31)
Male, n (%)	579 (97)
Fiebig stage, n (%) **I** **II** **III** **IV** **V**	 81 (14) 132 (22) 278 (47) 72 (12) 32 (5)
ARS, n (%)	453 (76)
CD4+ T-lymphocytes (cells/ul)	359 (262-501)
CD8+ T-lymphocytes (cells/ul)	511 (336-860)
CD4/CD8 ratio	0.71 (0.43-1.04)
Plasma HIV-1 RNA (log_{10} copies/ml)	5.93 (5.23-6.74)
Estimated duration of HIV-1 infection, days	19 (14-25)
Anti-HCV positive, n (%)	11 (2)
NPZ-4 Composite Score#	0.05 (-0.52-0.54)
Low Cognitive Performance, n (%)&#	149 (27)

*Median (IQR) are shown unless specified; &Defined as test performance below -1SD in at least two NP tests or below -2SD in at least one NP test; #n=561.
Abbreviation: ARS = Acute retroviral syndrome

Table 2. Clinical Parameters by Syphilis Status at Acute HIV Infection					
	o Syphilis (n=476)	**Any Syphilis (n=119)**	**Untreated Syphilis (n=51)**	**Any Syphilis vs. No Syphilis p value**	**Untreated Syphilis vs. No Syphilis p value**
Fiebig stage I or II, n (%)	171 (36)	42 (35)	21 (41)	0.898	0.459
Acute Retroviral Syndrome, n (%)	359 (75)	94 (79)	38 (75)	0.414	0.886
CD4+ T-lymphocytes (cells/ul)	358 (261-495)	364 (264-525)	411 (259-534)	0.272	0.109
CD8+ T-lymphocytes (cells/ul)	508 (334-832)	528 (352-960)	541 (352-1170)	0.279	0.099
CD4/CD8 ratio	0.71 (0.45-1.06)	0.71 (0.41-1.00)	0.67 (0.37-1.12)	0.823	0.852
Plasma HIV-1 RNA, (Log_{10} copies/ml)	5.92 (5.23-6.73)	6.00 (5.16-6.75)	5.79 (5.16-6.43)	0.929	0.303
Estimated Duration of HIV-1 Infection, Days	19 (14-26)	18 (13-23)	18 (13-23)	0.454	0.470
NPZ-4 composite score	0.07& (-0.45-0.55)	-0.15* (-0.77-0.44)	-0.02 (-0.67-0.58)	0.018	0.472
Impaired Cognitive Performance, n (%)	105& (22)	44* (38)	18 (35)	0.002	0.067

Definition of syphilis status: 1. No syphilis: negative serum TPHA and VDRL; 2. Any syphilis: positive serum TPHA; 3. Untreated syphilis (at the time of AHI): Positive serum TPHA and VDRL without history of prior syphilis or syphilis treatment. Median (IQR) are shown unless specified. &n=445; *n=116; #n=85.
Impaired Cognitive Performance = if individual's neuropsychological test performance was below -1SD in at least two tests or below -2SD in at least one test.
Abbreviations: ARS = Acute Retroviral Syndrome; NPZ-4 = Composite Score of 4 Neuropsychiatric Tests.

Table 3. Factors Correlating with log10 CD8+ T-lymphocyte Level

	Univariate Analysis		[&]Multivariate analysis	
Characteristic	% change (95% confidence interval)	p value	% change (95% confidence interval)	p value
Age	-0.3% (-1% to 0.5%)	0.523		
Sex				
Male	Ref			
Female	-12.7% (-39.1% to 25.5%)	0.465		
Plasma HIV-1 RNA (Log_{10} copies/ml)	0.07%* (-0.44% to 0.57%)	0.799		
Estimated Duration of HIV-1 Infection, days	2.1% (1.5% to 2.7%)	<0.001	1.3% (0.7% to 1.9%)	<0.001
Acute Retroviral Syndrome				
No	Ref			
Yes	19.7% (4.2% to 37.6%)	0.011		
Fiebig Stage				
I & II	Ref		Ref	
III to V	79.9% (60.3% to 101.8%)	<0.001	65.5% (46.9% to 86.5%)	<0.001
[#]**Untreated Syphilis**				
No	Ref		Ref	
Yes	15.5% (-5.7% to 41.6%)	0.164	20.2% (0.1% to 44.4%)	0.049
Anti-HCV Positive				
No	Ref			
Yes	41.6% (-23.9% to 163.3%)	0.272		
Chlamydia Infection				
No	Ref			
Yes	-12.1% (-26.2% to 4.7%)	0.148		
Gonorrheal Infection				
No	Ref			
Yes	-10.7% (-24.4% to 5.5%)	0.187		

#Only participants of negative serum TPHA (n=476) and untreated syphilis (n=51) were included.
*For every 10% of log10 HIV-1 RNA change. [&]Factors with p<0.2 in univariate analyses were included in the multivariate analysis (Linear regression, stepwise calculation model)
Abbreviation: Ref = Reference; HCV = viral hepatitis C.

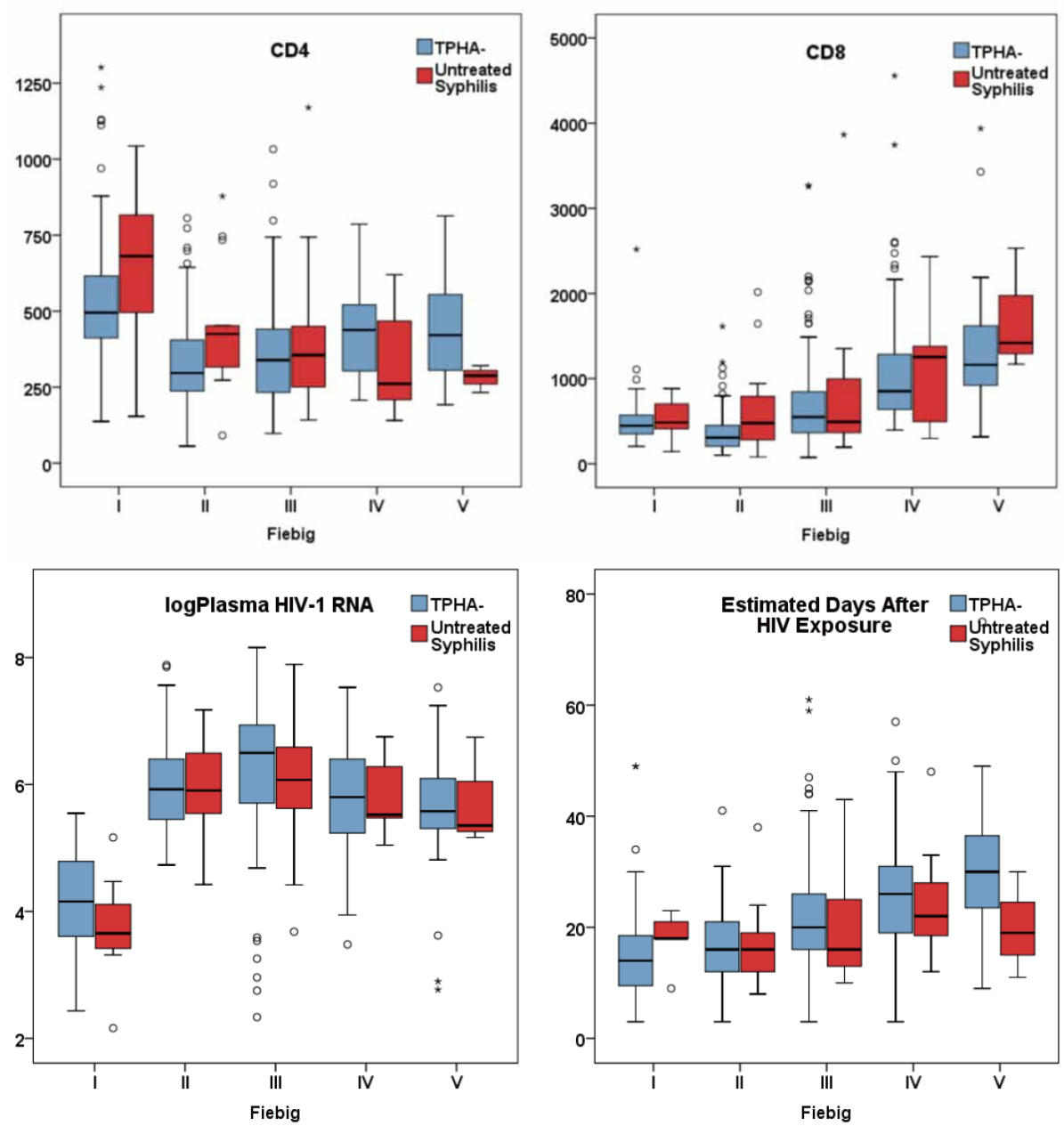
Table 4. Factors Correlating with Impaired Cognitive Performance

	Univariate Analysis		&Multivariate Analysis	
Characteristic	Exp(B) / OR (95% Confident Interval)	p value	Exp(B) / Adjusted OR (95% Confident Interval)	p value
Sex				
Male	Ref			
Female	2.12 (0.72 to 6.21)	0.171		
Plasma HIV-1 RNA (Log_{10} copies/ml)	1.10 (0.93 to 1.30)	0.264		
Estimated Duration of HIV-1 Infection	1.01 (0.99 to 1.03)	0.592		
Fiebig Stage				
I & II	Ref			
III and later	1.09 (0.73 to 1.61)	0.672		
CD4+ T-lymphocyte (per 100cells/µL)	0.93 (0.84 to 1.03)	0.183		
CD8+ T-lymphocyte (per 100cells/µL)	0.99 (0.96 to 1.03)	0.723		
CD4/CD8 ratio	0.80 (0.54 to 1.02)	0.286		
TPHA				
Negative	Ref		Ref	
Positive	1.98 (1.28 to 3.06)	0.002	2.00 (1.30 to 3.09)	0.002
Anti-HCV Positive				
No	Ref			
Yes	1.60 (0.46 to 5.53)	0.461		

&Factors with p<0.2 in univariate analyses were included into the multivariate analysis (Logistic regression, stepwise calculation model). Impaired Cognitive Performance = if individual's neuropsychological test performance was below -1SD in at least two tests or below -2SD in at least one test.

Abbreviation: TPHA = Treponema pallidum hemagglutination assay; HCV = viral hepatitis C; OR = odds ratio.

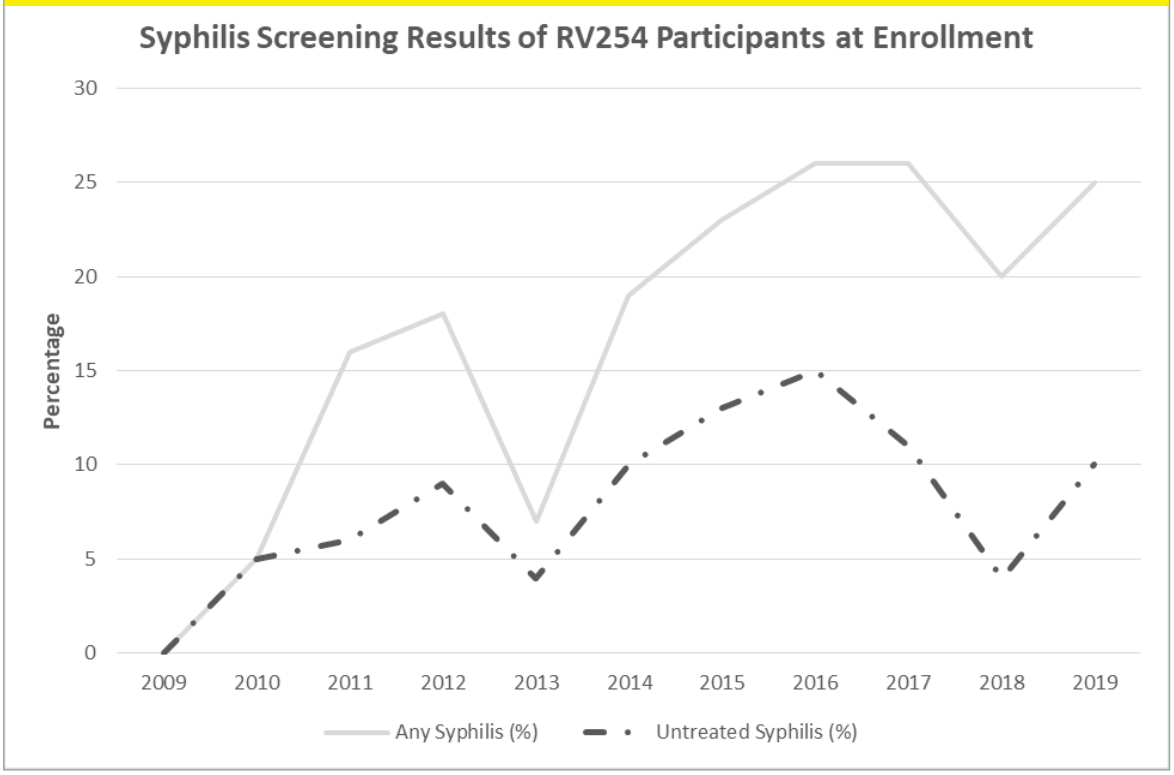
Figure 1. Various HIV-1 Infection parameters by Fiebig Stages and Untreated Syphilis Status



Untreated Syphilis: Positive TPHA and VDRL without history of prior syphilis or syphilis treatment

Supplementary Table 1. Syphilis Screening Results of RV254 Participants at Enrollment			
Year	**Participants Enrolled**	**TPHA +, n (%)**	**Untreated Syphilis, n (%)**
April 2009-	10	0 (0)	0 (0)
2010	22	1 (5)	1 (5)
2011	31	5 (16)	2 (6)
2012	22	4 (18)	2 (9)
2013	55	4 (7)	2 (4)
2014	91	17 (19)	9 (10)
2015	103	24 (23)	13 (13)
2016	97	25 (26)	15 (15)
2017	89	23 (26)	10 (11)
2018	55	11 (20)	2 (4)
-March 2019	20	5 (25)	2 (10)
Total	595	119	51

Supplementary Figure 1. Percentage of Syphilis Seropositivity at Acute HIV infection from 2009 to 207



SECTION 3
Characterizing Longitudinal Neurological Outcomes in the RV254 cohort

Chapter 8:
Cognitive Trajectories after Treatment in Acute HIV Infection

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AIDS. 2021 Jan 29.
doi: 10.1097/QAD.0000000000002831.

ABSTRACT

Objective: People with HIV continue to exhibit cognitive symptoms after suppressive antiretroviral therapy (ART). It remains unclear if initiating ART during acute HIV-1 infection (AHI) uniformly improves cognitive outcomes.

Methods: Sixty-seven individuals (96% male, median age 28 years) initiated ART immediately after AHI diagnosis and maintained viral suppression for six years. They underwent a four-test neuropsychological battery that measured fine motor speed and dexterity, psychomotor speed, and executive functioning at baseline (pre-ART AHI), weeks 12, 24, and 96, and annually thereafter through week 288. Performances were standardized to calculate an overall (NPZ-4) score and frequencies of impaired cognitive performance (≤-1 SD on ≥2 tests, or ≤-2 SD on ≥1 test). Group-based trajectory analysis (GBTA) was applied to identify distinct neuropsychological trajectories modeled from baseline to week 288. Post-hoc analyses examined HIV-1 and demographic factors that differed between trajectory subgroups.

Results: NPZ-4 scores improved from baseline to week 96 ($p<0.001$) and from weeks 96 to 288 ($p<0.001$), with frequencies of impaired performance of 30%, 6% and 2% at the respective time-points. The amplitude of NPZ-4 improvement throughout the period was >0.5 SD and beyond practice effects. GBTA identified three NPZ-4 trajectory subgroups that all showed improvement over-time. The subgroup with lowest baseline performance exhibited worse depressive symptoms at baseline ($p=0.04$) and the largest improvement among the three. HIV-1 indices did not differ between the subgroups.

Conclusions: Cognitive performance improved in a sustained and stable manner after initiating ART during AHI. Largest improvements were seen in participants with worst baseline cognitive performance.

INTRODUCTION

People living with HIV-1 (PLWH) remain at risk for cognitive impairment despite suppressive antiretroviral therapy (ART). Trajectory analysis revealed heterogeneous trajectories of cognitive performance among PLWH over 3 years [1], suggesting a subset of PLWH are more prone to cognitive decline. Contrarily, in a cross-sectional study of 26 participants who initiated ART within a year after HIV-1 transmission, only one was cognitively impaired after a median duration of 5.7 years of ART [2]. This low rate of cognitive impairment suggests that early ART may contribute to the long-term stability of cognitive function.

We previously reported cognitive performance of 36 individuals during acute HIV-1 infection (AHI) and 24 weeks after immediate initiation of ART [3]. This study extended the time frame to 288 weeks post-ART to determine the frequency of cognitive impairment among these research participants who maintained suppressive ART. Group-based trajectory analysis (GBTA) [4, 5] was applied to identify different cognitive trajectories and subsets of participants with cognitive decline. The final aim was to identify HIV-related and psychosocial determinants of trajectory subtypes.

METHODS

Study Design and Participants

Study participants were from the RV254 Thai AHI cohort [6]. They were diagnosed Fiebig I-V AHI at study enrollment (baseline) [7]. They underwent plasma HIV-1 RNA level, CD4+ and CD8+ T-lymphocytes counts testing at pre-ART baseline and each follow-up visit post-ART. They completed neuropsychological assessments at specified study visits in parallel with blood sampling, at baseline and visits at weeks 12, 24, 96 and every 48 weeks thereafter. The manifestation of acute retroviral syndrome (ARS) was defined as ≥3 qualifying symptoms using a standardized checklist [8]. Ninety-eight HIV-negative Thais who completed the same neuropsychological battery 24 weeks apart, provided a reference for practice effects in repeated assessments [9]. All participants provided written informed consent. The study protocol was approved by the institutional review boards of Chulalongkorn University in Bangkok, Thailand and participating organizations.

Neuropsychological Assessment

The four-test battery measured fine motor speed and dexterity (non-dominant hand Grooved Pegboard test; GPB; Lafayette Instrument Company, Lafayette, USA), psychomotor speed (Color Trails 1; CT1 and Trail Making A; Trails A; PAR, Inc., Lutz, USA) and executive functioning (Color Trails 2; CT2; PAR, Inc., Lutz, USA). Raw scores were standardized to z-scores using Thai normative data [10], which were averaged to create an overall performance (NPZ-4) score. Consistent with prior work [3], impaired cognitive performance was defined by having z-scores ≤-1 on ≥2 tests or ≤-2 on ≥1 test

in the four-test battery.

Depressive symptoms were measured using the Thai version of the 9-item Patient Health Questionnaire (PHQ-9, score 0-27) [11]. Moderate and moderate-severe depressive symptoms were defined by PHQ-9 scores ≥10 and ≥15, respectively [12]. The latter cut-off was employed in this study.

The current analysis included RV254 participants who: 1) initiated ART immediately after enrollment and maintained viral suppression (<50cps/ml) for 6 years (288 weeks); 2) completed neuropsychological assessment at both baseline and week 288; 3) were without overt HIV-1 blips during the study period, defined as plasma HIV-1 RNA >400cps/ml; 4) did not participate in treatment interruption studies[13-16].

Statistical Analysis

Longitudinal cognitive outcomes were examined as follows: 1) frequencies of impaired cognitive performance at baseline, weeks 96 and 288; 2) NPZ-4 and z-scores for each neuropsychological test at the same three time-points; and 3) GBTA, a latent analysis that identifies subsets of participants of different longitudinal trajectories of test performance. Briefly, GBTA is a data-driven method that identifies clusters of individuals from heterogeneous samples that follow distinct trajectories on a defined variable [4, 5]. GBTA determined whether subgroups of RV254 participants exhibited distinct trajectories of cognitive performance during the study period.

Statistical analysis was conducted in Stata 16 (Statacorp, College Station, TX, USA). Categorical and continuous parameters at various time-points were compared by McNemar and Wilcoxon tests as appropriate. GBTA was performed using a STATA plug-in, and the best fit of trajectory group number was determined by Bayesian information criterion (BIC) and model parsimony [17]. Individuals were assigned to the group for which posterior probability of membership was highest. Baseline characteristics were summarized by trajectory group; categorical covariates were compared with Fisher's Exact test, and continuous covariates were compared using a Kruskal-Wallis test. The changes in NPZ-4 from baseline to week 288 between trajectory subgroups were compared using random effects regression.

RESULTS

By July 2019, 67 RV254 participants (94% male, median age 28) fulfilled the selection criteria. Thirty-six (54%) presented at Fiebig stage I-II. Fifty-two (78%) had ARS. All commenced an efavirenz+2NRTI regimen. Thirty-four (51%) were randomized into a MEGA-ART substudy that provided 24 weeks of raltegravir and maraviroc in addition to their 3-drugs regimens. The duration between study enrollment and ART initiation was 0; IQR 0-2 days. Supplementary table 1 listed the laboratory outcomes at baseline, weeks

96 and 288.

Outcomes in Neuropsychological Assessment

The frequencies of impaired cognitive performance at baseline, weeks 96 and 288 were 30%, 6% and 2% respectively, revealing a significant decrease from baseline to week 96 ($p<0.001$) but not between weeks 96 and 288 ($p=0.38$). ART and MEGA-ART users showed similar rates of impaired cognitive performance at all three time-points. NPZ-4 and z-scores of the four tests (i.e. CT1, CT2, GPB, Trails A) improved between baseline and week 96 (all $p<0.01$). Between weeks 96 and 288, NPZ-4 and z-scores of CT1, CT2, Trails A further improved (all $p<0.01$) but the z-scores of GPB remained similar ($p=0.41$) (Table 1). Compared to the HIV-negative control, RV254 participants had a greater NPZ-4 improvement at week 24 (mean difference: 0.47, 95%CI 0.27-0.66; $p<0.001$) (Supplementary table 2). The mean NPZ-4 improvement of RV254 participants from baseline to week 288 was 1.04 times its standard deviation (SD).

Group-Based Trajectory Analysis

GBTA of the NPZ-4 revealed three distinct trajectories. They were labelled accordingly, from the lowest to the highest mean baseline NPZ-4 score, as subgroup 1 (n=15, 23%), 2 (n=40, 58%) and 3 (n=12, 19%) (Figure 1a). All subgroups showed interval improvement towards week 288. The highest-performing subgroup scored the highest in all four tests at baseline across subgroups (all $p<0.001$; Supplementary Table 3). Compared to the highest-performing trajectory subgroup, the relative gain in NPZ-4 was greatest for the lowest-performing subgroup (1.62, 95%CI 1.43-1.79; $p<0.001$), followed by the intermediate subgroup (0.92, 95%CI 0.78-1.05; $p<0.001$).

Analysis of individual test trajectories revealed 3 trajectory subgroups which were labelled the same way: subgroups 1 to 3 from the lowest to the highest median z-scores at baseline (Figure 1b-e). Every set of trajectories showed either improving or stable trend, except a transient decline in performance at week 144 in the lowest-performing subgroup of GPB (Figure 1d). Review of data and clinical history revealed an extreme outlier in that subgroup, who presented with 2 episodes of non-disabling embolic stroke which affected his dominant (right) side prior to weeks 96 and 132. His strokes recovered well with improving GPB performance towards normal range at week 288 (z-GPB=-0.36). The dip in test performance is therefore likely due to the combined effect of an extreme but transient decline in performance within a small subgroup and unrelated to HIV-1.

Factors Associated with Group-Based Trajectories

Comparing baseline characteristics including sex, age, Fiebig stage, CD4+ and CD8+ T-lymphocyte counts, CD4/CD8 ratio, plasma HIV-1 RNA, presence of ARS and PHQ-9 scores between trajectory subgroups revealed PHQ-9 score as the only significant factor (Supplementary table 3). The frequency of moderate-severe depression was highest in

the lowest-performing subgroup but not significantly different across the subgroups (subgroup 1: 40%; subgroup 2: 20%; subgroup 3: 8%, p=0.15).

DISCUSSION

We previously reported impaired cognitive performance in approximately one quarter of RV254 participants at AHI and week 24 post-ART[3], compared to 30% at baseline in current report. Contrary to an unchanged rate of impaired performance 24 weeks post-ART in the former report, the rate declined to 6% and 2% at weeks 96 and 288 in this analysis, indicating that a subset of individuals require a longer interval of viral suppression to more completely reverse initial cognitive dysfunction caused by AHI.

Using a latent trajectory modeling approach, we identified no evidence of cognitive decline among this cohort of virally suppressed AHI throughout 288 weeks. Despite variation in subgroup composition, the GBTA analyses demonstrated: 1) the greatest performance difference across trajectory subgroups at baseline; 2) greatest extent of improvement in the lowest-performing subgroups; and 3) continual improvement between weeks 96 and 288, except the transient decline in the lowest-performing subgroup in the GPB test driven by the participant with strokes. This unexpected finding reminds about the importance of co-morbidities on long-term cognitive function.

Practice effects, most readily seen during the first two assessments [9], account for at least part of the observed improvement in current study. Yet, the improvement in NPZ-4 in RV254 participants exceeds that of the HIV-negative control, suggesting the reversal of AHI-related cognitive dysfunction after ART in addition to the practice effects. The magnitude of NPZ-4 improvement is well above 0.5 SD, a frequently-used cut-off of clinically relevant change [18]. Further, the greater extent of improvement occurred in the subgroup with poorest baseline performance likely represents this group's greatest propensity for improvement given its degree of baseline abnormality. This contrasts with a greater risk of cognitive decline among treated PLWH of chronic HIV-1 infection with pre-existing cognitive deficit [5].

A higher PHQ-9 score was associated with the lowest-performing trajectory subgroup. Although chronic depression negatively impacts cognitive performance [19], pre-existing endogenous depression is unlikely the cause of impaired cognitive performance here because major psychiatric disorder is an exclusion criterion per RV254 protocol. Given stable improvement in participants' PHQ-9 scores post-ART (Table 1), the depressive symptoms at AHI are more likely related to the acute psychological stress of an HIV-1 diagnosis and the physical symptoms of substantial HIV-1 viremia during AHI. The former is largely governed by individual stress-coping skills; the latter is biologically linked to HIV-1 replication. As depressive symptoms worsened with elevated levels of plasma HIV-1 RNA and inflammatory markers at AHI

[20], cognitive impairment present during AHI could be mediated through depression symptoms driven by HIV-1 viremia. However, further study is necessary to disentangle such an interactive relationship.

Our study is limited by the breadth of the neuropsychological battery to fulfil the Frascati criteria of HIV-associated neurocognitive disorder [21]. Although it may overlook subtle deterioration in other untested cognitive domains, results of prior analysis revealed its sensitivity to identify variability in performance. Most RV254 participants switched to Dolutegravir-based ART in 2017, but such transition did not adversely affect cognitive test performance [22]. Our participants were mostly young males, with high ART adherence and without major co-morbidities, making our findings less generalizable to other settings with confounders [23].

Among individuals who initiated ART during AHI and maintained viral suppression for 6 years, persistent impairment or decline in cognitive performance within this 4-test neuropsychological battery is uncommon. In contrast to PLWH with chronic HIV-1 infection, GBTA reveals that even the cognitive performance of the worst-performing subgroup at AHI improved with stability, suggesting that cognitive dysfunction present during AHI is reversed by early ART with stability.

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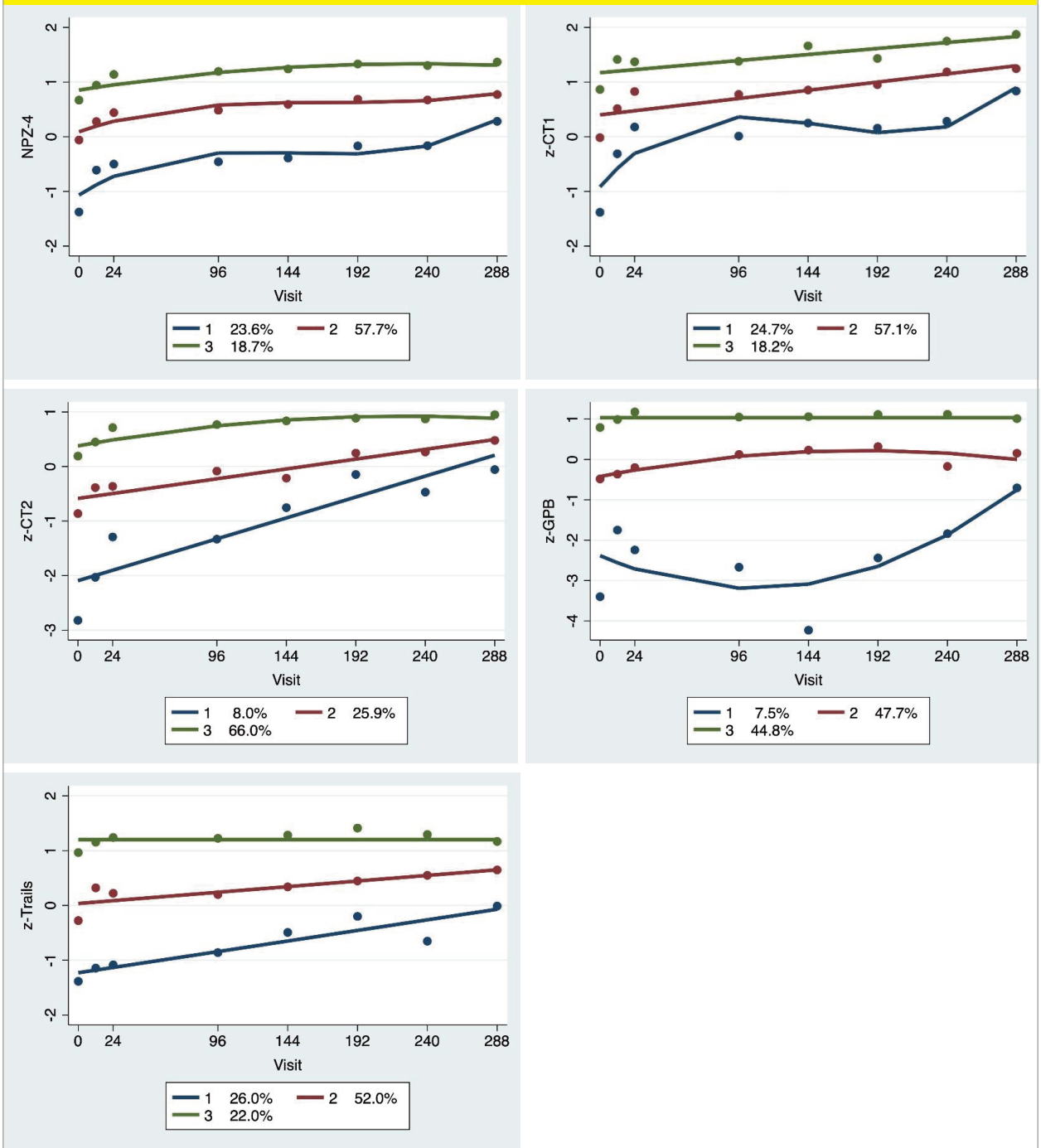
Authors' contributions:

Cohort Design:	VV, SS, JA
Study Design:	RP, VV, PC, SK, PR
Statistical Analysis:	PC, SK, RP
Participants/Patients Care:	PC, DC, EK, CS, JH, JA
Manuscript Preparation:	PC, SK, EK, DC, CS, JH, PR, SV, JA, VV, SS, RP

Contribution of work: We affirm that all authors have contributed to and approved the manuscript.

Table 1. Outcomes of Neuropsychological Assessment and Patient Health Questionnaire-9 (PHQ-9)					
	Week 0	**Week 96**	**Week 288**	**Week 0 vs. 96 P-value**	**Week 96 vs. 288 P-value**
Cognitive Assessment					
Impaired Cognitive Performance, n (%)	20 (30)	4 (6)	1 (2)	<0.001	0.38
NPZ-4	-0.12 (-0.59 to 0.42)	0.39 (0 to 0.93)	0.80 (0.38 to 1.17)	<0.001	<0.001
z-Color Trail 1	-0.05 (-0.94 to 0.51)	0.73 (0.22 to 1.17)	1.30 (0.92 to 1.65)	<0.001	<0.001
z-Color Trail 2	0.00 (-0.90 to 0.55)	0.52 (-0.02 to 0.85)	0.76 (0.37 to 1.16)	<0.001	<0.001
z-Grooved Pegboard Test	0.29 (-0.73 to 0.88)	0.51 (-0.04 to 1.12)	0.58 (0.14 to 1.03)	0.002	0.41
z-Trail Making A	-0.24 (-1.18 to 0.69)	0.23 (-0.58 to 1.02)	0.70 (0 to 1.18)	0.002	0.005
PHQ-9					
Total Score	10 (8 to 14)	6 (3 to 8)	5 (2 to 8)	<0.001	0.54
Moderate-Severe Depression, (PHQ-9≥15), n (%)	15 (23)	1 (2)	3 (5)	0.001	0.63
Median and (IQR) are shown.					

Figure 1. Outcomes of Group-based Trajectory Analysis.



1a. NPZ-4; 1b. Color Trail 1; 1c. Color Trail 2; 1d. Non-dominant hand grooved pegboard test; 1e. Trail Making A test. Numbers at X-axis represent the week of assessment. The second dot in each figure represents the assessment at week 12. Boxes at the bottom of each figure show the percentage of each trajectory subgroup.

Supplementary Table 1. Demographic and Assessment Outcomes at Baseline and Follow-ups

	Baseline (AHI)	Week 96	Week 288
Sex, male, n (%)	63 (94)	-	-
Age, years	28 (23 to 33)	-	-
Fiebig Stage, n (%) **I** **II** **III** **IV** **V**	 12 (18) 24 (36) 23 (34) 2 (3) 6 (9)	-	-
CD4+ T-lymphocyte, cells/mm3	354 (240 to 497)	633 (490 to 864)	623 (532 to 850)
CD8+ T-lymphocyte, cells/mm3	425 (257 to 728)	610 (461 to 793)	629 (462 to 774)
CD4/CD8 Ratio	0.86 (0.49 to 1.26)	1.10 (0.83 to 1.29)	1.10 (0.89 to 1.35)
Plasma HIV-1 RNA, log_{10} copies/ml	5.61 (5.02-6.69)	Suppressed	Suppressed
ARS, n (%)	52 (78)	-	-
MEGA-ART, n (%)	34 (51)	-	-
Days from Enrollment to Initiation of ART	0 (0 to 2)		

Median (IQR) are displayed unless specified.
Abbreviations: AHI = Acute HIV-1 Infection; NPZ-4 = Composite score of 4 neurocognitive tests; PHQ-9 = Patient Health Questionnaire-9; ARS = Acute Retroviral Syndrome
Remarks: Fiebig Staging of HIV-1: 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; MEGA-ART: Addition of raltegravir and maraviroc to the 3-drugs ART regimens between weeks 0 and 24.

Supplementary table 2. NPZ-4 scores between RV254 participants and HIV-negative controls				
	RV254 (n=67) Mean (SD)	**Control[#] (n=98) Mean (SD)**	**Mean difference (95% CI)**	**p-value**
NPZ-4 at Week 0	-0.23 (0.96)	0.09 (0.70)	-0.32 (-0.052; -0.94)	0.020
NPZ-4 at Week 24	0.35 (0.70)	0.23 (0.79)	0.12 (-0.12; 0.35)	0.340
NPZ-4 change between Weeks 0 and 24	0.61 (0.74)	0.14 (0.54)	0.47 (0.27; 0.66)	<0.001
[#]The HIV-negative control participant belongs to a larger HIV-negative group which formulates the Thai normative NP data for NP test scores standardization.				

Supplementary Table 3. Characteristic at AHI by Trajectory Group of NPZ-4

	Trajectory Group 1 (n=15)	Trajectory Group 2 (n=40)	Trajectory Group 3 (n=12)	p-value
Gender, male, n(%)	14 (93)	38 (95)	11 (92)	0.81
Age, year	31 (25 to 34)	28.5 (24 to 33)	24 (21 to 30)	0.13
Feibig Stage				0.66
I and II	9 (60)	22 (55)	5 (42)	
III - V	6 (40)	18 (45)	7 (58)	
CD4+ T-lymphocyte, cells/mm3	313 (214 to 395)	384 (236 to 512)	366 (269 to 470)	0.53
CD8+ T-lymphocyte, cells/mm3	344 (219 to 567)	434 (306 to 786)	474 (259 to 799)	0.51
CD4/CD8 ratio	0.97 (0.49 to 1.26)	0.81 (0.47 to 1.21)	0.81 (0.55 to 1.25)	0.84
Plasma HIV-RNA, log_{10} copies/ml	5.67 (5.45 to 6.84)	5.60 (4.64 to 6.63)	5.68 (4.93 to 6.63)	0.46
ARS, n(%)	14 (93)	29 (72)	9 (75)	0.29
Mood Questionnaires				
PHQ-9 Total Score, Median(IQR)	13.5 (10 to 17)	9 (7 to 14)	9 (7 to 13)	0.04
Moderate Severe Depression (PHQ-9≥15)	6 (40)	8 (20)	1 (8)	0.15
Neuropsychological Test Performance				
NPZ-4	-1.2 (-2.33 to -0.51)	-0.12 (-0.39 to 0.28)	0.75 (0.5 to 1.02)	<0.001
z-Color Trail 1	-0.99 (-1.7 to -0.05)	0.04 (-0.85 to 0.51)	0.71 (0.01 to 1.28)	<0.001
z-Color Trail 2	-0.75 (-1.52 to 0.02)	-0.18 (-0.93 to 0.44)	0.71 (0.56 to 1.05)	<0.001
z-Grooved Pegboard Test	-1.19 (-2.68 to 0.09)	0.4 (-0.26 to 0.83)	0.98 (0.54 to 1.22)	<0.001
z-Trail Making A	-1.51 (-2.46 to -0.46)	-0.22 (-1.09 to 0.64)	0.79 (0.26 to 1.55)	<0.001

Median (IQR) are shown unless specified.
Abbreviations: ARS = Acute Retroviral Syndrome; PHQ-9 = Patient Health Questionnaire-9; NPZ-4 = Composite score of the z-scores in the 4 neuropsychological tests.

Chapter 9:
Minimal Incidence of Cerebrospinal Fluid Escape After Initiation of Antiretroviral Therapy in Acute HIV Infection

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AIDS. 2020 Dec 9.
doi: 10.1097/QAD.0000000000002786.

ABSTRACT

Objective: Despite suppression of HIV-1 replication in the periphery by antiretroviral therapy (ART), up to 10% of treated individuals have quantifiable HIV-1 in the CSF, termed CSF escape. CSF escape may be asymptomatic but has also been linked to progressive neurological disease, and may indicate persistence of HIV in the central nervous system (CNS). CSF escape has not yet been assessed after initiation of ART during acute HIV-1 infection (AHI).

Design: Prospective cohort study.

Subjects: Participants identified and initiated on ART during AHI who received an optional study lumbar puncture at pre-ART baseline or after 24 or 96 weeks of ART.

Main outcome measures: Paired levels of CSF and plasma HIV-1 RNA, with CSF > plasma HIV-1 RNA defined as CSF escape.

Results: 204 participants had paired blood and CSF sampling in at least one visit at baseline, week 24, or week 96. 29 participants had CSF sampling at all three visits. CSF escape was detected in 1/90 at week 24 (CSF HIV-1 RNA 2.50 log10 copies/mL, plasma HIV-1 RNA < 50 copies/mL), and 0/55 at week 96.

Conclusions: While levels of CSF HIV-1 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 treatment. Early treatment may improve control of HIV-1 within the CNS compared with treatment during chronic infection, which may have implications for long-term neurological outcomes and CNS HIV-1 persistence.

BACKGROUND

The central nervous system (CNS) is an important site during early HIV-1 infection, chronic replication, and viral persistence, especially as a unique compartment sequestered by the blood-brain barrier that influences inflammatory response, immune cell trafficking, and antiretroviral drug penetration. Even while on suppressive antiretroviral therapy (ART), the CNS may continue to be affected uniquely in the phenomenon of cerebrospinal fluid (CSF) escape, in which viral presence is undetectable in the periphery but is detectable in CSF. During chronic HIV-1 infection, CSF escape may occur in up to 10% of individuals [1]. Recent international consensus guidelines have provided definitions [2].

CSF HIV escape may give some insight into interrogating long-term persistence of the CNS reservoir, which remains an important target for achieving ART-free remission [3]. Persistent CSF escape likely points to HIV-1 replication from CNS resident cells, i.e. a reservoir. However, some cases of CSF escape appear to be episodic [4], though this is difficult to know given the paucity of longitudinal CSF data [3]. Episodic CSF escape, also termed CSF HIV viral blips, could stem from CNS reservoirs but may also be explained by transient immune cell trafficking into the CNS that supports local viral replication [3].

In as early as eight days of AHI, CNS involvement can be detected by the presence of HIV-1 RNA in CSF, which is consistently lower than concurrent plasma HIV-1 RNA [5]. Given this and the proposed mechanism of immune cell trafficking that may introduce HIV-1 into the CNS, it is likely that CSF HIV-1 RNA has a delayed appearance behind that in plasma [6]. Additionally, markers of immune activation and inflammation, but not neuronal injury, appear early in the CNS in AHI [5, 7]. Prompt initiation of ART in AHI suppresses HIV-1 replication in the CNS and reverses neuroinflammation [6, 8].

Early ART in AHI appears to mitigate neuroinflammation and neuronal injury but whether it reduces events of CSF escape, a potential window into CNS HIV persistence, remains unknown [6]. We investigated the frequency of CSF escape and 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 in Bangkok were enrolled in the ongoing RV254/SEARCH010 study (clinicaltrials.gov NCT00796146) [9], followed by immediate initiation of ART via a standardized 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 [10]. The majority were switched to dolutegravir starting in 2017 as the first-line regimen. Participants underwent serial interviews, examinations, and phlebotomy, with optional lumbar puncture (LP). 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 Walter Reed Army Institute of Research.

This analysis 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-1 RNA greater than plasma HIV-1 RNA at week 24 or week 96, as per recent international consensus definitions [2].

Sampling and laboratory testing

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-1 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), with lower limits of quantification (LLQ) of 50 and 20 copies/mL, respectively. CSF samples were run in batch and diluted fourfold for volume requirements for detection of HIV-1 RNA, with an LLQ of 80 copies/mL using the TaqMan platform. HIV-1 RNA measurements below the LLQ that tested positive were designated detectable but not quantifiable. Neuropsychological testing was performed at standardized intervals at weeks 0, 12, 24, and 96 [11].

RESULTS

Study participant characteristics

Of 627 participants enrolled to date, 204 participants with AHI followed by prompt ART initiation were identified with paired blood and CSF sampling in at least one visit at baseline, week 24, or week 96 (Table 1). Among these, 29 participants had CSF sampling at all three visits. 98% 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/mm3 (range 91-1302) and median plasma HIV-1 RNA 5.87 log10 copies/mL (range 2.43-7.89). Study enrollment was at median 19 days post-estimated infection (range 3-49). Among clinical and laboratory characteristics compared at enrollment, the only differences were earlier Fiebig stage and higher CD4+ T cell count among those who consented to LP versus those who declined (Supplemental Table 1).

At baseline, 126 of 165 participants (76%) had quantifiable CSF HIV-1 RNA with

median 3.13 log10 copies/mL (range <1.90-6.61). There were no cases where CSF HIV-1 RNA exceeded the paired plasma HIV-1 RNA level. At week 24, of 90 participants with paired blood and CSF samples, four (4%) had detectable CSF HIV-1 RNA, of which two (2%) were quantifiable. Three of these four cases with detectable CSF HIV-1 RNA were associated with plasma virological failure. The remaining one met criteria for CSF escape, with plasma HIV-1 RNA < 50 copies/mL and CSF HIV-1 RNA at 2.50 log10 copies/mL. At week 96, of 55 participants with paired blood and CSF samples, one (2%) had detectable and quantifiable CSF HIV-1 RNA, which was associated with plasma virological failure (Figure 1). This participant did not have quantifiable plasma or CSF HIV-1 RNA at week 24.

Clinical course of CSF escape after treatment in acute HIV-1 infection

From 145 on-ART CSF samples, one participant at week 24 post-treatment initiation met criteria for CSF escape. This 23-year-old male identified as bisexual and presented with acute retroviral syndrome. He was diagnosed with AHI in Fiebig stage IV at estimated 21 days post infection and was immediately started on efavirenz, tenofovir, and emtricitabine. Plasma HIV-1 RNA and CD4+ T cell count responded rapidly to ART (Supplemental Figure 1).

This participant had no study lumbar punctures or neuroimaging performed other than that at week 24. CSF white blood cell count was 4 cells/mm3, protein 30 mg/DL, and glucose 62 mg/dL. The participant did not endorse any neurological symptoms at week 24. An MRI performed at this time showed a small nonspecific hyperintense focus in the right high frontal white matter. Performance on neuropsychological testing was lowest at week 12 with a composite z-score of -1.8. At subsequent visits, he returned to a z-score within ±1.0 (Supplemental Figure 1). 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 baseline, the participant had nonreactive venereal disease research laboratory test (VDRL) and Treponema pallidum hemagglutination assay (TPHA). At week 24, TPHA was nonreactive, yet VDRL was not performed. At week 48, the participant had a reactive VDRL with a titer of 1:32 and reactive TPHA.

DISCUSSION

After immediate ART initiation in Thai participants with AHI, we detected a very low frequency of CSF escape of 1% after 24 weeks and 0% after 96 weeks. Moreover, the single case of CSF escape was a low CSF viral load of 316 copies/mL and was not associated with any neurological findings or dysfunction. This represents asymptomatic escape that was detected incidentally in a research setting. Prevalence of HIV CSF escape has been estimated in 10-20% of individuals who started ART during chronic HIV [1, 12]. Thus, initiation of ART during AHI may produce a beneficial control of HIV-1

within the CNS compared with treatment during chronic infection.

CSF escape has been described in the context of symptomatic neurological and neurocognitive impact in some patients, along with neuroinflammation and neuroimaging abnormalities [1, 13]. Additionally, persistent CSF escape may indicate the presence of a CNS reservoir due to HIV-1 replication from CNS resident cells. Episodic CSF escape, otherwise called CSF HIV viral blips, can stem from CNS reservoirs, but may also be explained by transient immune cell trafficking into the CNS that supports local HIV-1 replication or release [3]. Many cases of detectable CSF HIV-1 RNA are likely better described as CSF viral blips since they are not elevated on longitudinal measurements [4]. Of note, even with CSF viral blips, there may be an association between low-level CSF HIV-1 RNA and increased neuroinflammation as measured by elevated CSF neopterin levels [4].

Prior characterizations of this AHI cohort have described pre-ART characteristics of acute CNS infection. At baseline, most participants have detectable CSF HIV-1 RNA, which is consistently lower than that in plasma [5]. A higher ratio of CSF to plasma HIV-1 RNA associates with a greater degree of systemic immunodysregulation and neuroinflammation [14]. We now demonstrate that despite high pre-ART HIV-1 RNA levels (up to 106 copies/ml), CSF HIV-1 RNA is almost always suppressed to undetectable levels when initiating ART in AHI. This rarity of CSF escape may suggest that very early treatment reduces neuroinflammation, CNS persistence, and possibly viral reservoirs since the CNS harbors unique viral entry dynamics and compartmentalization [6]. Further investigations are needed to interrogate levels of HIV-1 DNA in CSF cells and other markers of CNS HIV-1 persistence.

The case of CSF escape was identified in a participant who has undergone only one study lumbar puncture to date. Thus, it is impossible to distinguish between persistent CSF escape and a CSF viral blip. Secondary CSF escape is unlikely given the nonreactive TPHA at time of lumbar puncture [15]. The level of 316 copies/mL is too low to perform sequencing or resistance testing using available assays. Applying novel assays with low viral copy number input could be useful in determining whether drug resistance mutations drive this case of CSF escape, degree of compartmentalization between blood and the CNS, and cellular tropism (macrophage versus CD4+ T cell). Such information would provide additional clues about whether the population is produced by resident CNS cells versus transient immune cell trafficking [3], though does not exclude the possibility of macrophage migration to and from the CNS through meningeal lymphatics [16].

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 [8]. Due to sample dilution for volume requirements for CSF, the LLQ of HIV-1 RNA differs between CSF and plasma, and thus our study cannot capture low-level CSF HIV-1 RNA < 80 copies/mL. Recent work has demonstrated even low-level CSF HIV-1 RNA < 20 copies/mL associates with decreased blood-brain barrier integrity and executive function [17]. Participants who consented for LP had small differences in CD4+ T cell count and Fiebig stage at enrollment compared with those who declined LP, and thus our study population may not be representative of all individuals presenting with AHI.

In conclusion, CSF escape is rare (1%) following initiation of ART during AHI in this cohort. Future work should corroborate the low incidence of CSF escape over longer follow-up and investigate the virological features of any CSF escape viruses detected after treatment in AHI.

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Table 1. Characteristics of participants treated in acute HIV infection with available study lumbar puncture.

	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 MSW MSM	 5 (2) 7 (3) 192 (94)	 5 (3) 6 (4) 154 (93)	 4 (4) 1 (1) 85 (94)	 2 (4) 2 (4) 51 (93)
Fiebig stage at enrollment[a], n (%) Stage I Stage II Stage III Stage IV Stage V Stage VI	 33 (16) 47 (23) 96 (47) 19 (9) 8 (4) 1 (0)	 27 (16) 37 (22) 76 (46) 17 (10) 7 (4) 1 (1)	 13 (14) 20 (22) 42 (47) 10 (11) 4 (4) 1 (1)	 8 (15) 11 (20) 29 (53) 3 (5) 3 (5) 1 (2)
Infection duration[b] (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-1 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)
Plasma HIV-1 RNA < 50 copies/mL, n (%)	0 (0) †	0 (0)	86 (96)	53 (96)
CSF HIV-1 RNA, log_{10} copies/mL, (range)	-	3.13 (<1.90-6.61)	<1.90 (<1.90-3.84)	<1.90 (<1.90-3.14)
CSF HIV-1 RNA < 80 copies/mL, n (%)	-	39 (24)	88 (98)	54 (98)
CSF viral escape, n (%)	-	-	1 (1)	0 (0)

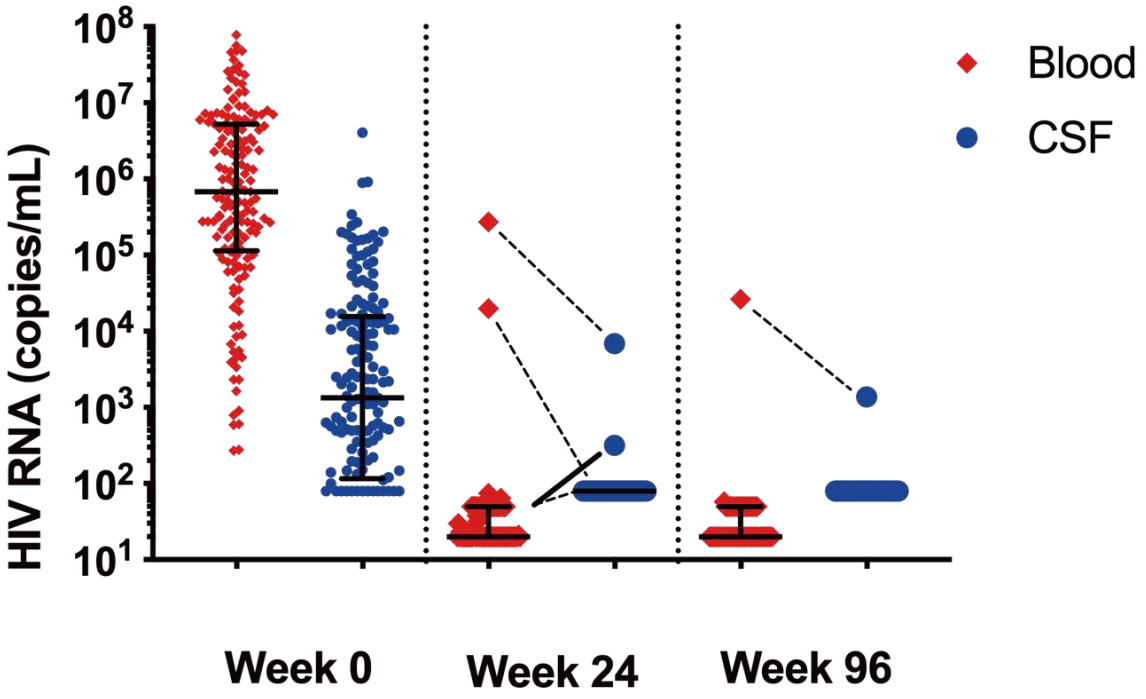
Abbreviations: HIV-1, human immunodeficiency virus-1; WSM, women who have sex with men; MSW, men who have sex with women; MSM, men who have sex with men including men identifying as bisexual; CSF, cerebrospinal fluid.

[a]Stage I: RNA+, p24 antigen–; Stage II: p24 antigen+, IgM–; Stage III: IgM+, Western blot–; Stage IV: Western blot indeterminate; Stage V: Western blot+ without p31 protein band; Stage VI: Western blot+.

[b]Interval between estimated time of exposure and enrollment. If a range of dates was provided, the mean was used.

†At time of enrollment.

Figure 1. Paired blood and CSF HIV-1 RNA at weeks 0, 24, and 96.



Medians and interquartile ranges are indicated by black bars. Dashed or solid lines represent paired blood and CSF HIV-1 RNA from participants who had either blood or CSF HIV-1 RNA greater than the respective limit of detection at week 24 (four participants) or 96 (one participant), with the solid line representing CSF escape.

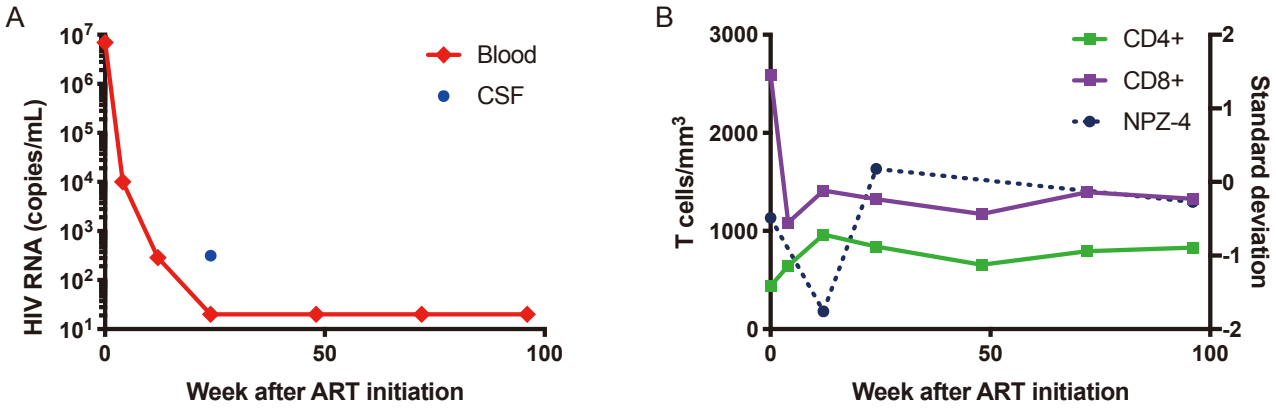
Supplemental Table 1.

	Participants with at least one lumbar puncture (n=204)	Participants declining lumbar puncture (n=423)	P-value[†]
Age at enrollment, median (IQR)	26 (23-31)	26 (22-31)	0.8
Male, n (%)	199 (98)	413 (98)	>0.9
Risk behavior, n (%)			>0.9
WSM	5 (2)	10 (2)	
MSW	7 (3)	15 (4)	
MSM	192 (94)	398 (94)	
Fiebig stage at enrollment[a], n (%)			0.03
Stage I	33 (16)	51 (12)	
Stage II	47 (23)	86 (20)	
Stage III	96 (47)	203 (48)	
Stage IV	19 (9)	56 (13)	
Stage V	8 (4)	24 (6)	
Stage VI	1 (0)	3 (1)	
Infection duration[b] (days), median (IQR)	19 (15-24)	19 (14-26)	0.3
Baseline CD4+ T-cells, cells/mm^3 (IQR)	386 (276-533)	348 (253-477)	0.03
Baseline CD8+ T-cells, cells/mm^3 (IQR)	517 (339-848)	511 (337-914)	0.7
Baseline plasma HIV-1 RNA, $\log_{10}$ copies/mL, (IQR)	5.87 (5.11-6.75)	5.92 (5.26-6.69)	0.7

Abbreviations: HIV-1, human immunodeficiency virus-1; IQR, interquartile range; WSM, women who have sex with men; MSW, men who have sex with women; MSM, men who have sex with men including men identifying as bisexual.
[a]Stage I: RNA+, p24 antigen–; Stage II: p24 antigen+, IgM–; Stage III: IgM+, Western blot–; Stage IV: Western blot indeterminate; Stage V: Western blot+ without p31 protein band; Stage VI: Western blot+.
[b]Interval between estimated time of exposure and enrollment. If a range of dates was provided, the mean was used.
[†]Comparisons between those consenting to versus declining lumbar puncture were performed using the Mann-Whitney *U* test for continuous and ordinal variables and the χ^2 test for categorical variables. Statistical analyses were performed using R (version 3.6.1; R Foundation for Statistical Computing).

Characteristics of participants treated in acute HIV infection stratified by consent for study lumbar puncture. Participants who consented for study lumbar puncture were no different in age, sex, risk behavior, and estimated acute infection duration, nor in CD8+ T cell count or plasma viral load at time of study enrollment, compared with participants who declined study lumbar puncture. Participants who consented for study lumbar puncture were more likely have earlier Fiebig stage of acute HIV infection and had higher CD4+ T cell count at study enrollment, compared with participants who declined study lumbar puncture.

Supplemental Figure 1.



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. The NPZ-4 score was a neuropsychological test composite z-score in four domains (Color Trails 1, Color Trails 2, Trail Making A, Grooved Pegboard), standardized to healthy Thai control data.

Chapter 10:
Neuropsychiatric Outcomes Before and After Switching to Dolutegravir-based Therapy in an Acute HIV Cohort

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AIDS Res Ther. 2020 Jan 7;17(1):1.
doi: 10.1186/s12981-019-0257-8.

ABSTRACT

Introduction: Dolutegravir (DTG)-based antiretroviral therapy (ART) is currently the first-line treatment for people living with HIV. Neuropsychiatric adverse events (NP-AEs) have been reported with DTG but neuropsychiatric symptoms have not been systemically quantified using structured scales. This study examined mood and cognitive parameters before and after a planned transition from non-DTG to DTG-based ART within a longitudinal study of acute HIV infection (AHI).

Methods: RV254 AHI cohort participants on ≥ 24 weeks of ART initiated at AHI underwent sequential assessments before and after the switch including: (1) Patient Health Questionnaire-9 (PHQ-9), a 9-item survey (scores 0-27) that evaluates somatic and affective/cognitive symptoms of depression; (2) a 2-Questions screening that has been validated locally for depression; (3) Distress Thermometer (scores 0-10); and 4) administration of a 4-test neurocognitive battery sensitive to HIV.

Results: 254 individuals (95% male, median age 30) switched to a DTG-based regimen after a median 144 weeks of ART. Serial assessments were completed at a median of 19 weeks before and 37 weeks after DTG. There was a modest but statistically significant increase in PHQ-9 scores after DTG (pre-switch: 5 [IQR 1-7] vs. Post-switch: 5 [IQR 2-8], p = 0.009). The percentage of participants with at least moderate depression (PHQ-9 ≥ 10) increased from 10 to 16% (p = 0.006), but the frequency of moderate-severe depression (PHQ-9 ≥ 15) remained unchanged (3%). No volunteer reported NP-AEs within the study period. Somatic symptoms of depression increased more than cognitive/affective symptoms. Plasma viral suppression (HIV-1 RNA < 50; p = 0.005) and PHQ-9 ≥ 10 (p < 0.001) before switch were linked to lower PHQ-9 scores after DTG in multivariable analysis. Performance on all neuropsychological tests, except grooved pegboard test, improved modestly after DTG (all p < 0.05).

Conclusions: After a median duration of 37 weeks of DTG use, there was a modest increase in the higher quartile of PHQ-9. This increase was associated with a rise in moderate depression symptoms but not the more severe forms of depression on PHQ-9. No clinically relevant NP-AEs were reported. Pre-existing depression was not associated with subsequent worsening of symptoms after DTG. Cognitive test performance improved post-DTG but could be due to practice effect.

INTRODUCTION

Dolutegravir (DTG) is a potent second-generation HIV integrase strand inhibitor with a high genetic barrier to resistance and favourable tolerability [1]. The World Health Organization recently recommended the use of DTG-based regimens as first-line antiretroviral therapy (ART) for people living with HIV (PLWH). However, clinical reports have raised concern regarding the risk of DTG-associated neuropsychiatric adverse events (NP-AEs) [2-4]. The rate of DTG discontinuation for NP-AEs ranged from 1 to 6% in previous studies [2-4].

Prior studies including self-reported NP-AEs described increased rates of insomnia in a minority of individuals after starting a DTG-based regimen. Increased depression has also been reported, particularly among individuals with a history of depression before DTG initiation [3]. However, the impact of DTG-based ART on the dimensional characterization of depression has not been explored. Additionally, neurocognitive performance before and after DTG-based ART is not well defined [2]. We previously reported that DTG was well tolerated with few discontinuations among young men who switched from a non-DTG to a DTG-based regimen [5]. This follow-up report focuses on affective and somatic dimensions of depression, and cognitive performance before and after switch to DTG.

METHODS

Study Design

We examined prospective data from the SEARCH010/RV254 cohort, an ongoing study of long-term outcomes following ART initiation during acute HIV infection (AHI) started in April 2009 (NCT00796146 and NCT00796263) [6]. Almost all participants initiated Efavirenz (EFV)-based ART within days (median=0; [IQR: 0-1]) after AHI diagnosis. They underwent regular clinical follow-up, laboratory blood tests, neurocognitive assessment and self-reported mood symptoms questionnaires (see below). The study protocol was approved by the institutional review boards of all relevant collaborating institutions. All participants provided written informed consent. Starting in March 2017, cohort participants systematically switched to a DTG-based regimen (Figure 1). Participants with elevated liver enzymes (grade III or above) or unstable liver disease were excluded from switching.

Participants Selection

Participants who fulfilled the following criteria by 30th April 2018 were included for analysis: 1/ completed structured assessments of mood, neurocognitive assessment and HIV-related laboratory tests (plasma HIV-1 RNA, CD4+ and CD8+ T-cell levels) before and after the switch; 2/ pre-DTG assessments were completed at least 24 weeks after cohort enrolment (AHI) and post-DTG assessments were completed at least 3 months after the switch; 3/ stable virologic control with undetectable (<50 copies/ml) or

declining (<200 copies/ml) plasma HIV-1 RNA; and 4/ without objective or subjective side effects on their pre-DTG ART regimen. The second and third criteria aimed to prevent impact from depression and anxiety associated with AHI diagnosis and the biological effect driven by plasma viremia [7], whereas the last criterion aimed to prevent cognitive and psychological benefits gained from switching from an ART regimen with known side effects.

Neuropsychiatric Assessment

Mood assessments included the Patient Health Questionnaire-9 (PHQ-9), 2Q-Depression screen and Distress Thermometer (DT), which have been validated for use in Thailand [8-11]. The PHQ-9 is a 9-item survey (score range 0-27) derived from DSM-IV criteria for depression [12]. It can be further categorized into somatic (sleep/appetite/energy level, questions 3-5) and affective/cognitive (questions 1, 2, 6-9) components of depression. PHQ-9 total scores ≥10 and ≥15 have been used to detect moderate and moderate-severe depression, respectively [12]. The 2Q-Depression screen was developed and validated by the Thai Ministry of Public Health to serve as a rapid assessment of clinically relevant depression [8]. The 2Q-Depression screen asks participants two yes/no questions related to sadness and loss of interest or pleasure in daily activities [8]. The DT is a self-report measure of emotional stress that utilizes an image of a thermometer to guide severity ratings of stress and anxiety from 0-10 [10, 11].

Neurocognitive Assessment

Neurocognitive tests included measures of fine motor speed and dexterity (non-dominant hand Grooved Pegboard test (GPB; Lafayette Instrument Company, Lafayette, USA), psychomotor speed (Color Trails 1 and Trail Making A; PAR, Inc., Lutz, USA) and executive functioning/set shifting (Color Trails 2; PAR, Inc., Lutz, USA; see [13] for complete information). In the parent study cohort (RV254), participants are regularly assessed by this battery longitudinally, since enrolment at pre-treated AHI. As all the selected participants had to be followed for more than 24 weeks after enrolment (2nd criterion), they would have completed the neurocognitive test battery on at least 3 occasions (baseline, week 12 and 24) prior to the DTG switch. This design consideration minimized the potential confound of practice effect before and after the switch, which is most obvious between the first and second assessment [14]. Raw scores were standardized to Thai normative data [13] and z-scores for each test were averaged to provide a measure of overall neuropsychological performance (NPZ-4).

Data Analysis

Results were reported as median and interquartile range (IQR) or frequency and percentage, as appropriate. Plasma viral suppression was defined as HIV-1 RNA < 50 copies/ml. McNemar and Wilcoxon signed-rank tests were used, as appropriate, to compare the outcomes before and after DTG. Multivariate linear regression examined

factors that were correlated with the change in PHQ-9 scores between the 1st and 2nd assessment. Statistical analyses were performed using SPSS Version 18.0 (IBM Corp., Armonk, NY).

RESULTS

At the time of analysis, 260 participants who had switched to a DTG-based regimen fulfilled the selection criteria. Of note, 6 participants preferred not to switch to a DTG-based regimen due to pill burden (n=5) or for unknown reason (n=1). Six participants discontinued DTG before post-DTG assessment due to acute hepatitis C-related elevated liver enzymes; these individuals were excluded. No participants discontinued DTG because of subjective or elicited NP-AEs within the analysis period. Among the 254 participants included in this study, nearly all were Thai (99%) and male (95%), with a median age of 30 [IQR 25-36]. Participants switched to dolutegravir/abacavir/lamivudine (85%) or dolutegravir/tenofovir disoproxil fumarate/lamivudine in case of a positive HLA-B*5701 assay or chronic hepatitis B infection. The median duration of ART prior to the planned switch was 144 [IQR 24-192] weeks; 82% were previously on EFV-based ART, 13% were on a boosted protease inhibitor (mostly lopinavir)-based ART, 5% were on rilpivirine-based ART, and one individual was on a raltegravir-based regimen.

The median duration from pre-switch assessment to the switch to DTG was 19 [IQR 9-34] weeks, and from the switch to the follow-up assessment was 37 [IQR 24-48] weeks. Table 1 shows all the tested parameters before and after the switch. At follow-up, the frequency of plasma viral suppression increased from 96% to 98% (p=0.070). Additionally, CD4+ T-cell count (pre-switch: 624 [IQR 512-783] vs. post-switch: 662 [IQR 530-833], p<0.001) and CD4/CD8 ratio (pre-switch: 1.09 [IQR 0.85-1.41] vs. post-switch: 1.12 [IQR 0.87-1.43], p=0.026) were higher at follow-up.

Mood Symptoms Before and After Dolutegravir

Scores on the DT and the 2Q-Depression screen did not change after DTG (both p>0.10). At follow-up, the total PHQ-9 score increased in 48% of participants, decreased in 31%, and remained unchanged in 21%, resulting in a modest but statistically significant increase in the PHQ-9 score after DTG, with an increase in the upper IQR (pre-switch: 5 [IQR 1-7] vs. post-switch: 5 [IQR 2-8], p=0.009). The percentage of participants with at least moderate depression (PHQ-9≥10) increased from 10% (n=24) to 16% (n=40, p=0.006), whereas the percentage of participants with moderate-severe depression (PHQ-9≥15) remained unchanged (3%).

Comparing the changes in the somatic and cognitive/affective subset scores of PHQ-9 showed a more prominent but modest change in the somatic sub-scores similar to that in the total PHQ-9 score. There were increases in somatic sub-scores (pre-switch: 2 [IQR

0-3] vs. post-switch: 2 [IQR 1-3], p=0.007) and the cognitive/affective sub-scores (pre-switch: 2 [IQR 0-4] vs. post-switch: 2 [IQR 0-5], p=0.064) at the lower and upper IQR respectively with unchanged median scores.

Factors Associated with PHQ-9 Change after Dolutegravir

Linear regression was employed to evaluate potential factors that were associated with the PHQ-9 change (i.e. PHQ-9 at 2nd assessment minus PHQ-9 at 1st assessment) (Table 2). In the univariate analyses, plasma viral suppression and PHQ-9 ≥10 before DTG were associated with lower PHQ-9 scores at follow-up (p=0.003 and p<0.001, respectively). Older age trended towards association with lower PHQ-9 scores after DTG (p=0.096). CD4+ T-cell count and EFV use before DTG were not associated with subsequent change in PHQ-9 score. In the multivariate analysis, only pre-existing viral suppression (mean difference -3.2, 95%CI [-0.9 to -5.4], p=0.006) and PHQ-9≥10 pre-switch (mean difference -2.7, 95%CI [-1.2 to -4.2], p<0.001) remained independently associated with a decrease in PHQ-9 score.

Neurocognitive Tests Performance

The mean neurocognitive performance, measured by the NPZ-4, increased modestly at follow-up (pre-switch: 0.70 [IQR 0.31-1.10] vs. post-switch: 0.88 [IQR 0.37-1.19], p<0.001). Z-scores on the Color Trails 1 and 2 and Trail Making A were higher after DTG (all p<0.05) while the performance on the GPB remained statistically similar (Table 1). Additional comparison between EFV (n=207) and non-EFV users (n=47) pre- and post-switch revealed statistically similar test performance (Table 3).

DISCUSSION

This study provides an integrated evaluation of mood symptoms and cognitive performance before and after a planned switch to DTG in individuals on stable ART initiated during AHI. All participants were maintained on stable ART before the switch with a median duration of 144 weeks. The current study leveraged an organized change in treatment regimen to a standardized DTG-based regimen among a large cohort who had almost universally achieved viral suppression using non-DTG ART. This is an important distinction to other studies that reported worsening of cognitive performance among those on DTG. All participants were stable on their pre-switch ART without objective or subjective side-effects, which helped to prevent additional benefit from switching to a DTG-based ART. After a median duration of 37 weeks of DTG, there were no discontinuations because of NP-AEs. The proportion of participants with moderate depression (PHQ-9≥10) increased after DTG, but the median total PHQ-9 score and the percentage of participants with moderate-severe depression (PHQ-9≥15) remained unchanged. Scores on the DT and the 2Q-Depression screen were similar after DTG. Taken together, our findings do not support a clear association between the use of

DTG and worsening of clinically relevant mood symptoms in young male HIV-positive population.

The multivariable analysis aimed to identify potential contributors linked to PHQ-9 changes. It revealed that individuals who achieved viral suppression pre-switch were less likely to experience worsening of depression symptoms following DTG. Further, those with higher PHQ-9 scores pre-switch were not linked to higher scores at follow-up.
In subset analyses, both somatic and affective/cognitive dimensions of the PHQ-9 modestly worsened after DTG, but the magnitude of change was only statistically significant for the somatic subscale. Yagura et al. previously reported a potential link between supratherapeutic DTG levels and CNS side-effects [15], in which 88% of adverse events were somatic in nature (e.g. headache, dizziness, insomnia and restlessness). Elliot et al., however, reported no association between DTG pharmacokinetics (PK) and changes in sleep parameters or neurocognitive performance in PLWH aged 60 or older [4]. Additional work is needed to determine whether a subgroup of PLWH may be prone to DTG-related NP-AEs.

Modest improvements were observed in 3 out of 4 neurocognitive test scores and HIV clinical indices (CD4+ T-cell count and CD4/CD8 ratio). However, the improvement in neurocognitive test is unlikely driven by the switch to DTG. First, the pre-DTG z-scores in all 4 tests were within normal range and did not support any cognitive impairment among our participants pre-switch. Second, albeit multiple groups have reported a possible association between EFV use and worse cognitive performance [16-18] and the majority of our participants were on EFV pre-switch, pre-DTG cognitive test performances were similar between EFV and non-EFV users in our study. Thus, the improvement in test performance was less likely driven by elimination of any negative effects from EFV-related cognitive symptoms. Practice effect remains a plausible explanation [14], although the participants had already undergone repeated testing before the post-DTG assessment. The improvement in cognitive function may have also allowed greater insight into their bodily condition that led to the modest worsening of depression symptoms. However, as our participants were on average not cognitively impaired pre-switch and insight ability was not evaluated in the neurocognitive test battery, we are unable to examine this potential linkage. In any case, the observation that neurocognitive performance post-DTG was stable among this cohort of AHI is reassuring and is in line with previous work [4] that utilized different neurocognitive tests [19].

LIMITATIONS

Our participants were mostly young males without additional complicating medical problems, who started ART during AHI and thus had few comorbidities and a relatively high CD4 nadir. Individuals with major psychiatric illness were not enrolled in the main protocol. These factors may restrict the generalizability of the findings to other settings,

where PLWH of older age, with multiple co-morbidities, and/or more advanced immunosuppression are common. Our findings are also limited by the lack of a control group comprised of individuals who did not switch to DTG, which would allow a more robust assessment of DTG-related changes in mood and cognitive performance.

CONCLUSION

Although there was a modest increase in the higher quartile of PHQ-9 after the switch, transition to a DTG-based ART is associated with relative stability in neuropsychiatric symptoms in a group of predominantly young male PLWH. The modest increase in somatic symptoms may explain the anecdotal reporting of increased neuropsychiatric side effects following use of DTG in large clinical trials. Additional studies are needed to inform outcomes among females, older PLWH, and individuals with chronic disease receiving a DTG-based regimen for HIV.

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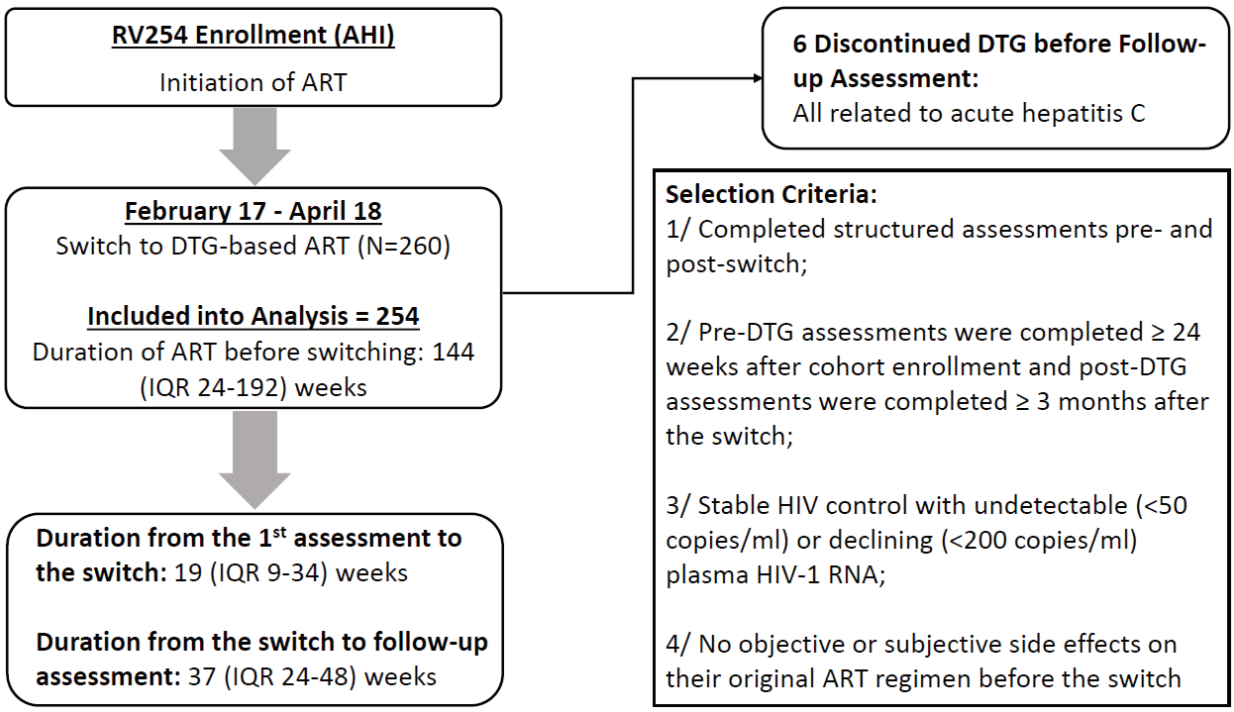
Parent Protocol Design and Operation:	JA, VV, SS
Study Design:	PC, OG, PR, RP
Clinician Follow-up and Assessment:	PC, OG, EK, DC, CS
Nurse Assessment and Mood Questionnaires:	PP
Statistical Analysis:	PC, SP, RP
Manuscript preparation:	PC, OG, EK, DC, CS, SP, PP, PR, JA, VV, SS, RP

This is a pre-copy editing, author-produced PDF of an article accepted for publication in *AIDS Research and Therapy* follwing peer review. The definitive published article is available online at: https://doi.org/10.1186/s12981-019-0257-8

Figure 1: Study Design and Selection Criteria



Abbreviations: AHI = Acute HIV infection; DTG = Dolutegravir; ART= Antiretroviral therapy.

Table 1. Parameters Before and After Transition to Dolutegravir (N=254)

	Pre-switch**	Post-switch**	P value
CD4+ T-cells (cells/ul)	624 (512-783)	662 (530-833)	< 0.001
CD8+ T-cells (cells/ul)	574 (449-787)	618 (482-797)	0.155
CD4/CD8	1.09 (0.85-1.41)	1.12 (0.87-1.43)	0.026
NPZ-4	0.70 (0.31-1.10)	0.88 (0.37-1.19)	< 0.001
Color Trails 1 z-score	1.15 (0.59-1.56)	1.30 (0.64-1.74)	0.001
Color Trails 2 z-score	0.61 (0.14-1.11)	0.86 (0.40-1.22)	< 0.001
Grooved Pegboard Test z-score	0.54 (-0.20-1.05)	0.64 (-0.09-1.10)	0.149
Trail Making A z-score	0.75 (0.14-1.15)	0.80 (0.07-1.33)	0.037
PHQ-9 score	5 (1-7)	5 (2-8)	0.009
PHQ-9 ≥ 10, n(%)	24 (10)	40 (16)	0.006
PHQ-9 ≥ 15, n(%)	8 (3)	8 (3)	1.000
^ PHQ-9 Somatic Sub-score	2 (0-3)	2 (1-3)	0.007
# PHQ-9 Cognitive/Affective Sub-score	2 (0-4)	2 (0-5)	0.064
Major Depression by 2Q-Depression Screening, n(%)	2 (1)	3 (1)	1.000
Distress Thermometer Score	2 (1-5)	2 (1-4)	0.898
***Viral Suppression, n(%)**	244 (96)	250 (98)	0.070

**Median (IQR) is presented unless specified; Wilcoxon and McNemar Test were used accordingly.
^ Questions 3, 4, 5; # Questions 1, 2, 6, 7, 8, 9.
* Defined as plasma HIV RNA < 50 copies/ml.
Abbreviations: NPZ-4 = Composite z-score of the 4 neuropsychiatric tests; PHQ-9 = Patient Health Questionnaire-9.

Table 2. Factor Correlation with PHQ-9 Changes*

	Univariable		Multivariable	
	PHQ-9 mean difference (95% CI)	**P-value**	**PHQ-9 mean difference (95% CI)**	**P-value**
Age	-0.05 (-0.01 to 0.008)	0.096		NS
Sex (male)	0.64 (-1.4 to 2.70)	0.537		
CD4, every 100 cells/ul	-0.03 (-0.2 to 0.2)	0.713		
Viral suppression	-3.4 (-5.7 to -1.2)	0.003	-3.2 (-0.9 to -5.4)	0.006
PHQ-9 ≥ 10 before DTG	-2.8 (-4.3 to -1.3)	<0.001	-2.7 (-1.2 to -4.2)	<0.001
PHQ-9 ≥ 15 before DTG	-6.2 (-8.6 to -3.7)	<0.001		
EFV use before DTG	0.11 (-1.1 to 1.3)	0.859		

*PHQ-9 change = PHQ-9 at 2nd assessment minus PHQ-9 at 1st assessment
Statistical method: Linear regression with PHQ-9 change as dependent variable
Factors with p<0.1 in the univariable model were included into multivariable analysis
Abbreviation: NS = not significant; PHQ-9 = Patient Health Questionnaire-9; EFV = Efavirenz

Table 3. Neuropsychological Tests Performance before and after DTG by EFV Use at Pre-switch

	EFV-based ART	**Non-EFV-based ART**	**P-value**	**EFV-based ART**	**Non-EFV-based ART**	**P-value**
NPZ-4	0.69 (0.32 to 1.10)	0.83 (0.27 to 1.14)	0.776	0.87 (0.37 to 1.19)	0.89 (0.31 to 1.21)	0.747
zCT1	1.13 (0.59 to 1.54)	1.25 (0.53 to 1.63)	0.667	1.29 (0.63 to 1.74)	1.36 (0.89 to 1.81)	0.689
zCT2	0.59 (0.12-1.10)	0.65 (0.26 to 1.15)	0.525	0.86 (0.40 to 1.20)	0.81 (0.17 to 1.33)	0.916
zGPB	0.55 (-0.15 to 1.07)	0.44 (-0.40 to 1.00)	0.379	0.66 (-0.08 to 1.14)	0.56 (-0.45 to 1.04)	0.857
zTrail A	0.75 (0.09 to 1.15)	0.79 (0.37 to 1.13)	0.902	0.84 (0.11 to 1.37)	0.65 (-0.21 to 1.15)	0.126

Median (IQR) is presented.
Abbreviations: ART = Antiretroviral Therapy; EFV = Efavirenz; NPZ-4 = Composite z-score of the 4 neuropsychiatric tests; CT1 = Color Trails 1; CT2 = Color Trails 2; GPB = Grooved Pegboard Test; TrailA = Trail Making A

SECTION 4
RV254 in Connection with HIV-1 Cure and Analytical Treatment Interruption

Chapter 11:
Perspective on potential impact of HIV central nervous system latency on eradication

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AIDS. 2019 Dec 1;33 Suppl 2:S123-S133.
doi: 10.1097/QAD.0000000000002264.

ABSTRACT

HIV remission and eradication are desirable goals for people living with HIV given the challenges of life-long adherence and comorbidities such as HIV association neurocognitive disorder despite suppressive antiretroviral therapy (ART). There is evidence that HIV persists and replicates in the CNS in at least some individuals which would impact the success of HIV remission interventions. This article addresses the role of the HIV CNS latency on HIV eradication, examine the effects on the CNS of early ART, latency modifying agents, antibody-based and T-cell enhancing therapies as well as ART interruption in remission studies. We propose the integration of CNS monitoring into such studies in order to clarify the short- and long-term neurological safety of experimental agents and treatment interruption, and to better characterize their effects on HIV CNS persistence.

INTRODUCTION

Antiretroviral therapy (ART) has transformed HIV-1 infection from a life-threatening disease to a manageable yet sometimes complex chronic illness. Latent HIV-1 resides mostly in long-lived memory CD4+ T-lymphocytes in blood and lymphoid tissues, and poses a barrier to curing HIV-1. Reactivation of replication-competent virus in these cells can fuel a new round of cell infection in the absence of ART. HIV-1 cure is an important goal given the challenges of lifelong ART adherence, the financial burden to healthcare systems, chronic immune activation [1, 2], and non-AIDS related co-morbidities including HIV-associated neurocognitive disorders (HAND) despite successful therapy [3-6].

The ultimate and near impossible goal of HIV-1 cure research is sterilizing cure or eradication, which aims to abolish all cells that harbor replication-competent provirus within the host cellular DNA. A functional cure or sustained virologic remission is a difficult but more attainable goal. It is the ability to maintain plasma viral load below detection limit without ART despite the persistence of replication-competent HIV-1. The potential HIV-1 eradication in the Berlin patient and lately, the London patient, required extreme means of cancer treatment and CCR5-negative stem cell transplantation [7-9]. Remission cases are unusual and mostly described in people who initiated ART during acute HIV-1 infection (AHI). There are several investigative approaches to reduce and control latently infected cells, namely, early ART, latency reactivation or suppression, and immune-based, cell-based and gene-editing therapies [10].

This article addresses three questions relating to the role of the HIV-1 central nervous system (CNS) latency on HIV-1 eradication. First, how is HIV-1 CNS infection and persistence relevant to HIV-1 cure efforts? Second, how do HIV-1 remission strategies affect the CNS? Third, what future investigations could be considered in HIV-1 remission studies?

How is HIV-1 CNS infection and persistence relevant to HIV-1 cure efforts?

HIV-1 clearly involves the CNS. Millions living with HIV-1 suffer from HAND that affects their lives in substantial ways. Although ART has dramatically reduced HIV-associated dementia, milder forms of HAND are prevalent in 15% to 55% of people with HIV [4, 5, 11]. Moreover, new onset and ongoing deterioration of cognitive impairment occur despite ART [6, 12, 13]. HIV-1 RNA is readily detected in cerebrospinal fluid (CSF) samples within days of infection [14, 15]. Among individuals on suppressive therapy, CSF examination shows evidence of ongoing immune activation [16], whereas positron emission tomography (PET) study shows an association between microglial activation and lower cognitive performance [17].

Whether the HIV-1 virions in the CNS are replication-competent is unproven. However, some treated individuals with plasma viral suppression still show low levels of HIV-1 RNA

in their CSF with elevated levels of CSF inflammatory biomarkers [18]. In the systemic circulation, infected CD4+ T-lymphocytes with defective HIV-1 DNA still generate incomplete RNA transcripts that could contribute to the persistent immune activation [19, 20]. Future studies should clarify if such phenomenon co-exists in the CNS as HIV-1 DNA is frequently present in the brain cells of people who have been on years of suppressive ART [21].

Controlling CNS infection and persistence is integral to the efforts in curing HIV-1. First, HIV-1 remission strategies should reduce frequencies of HIV-infected cells in the CNS. HIV-1 infected brain cells are long lived residential cells including perivascular macrophages, microglia and astrocytes. Pathological studies suggest their ability to harbor HIV-1 DNA with or without the development of HIV encephalitis [22-27], and the frequencies of HIV-1 DNA+ cells correlate with reduced cognitive function [28, 29]. Despite long-term suppressive ART, brain macrophages/microglia from simian immunodeficiency virus (SIV)-infected macaques still harbor replication-competent virus that could be reactivated by latency-reversing agents (LRA) [30, 31].

Second, strategies should improve control of viral replication in the CNS. CNS viral escape is when HIV-1 RNA is detected only in the CSF, or CSF viremia is $\geq$ 1 log10copies/ml above a fairly well controlled HIV-1 RNA in blood [32-34]. Although mostly asymptomatic, CNS viral escape is linked to elevated CSF soluble inflammatory markers [18, 33, 35]. Moreover, patients with symptomatic CNS viral escape can present with a spectrum of neurologic issues ranging from a mild headache or sensory disturbance to encephalopathy or coma [36-38]. Symptomatic viral escape typically responds to ART adjustment guided by genotyping of CSF HIV-1, or by selecting ART regimens with higher CNS penetration-effectiveness (CPE) scores [36, 39]. However, the latter does not necessarily improve the cognitive outcome of individuals with HAND [40-42]. Furthermore, studies have reported ART-resistant strains in the CSF of treated individuals with plasma viral suppression [36, 38, 39].

Third, remission strategies should be able to tackle potential viral quasispecies that may be different in CSF compared to blood. HIV-1 CNS compartmentalization can occur as early as the first year of infection in untreated individuals [43]. In one report, it persists during ART and returns during viral rebound post ART interruption [44]. The repertoires of HIV-specific CD8+ T-lymphocytes receptor are different between CSF and blood even at AHI [45]. Such phenomenon could affect the responses to immune-based HIV-1 remission strategy.

How do HIV remission and cure strategies affect the CNS?
Table 1 summarizes human and non-human primate (NHP) studies that evaluate the CNS outcomes of 1) stand-alone treatment interruptions (TI) and 2) remission strategies with or without TI.

Early ART

ART initiated during AHI markedly reduces frequencies of cells that harbor HIV-1 DNA in blood and lymphoid tissues [46, 47], and associates with higher CD4+ T-lymphocytes and CD4/CD8 ratio [48]. Compared to ART initiation at chronic infection, early ART leads to lower levels of CSF microglial activation [49] and neuronal damage markers [50], and normalization of intracranial inflammation in magnetic resonance spectroscopy (MRS) studies [51, 52]. People received early ART (20-132 estimated days of exposure) also have markedly reduced CSF HIV-1 antibody levels likely due to shorter HIV-1 antigens exposure [53]. In short, early ART initiation could contribute to a dramatic reduction in systemic, and likely, CNS reservoirs. Together with the preserved adaptive immunity, early treated individuals could be better candidates for HIV-1 remission strategies.

Latency reversal agents

Latency reversal is part of the "shock and kill" concept [54]. It aims to "shock" infected cells out of latency, forcing them to express viral antigens and thereby exposing them to killing by direct viral cytopathic action and HIV-specific cytotoxic T-lymphocytes (CTLs). Early trials combining anti-CD3 antibody and recombinant human IL-2 that induced global T-lymphocytes activation validated the concept of latency reversal but also induced significant toxicity from cytokine release [55, 56]. Subsequent research has focused on small molecule LRAs that do not cause overt T-lymphocytes activation and cytokine release [57].

Such LRAs include 1) histone deacetylation inhibitors (HDACi) that block the epigenetic control of proviral gene silencing (e.g. vorinostat, panobinostat and romidepsin); 2) protein kinase C (PKC) agonists that induce transcription factor NF-κB (e.g. prostratin, bryostatin-1, and ingenol compounds); and 3) toll-like receptor (TLR) agonists that reactivate viral latency through cellular signaling [58, 59].

Most LRAs appear to be non-toxic to primary CNS cells at therapeutic concentrations [60]. Some LRAs including romidepsin, JQ-1, and panobinostat can induce viral transcription in infected astrocytes in-vitro [60-62]. One study shows bryostatin-1 to reactivate infected astrocytes with p24 antigen upregulation [62]. However, another LRAs study including bryostatin-1 fails to reactivate the latency of infected astrocytes after initial active viral productive phase [63]. In-vitro macrophage/microglia studies demonstrate that combination of LRA reactivates latent virus with increased HIV-1 mRNA and protein levels [64, 65].

Non-human primate (NHP) studies provide insight into the viral dynamics of the HIV-1 reservoir in the CNS. After administration Ingenol-B and vorinostat, one out of two SIV-infected macaques on long-term suppressive ART developed SIV encephalitis with neurological symptoms; subsequent CSF examination showed a viral load 10-fold higher

than that in plasma [30]. Post-necropsy investigation suggest that the CSF viral transcripts originate from resident CD68+ macrophages/microglia within the occipital lobe of the brain. Importantly, replication-competent virus was isolated from infected brain macrophages in 7 out of 8 macaques under similar experimental conditions [31]. Therefore, a balance between CNS desired effects and safety requires careful consideration.

More than 15 clinical trials have evaluated LRAs, and some showed a modest increase in blood cell-associated and plasma HIV RNA after LRAs without a decrease in cell-associated HIV DNA [59]. Two assessed the CNS outcomes and showed no adverse effects [66, 67]. One panobinostat study exhibited undetectable CSF HIV-1 RNA in 11 participants despite six having measurable plasma HIV-1 RNA after the last dose of LRA [68]. There were also no changes in CSF inflammatory and neuronal injury markers. Perhaps these findings are unsurprising given the undetectable CSF levels of panobinostat. Our group previously examined the effect of vorinostat, hydroxychloroquine and maraviroc (VHM) in 10 acutely treated AHI participants who have maintained viral suppression for at least 48 weeks [67]. VHM was given under ART coverage, followed by ART interruption. Low-grade CSF HIV-1 viremia was detected in 2 out of 8 CSF samples (25 and 42 copies/ml) at systemic viral rebound (plasma HIV RNA 25796 and 329 copies/ml respectively) after TI. There was no adverse CNS outcome observed from serial neuropsychiatric tests, CSF inflammatory markers measurements and MRS.

Multiple factors could affect the outcomes of LRAs. Apart from CNS penetration, LRAs may alter the blood-brain barrier (BBB) permeability and immune cell functions [69]. Moreover, inconsistent reactivation responses [61] and LRAs-induced reactivation of defective HIV-1 provirus reservoir [20, 70] may hinder the efficacy of subsequent killing of the "true" reservoir.

Latency suppression agents

The improved understanding of HIV-1 latency also gives rise to the "block and lock" strategy that aims to disarm the reactivation ability of the HIV-1 reservoir. HIV-1 transactivator of transcription (Tat) protein, which expresses early in the viral life cycle and promotes subsequent HIV transcription, appears to be a potential target for intervention due to the absence of a human homolog. Didehydro-cortistatin A (dCA) is an analog of the natural compound cortistatin A. It potently inhibits Tat production from infected CD4+ T-lymphocytes isolated from viraemic and aviraemic patients [71]. In combination with ART, dCA potentiates the inhibition of viral production during cellular expansion in comparison to ART alone [72]. dCA further inhibits viral reactivation in T-lymphocytes exposed to LRAs and ART interruption [72, 73]. dCA crosses the BBB in animal models and inhibits Tat uptake in microglia-like and astrocytes cell lines [74].

In the CNS, Tat may directly contribute to HAND through pro-inflammatory and direct cytotoxic effects [75]. Tat induces neurobehavioral in an animal model [76]. It upregulates platelet-derived growth factor in astrocytes [77], which eventually leads to CCL2 secretion and monocytes infiltration across BBB [78]. It may also cause direct neuronal loss through astrocytosis-related excitotoxicity [79]. As a result, if dCA shows a similar inhibition of latency reactivation in CNS infected cells, it may alleviate intracerebral immune activation and perhaps offer a viable alternative for HIV remission.

Immune-based therapies

Aside from the complexity of the CNS response during "shock," the lack of an effective "kill" mechanism marks another major obstacle to a cure. Human and NHP trials have evaluated interventions to enhance antibody and T cell functions. Their mechanisms and study outcomes were recently reviewed [80].

Broadly Neutralizing Antibodies

Broadly neutralizing antibodies (bNAbs) can neutralize diverse, multi-clades circulating HIV-1 strains. BNAbs binds cell-free virus, blocks new infection [81], and reduces infected cells through FcϒR receptor-dependent mechanisms in animal models [82]. The effect of bNAbs in CNS is unclear. First, bNAbs are theoretically too large to cross the BBB and gain access to locally infected cells. Second, bNAbs may not target virus-containing compartment in monocyte-derived macrophages according to in vitro experiments [83, 84]. Antibody-treated macrophages remain infectious and could transmit HIV-1 to CD4+ T-lymphocytes via macrophage-T-lymphocytes virological synapse [83, 85]. The role of bNAbs in reducing infection in microglia and astrocytes requires further studies. Our group did not observe changes in neuropsychological tests or MRI parameters in acutely treated participants who either received VRC01 bNAb or placebo at time of TI [86]. A subset with CSF samples did not reveal viremia post-TI despite detectable plasma viremia [86, 87].

Anti-α4β7 integrin Ab

α4β7 integrin is a gut-homing receptor and target of HIV-1 gp120 [88, 89]. It is extensively expressed on memory CD4+ T-lymphocytes [90], resulting in massive infection and depletion of gut α4β7+ CD4+ T-lymphocytes during productive HIV-1 infection. Sustained SIV remission has been reported in macaques treated with primatized α4β7 integrins monoclonal antibody (mAb) in addition to ART during primary infection [91]. Vedolizumab, an approved α4β7 integrin mAb treatment for inflammatory bowel diseases, is being investigated for HIV remission in chronically HIV-infected people (NCT02788175 and NCT03147859). Vedolizumab does not affect T-lymphocytes migration to the CNS like the α4β1 mAb (Natalizumab) [92]. Although vedolizumab is not anticipated to impact CNS immune surveillance and cause neurologic diseases, CNS safety monitoring is prudent as being performed in one study (NCT03147859).

T-lymphocytes enhancing strategies

Boosting T-lymphocytes immunity against HIV-infected cells is another killing strategy. Learning from the cancer field, several agents including dual affinity retargeting molecules (DARTs), immune checkpoint inhibitors and therapeutic vaccines are now being investigated for HIV-1 infection.

DARTs are bispecific, antibody-based molecules that recognize two different epitopes and redirect T cells to target cells [93]. Applications of DARTs in hematological malignancies are actively explored [94], but little is known about their effects on the CNS residential cells including macrophages, microglia and astrocytes.

Immune checkpoint inhibitors aim to reverse the immune exhaustion and dysfunction of HIV-specific CD8+ T-lymphocytes during chronic HIV-1 infection. Anti-programmed death ligand 1 (PD-L1) mAb was well tolerated in a phase 1 trial involving 8 HIV-1 infected individuals on suppressive ART [95] but one developed asymptomatic hypophysitis 266 days after the infusion. Endocrine complications including hypophysitis and hypothyroidism/thyroiditis are observed in cancer trials with immune checkpoint inhibitors such as anti-cytotoxic T-lymphocyte-associated molecule 4 (8% and 6% respectively, n=256) [96], and to a lesser extent, anti-PD-L1 [97]. Neurological complications such as myopathy, neuropathies and cerebellar ataxia were reported in 2.9% of cancer patients (n=347) who received anti-PD-L1 mAb [98]. Given these infrequent but severe toxicities, close systemic and CNS monitoring is warranted in trials using immune checkpoint inhibitors.

Therapeutic vaccinations induce systemic immune responses against target cells including brain cells. Dendritic cells (DC)-based vaccines show promising results in cancer trials of CNS glioma and glioblastoma [99, 100]. Severe immune-related toxicity is less frequent in DC vaccines compared to antibody-based treatment for cancers [99]. HIV-1 antigen-pulsed DC vaccines can induce HIV-specific T-lymphocytes responses in HIV-infected individuals on suppressive ART [101]. Boosted DCs may also reactivate latent HIV-1 provirus [102, 103]. In one study, 4 out of 10 participants on ART had a transient increase in plasma HIV-1 RNA levels following autologous DC vaccines [104]. Although the vaccination did not prevent viral rebound during ART interruption, it showed a modest reduction in peak plasma viremia [104]. Other therapeutic HIV-1 vaccines combination such as ChAdV63.HIVcons and MVA.HIVconsv showed potential in viremic control potential among participants who started ART within first year of HIV-1 infection [105].

The chimeric antigen receptor (CAR) engineered T-lymphocytes offers a new option for difficult-to-treat cancers and possibly HIV-1. FDA approved CD19-targeted CAR-T therapy in 2017 for relapsed or refractory B cell acute lymphoblastic leukemia and lymphoma. First generation CAR-T therapy for HIV used CD8+ T cells CAR expressing

CD4 infused with a CD3 zeta signaling domain showed good safety profile and partial effect on plasma viremia [106-108]. Second generation CAR-T therapy are based on bNAb expression to enhance specific killing of infected cells, and disruption of CCR5 to reduce cell infectivity [109]. Primary human T-cell model of bNAb CARs shows elimination of HIV-infected CD4+ T-lymphocytes in the absence of active viral replication [109]. The induced mutation of CCR5 locus protects these CAR-T cells from HIV-1 infection [109]. Phase I/II CAR-T study in chronically HIV-infected individuals is ongoing (NCT03240328). Toxicities are less likely to happen in HIV-infected individuals than in cancer patients as they have large tumor burden and hence a higher risk of cytokine releasing syndrome [110]. The incidence of neurotoxicity in cancer settings ranges from 0-50% [111]. Their manifestations are diverse and non-localizing, including headaches, confusion, and occasionally life-threatening convulsion [111].

Taken together, both antibody-based and T-lymphocytes enhancing strategies offer compelling evidence to decrease HIV-1 reservoirs and promote virologic remission [82, 91, 112, 113]. The potential for antibodies to affect CNS viral latency is uncertain given their general lack of CNS penetration. Enhanced T-lymphocytes could readily cross the BBB and perhaps target the infected CNS cells. Whether they are beneficial in eliminating HIV-infected cells in the CNS remain to be clarified. Regardless, the extent and duration of such T-lymphocytes driven cytotoxicity need monitoring.

What future investigations could be considered in HIV remission studies?

There are two primary goals to perform CNS investigations in HIV remission studies. First is to determine whether experimental agents and TI are safe. Second is to understand the effects of remission interventions on CNS HIV persistence.

Experimental agents in HIV remission trials can have direct or indirect effects on the CNS. Those that cross the BBB may reactivate or kill target infected cells resulting in localized immune activation. The systemic effects of agents that do not cross BBB could indirectly affect the CNS through systemic viremia and immune activation. TI is included in remission trials to measure the efficacy of interventions because there are no biomarkers for post-treatment control. Short TIs with frequent HIV-1 RNA monitoring has been clinically safe [67, 86, 114], but severe complications could occur in rare cases. Both Boston patients with CCR5+ allogeneic stem cell transplantation from HIV-uninfected donors had low level CSF viremia during systemic viral rebound post-TI, but one subsequently developed HIV-meningitis [115]. The lack of pre-existing HIV-1 immunity likely contributed to the meningitis. Further, life-threatening encephalitis secondary to massive immune activation and CD8+ T-lymphocytes infiltration can occur after CNS viral escape in unplanned TI [116], highlighting the crucial role of CNS monitoring in remission studies.

We propose CNS monitoring based on our experience conducting four HIV-1 remission

trials with TI at the Thai Red Cross AIDS Research Centre in Bangkok, Thailand. All participants initiated ART during AHI and sustained at least 12 months of viral suppression before entering the remission trials which included shock and kill (NCT02475915, n=14), VRC01 bNAb infusion (NCT03036709, n=18), and very early ART initiation in Fiebig 1 AHI (NCT02614950, n=8). Plasma viral load was monitored every 3-7 days in these trials. ART was resumed for confirmed plasma viral load > 1000 copies/ml, fall in CD4+ T-lymphocyte count, development of clinical HIV disease or acute retroviral syndrome, pregnancy, or at volunteer request. No participant has experienced severe neurological adverse events or change in cognitive function as a result of interventions and TI. CSF viremia and elevated inflammatory markers during ATI were uncommon despite detectable plasma viremia [67, 86, 114].

HIV remission studies should incorporate clinical neurologic monitoring at a minimum. This includes self-reported questionnaires, standardized neurological examination, and neuropsychological testing. Serial CSF sampling throughout the course of intervention is ideal but less feasible to study participants, yet lumbar punctures at systemic viral rebound and upon achieving plasma aviraemia off ART are especially important. Comparing the phylogenetics and HIV RNA levels in the former scenario would help deciphering the dynamics between the 2 compartments, whereas measuring CSF HIV RNA in the latter case could exclude the possibility of asymptomatic CSF viral escape. Measurement of immune markers in both plasma and CSF samples is equally essential to clarify whether people in remission have heightened immune activation similar to EC, which is linked to unfavorable clinical outcomes [117, 118] and cognitive decline in neurodegenerative diseases [119]. Plasma and CSF neurofilament light chain protein (NFL) assay could provide additional information for subclinical neuronal damage [120].

Neuroimaging is another key player in neurological monitoring given the non-invasiveness and likely superior sensitivity for pre-clinical changes. MRI sequences such as MRS and diffusion-tensor imaging (DTI) are useful to detect intracerebral inflammation and microstructure change, respectively. Other potential MRI technique includes diffusion basis spectrum imaging (DBSI) which provides paired axonal integrity and pathological information through additional evaluation of vasogenic edema and cellularity [121, 122].

Figure 1 illustrates the proposed CNS data acquisition at different phases of a remission study: (1) pre-intervention preparation: document baseline neurologic functions, neuroimaging, CSF viremia, immune activation markers and viral sequences, (2) during and post-intervention: assess changes from baseline for clinical and laboratory markers, measure concentration of experimental agents if appropriate, (3) post-TI: evaluate neurologic functions for safety, and measure CSF viremia and inflammatory markers in individuals who experience plasma viral load rebound, and in those with sustained

aviremia. Phylogenetic analysis can be performed in CSF viremic individuals to compare to pre-ART or baseline sequences, which will inform the source of rebound viremia and possible selective immune pressure from interventions, and (4) post ART resumption and viral re-suppression: safety monitoring to ensure that any neuropsychiatric manifestations, CSF viremia and inflammation normalizes or return to baseline values.

Given the multi-system involvement of HIV and the impracticability of multi-tissue sampling (especially the brain) for disease evaluation, priority should be given to developing non-invasive methods to measure HIV disease activity. Molecular imaging using PET has been increasingly used in HIV researches. In an immuno-PET study, 64Cu-labeled SIV Gp120-specific antibody ligand illustrated locations of infected cells throughout the body apart from the brain in SIV-infected monkeys [123]. PET imaging based on 18kDA translocator protein (TPSO)-targeted ligand measures activation of microglia activation and has become a valid choice for neuroinflammation research [17, 124]. Vascular-PET that evaluates atherosclerotic vascular inflammation [125, 126] would be helpful for predicting long-term co-morbidities. Dual-tracer PET imaging [127] in HIV infection should be explored given its success in caner researches.

CONCLUSION

HIV persistence in the CNS is an obstacle to curing HIV. Strategies towards a remission and cure should reduce the frequencies of HIV-infected cells, improve control of viral replication and target unique viral quasispecies within the CNS. HIV remission trials include experimental agents and TI that can directly and indirectly impact the CNS, but CNS monitoring is often not done. Latency modifying agents, therapeutic vaccines and cell-based therapies can cross the BBB, and potentially target infected cells resulting in local immune activation. Antibody-based therapies generally do not cross the BBB but its systemic effects on viremia and immune activation could affect the CNS. Therefore, HIV remission studies should incorporate CNS investigations to determine if experimental agents and TI are safe, and to understand the effects of interventions on CNS HIV persistence.

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This is a pre-copy editing, author-produced PDF of an article accepted for publication in *AIDS* follwing peer review. The definitive published article is available online at: https://doi10.1097/QAD.0000000000002264.

Table 1. Neurological outcomes in treatment interruption and remission intervention studies

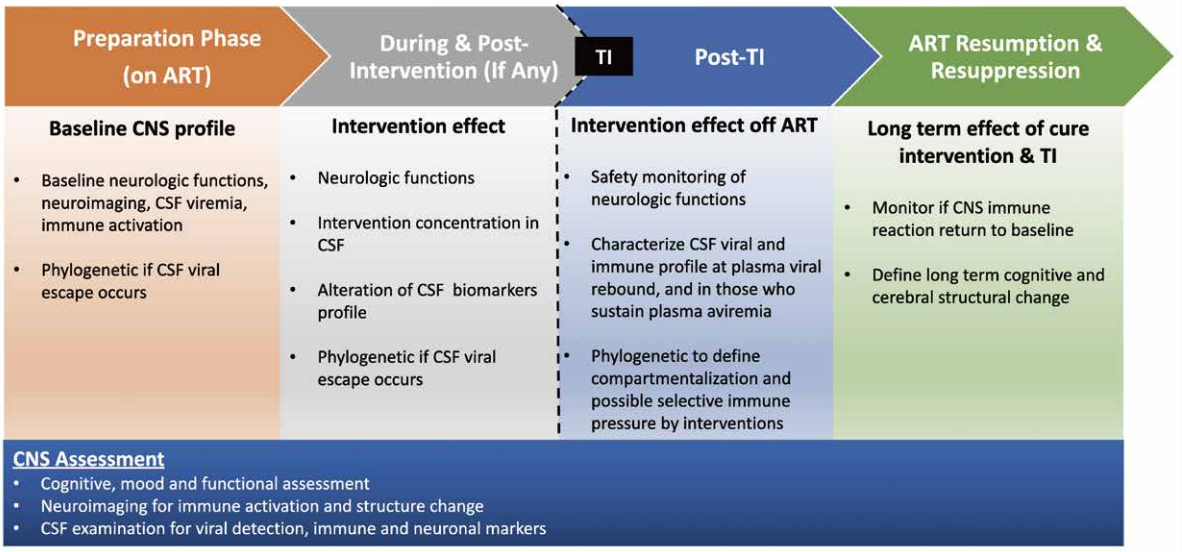
	Author, year of publication	Participants characteristics, study setting, CNS parameters tested	CNS outcome
ART interruption alone	Price et al. 2001	HIV-infected, with treatment failure (n=5) Serial CSF analyses during TI	Four participants showed a rapid increase in CSF viral load than that of the plasma, with marked changes in plasma to CSF viral load ratio Three showed asymptomatic CSF lymphocytic pleocytosis.
	Price et al. 2004	HIV-infected, on ART (aviremic/viremic = 3/9) Retrospective CSF and neurological evaluations after TI	Five participants developed asymptomatic CSF lymphocytic pleocytosis, with white cell counts up to 30-60 cells/microL.
	Gisslén et al. 2005	HIV-infected, on suppressive ART (n=8) Retrospective CSF parameters measurements before and after TI (HIV viral load, immune activation and neuronal damage markers)	Beside viral rebound in plasma and CSF, 7 showed intrathecal immune activation, 3 demonstrated progressive increase in neuronal injury marker from below cutoff. All participants were neurologically asymptomatic without functional decline. Five underwent brief quantitative neurological testing with stable performances.
	Robertson et al. 2010	HIV-infected, on suppressive ART (n=167) Neurocognitive performance before and after TI up to 96 weeks	At 96 weeks, 95 participants remained off ART, 46 resumed ART before end of study. No cognitive decline among those who remained off ART at 96 weeks
	Gianella et al. 2016	HIV-infected, on ART (n=14, viral suppression = 10) Retrospective phylogenetic comparison of rebound virus between plasma and CSF	CSF compartmentalized HIV with phylogenetic difference between blood and CSF in 9/14 participants.
	Chan et al. CROI abstract 2017	Acutely treated HIV (Fiebig I), viral suppressive > 2 years (n=8) Prospective multi-disciplinary CNS monitoring across TI	At plasma viral rebound, only low levels of CSF HIV RNA were detected in 2 out of 4 CSF samples using ultra-sensitive assay (all negative in conventional assay). No significant increase in CSF neopterin, CXCL10, CCL2 across TI, no major change
			in MRS and Flanker cognitive test performance

Remission intervention	Henrich et al. 2014	TI in HIV infected, post allogeneic stem cell transplantation on suppressive ART (n=2) Observation on clinical outcomes, before and after TI	One participant developed HIV meningitis symptoms with low grade CSF lymphocytic pleocytosis after plasma HIV rebound. The other participant had plasma viral rebound on day 225 after TI (1.9 million copies/ml). CSF HIV RNA was 269 copies/ml on day 238 after TI (5 days after ART resumption).
	Rasmussen et al. 2015	HIV infected, on suppressive ART > 2 years (n=15) CSF analyses before the 1st course and after the last course of panobinostat, no TI (n=11)	Measurable plasma HIV RNA in 6 out of 11 participants CSF Panobinostat concentration was below detection. CSF HIV RNA was not detected; neuroinflammatory or degenerative biomarkers did not change.
	Kroon et al. IAS abstract 2016	Acutely treated HIV, viral suppression > 48 weeks 10 received VHM with ongoing ART, followed by TI 8 underwent serial CSF sampling and neuroimaging	HIV RNA was undetectable in plasma and CSF upon the end of VHM on ART. After TI, 6 had undetectable CSF HIV RNA at plasma viral rebound. Two had low levels of CSF HIV RNA (25 and 42 copies/ml, plasma HIV RNA 35,796 and 329 copies/ml respectively). CSF inflammatory marker levels did not change before and after intervention.
	Hellmuth et al. CROI abstract 2018	Acutely treated HIV, viral suppressive > 2 years (n=24) Combined neurological outcomes from 3 TI studies including with either no intervention, bNAb or latency reversing agents)	No significant change in neurocognitive test performances and MRI DTI imaging before and after TI

Abbreviations: CSF = Cerebrospinal fluid, ART = Antiretroviral therapy, LRA = Latency reversing agent, TI = Treatment interruption DTI = Diffusion Tensor imaging, VHM = Vorinostat, Hydroxychloroquine, Maraviroc

Figure 1.

CNS monitoring in cure studies. Top row: Different phase of cure studies. Middle row: Information of interest to better understand CNS latency and intervention effect. Lower row: Modalities of CNS assessment.



Chapter 12:
Central nervous system safety during brief analytic treatment interruption of antiretroviral therapy within four HIV remission trials: an observational study in acutely treated people living with HIV

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Clin Infect Dis. 2020 Sep 11;ciaa1344. (Online ahead of print)
doi: 10.1093/cid/ciaa1344.

ABSTRACT

Background: The central nervous system (CNS) is a likely reservoir of HIV, vulnerable to viral rebound, inflammation, and clinical changes upon stopping antiretroviral therapy (ART). It is critical to evaluate the CNS safety of studies using analytic treatment interruption (ATI) to assess HIV remission.

Methods: Thirty participants who started ART during acute HIV infection underwent CNS assessments across four ATI remission trials. ART resumption occurred with plasma viral load >1000 copies/mL. CNS measures included paired pre- vs. post-ATI measures of mood, cognitive performance, and neurologic examination, with elective cerebrospinal fluid (CSF) sampling, brain diffusion tensor imaging (DTI) and magnetic resonance spectroscopy (MRS).

Results: Median participant age was 30 years old and 29/30 were male. Participants' median time on ART prior to ATI was 3 years, and ATI lasted a median of 35 days. Post-ATI, there were no differences in median mood scores or neurologic findings and cognitive performance improved modestly. During ATI, a low level of CSF HIV-1 RNA was detectable in six of 20 participants with plasma viremia, with no group changes in CSF immune activation markers or brain DTI measures. Mild worsening was identified in post-ATI basal ganglia total choline MRS, suggesting an alteration in neuronal membranes.

Conclusions: No adverse CNS effects were observed with brief, closely monitored ATI in participants with acutely treated HIV, except a MRS alteration in basal ganglia choline. Further studies are needed to assess CNS ATI safety in HIV remission trials, particularly for studies using higher thresholds to restart ART and longer ATI durations.

INTRODUCTION

Analytic treatment interruption (ATI) is component of HIV remission studies that evaluates the capacity to sustain viral suppression off antiretroviral therapy (ART). With advancing efforts towards HIV remission and cure, we must determine the clinical safety of ATI as a scientific approach considering the potential risks.1 Discontinuing ART was once thought to reduce associated toxicities, but the risks of this approach ultimately outweighed the benefits. Poor outcomes after prolonged and CD4+-guided ART interruption included increased morbidity, drug resistance, recurrent acute retroviral syndrome (ARS), and HIV transmission.2–6 Recent, safer HIV remission trials use intensive monitoring and low, pre-defined plasma HIV RNA parameters for restarting ART, with the time to rebound as a study outcome.7–11

Emerging work suggests that short periods of ATI may have minimal influence on systemic HIV reservoirs.12-14 However, the impact of brief ATI on the central nervous system (CNS) remains unknown. Concern persists as the CNS is a possible site of viral resurgence, vulnerable to adverse laboratory and clinical effects with ART withdrawal. Studies during prolonged ATI with neurologic monitoring have demonstrated viral rebound in the cerebrospinal fluid (CSF), emergence of neuroinflammation, elevations in neuronal injury markers, and rare clinical meningitis after ART cessation. 5,15-20 These CNS effects occurred with ART interruption lasting up to five months, often in individuals with low CD4+ nadirs and, in some cases, virologic failure. It is unknown whether participants with healthier immune status experience CNS risks when undergoing limited ATI with low level plasma viral rebound. This is an urgent clinical issue for individuals on stable, suppressive ART deciding whether to participate in HIV remission studies.

To evaluate the CNS safety of closely monitored ATI in individuals with HIV with relatively preserved immunity, we prospectively examined neurologic and behavioral measures in four state-of-the-art HIV remission trials conducted at single center in a cohort of participants who originally started ART during acute HIV infection (AHI).8,11 Where available, we analyzed paired CNS assessments of mood, cognitive functioning, neurologic findings, CSF, and neuroimaging. An additional objective was to identify whether rebounding virus found in the CSF during ATI derived from the CNS or from systemic HIV reservoirs.

METHODS

Participants and ATI studies. Participants in Bangkok, Thailand were initially enrolled in the parent RV254/SEARCH 010 study of individuals diagnosed during AHI (Fiebig stage I-V), rapidly started on ART, then followed longitudinally (NCT00796146/ NCT00796263).21,22 Most participants in this study (83%, 25/30) started on efavirenz/tenofovir/lamivudine. Participants with stable plasma viral suppression who met specific study criteria were offered entry into one of four trials involving ATI that

assessed the efficacy of interventions to achieve HIV remission without ART or delay time to viral rebound (Very early ART RV411/NCT02614950; Vorinostat RV409/NCT02475915; VRC01 antibody RV397/NCT02664415; Ad26.Mos.HIV and MVA-Mosaic vaccine RV405/NCT02919306; Figure 1; Table 1).8,11 Participants in RV411 and RV397 were switched from efavirenz to ritonavir-boosted darunavir four weeks prior to ATI. Participants in RV409 and RV405 had efavirenz switched to a boosted protease inhibitor two weeks prior to ATI. All studies had viral load monitoring every three to seven days. ART was resumed with confirmed plasma HIV-1 RNA >1000 copies/mL with additional trial conditions (Table 1). Included participants had paired CNS data collected at baseline prior to ATI ("pre-ATI") while on stable ART with plasma viral load below the level of detection, and then a comparison measure either during ATI (off ART) or after ATI ("post-ATI") when ART was restarted (Figure 1). During ATI measures included assessments prior to and during viral rebound, except for lumbar punctures (LP), which were all performed during plasma viral rebound. Coordination challenges across the four ATI studies limited standardized timing of all CNS measures. We received study approval from institutional review boards at UCSF, Yale University, Chulalongkorn University, the Walter Reed Army Institute of Research, and all other participating institutions. All participants provided written, informed consent for research.

Neurologic and behavioral measures

The parent RV254 study included neurologic and behavioral measures integrated as possible into the four ATI trials. Mood measures included validated Thai versions of the Patient Health Questionnaire-9 (PHQ-9), the Hospital Anxiety and Depression Scale (HADS), and a Distress Thermometer.23 Not every ATI study could incorporate each CNS measure (Supplemental Table 1). Neuropsychological testing included four assessments sensitive to CNS effects of HIV: Color Trails 1 and Trail-making A (psychomotor speed); Color Trails 2 (executive functioning/set-shifting); and Grooved Pegboard, non-dominant hand (fine motor function).24 We computed a composite neurocognitive performance (NPZ-global) from the mean z score of each test. Several ATI studies incorporated the NIH ToolBox Flanker subtest (reaction time during response inhibition) before, during, and after ATI.25 Participants underwent a standardized neurologic assessment based on the AIDS Clinical Trials Group Macro Neurological Examination.24 Participants underwent optional LP pre-ATI and during ATI when plasma HIV RNA was >20 copies/mL. CSF HIV RNA analysis occurred at two different laboratories: CSF from RV409 at the HIV Netherlands Australia Thailand Research Collaboration laboratory in Bangkok (lower level of quantification (LLQ) of 20 copies/mL); and the other three studies at the Military HIV Research Program (MHRP) laboratory in Silver Spring, Maryland (LLQ of 80 copies/mL, Roche COBAS AmpliPrep/COBAS TaqHIV-1 Test v2.0 diluted 4-fold due to low CSF volumes). CSF immune activation markers measured at MHRP include neopterin (EIA), a marker of macrophage activation; chemokines CCL2/ MCP-1 and CXCL10/IP-10 (Luminex platform), which regulate trafficking of immunologic cells; CD163 (Luminex), a marker

of monocyte/macrophage lineage cells; and s100B (Luminex), a marker of glial activation. Brain MRI scans were optional pre-ATI and during ATI, including diffusion tensor imaging (DTI) assessing white matter tract integrity performed on a Philips Ingenia 3T MRI scanner with a 15-channel volume head coil for signal excitation and reception following previous procedures.26 Brain proton magnetic resonance spectroscopy (MRS) was acquired pre-ATI and either during ATI (RV411) or post-ATI (RV409) measuring CNS inflammation and glial function using vendor-specific single voxel 1H-MRS PRESS sequence with the following acquisition parameters: TE/TR = 35/1500 ms; 2048 data points; 128 total averages; 20x20x20 mm3 voxel at the left basal ganglia. Limited samples prevented other voxel analysis. Water-unsuppressed spectra were acquired with similar parameters and 16 averages. LCModel (version 6.2) was used to quantify brain metabolites using GAMMA simulated reference basis sets.28 Fittings were performed between 4.0 - 0.5 ppm. Quantified metabolites total n-acetyl aspartate, total choline, myo-inositol, and glutamate + glutamine were included only if the signal to noise ratio was >4 and their percent standard deviations were <20%. Metabolites were expressed as a ratio to stable total creatine levels.

Statistical analyses

Numerical results were presented as medians, interquartile ranges (IQR) and total ranges. Paired data was analyzed using two-tailed Wilcoxon matched signed rank test, and ANOVA for multiple time points. Approximate 95% confidence intervals for median values were included to account for variability of the data and small sample size, with actual median confidence level stated. Chi-squared analyses were used to compare proportions.

RESULTS

Thirty participants (29 males) with paired CNS data were included. Pre-ATI, all were stable on ART for a median of 3 years, had a plasma HIV-1 HIV RNA <20 copies/mL, with a median CD4+ cell count of 695 cells/uL (Table 2). Median ATI duration was 35 days until ART was restarted and none experienced ARS (Table 2; Supplemental Table 1).

Neurologic, cognitive, and mood measures pre- and post-ATI

There were no differences in the occurrence of neurologic symptoms (n=28, Table 3). The median NPZ-global score improved post-ATI, with increases in Color Trails 1 and 2 (n=18; Table 3; Supplemental Figure 1A–C) but not in other assessments. Flanker metrics did not change in computed score or average response time (n=16). Comparing pre- vs. post-ATI mood assessments (n=14), there was no group change in PHQ-9 depression score, HADS depression or anxiety scores, or Distress Thermometer rating (Table 3, Supplemental Figure 1D-E).

Detection of CSF HIV RNA during ATI

CSF HIV RNA assays were performed for all 20 participants who underwent LP during plasma viral rebound; of these, six (30%) revealed detectable HIV RNA. Two were in the active drug arm of RV409 (vorinostat, hydroxychloroquine, maraviroc prior to ATI): one with CSF HIV RNA of 25 copies/mL at 29 days into ATI (plasma HIV RNA 329 copies/mL) and the other CSF HIV RNA 42 copies/mL 32 days into ATI (plasma HIV RNA 35,796 copies/mL; LLQ=20 copies/mL). Additionally, four participants in RV405 had detectable CSF HIV RNA at the time of restarting ART, although three were unquantifiable (LLQ=80 copies/mL). One participant who received placebo had CSF HIV RNA <80 copies/mL 31 days into ATI (plasma HIV RNA 111,812 copies/mL). Three received the Ad26.Mos.HIV and MVA-Mosaic heterologous vaccine regimen prior to ATI. Two of these had CSF HIV RNA <80 copies/mL 36 and 44 days into ATI (plasma HIV RNA 6,585 and 83,014 copies/mL, respectively) and one had a quantifiable CSF HIV RNA of 424 copies/mL at 50 days into ATI (plasma HIV RNA 59,890 copies/mL). Due to low CSF HIV RNA levels, viral sequencing could not be performed to identify origin of the CSF virus during ATI. Participants with detectable CSF HIV RNA during ATI had no change in neurological findings (n=6) or neuropsychological test scores (n=5). Other CNS metrics for this subgroup were not analyzed due to limited data (n<3).

Assessments of CNS inflammatory and neuroimaging changes with ATI

There were no alterations in CSF white blood cell count or protein levels pre- and during ATI (n=12). There were also no differences in soluble CSF immune activation markers pre-ATI vs. during ATI for neopterin, CCL2/ MCP-1, CXCL10/IP-10, CD163, s100B (n=8; Table 3, Supplemental Figure 1F-H). None of the participants with detectable CSF HIV RNA were in the subset (n=8) of those with measured CSF immune activation markers in this study. There were no differences in brain DTI measures pre- vs. during ATI in fractional anisotropy or mean, radial, or axial diffusivity (n=12, Figure 2A). MRS data from the single basal ganglia voxel identified a mild worsening of total choline from pre-ATI to either during ATI (n=5) or post-ATI (n=3) (p=0.047; median 0.217 [IQR: 0.202, 0.228] vs. 0.237 [IQR: 0.224, 0.242]; Figure 2B), with no differences in other metabolites.

DISCUSSION

In participants with very early ART intervention sustained for a median of three years, we identified that a brief, closely monitored ART interruption resulted in no adverse clinical CNS complications. Neurocognitive performance improved modestly after ATI on tests of psychomotor speed and executive functioning/set-shifting. In 30% of sampled participants we observed low levels of CSF HIV RNA during plasma viral rebound in ATI. We found a modest alteration in one MRS measure of cellular turnover during/post-ATI without a change in neurologic findings, highlighting the need for additional ATI studies examining brain MRS studies.

In previous literature, treatment interruption has been associated with detectable HIV in CSF and elevations in CSF lymphocytic cell count preceded by increases in chemokine CCL2/MCP-1.15,16 One study suggests that axonal injury can occur in asymptomatic individuals after stopping ART, measured via elevations in CSF neurofilament light chain protein.18 Similar to our findings, one long ATI study found participants' neuropsychological test performance actually improved over two years off treatment.19 Likewise, a study of five patients who underwent 12 weeks of ATI did not find any decline on standardized neurologic assessments.20 More fulminant neurologic sequelae during ART interruption, including meningitis or meningoencephalitis, have been reported but are uncommon.5

In terms of the improved neurocognitive performance, participants were exposed to this testing battery multiple times in the parent study, minimizing practice effects. While viral suppression is broadly thought to improve cognitive functioning, cessation of efavirenz may have contributed to this improvement and may have masked adverse CNS effects. Self-reported mood assessments showed no group level increase post-ATI, with few participants scoring above clinically relevant thresholds. Some individuals exhibited increased mood symptoms after viral resurgence, consistent with work describing disappointment with rapid viral rebound in the RV411 HIV remission trial.29

Participants with paired MRS data (n=8) revealed evidence of mild cell membrane damage in the basal ganglia with ATI. There was no extended MRS follow up and we do not know whether this resolved with additional time on ART. However, we measured no increase in five CSF markers of neuroinflammation during ATI or any increase in CNS clinical sequelae. A similar stability in CSF inflammatory markers across ATI was observed in n=7 participants in RV409, assessed using different assays in a separate study.30 Post-ATI CSF inflammatory markers were not measured in this work; however rhesus macaques inoculated with SHIV-1157ipd3N4 who underwent treatment interruption had no elevations in CSF IL15, neopterin, CCL-2/MCP-1, or CXCL-10/IP-10 at 12 weeks after plasma viral rebound.31 Further, higher numbers of CD3+ cells were seen in the brains of three of five macaques and greater CD68+/CD163+ was seen in one macaque prior to sacrifice, compared to four infected macaques that did not undergo ATI.31 This contrasts with a brain FDG-PET analysis in SIV-infected macaques (SIVmac251, n=4; SIVE660, n=3) who underwent ATI: within one month, brain glucose metabolism and CSF inflammatory markers IL2 and IL15 increased, suggesting neuroinflammation in the context of viral rebound.32 These findings, along with older studies of sustained ATI, imply that HIV remission studies with more liberal parameters for ART resumption or longer periods off treatment may have adverse CNS effects. This also highlights the value of planning parallel animal model studies with tissue-based CNS measures with HIV remission trials.1

The absence of clinically relevant CNS alterations in these studies is reassuring, but the

relative CNS safety of ATI is unknown for other contexts. These study participants uniquely had early initiation of ART during Fiebig I-V, likely associated with lower viral reservoir size and potentially distinct virologic and immunologic characteristics limiting CNS sequelae with ATI. To more fully characterize the CNS safety of ATI, longitudinal CNS evaluations should include participants who started ART during the chronic phase of HIV infection, and should assess remission trials with longer durations off ART and with higher plasma HIV RNA thresholds for restarting ART. Potential effects on inflammation, viral replication, and tissue injury in target organs such as the CNS remain a significant concern to stakeholders.1 We provide proof of concept for implementing prospective CNS assessments in future HIV remission trials involving ATI.
A potential study contribution was to examine sources of CNS viral rebound during ATI. The rebounding CSF virus detected may derive from the CNS rather than peripheral systemic reservoirs, as unique CNS viral populations have been identified under this circumstance.17 We also questioned whether any participants would display higher CSF than plasma HIV RNA levels during ATI, suggesting CNS viral reservoirs may seed plasma viral rebound; this was not observed. Less than one-third of sampled participants had relatively low levels CSF HIV RNA during plasma viral rebound, consistent with earlier observations that CSF tends to rebound later than in plasma (in one study, a mean of 8 days later) and at lower levels.15,18 With low CSF HIV RNA levels, standard sequencing techniques could not be used to compare with plasma HIV RNA for viral reservoir origin/s or viral compartmentalization. The conservative criterion to restart ART in these HIV remission studies may have restricted the host immune system from independently controlling the virus, thus limiting the ability to assess the full picture of CSF viral rebound and CNS HIV reservoirs. Future HIV remission studies using ATI will provide an unmatched opportunity to assess these viral rebound dynamics and reservoirs in the CNS compartment.

There are a number of limitations to the present work. The sample size was small and further limited by the lack of inclusion of all CNS measures across the four ATI studies. The CNS measures were not collected at uniform time intervals in all studies (Supplemental Table 1), potentially introducing bias through different immunobiological states among participants, particularly in the post-ATI time point where individuals had either a declining or undetectable plasma HIV RNA. These differences introduced variability and potentially obscured transient, adverse CNS findings during ATI. Another consideration is that three of four ATI studies exposed some participants to a drug intervention that potentially influenced the CNS measures, perhaps through viral escape or neuroinflammation. The VRC01 broadly neutralizing antibody intervention in RV397 may have caused CNS immune or virologic effects; however, this drug is not likely to penetrate into the CNS, and in the parent study VRC01 did not result in HIV remission at 24 weeks (the primary outcome) or lead to significant systemic immunological benefits.11,33 There were no differences in CNS measures when analyzing data only from participants who received no study drug intervention (n=10). Despite the clinical

heterogeneity in the ATI trial conditions, the reported confidence intervals include this variability and rule out large effect sizes. This work included only one female, which obscured detection of sex differences. Almost all participants were young in age, with high CD4+ cell counts who started treatment very early in the course of HIV infection and likely a limited HIV reservoir size; these issues restrict the generalizability to other populations. Though the brief neuropsychological test battery has been sensitive to detecting impairment during acute infection, it may overlook subtle deterioration in untested cognitive domains.34 Collectively, these issues limit our ability to definitively state there are no adverse CNS outcomes with brief, closely monitored ATI, and highlight the need for future work in the field. Despite these limitations, we hope these findings encourage future ATI trials to incorporate coordinated neurologic measures to further assess safety and efficacy of CNS viral eradication strategies.35

CONCLUSIONS

CNS assessments spanning brief, monitored ATI in HIV remission studies revealed no adverse clinical outcomes and only low levels of CSF HIV RNA rebound in a minority of participants originally diagnosed and treated during acute HIV infection. A subset of participants with MRS data revealed a mild worsening in a measure of cell turnover in the basal ganglia, suggesting injury. CNS assessments are feasible to integrate in HIV remission studies involving ATI and are critical to incorporate moving forward, particularly in studies utilizing higher plasma HIV RNA thresholds for ART resumption, longer durations of ATI, and study populations where ART was not initiated very early.

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This is a pre-copy editing, author-produced PDF of an article accepted for publication in *Clinical Infectious Diseases* follwing peer review. The definitive published article is available online at: https://doi.org/10.1093/cid/ciaa1344.

Figure 1.

Timeline of HIV infection, antiretroviral therapy (ART), and analytic treatment interruption (ATI) by study. Study intervention is listed in blue, with RV409, RV397 and RV405 also including a placebo arm detailed in Table 1. Information at the bottom of the figure indicates median timing between events across all ATI studies, as well as ART resumption criteria. bNAb = broadly neutralizing antibody.

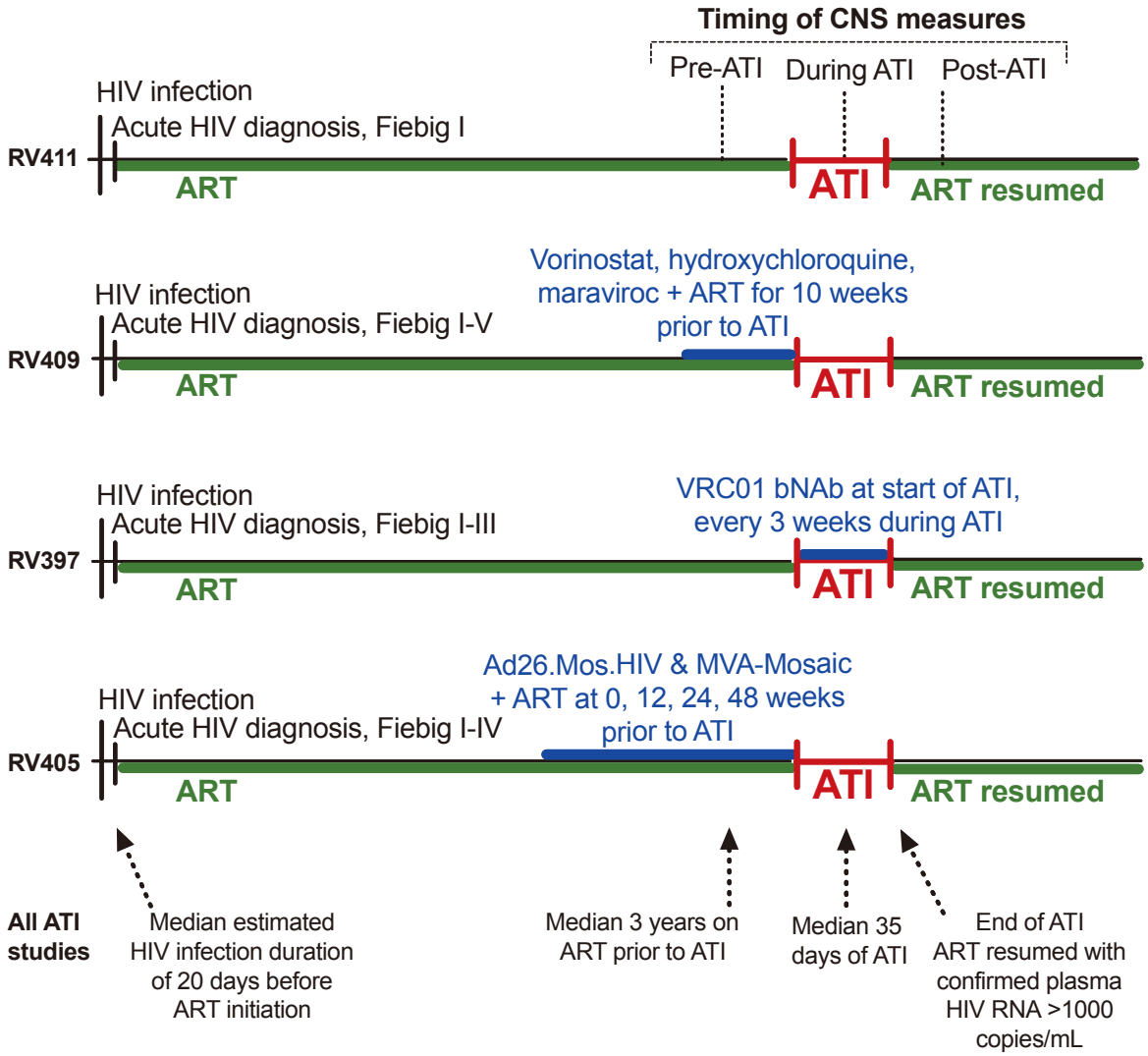

Figure 2.

Neuroimaging findings in ATI. A. Brain diffusion tensor image MRI measures in participants (n=12) on treatment (initial acquisition) compared to a time point during or after treatment interruption (follow-up). Top to bottom images are representative coronal, axial, and sagittal slices revealing no significant voxels (no red) found between the two time points. Mean fractional anisotropy (FA) skeleton (cyan) is shown superimposed on the mean FA template for entire ATI group to demonstrate the coverage of the tested white matter tracts. B. Magnetic resonance spectroscopy basal ganglia for total choline (n=8). Red dots indicate paired MRS data with end point collected post-ATI (n=3, mean of 66 days after resuming ART). Larger red dot indicates the only participant with MRS data where CSF HIV RNA was detected during ATI; MRS was performed 49 days after resuming ART.

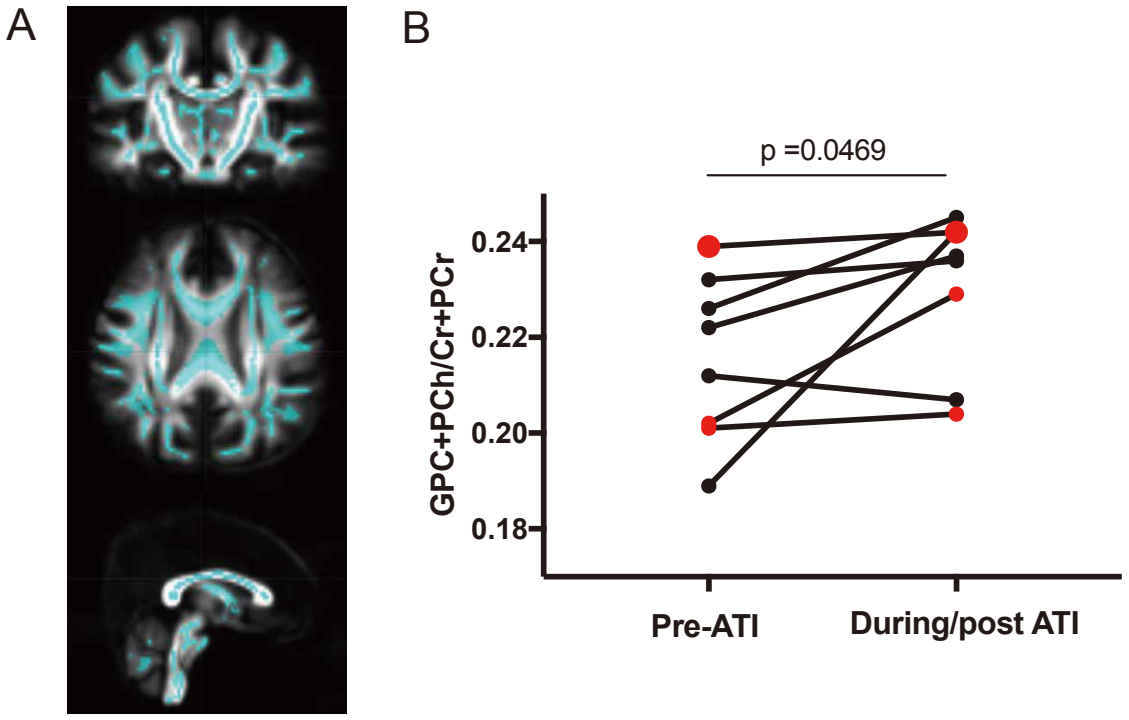

Table 1. Characteristics of HIV remission studies incorporating ATI with paired CNS measures

Study	Intervention	n	Inclusion criteria
RV411 NCT02614950	Observational ATI in Fiebig I, no study drug intervention	6	Started ART during Fiebig I acute HIV On ART for 96+ weeks Plasma HIV-1 RNA <50 cps/mL for 48+ weeks CD4+T cells ≥400 cells/uL Integrated HIV DNA <10 cps/10^6 in PBMCs
RV409 NCT02475915	2:1 randomized, placebo-controlled open-label vorinostat, hydroxychloroquine, maraviroc for 10 weeks before ATI	8 (7 study drug; 1 placebo)	Started ART during Fiebig I-V acute HIV On ART for 48+ weeks Plasma HIV-1 RNA <50 cps/mL for 28+ weeks CD4+T cells ≥450 cells/uL, 2x in last 6 months
RV397 NCT02664415	3:1 randomized, placebo-controlled, double blind VRC01 broadly neutralizing HIV antibody at start of ATI and every 3 weeks during ATI	10 (8 study drug; 2 placebo)	Started ART during Fiebig I-III acute HIV On ART for 24+ months Plasma HIV-1 RNA <50 cps/mL for 3x measures CD4+ T cells of ≥400 cells/uL Integrated HIV DNA <10 cps/10^6 in PBMCs
RV405 NCT02919306	2:1 randomized, placebo controlled Ad26.Mos.HIV & MVA-mosaic vaccine at 0, 12, 24, 48 weeks before ATI	6 (5 study drug; 1 placebo)	Started ART during Fiebig I-IV acute HIV On stable ART for at least 4 weeks Plasma HIV-1 RNA <50 cps/mL for 48+ weeks CD4+ T cells of ≥400 cells/uL

ATI = analytic treatment interruption; ART = antiretroviral therapy; PBMCs = peripheral blood mononuclear cells; cps/mL = copies per milliliter

Table 2. Demographics of n=30 participants in ATI trials with CNS monitoring.

Age at ATI (years)	30 (25,35; 21–52)
Sex	29 male, 1 female
%Fiebig I at ART initiation (n)	26.7 (8)
%Fiebig II at ART initiation (n)	23.3 (7)
%Fiebig III at ART initiation (n)	36.7 (11)
%Fiebig IV at ART initiation (n)	13.3 (4)
Log_{10} plasma HIV RNA at ART initiation	5.5 (4.6,6.4; 3.3–7.5)
Estimated infection duration at ART start (days)	20 (15,25; 9–32)
ART duration prior to ATI (years)	3.0 (2.5,4.7; 1.8–5.9)
Plasma HIV RNA (copies/mL) prior to ATI	all <20
CD4+ T cells/uL prior to ATI	695 (555,812; 402–1211)
Highest plasma HIV RNA during ATI (log_{10} copies/mL)	3.8 (3.5,4.4; 3.1–5.1)
Days of ATI until ART resumption	35 (22,38; 14–69)

Unless otherwise described, data are presented as median (IQR; range). ATI = analytic treatment interruption. ART = antiretroviral therapy.

Table 3. Paired neurologic, neuropsychological, mood, and CSF immune activation marker data across ATI.

CNS measure	Sample	Pre-ATI	During ATI	Post-ATI	p value
% with any neurologic findings	n=28	25%	–	18%	p=0·515
Median # neurologic findings (IQR, range)	n=28	0 (0–0·75; 0–7)	–	0 (0–0·75; 0–5)	p=0·305
Median composite NPZ-global (96•9% CI)	n=18	0·95 (0·33–1·30)	–	1·13 (0·61–1·52)	*p<0·001
Median Color Trails 1 z score (96•9% CI)	n=18	1·47 (0·64–1·73)	–	1·54 (1·16–2·01)	*p=0·040
Median Color Trails 2 z score (96•9% CI)	n=18	1·06 (0·57–1·25)	–	1·20 (0·90–1·62)	*p=0·007
Median Grooved Pegboard z score (96•9% CI)	n=18	0·71 (0·41–1·10)	–	0·86 (0·23–1·23)	p=0·064
Median Trails A z score (96•9% CI)	n=18	1·45 (0·73–1·80)	–	1·36 (1·01–1·75)	p=0·890
Flanker computed score (97·9% CI)	n=16	8·87 (8·27–9·30)	8·96 (8·44–9·56)	9·14 (8·36–9·59)	p=0·143
Flanker average response time (97•9% CI)	n=10	0·76 (0·56–0·94)	0·75 (0·60–0·88)	0·78 (0·55–0·88)	p=0–657
Median PHQ-9 depression score (98•7% CI)	n=14	3·5 (0–8)	–	5·0 (2–7)	p=0·510
Median HADS depression score (98•7% CI)	n=14	1·0 (0–3)	–	1·5 (0–6)	p>0·999
Median HADS anxiety score (98•7% CI)	n=14	3·0 (2–7)	–	3·0 (2–9)	p=0·677
Median Distress Thermometer rating (98•7% CI)	n=14	1·5 (0.5–4.0)	–	2·5 (1·5–6·3)	p=0·252
CSF neopterin pg/mL (99•2% CI)	n=8	527 (332–1284)	504 (221–909)	–	p>0·999
CSF CCL2/MCP-1 pg/mL (99•2% CI)	n=8	690 (448–1024)	644 (431–1281)	–	p=0·461
CSF CXCL10/IP-10 pg/mL (99•2% CI)	n=8	428 (170–2220)	361 (216–709)	–	p=0·742
CSF CD163 pg/mL (99•2% CI)	n=8	5991 (5286–9851)	6238 (5264–6939)	–	p=0·945
CSF s100B pg/mL (99•2% CI)	n=8	1137 (887–1998)	1214 (896–2357)	–	p=0·078

Unless otherwise described, data are presented as median values with approximate 95% confidence interval (CI) of the median. NPZ-global = composite neuropsychological test z score; PHQ-9=Patient Health Questionnaire HADS = Hospital Anxiety and Depression Scale.

PUTTING FINDINGS OF THIS THESIS IN PERSPECTIVE

Chapter 13:
General Discussion

INTRODUCTION

Neurobehavioral complications, including cognitive and mood disorders, are common in people living with HIV-1 (PLWH) since the recognition of acquired immunodeficiency syndrome (AIDS) in the 1980s. Mood disorders in PLWH are multifactorial, including likely contributions from the distress of baring an incurable disease, a lower quality of life due to general ill-health, increased substance use in certain subsets of PLWH, and a contribution of inflammation to mood disorders during acute HIV [1]. Cognitive disorders in PLWH, on the other hand, link more directly to HIV-1 infection and particularly, the severity of current and past immunodeficiency [2]. Complications of HIV-1 in the central nervous system (CNS), including opportunistic infections (OI), progressive encephalopathy termed HIV-associated dementia (HAD), and myelopathy, are common in individuals with AIDS. OIs arise from co-infections with other pathogens due to overt immunodeficiency. However HAD and myelopathy are directly related to uncontrolled HIV-1 infection. Subsequent research confirms the linkage between HAD and HIV-1 encephalitis (HIVE), which manifests as a progressive subcortical dementia syndrome with cognitive, motor and behavioral abnormalities.

Table 1. Neuropsychiatric and functional features of HIV-associated neurocognitive disorders

	Cognitive Performance in Standardized Neuropsychiatric Tests	Impact on Daily Functioning
ANI	Affects at least two cognitive domains, with performance of at least 1 SD below the mean of matched norms	No impact
MND	Affects at least two cognitive domains, with performance of at least 1 SD below the mean of matched norms	Mild interference
HAD	Affects at least two cognitive domains, with performance of at least 2 SDs below the mean of matched norms	Marked interference

Abbreviations: ANI: asymptomatic neurocognitive impairment; MND: mild neurocognitive disorder; HAD: HIV-associated dementia; SD: standard deviation.

The introduction of combination antiretroviral therapy (ART) converts HIV-1 infection from a lethal disease to a manageable chronic condition. Today, most PLWH on ART achieve plasma HIV-1 suppression and substantial restoration of T-cell mediated immunity. OIs and AIDS-related complications are uncommon. There is evidence that ART benefits the CNS. HIVE and HAD are rare in the post-ART era. The understanding and terminology of HIV-related neurocognitive impairment has been gradually refined in the past 30 years (**Figure 1**). In 2007, the American Academy of Neurology (AAN) published its updated recommendations for diagnosing HIV-associated neurocognitive disorders (HAND) based on neurocognitive disturbance defined in the post-ART era [3]. The

"Frascati criteria" categorized HAND into three groups, namely, asymptomatic neurocognitive impairment (ANI), mild neurocognitive disorder (MND) and HIV-associated dementia (HAD). Their neuropsychiatric and functional features are shown in **Table 1**.

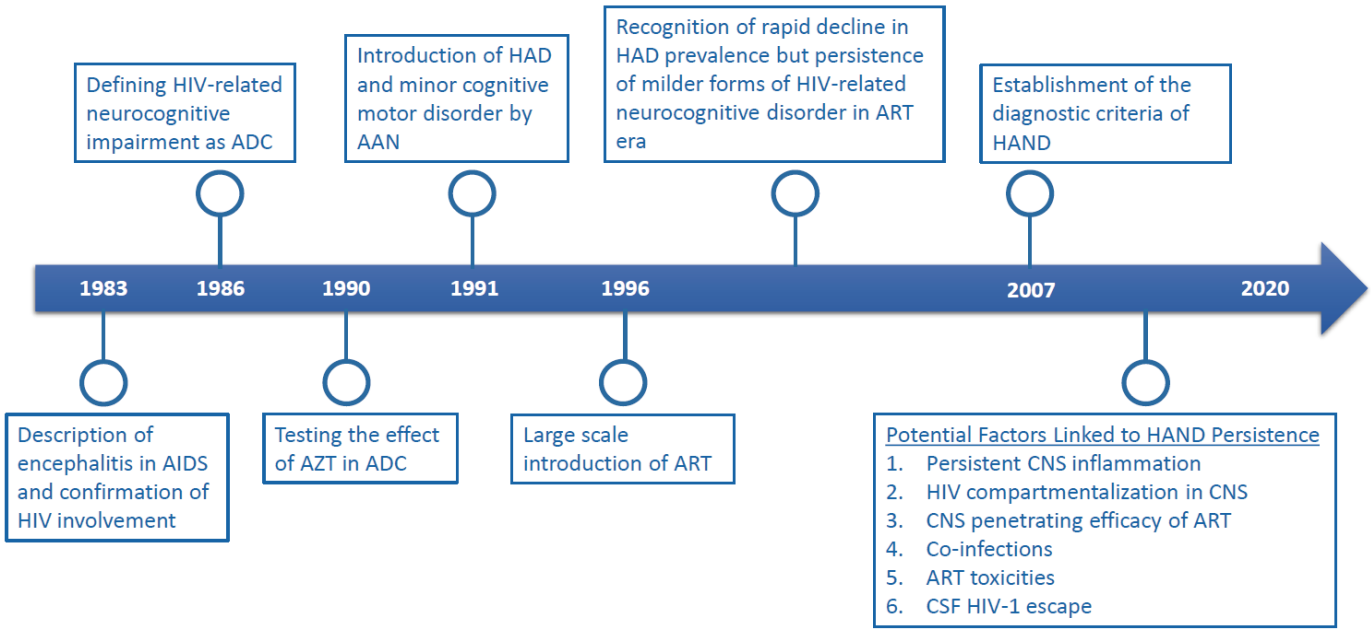

Table 1. Timeline of the evolving understanding and nomenclature of HIV-related neurocognitive impairment.
Abbreviations: AAN: American Academy of Neurology; ADC: AIDS dementia complex; ART: antiretroviral therapy; AZT: Zidovudine; HAD: HIV-associated dementia; HAND: HIV-associated neurocognitive disorder.

Although the diagnostic criteria of HAND have evolved over the years, researchers generally agree that the prevalence of more severe forms of HAND has dropped significantly after the introduction of ART, from about 20% to less than 5% [4]. However, the incidence of less severe forms of HAND, including ANI and MND, remains stable in the ART era, ranging from 20% to 50% in various studies [4-6]. Several factors account for the massive reduction of HAD in ART era. In untreated individuals with HIVE, ART benefits by suppressing HIV-1 replication in both systemic circulation and the CNS, leading to degrees of recovery in cognitive function. An earlier diagnosis of HIV-1 infection due to test availability, and the prompt ART initiation regardless of the severity of immunodeficiency determined by the blood CD4+ T-cell count, prevent the development of major cognitive disability.

The outcomes in **Chapter 1** serve as a real-world example of HAND in ART era. The study recruited ninety-eight consecutive treatment-naïve new clinic attendees who were free from CNS OI and active neurological complaints. They were tested with the International HIV Dementia Scale (IHDS) [7] and the Montreal Cognitive Assessment (MoCA) [8] screening tools (**Box 1**) before and after ART. At pre-ART baseline, despite their young age (median age = 30) and high frequency of tertiary education (50%), 39% and 26% of them scored below the cut-off scores of IHDS and MoCA respectively, suggesting the likely presence of cognitive deficit in a considerable proportion of them. The frequencies of impaired test performance are in line with another two cross-sectional studies of HAND conducted in Singapore (22.7%) and South Korea (26.3%) [9, 10].

At 6-months post-ART, only a minor proportion of participants with poor IHDS performance at baseline improved and scored above the cut-off. Similarly, neither the total MoCA score nor the percentage of participants who scored above the cut-off (25/26) showed statistically significant improvement. Several methodological limitations could lead to the failure of detecting cognitive improvement in the study. First, the process of neurological recovery post-ART may take longer than 24 weeks. Existing reports that examined neurocognitive benefit of ART usually take durations of reassessment between 24 to 48 weeks. Neurocognitive improvement post-ART is inconsistently observed at 3 months [11-13] but frequently reported with a duration of 6 months or longer [12, 14-18]. Second, while MoCA has been validated in patients with Alzheimer's disease [19] and small vessel disease [20] in Hong Kong, the use of IHDS has not been validated locally. Besides, IHDS and MoCA may not be sensitive enough to detect mild neurocognitive improvement. IHDS is originally designed for HAD screening instead of monitoring in resource-limited settings. MoCA has been used for monitoring progression of Alzheimer's disease [18, 21] but its usefulness in HAND is not well defined [22, 23]. Another issue is whether deficits in all cognitive domains could benefit equally from ART. In a review that included 23 studies

Box 1

The International HIV dementia scale (IHDS) is a brief 3-min screening test, examining motor speed, psychomotor speed and memory-recall function. A cut-off score ≤10 (out of 12) shows a reasonable sensitivity (64–74%) and specificity (55–66%) in identifying moderate to severe HAND

The Montreal Cognitive Assessment (MoCA) examines visuospatial/executive, naming, memory, attention, language, abstraction, delayed recall, and orientation function. In Hong Kong, a cut-off at 21/22 (out of 30) has been validated for evaluating mild cognitive impairment in elderly and stroke patients. A cut-off of 25/26 is used in the original English version and previous HAND-related studies in Singapore and Korea.

examining ART and cognitive performance, ART usage was associated with modest improvements in attention, executive function, and motor function but did not improve language, verbal memory, visual memory or visuospatial function [24].

Apart from the methodological factors, the findings could represent a true lack of improvement in the CNS after ART due to various reasons, simultaneously present and not mutually exclusive. First, the deficit may be due to permanent neuronal damage which is irreversible but remains static post-ART. Second, the persistent deficit may be part of an irreversible degenerative process independent of HIV-1 replication and immunological recovery post-ART. Third, it may be due to a dynamic process contributed by host viral, immune and behavioral factors. Lastly, neurotoxicity and non-equivalent CNS benefit between different ART regimens may play a role, resulting in an initial cognitive improvement due to viral suppression but a later decline due to toxicity.

Chapter 2 reviews the aforementioned cognitive impairment and persistent CNS Injury in treated PLWH. Briefly, inflammatory marker studies based on plasma, CSF [25, 26], and positron emission tomography (PET) [27, 28] that targeted microglial activation suggest that PLWH on suppressive ART still endure persistent low-level systemic and intracerebral immune activation. Chronic inflammation is known to exacerbate neurological symptoms and drive the progression of neurodegeneration in both chronic cerebral inflammatory diseases and neurodegenerative diseases [29-31]. As a result, such chronic inflammation, even at a low-level, could be neurologically harmful because of the long-term exposure of such inflammation. Several host and viral factors are tied to persistent cerebral inflammation and potentially HAND, including nadir CD4+ T-cell level, co-infections, viral compartmentalization and CSF viral escape.

Nadir CD4+ T-cell level representing an individual's pre-ART immune status is one of the well-reported associating factors of HAND [2]. A nadir CD4+ T-cell level <200 cells/mm3 and a past history of clinically-defined AIDS are associated with onset of cognitive decline before age of 50 [32-35]. Co-infections are similarly associated with HAND development. Among them, hepatitis C (HCV) and syphilis are frequently examined due to their potential to cause neurological complications and their high prevalence in PLWH. PLWH co-infected with HCV have an increased risk of cognitive impairment compared to those without [36], but such correlation is probably confounded by issues of substance misuse [37] and severity of liver disease [38, 39] in HCV-positive individuals. Similarly, syphilis has been inconsistently associated with cognitive impairment in PLWH [40-42].

The phenomenon of CNS viral compartmentalization and CSF viral escape are probably inter-related in PLWH. The former refers to the diversification of HIV-1 replication in different body compartments including the CNS [43-45]. The latter refers to the detection of HIV-1 RNA in CSF despite plasma HIV-1 suppression based on commercially

available viral assays. Although CSF viral escape is mostly asymptomatic and can be detected in around 10% of PLWH on stable ART [46], individuals with CSF viral escape demonstrate elevated levels of immune activation markers in their CSF, suggesting the contribution of intracerebral inflammation [47, 48]. Further, some individuals with CSF viral escape develop neurological complications of wide spectrum of severity, from simple headache, neuropathy, seizure, cognitive decline to overt encephalitis [49-51]. In symptomatic CSF viral escape, CSF viral strains frequently have resistance against their current ART regimen that effectively suppresses plasma viral replication [52-55]. Whether asymptomatic and symptomatic CSF viral escapes share the same pathogenesis remains to be clarified. For instance, whether development of a resistant viral strain detected in CSF occurs before or after the ART introduction is unclear. Regardless of the above scenarios, the phenomenon highlights the potential of CNS to generate alternate HIV-1 quasispecies independently in certain subsets of PLWH.

In summary, studies in the post-ART era suggest that HAND remains common in PLWH on stable ART. The persistence of HAND could be partly due to residual neurological damage that occurred before ART initiation. However, persistent immune activation is detected in both plasma and CSF from PLWH on stable ART started in chronic HIV-1 infection, highlighting the inadequacy of ART to overcome all the HIV-1 related pathologies. Systemic and CNS-specific factors have been identified that contribute to cognitive decline and persistent immune activation. Given that the onset of infection is unknown in most PLWH, it remains unclear whether prevention or reversibility of such abnormalities depends on timing of ART. The setting of RV254 study offers a unique opportunity to examine the CNS and immunological features during acute HIV-1 infection and after immediate ART. Absence of such complications would indicate the benefit of early treatment for brain health in PLWH. For instance, while large clinical trials, including the SMART [56] and START [57] studies, confirm the benefit of immediate ART in reducing risks of opportunistic disease or death from any cause in PLWH, early ART does not show superior cognitive outcomes in a subgroup analysis of the SMART study [58]. Further, comparing the biological samples from RV254 participants and individuals who initiated ART during chronic HIV-1 infection would provide insight for optimizing ART. Lastly, with a restricted HIV-1 reservoir [59] and excellent clinical outcomes associated with early ART, RV254 provides an ideal setting for cure research. The following sections will discuss the CNS data generated from RV254 cohort during AHI, after stable ART and after a short course of analytical treatment interruption (ATI) in cure substudies.

ACUTE HIV-1 INFECTION AND CNS IN RV254

RV254 is amongst the first cohorts to systematically evaluate the neurological profile of HIV-1 neuroinvasion during acute infection. Abnormal neurological signs [60], abnormal neurocognitive performance [61] and depression symptoms [1] are common during early acute infection, associated with HIV-1 invasion of the CNS within days after

transmission [62]. In a follow-up report (**Chapter 4**) of 117 CSF samples collected at AHI, the rate of HIV-1 RNA detection in CSF exceeds 90% in people with later Fiebig stages (III-V) of AHI [63]. Further, CNS immune activation, reflected by the levels of CSF immune activation markers (neopterin, sCD14, IL-6, CXCL10, CCL2 and sCD163) increases concurrently with CSF HIV-1 RNA levels. Phenotypic and gene expression analyses of the expanding CSF CD8+ T-cells suggest that they are functional and directed against HIV antigens [64]. Yet, MRI analysis suggests that the neuroinvasion at AHI leads to limited disruptions to microstructural white matter and resting-state functional connectivity (rsFC) [65]. Magnetic resonance spectroscopy (MRS) detects cellular inflammation without measurable neuronal injury during AHI [66]. Follow-up scans reveal resolution of such abnormalities 6 months after immediate ART, hinting that the clinical abnormalities are potentially reversible with appropriate management.

Data from RV254 also enhances the understanding of HIV-1 compartmentalization in the CNS. Previously, subpopulations of HIV-1 compartmentalized in CSF were reported in primary HIV-1 infection (PHI, i.e. within first year of HIV-1 infection). Phylogenetic analyses further demonstrate that the independent evolution of CSF variants begins as early as four months after estimated HIV-1 transmission [67, 68]. Phylogenetic analysis based on RV254 CSF samples collected at AHI identifies such diversification of HIV-1 in a subset of participants, suggesting that HIV-1 CNS compartmentalization takes place during acute infection under certain conditions [69]. The study reveals that RV254 participants with single transmitted/founder (T/F) infections have essentially no differences between the CSF and blood compartments. In contrast, all participants with multiple T/F infections showed differences in the proportions of HIV-1 variants between the two compartments. Further, the differences could be highly variable. Some of them demonstrated similar proportions of major HIV-1 variants in both compartments, while others showed either a lower or higher proportion of major HIV-1 variants in plasma compared to CSF. These findings imply that unknown mechanisms for HIV-1 trafficking into tissues outside of the blood compartment may lead to an initial genetic bottleneck or selection of HIV-1 variants at the very early stage of infection.

The variability noted in plasma to CSF HIV-1 RNA ratio (PCratio) in **Chapter 4** supports the existence of mechanisms that could modify HIV-1 trafficking into the CNS. The ratio is established by calculating the difference between plasma and CSF HIV-1 RNA in log10 scale. The median PCratio during AHI was 2.36 log10 copies compared to 1.48 log10 copies in PHI [70] and between 0.64 and 1.14 log10 copies in chronic HIV-1 infection among individuals with differing severities of immunodeficiency [70]. The smaller PCratio values in later stages of HIV-1 infection is likely due to local viral replication associated with CNS compartmentalization. Interestingly, a small percentage of RV254 participants demonstrated a lower-than-expected PCratio of less than one log, similar to that in chronic HIV-1 infection. Given that compartment-specific HIV-1 RNA replication within the CNS should be minimal at the stage of AHI, a

smaller-than-expected PCratio would suggest either a greater degree of viral transmigration from systemic circulation to the CNS or an early establishment of CNS HIV-1 RNA replication. Correlating low PCratio with clinical parameters reveals that individuals' immune response, denoted by a lower plasma CD4/CD8 ratio, is associated with lower PCratio. Taken together, these CSF studies confirm that HIV-1 invades the CNS during AHI. Further, variations in PCratio and phylogenetic outcomes support the heterogeneity of HIV neuropathogenesis during AHI. It remains unclear whether these alterations lead to different trajectories of disease progression and development of neurological complications if treatment is delayed.

Examining the Potential Effects of Untreated Syphilis in AHI

Chapter 5 and 6 examine the potential of Treponemal pallidum infection (syphilis), a common co-infection in PLWH, in moderating the immunological and virological outcomes of AHI. Experimental model demonstrate that Treponemal pallidum can invade the CNS within days after transmission [71, 72]. Neurosyphilis can occur at all stages of infection, ranging from early meningitis, meningovasculitis with cranial nerve palsies and stroke, to parenchymal diseases that present as dementia and myelopathy during late-latent stage. HIV-1 infection may facilitate the development of neurosyphilis. A recent study reported neurosyphilis, defined by positive CSF syphilis serology and elevated white CSF white blood cell count, in 90% of HIV-positive LP participants with early syphilis [73]. Neurosyphilis is especially more common in PLWH of advanced HIV-1 infection compared to HIV-negative populations [74]. Syphilis may augment HIV-1 replication during untreated chronic infection: incident syphilis is associated with elevation of plasma HIV-1 RNA and decline in CD4+ T-cell level in chronic HIV-1 infection [75-77]. HIV-1 RNA in CSF was highest in HIV-positive participants with neurosyphilis, followed by HIV / systemic syphilis, and lowest among with HIV-1 mono-infection without syphilis [78]. Syphilis may therefore worsen HIV-1 replication in both systemic circulation and CNS. By far, the understanding of HIV-1 and syphilis co-infection is limited to chronic HIV-1 infection. The effect of syphilis during AHI or early HIV-1 infection remains unclear because of the diagnostic challenge of AHI and the lack of comprehensive neurological characterization in usual clinical setting.

Chapter 5 reports the clinical features of a RV254 participant with concomitant neurosyphilis during AHI. Apart from the overt CSF pleocytosis and abnormal brain MRI findings, the study participant had a grossly reduced PCratio, suggesting an increased HIV-1 transmigration into the CSF compartment and perhaps an earlier-than-usual establishment of local viral replication in the CNS/CSF compartment. The participant commenced ART immediately after study enrollment, and received a standard course of neurosyphilis treatment. Unfortunately, he discontinued ART four weeks before the follow-up lumbar puncture (LP) at week 24, resulting in plasma HIV-1 RNA rebound. Interestingly, comparing the PCratio at AHI and week 24 revealed a drastic increase of PCratio at week 24 (0.81 vs. 1.60). Without other interventions during the period, the

"reversal" or "normalization" of PCratio is possibly due to the resolution of neurosyphilis that enhances the HIV-1 CNS transmigration. This is in line with the elevation of CSF HIV-1 RNA level among PLWH who are co-infected with neurosyphilis [78]. Future investigations should examine the mechanisms by which neurosyphilis augments HIV-1 RNA level in the CSF. For instance, whether neurosyphilis directly augments the viral replication of the infected CNS residential cells, or it enhances lymphocytic infiltration into the CSF space including HIV-infected lymphocytes.

The effect of untreated syphilis on HIV-1 during acute infection is evaluated in **Chapter 6**. In the study, untreated syphilis was defined by positive blood T. pallidum hemagglutination assays (TPHA) and Venereal Disease Research Laboratory test (VDRL) in the absence of prior syphilis treatment, while the status of prior syphilis was defined by a positive blood TPHA regardless of the VDRL result and treatment status. Untreated syphilis was not associated with a higher plasma HIV-1 RNA level or a lower CD4+ T-cell level during AHI. Instead, the study reveals an association between untreated syphilis and higher CD8+ T-cell levels in a multivariate analysis. The analysis also revealed lower cognitive test performance among RV254 participants with prior syphilis but not untreated syphilis. Prior syphilis in the absence of neurosyphilis has been inconsistently associated with poorer cognitive performance in chronic HIV-1 infection [40-42]. In one study, heightened immune activation in CSF (e.g. monocyte activation and elevated CXCL10 and CCL2 levels) was associated with lower cognitive performance and neurosyphilis but the latter two showed an insignificant association [79]. One possible explanation is that neurosyphilis only augments the pre-existing HIV-related CNS inflammation, instead of an independent role in causing cognitive dysfunction.

One of the key confounders underlying the inconsistent study outcomes in HIV and syphilis co-infection is the heterogeneity around use and compliance with ART. For example, incident syphilis is not associated with change in plasma HIV-1 RNA in a study of participants predominantly on suppressive ART [80]. In that study, new syphilis is associated with transient lymphopenia with declines in absolute counts of B- and T-lymphocytes including CD4+ and CD8+ subsets. Future studies should clarify if syphilis causes lymphopenia in HIV-negative individuals as well. If it is the case, the transient lymphopenia secondary to incident syphilis may be indeed the reason for plasma HIV-1 RNA elevation in untreated PLWH in that it transiently suppresses the adaptive immunity that controls HIV-1 replication. Besides, it is important to investigate whether PLWH on stable ART would still show a higher risk of neurosyphilis than HIV-negative populations. Additional works are needed to decipher the possible association between cognitive impairment and prior syphilis in PLWH. As discussed in chapter 6, possible causes of such association include the direct biological consequence of neurosyphilis, a proxy effect of syphilis in association with other co-infections, and socioeconomic and behavioral factors including substance uses. Lastly, phenotypic and inflammatory marker analyses will be useful to validate the observation of CD8+ T-cell alteration by untreated

syphilis during AHI.

RV254 for Safety of Investigation Procedures

RV254 has published a number of safety reports regarding invasive procedures, including repeated blood sampling [81], sigmoid gut biopsy [82], lymph node biopsy [83], leukopharesis and lumbar puncture in **Chapter 3**. These optional procedures are performed regularly after study enrollment, offering biological samples paired with the clinical outcomes. Despite the complexity, all investigational procedures are conducted safely according to international standards. These published reports suggest that performing invasive procedures during AHI is feasible and associated with acceptable safety profiles. In Chapter 3, apart from evaluating the frequency of post-LP headache (PLPH), we examined correlations with the volume of CSF collected (10ml vs. 20ml). The analysis suggests that collecting a larger sample of CSF is not associated with an increased risk of PLPH compared to a more commonly employed smaller volume in CSF studies. Clarifying the safety of high volume CSF collection will be especially useful for researchers because CSF volume is crucial for the quality of CSF studies, particularly cellular investigations.

Neurological Outcomes after Initiating ART during Acute HIV-1 infection

The neuropsychological assessment of RV254 includes a 4-test battery that measures fine motor speed and dexterity (non-dominant hand Grooved Pegboard test, GPB), psychomotor speed (Color Trails 1, CT1 & Trail Making A, TrailA) and executive functioning (Color Trails 2, CT2). Raw scores are standardized to Thai normative data [84] to calculate z-scores, which are averaged to create an overall performance (NPZ-4) score. The first cognitive outcome report of RV254 examines the change of neuropsychological test performance between AHI (week 0) and week 24 [61]. It reported impaired cognitive performance in approximately a quarter of the 36 participants studied. Participants with impairment had higher CSF HIV-1 RNA levels and did not improve after ART, leading to a concern about limited reversibility even for cognitive impairment during AHI.

To better understand the issue, **Chapter 7** expands the analysis with a larger sample and extended time frame out to six years (288 weeks) post-ART initiation. It aims to understand the long-term cognitive trajectory and stability of RV254 participants by applying group-based trajectory analysis (GBTA). In particular, it aims to identify subsets of individuals who demonstrate persistent cognitive impairment or subsequent decline in cognitive performance despite initial improvement. As shown in the chapter, three subgroups are identified by GBTA. All trajectory subgroups show improvement with greatest improvements seen in the first 96 weeks. Moreover, none of the trajectories demonstrate subsequent decline in test performance during the study period. The findings support that neuropsychological testing abnormalities noted in AHI is reversible concurrent with immediate ART initiation but this improvement may take more than 24

weeks to occur. Improvement in depression symptoms follows a similar trend. Moderate depressive symptoms, measured by the Patient Health Questionnaire-9 (PHQ-9) (**Box 2**), were detected in 46% of RV254 participants during AHI [1]. The PHQ-9 score directly correlates with both plasma HIV-1 RNA and neopterin levels and improves after initiation of ART out to week 24 [1], and remains stable 6 years after ART initiation (unpublished data).

Box 2
The **PHQ-9** is a 9-item survey (score range 0-27) of depressive symptoms based on DSM-IV criteria. PHQ-9 total scores ≥10 and ≥15 define moderate and moderate-severe depression respectively

Examination of biomarkers in RV254 identify that neuronal injury is uncommon at untreated AHI and 24 weeks post-ART. Specifically, before ART, CSF neurofilament light chain (NFL), a neuronal damage marker, was elevated in 1 out of 32 (3%) samples tested, compared to 10 out of 32 (31%) of samples from chronic HIV-1 infection [85]. After 24 weeks of ART, 1 out of 25 (4%) and 4 out of 9 (44%) CSF samples showed elevated NFL levels in AHI and chronic HIV-1 infection, respectively [85]. CSF inflammatory markers including neopterin, CXCL10, CCL2, interleukin 6 and YKL-40 markedly reduce by week 24 of ART [86, 87], and trend towards normalization by week 96 [87], highlighting the resolution of overt intracerebral inflammation after AHI. Further, RV254 participants also show a lower frequency of CSF HIV-1 escape than PLWH who started ART during chronic HIV-1 infection (Chapter 8). These CSF findings are summarized in **Table 2**. Taken together, CSF studies identify that neuronal injury is uncommon during AHI and remains so after immediate ART. Treating HIV-1 at AHI leads to reduced parenchymal immune activation and likely a smaller pool of activated or infected resident cells in the CNS than in chronic HIV-1 infection, allowing for the reversibility of the initial CNS inflammation and cognitive deficits present during AHI. Most importantly, such benefit remains stable over a long period of time.

Table 2. CSF Features of HIV-1 Infection before and after Treatment

	Acute HIV Infection		#Primary HIV Infection		Chronic HIV Infection	
	Pre-ART	**Post-ART**	**Pre-ART**	**Post-ART**	**Pre-ART**	**Post-ART**
Abnormal ^Neopterin	Elevated	Absent	Elevated	Absent/Rare	Elevated	Elevated in ~50%
***NFL Elevation**	Absent	Absent	~40%	Unknown	10-75%	16%
PCratio	≥2	N/A	1-2	N/A	0.6-1.1	N/A
HIV-1 Escape	N/A	Rare (1%)	N/A	Unknown	N/A	10%

Abbreviations: NFL: ART: antiretroviral therapy; CSF: cerebrospinal fluid; neurofilament light chain; PCratio: ratio between CSF and plasma HIV-1 RNA level
Primary HIV infection: within first year after HIV-1 transmission
^ Neopterin: A marker of cellular immune system activation, synthesised by human macrophages upon stimulation
* NFL: A marker of neuronal damage

The absence of immunodeficiency-related complications in RV254 participants, as compared to PLWH diagnosed and treated during chronic HIV-1 infection, makes RV254 a desirable model to further examine any potential adverse neuropsychiatric effects of ART. In 2018, the World Health Organization (WHO) updated the ART guideline and recommended the use of Dolutegravir (DTG)-based regimens as first-line ART. Although DTG is well tolerated in the original research, post-marketing clinical reports raise concern regarding the risk of DTG-associated neuropsychiatric adverse events (NP-AEs), which leads to a discontinuation rate up to 6% [88, 89]. Increased insomnia and depression symptoms have been reported, particularly among individuals with a history of affective symptoms before DTG initiation [89]. Starting from Feb 2017, all new RV254 participants commenced DTG-based ART during AHI, while the existing participants were systematically switched to DTG-based ART from their original ART regimen.

Two safety reports about Dolutegravir-based ART are published based on the cohort's experience. One addresses general safety [90] and the other examines mood and neurocognitive side-effects (**Chapter 9**). The reports suggest that DTG-based ART is safe and well-tolerated. In **Chapter 9**, we examine participants' NP performance and PHQ-9 score before and after the switch. NP testing performance remained stable after the switch. PHQ-9, particularly the somatic subset scores (sleep/appetite/energy level, questions 3-5), are modestly higher after the switch. However, participants of higher PHQ-9 pre-switch did not show higher PHQ-9 scores at follow-up, speaking against the notion that DTG would worsen depression symptoms among individuals with pre-existing depression. Although a previous study reported no association between DTG pharmacokinetics and changes in sleep parameters or neurocognitive performance in PLWH aged 60 or older [91], additional work is needed to determine whether a subgroup of PLWH may be prone to DTG-related NP-AEs, including concomitant medicine and substance use.

Examining the CNS impact in Analytical Treatment Interruption

The residual immune dysfunction and morbidities after ART as well as the need for life-long ART compliance signify the need of cure for HIV-1 infection. Further, the stigmatization of the disease continues to negatively affect the social and psychological wellbeing of PLWH, highlighting the clinical and strategical importance of HIV cure. Several cure strategies, such as viral latency reversal, broadly-neutralizing antibodies and vaccines, have been tested; however, major breakthroughs have not been achieved. To test the effectiveness of cure strategies, analytical treatment interruption (ATI) is needed and its safety remains a critical concern for both investigators and research participants. CNS safety has been one of the biggest concerns for ATI because of this compartment's vulnerability, its unique model of immune surveillance and the known variation in drug penetration. Moreover, CNS may serve as an isolated HIV-1 compartment with CSF viral escape during prolonged ATI. **Chapter 10** reviews the

literature around ART interruption in PLWH in both clinical and research settings. It also reviews the CNS effects of different cure strategies in both the human and primate settings.

Since 2015, SEARCH has conducted a number of ATI/cure studies [92-95], of which three included interventional agents. The participants were co-enrolled from the RV254 cohort. The ATI studies were conducted under close monitoring of plasma HIV-1 RNA and ART was resumed if plasma HIV-1 RNA exceeded 1000copies/ml twice. There are several key questions about CNS safety during the initial design of these ATI studies. First, whether CSF HIV-1 RNA will be detected in the absence of detectable plasma HIV-1 RNA during ATI required examination. Second, upon plasma HIV-1 RNA rebound, it is not clear if immune activation would occur in the CNS, similar to that in AHI, leading to a similar degree of cognitive dysfunction and depression symptoms. Third, if CNS inflammation occurred, it was important to understand the timeline needed to return to pre-ATI levels. To settle these questions, a full panel of neurological monitoring, including NP testing and mood assessment, optional LP and brain MRIs were included in the course of the studies, before, during and after ATI.

With brief and closely monitored ATI after early initiation of ART in the RV254 cohort, there were no major adverse event during all ATI studies. The neurological data collected from them were combined and analyzed in **Chapter 11**. Low level of CSF HIV-1 RNA (<80 copies/ml) was detected in 6 out of 20 participants who provided CSF at the time of initial return of plasma viremia during ATI. However, there were no differences in median depression questionnaire scores and neurocognitive battery performance. Magnetic resonance spectroscopy (MRS) showed only alteration of choline level, a marker that is thought to reflect cellular inflammation and cell membrane changes in the basal ganglia after ATI. However, the neurological data available from these studies was quite limited, and thus additional studies are needed to determine the CNS safety of ATI. In addition, these safety outcomes may not be directly applicable to other PLWH populations as RV254 participants in general have a much smaller HIV-1 reservoir than PLWH who are treated later. A smaller sized HIV-1 reservoir may lead to less robust viral rebound and weaker immune response reaction. Nonetheless, the findings provide an important reference for the design of future HIV-1 cure studies that the cohort continues to investigate.

LIMITATIONS

Similar to other Asian-based HIV cohorts, RV254 participants are predominantly young MSM who are healthy and highly adherent to ART, which may make the findings less generalizable to other cohorts with different demographic and disease characteristics. Further, it is challenging to obtain matched chronic HIV-1 infection and HIV-negative

controls especially in analyses that involve optional procedures. SEARCH is currently enrolling both treatment-naïve people with chronic HIV infection and HIV-negative control participants who will be followed for two years with clinical and optional procedures similar to those in RV254. These measures will strengthen the ability to ascertain effects of AHI and its treatment on clinical and neuropsychological outcomes.

CONCLUSIONS

The information gathered from the RV254 cohort study has vastly improved the understanding of HIV-1 pathogenesis at the earliest stage of infection. It highlights that HIV-1 invades the CNS during acute infection, and is associated with abnormal neurological signs, neurocognitive dysfunction and depression symptoms. Prompt initiation of ART is associated with reversal of most of these abnormalities as well the associated CNS inflammation observed. More importantly, individuals who initiate ART during AHI are generally free from neuropsychiatric complications frequently seen in other HIV-positive populations. The stable neurological outcomes suggest that ART neurotoxicity, if any, probably plays a lesser role than HIV-related factors in HAND development. Future studies should look at long-term durability of early ART in RV254, including protection against non-communicable diseases of HIV, stability of HIV-1 reservoir and trajectory of chronic inflammation. Single cell capabilities has been established on-site in Bangkok to allow detailed interrogation of immunologic perturbations and HIV-1 persistence in the CNS. PET imaging using novel markers will help identifying "hotspots" of inflammation. Clinical studies should also focus on the adverse neurological effects driven by common co-infections and substance use as they both show increasing frequency in PLWH globally.

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Chapter 14:
Summary & Nederlandse Samenvatting

This thesis aims to investigate the neurological impact of acute HIV infection (AHI) and its outcomes after immediate initiation of antiretroviral therapy (ART). Following a general introduction in **Chapter 1**, the thesis is divided into four sections. Section 1 is an introduction about the persistence of HIV-associated neurocognitive disorder (HAND) in ART era. It describes the involvement the central nervous system (CNS) during early stage of HIV-1 infection and highlights the importance to study AHI to understand HIV neuropathogenesis, illustrating the importance of RV254 Thai AHI cohort in filling this knowledge gap. Section 2 includes four RV254 reports that focus on AHI data. Two of them are about the impact of concomitant syphilis infection during AHI, one about the determinant of HIV-1 RNA level in cerebrospinal fluid (CSF) and the other one about safety of performing lumbar punctures during AHI. Section 3 includes three post-ART reports which examine the 6 years neurocognitive trajectory, the frequency of CSF HIV-1 escape and neuropsychological safety of switching to a Dolutegravir-based ART regimen. Section 4 explores the implications of CNS in HIV cure studies.

Chapter 2 is a cognitive screening study conducted at the HIV clinic of Queen Elizabeth Hospital of Hong Kong. During the study period, 98 consecutive antiretroviral therapy (ART)-naïve people living with HIV (PLWH) underwent the International HIV Dementia Scale (IHDS) and Montreal Cognitive Assessment (MoCA) screening before and 6 months after ART initiation. Before ART, 39% and 26% of them scored below the cut-offs of IHDS and MoCA respectively. At the 6-month follow-up, despite a high rate of ART adherence and plasma viral suppression, their MoCA performance did not show a significant improvement. Further, near a fifth of them persistently showed an impaired performance in IHDS. The high frequencies of persistently abnormal IHDS and MoCA performance suggest the presence of irreversible cognitive impairment despite effective ART.

Chapter 3 reviews the prevalence of cognitive impairment and persistent central nervous system (CNS) injury in treated PLWH in ART era. This notion is supported by the unchanged rate of cognitive impairment in PLWH in the ART era despite a significant decline in the frequencies of more severe forms of HIV-associated neurocognitive disorders (HAND). The article discusses the incomplete CNS protection by ART, highlighted by reports of persistent immune activation in the CNS despite plasma HIV-1 suppression. Moreover, neuroinvasion during early HIV-1 may set a stage for later cognitive manifestations, illustrating that acute HIV-1 infection (AHI) studies are essential to understand HIV-related cognitive disorders.

Section 2 includes reports that focused at the baseline (AHI) of the RV254 cohort. **Chapter 4** is a safety report of lumbar puncture (LP) in the study. The study includes 195 LP procedures of which 89 (46%) were performed during AHI before ART. This is the first report on the safety of performing LPs during AHI. We note that LP at AHI did not associate with a higher risk of post-LP headache (PLPH) compared to LPs performed after

ART. More severe complications such as persistent CSF leakage and wound infection were essentially absent. Apart from demonstrating the superiority of atraumatic LP needle to reduce PLPH, the study reports a statistically similar risk of PLPH between lower and larger volumes of CSF collection (i.e. 10ml vs. 20ml).

Chapter 5 examines the relationship between CSF HIV-1 RNA levels and other blood parameters during untreated AHI. HIV-1 RNA was quantifiable in 74 out of 114 (65%) CSF samples at study enrollment, but the rate of quantification at later Fiebig stages (III-V) was >90%, suggesting the invariable presence of HIV-1 neuroinvasion upon late stages of AHI. The degree of HIV-1 neuroinvasion, estimated by the ratio between plasma and CSF HIV-1 RNA (PCratio), was employed to understand associated factors among participants. Higher degree of neuroinvasion, represented by a PCratio<1, associated with higher levels of CSF immune activation makers including neopterin, soluble (s)CD163, interleukin-6, and sCD14. In multivariable analyses, plasma HIV-1 RNA and CD4/CD8 ratio independently correlated with CSF HIV-1 RNA, whereas CD4/CD8 ratio predicted PCratio, suggesting the link between immunologic response and viral transmigration into the CNS during AHI.

Chapter 6 reports the CNS profile of a RV254 participant who had concomitant neurosyphilis at the time of AHI. Despite being neurologically asymptomatic, his CSF demonstrated overt lymphocytic pleocytosis (CSF WBC 105 cells/mm3). His CSF venereal disease research laboratory (VDRL) titer was negative; however, his CSF Treponema pallidum hemagglutination (TPHA) was positive. A brain MRI demonstrated signal abnormalities supportive of meningitis along the sulci in bilateral occipital and high frontal regions. Cytometry of his CSF and PBMC showed a B-cell predominant lymphocytosis in CSF, instead of a T-cell lymphocytosis in typical acute HIV-1 mono-infection. After a standard course of neurosyphilis treatment, the aforementioned CSF and MRI abnormalities largely resolved. These findings highlight the potential of neurosyphilis to alter the immunological response towards HIV-1.

Chapter 7 investigates the effects of untreated systemic syphilis on AHI. Clinical and laboratory parameters of RV254 participants with concomitant untreated syphilis at baseline were compared to those with acute HIV-1 mono-infection. In chronic HIV-1 infection, incident syphilis causes elevation of plasma HIV-1 RNA level and decline in CD4+ T-cells level. Interestingly, such effects were not observed at AHI. Instead, CD8+ T-cell levels were elevated among those with concomitant syphilis. Moreover, a history of prior syphilis, but not untreated current syphilis, was associated with lower cognitive test performance.

Section 3 focuses on the longitudinal outcomes of RV254 cohort after ART. **Chapter 8** reports their longitudinal cognitive outcomes after six years of follow-up. It includes 65 RV254 participants who achieved stable viral suppression throughout the period.

Group-based trajectory analysis (GBTA) was applied to identify subsets of participants with differing cognitive trajectories. Our modeling identified three separate trajectories which all demonstrated an improving trend of performance over time. The trajectory subgroup with the worst initial test performance showed the greatest degree of improvement compared to the other two trajectory subgroups, highlighting that the former group may suffer from a greater virological impact in the CNS during AHI. Further, the group with the lowest initial performance evidenced more depression symptoms at baseline.

Chapter 9 determines the frequency of CSF HIV-1 escape in RV254 post-ART. At week 24 post-ART, 4 out of 89 (4%) participants had quantifiable CSF HIV-1 RNA but only one had CSF escape. Here, the CSF HIV-1 RNA was 320 copies/ml while the corresponding plasma HIV-1 RNA was below 50 copies/ml. At week 96, 1 out of 46 (2%) participant had quantifiable CSF HIV RNA but the case not meet the criteria for CSF escape because the plasma HIV-1 RNA was higher than that in CSF. The participant with CSF escape was treated with efavirenz/tenofovir/lamivudine and had a CD4+ T-cell level of 840 cells/mm3. The levels of CSF white blood cell and CSF protein were within normal limit. His MRI at week 24 showed a small nonspecific T2/FLAIR hyperintense focus in the right high frontal white matter. He did not undergo LP at untreated AHI nor at subsequent visits. The study suggests a very low rate of CSF escape in the first two years after starting ART during AHI.

Chapter 10 examines the potential effects of Dolutegravir on mood and cognitive performance. The analysis included 254 RV254 participants who underwent a planned transition from non-dolutegravir (DTG)- to DTG-based ART. Paired parameters, including 1) Patient Health Questionnaire-9 (PHQ-9); 2) a 2-Questions depression screening; 3) Distress Thermometer score; and 4) a 4-test neurocognitive battery, prior to and at least 3 months after the switch were compared. The analysis revealed a modest but statistically significant increase in PHQ-9 scores after DTG. The percentage of participants with at least moderate depression (PHQ-9≥10) increased from 10% to 16%, but the frequency of moderate-severe depression (PHQ-9≥15) remained unchanged (3%). In particular, somatic symptoms of depression increased more than cognitive/affective symptoms. Plasma viral suppression and PHQ-9≥10 before switch were linked to lower PHQ-9 scores after DTG in multivariable analysis. Performance on all neuropsychological tests, except grooved pegboard test, improved modestly after DTG. However, the improvement after the switch could be part of the longitudinal improvement in NP test as observed in Chapter 8, rather than a direct neurocognitive benefit of DTG over EFV.

Section 4 examines CNS contributions and impact in HIV-1 cure studies. **Chapter 11** reviews the role of CNS latency on HIV-1 eradication. It examines the effects of early ART, latency-modifying agents, antibody-based, T-cell enhancing therapies and ART

interruption with CNS parameters. The manuscript proposes the integration of CNS monitoring into HIV-1 remission studies in order to clarify the short- and long-term neurological safety of experimental cure agents and ART interruption, and to better characterize their interaction with HIV-1 in the CNS.

Chapter 12 described the CNS profile of 30 RV254 participants who completed ATI trials during which ART was resumed upon plasma HIV-1 RNA exceeded 1000 copies/mL. CNS measures included paired mood, cognitive performance, and neurologic examination, CSF and brain MRI analyses before and after ATI. There were no differences in median mood scores or neurologic findings after ATI and cognitive performance improved modestly compared to pre-ATI. During ATI, a low level of CSF HIV-1 RNA was detectable in 6 out of 20 participants with plasma viremia, with no group changes in CSF immune activation markers or brain DTI measures. Mild alteration of choline level at basal ganglia was detected by MRS post-ATI, suggesting an alteration in neuronal membranes. The findings support the absence of major adverse CNS effects in brief, closely-monitored ATI in RV254 participants.

General Discussion

The final chapter, **Chapter 13**, combines the findings in this thesis and other publications based on RV254 data together and put them into perspective. Briefly, HIV-1 invades the CNS during acute infection, and is associated with abnormal neurological signs, neurocognitive dysfunction and depression symptoms. Prompt initiation of ART is associated with reversal of these abnormalities as well the associated CNS inflammation observed. More importantly, individuals who initiate ART during AHI are generally free from neuropsychiatric complications frequently seen in other HIV-positive populations. The stable neurological outcomes suggest that ART neurotoxicity, if any, likely plays a lesser role than HIV-related factors in HAND development. Future studies should focus on the adverse neurological effects driven by common co-infections and substance use as they both show increasing frequency in PLWH globally.

Nederlandse samenvatting

Dit proefschrift heeft tot doel de neurologische gevolgen van een acute hiv-infectie (AHI) te onderzoeken, en de mate waarin deze beinvloed worden wanneer onmiddellijk begonnen wordt met antiretrovirale therapie (ART). Na een algemene inleiding in **Hoofdstuk 1**, is het proefschrift onderverdeeld in vier delen. **Deel 1** is een inleiding over het persisteren van hiv-geassocieerde neurocognitieve stoornis (HAND) in het ART-tijdperk. Het beschrijft de betrokkenheid van het centraal zenuwstelsel (CZS) vroeg na een hiv-1-infectie en benadrukt het belang van het bestuderen van AHI om de neuropathogenese van hiv beter te begrijpen, en illustreert daarmee het belang van RV254 AHI-cohort in Thailand voor het dichten van lacunes in onze kennis. **Deel 2** bevat vier artikelen op basis van gegevens verkregen tijdens AHI in het RV254-cohort. Twee daarvan gaan over de impact van een gelijktijdige syfilis-infectie tijdens AHI, één over de determinanten van de hiv-1 RNA-concentratie in de liquor cerebrospinalis en het vierde over het veilig uitvoeren van lumbaalpuncties tijdens AHI. **Deel 3** bevat drie artikelen met lange termijn data van RV254-cohortdeelnemers nadat zij met ART waren gestart. Eén artikel beschrijft diverse neurocognitieve parameters gedurende een follow-up van 6 jaar, één de frequentie van optreden van hiv-1 " escape" in de liquor en het laatste artikel de veiligheid van een switch naar een dolutegravir-bevattende combinatietherapie met betrekking tot mogelijke neuropsychologisch gevolgen daarvan. **Deel 4** gaat in op het belang van het CZS bij onderzoeken naar hiv-genezing.

Hoofdstuk 2 van **Deel 1** beschrijft een onderzoek naar cognitieve screening, uitgevoerd in de hiv-kliniek van het Queen Elizabeth Hospital in Hong Kong. Tijdens de onderzoeksperiode werden 98 opeenvolgende antiretrovirale therapie (ART) -naïeve mensen met hiv (PLWH) gescreend met behulp van de International HIV Dementia Scale (IHDS) en Montreal Cognitive Assessment (MoCA), zowel voor als 6 maanden na de start van ART. Vooraf aan de start van ART scoorde 39% en 26% van hen onder de cut-offs van respectievelijk de IHDS en de MoCA. Zes maanden na start van ART werd, ondanks een hoge mate van therapietrouw en goede virale suppressie in het plasma, geen significante verbetering in de MoCA gezien. Bijna een vijfde toonde ook aanhoudend verminderde prestaties gemeten met de IHDS. De hoge frequentie van aanhoudend abnormale prestaties gemeten met zowel de IHDS- als de MoCA suggereren de aanwezigheid van een onomkeerbare cognitieve stoornis ondanks effectieve ART.

Hoofdstuk 3 geeft een overzicht van de prevalentie van cognitieve stoornissen en persisterende schade aan het centraal zenuwstelsel (CZS) in met ART-behandelde mensen met hiv. Het concept dat een dergelijke schade persisteert wordt ondersteund door een ongewijzigde overall prevalentie van cognitieve stoornissen in het ART-tijdperk, ondanks een evidente afname in de frequentie van de meest ernstige vormen van HIV-geassocieerde neurocognitieve stoornis (HAND). Het artikel bespreekt de onvolledige bescherming van het CZS ondanks ART, hetgeen benadrukt wordt door

aanwijzingen voor aanhoudende immuunactivatie in het CZS ondanks adequate onderdrukking van hiv-1 in het bloed. Bovendien zijn er aanwijzingen dat penetratie van hiv-1 in het CZS kort na infectie de basis legt voor latere cognitieve manifestaties, wat illustreert dat onderzoeken tijdens een acute hiv-1-infectie (AHI) essentieel zijn om hiv-gerelateerde cognitieve stoornissen beter te begrijpen.

Deel 2 bevat de publikaties die gericht zijn op de bevindingen (AHI-fase) ten tijde van inclusie in het RV254-cohort. **Hoofdstuk 4** bespreekt de veiligheid van de lumbaalpuncties (LP) zoals die in het onderzoek zijn verricht. De studie omvat 195 LP's waarvan 89 (46%) werden uitgevoerd tijdens AHI vóór start van ART. Dit is de eerste keer dat de veiligheid van het doen van LP's tijdens AHI wordt gerapporteerd. LP's verricht ten tijde van AHI waren niet geassocieerd met een hoger risico op postpunctionele hoofdpijn vergeleken met LP's uitgevoerd na start van ART. Ernstiger complicaties zoals aanhoudende liquor-lekkage en wondinfectie waren nagenoeg afwezig. Naast het aantonen van de superioriteit van het gebruik van atraumatische LP-naalden in het verminderen van de kans op postpunctionele hoofdpijn, toonde de studie ook een statistisch vergelijkbaar risico op postpunctionele hoofdpijn bij het afnemen van een klein of groter volume (d.w.z. 10 ml versus 20 ml) aan liquor.

Hoofdstuk 5 onderzoekt de relatie tussen de concentratie hiv-1 RNA in de liquor en diverse parameters gemeten in bloed tijdens onbehandelde AHI. Hiv-1-RNA was kwantificeerbaar in 74 van de 114 (65%) liquor-monsters ten tijde van inclusie van deelnemers in het onderzoek, waarbij dit voor latere Fiebig-stadia (III-V) het geval was in > 90%, hetgeen wijst op het vrijwel uniform optreden van infectie in het CZS met hiv-1 in de latere stadia van AHI. De mate van CZS infectie, geschat aan de hand van de verhouding tussen plasma en CSF hiv-1-RNA (PCratio), werd gebruikt om te beoordelen welke factoren hiermee geassocieerd waren. Een hogere mate van CZS-infectie, weerspiegeld in een PCratio <1, was geassocieerd met een hogere concentratie van immuunactivatie-markers in de liquor , waaronder neopterine, soluble (s) CD163, interleukine-6 en sCD14. In multivariabele analyses waren plasma hiv-1 RNA en de CD4 / CD8 ratio onafhankelijk geassocieerd met de concentratie hiv -1 RNA in de liquor, terwijl de CD4 / CD8 ratio voorspellend was voor de PCratio. Dit suggereert een verband tussen de immunologische respons en de migratie van het virus naar het CZS tijdens AHI.

Hoofdstuk 6 beschrijft de CZS kenmerken van een RV254-deelnemer bij wie op het moment van AHI gelijktijdig sprake was van neurosyfilis. Hoewel hij neurologisch asymptomatisch was, was er sprake van een evidente lymfocytaire pleiocytose in de liquor (leukocyten in de liquor 105 cellen / mm3). De VDRL (venereal disease research laboratory) reactie in de liquor was negatief, maar de Treponema pallidum hemagglutinatie (TPHA) reactie was positief. Een MRI van de hersenen toonde tekenen

van meningitis, bilateraal langs de sulci in de occipitale en bovenste frontale gebieden. Cytometrie van de liquor en van PBMC toonde een overwegend B-cel lymfocytose in de liquor, in plaats van de gebruikelijke overwegend T-cel lymfocytose bij een typische acute hiv-1 mono-infectie. Na een standaardbehandeling voor neurosyfilis waren de eerder genoemde liquor- en MRI-afwijkingen grotendeels verdwenen. Deze bevindingen benadrukken hoe neurosyfilis potentieel de immunologische respons op hiv-1 kan veranderen.

Hoofdstuk 7 onderzoekt de effecten van een onbehandelde systemische syfilis op AHI. Klinische en laboratoriumparameters van RV254-deelnemers met een gelijktijdige onbehandelde syfilis ten tijde van inclusie werden vergeleken met die van deelnemers met een acute hiv-1-mono-infectie. Tijdens chronische hiv-1-infectie kan een incidente syfilis een toename van de plasma hiv-1 RNA-concentratie en een afname van het aan CD4 + T-cellen veroorzaken. Opvallend werden dergelijke effecten niet waargenomen tijdens AHI. In plaats daarvan werden verhoogde aantallen CD8 + T-cellen gezien bij degenen met gelijktijdige syfilis. Bovendien was een voorgeschiedenis van syfilis, maar niet een onbehandelde gelijktijdige actieve syfilis, geassocieerd met slechtere cognitieve testprestaties.

Deel 3 richt zich op de longitudinale langere termijn uitkomsten van de RV254-cohortdeelnemers tijdens ART. **Hoofdstuk 8** beschrijft de cognitieve uitkomsten van deelnemers na zes jaar follow-up. Het betreft 65 RV254-deelnemers die gedurende deze hele periode stabiel viraal onderdrukt waren. Group-based trajectory analysis (GBTA) werd toegepast om groepen van deelnemers met een verschillend cognitieve beloop te identificeren. Onze modellering kon drie afzonderlijke vormen van beloop onderscheiden, welke alle drie een trend van betere cognitieve prestaties in de tijd lieten zien. De groep met de aanvankelijk slechtste testprestaties vertoonde de grootste mate van verbetering in vergelijking met de andere twee te onderscheiden groepen, wat erop wijst dat bij de eerste groep er mogelijk een grotere impact van het virus op het CZS was tijdens AHI. Verder vertoonde deze groep met de bij aanvang slechtste testprestaties ook meer depressieve symptomen.

Hoofdstuk 9 beschrijft de frequentie van het optreden van hiv-1 "escape" in de liquor in het RV254 cohort na de start van ART. Op week 24 na de start van ART hadden 4 van de 89 (4%) deelnemers een kwantificeerbare concentratie hiv-1 RNA in de liquor, maar bij slechts één deelnemer was er sprake van "escape in de liquor". De concentratie hiv-1 RNA in de liquor bedroeg 320 kopieën / ml, bij een gelijktijdige plasma hiv-1 RNA concentratie van minder dan 50 kopieën / ml. Op week 96 had slechts 1 van 46 (2%) deelnemers een meetbaar hiv-1 RNA in de liquor, maar de casus voldeed niet aan de criteria voor "escape" aangezien de gelijktijdige plasma hiv-1 RNA concentratie hoger was dan die in de liquor. De deelnemer met hiv-1 "escape" in de liquor werd behandeld met efavirenz / tenofovir / lamivudine en had een CD4 + T-cel aantal van 840 cellen /

mm3. De concentratie leukocyten en eiwit in de liquor waren niet afwijkend. Een MRI van de hersenen op week 24 toonde een kleine niet-specifieke T2 / FLAIR hyperintense lesie rechts hoog frontaal in de witte stof. De deelnemer had geen LP ondergaan tijdens de fase dat zijn AHI nog niet behandeld werd, en evenmin bij volgende studiebezoeken. Deze studie suggereert een zeer geringe mate van hiv-1 "escape" in de liquor gedurende de eerste twee jaar na het starten van ART tijdens AHI.

Hoofdstuk 10 onderzoekt de mogelijke effecten van dolutegravir op de stemming en cognitieve prestaties. De analyse omvat 254 RV254-deelnemers met een geplande switch naar DTG-bevattende ART. Gepaarde parameters, waaronder 1) Patient Health Questionnaire-9 (PHQ-9); 2) een screening op depressie aan de hand van 2-vragen ; 3) Distress Thermometer-score; en 4) een neurocognitieve testbatterij van 4 testen, verkregen voorafgaand aan de ART-switch en ten minste 3 maanden na de switch werden vergeleken. Er was sprake van een bescheiden maar statistisch significante toename van PHQ-9-scores na switch naar DTG. Het percentage deelnemers met tenminste matige depressie (PHQ-9≥10) nam toe van 10% naar 16%, maar het percentage met matig tot ernstige depressie (PHQ-9≥15) bleef ongewijzigd (3%). Met name somatische symptomen van depressie namen meer toe dan cognitieve / affectieve symptomen. Virus suppressie in het plasma en een PHQ-9 score ≥10 vóór de switch waren geassocieerd met een lagere PHQ-9-score na switch naar DTG in de multivariabele analyse. Prestaties op alle neuropsychologische testen, behalve de grooved pegboard-test, verbeterden in beperkte mate na switch naar DTG. Deze verbetering na de switch zou echter ook een reflectie kunnen zijn van de longitudinale verbetering in neuropsychologische testen zoals beschreven in **Hoofdstuk 8**, en niet zozeer een rechtsreeks neurocognitief voordeel van DTG ten opzichte van EFV.

Deel 4 behandelt de bijdrage en rol van het CZS bij studies naar genezing van hiv-1. **Hoofdstuk 11** vat de betekenis samen van een latente hiv-1 infectie in het CZS bij pogingen tot eradicatie van een hiv-1 infectie. Het beschrijft aan de hand van CZS-parameters de effecten van vroegtijdige ART, latentie-modificerende middelen, op antilichamen gebaseerde, T-cel-versterkende therapieën en van het onderbreken van ART. Voorgesteld wordt om monitoring van CZS parameters te integreren bij het verrichten van hiv-1 remissiestudies, teneinde de korte en lange termijn veiligheid op neurologisch vlak van zowel experimentele middelen gericht op genezing als van het onderbreken van ART verder te verduidelijken, en hun interactie met hiv-1 in het CZS beter te karakteriseren.

Hoofdstuk 12 beschrijft de effecten op het CZS bij 30 RV254-deelnemers die diverse hiv-1 remissietrials voltooiden gevolgd door een analytic treatment interruption (ATI) van hun ART, waarbij ART werd hervat wanneer het plasma-hiv-1-RNA de grens van 1000 kopieën / ml overschreed. Gepaarde metingen van voor en na ATI betroffen metingen van stemming, cognitieve prestaties, neurologisch onderzoek, liquorparameters en MRI

van de hersenen. Er waren geen verschillen in mediane stemmingsscores of neurologische bevindingen na ATI en de cognitieve prestaties toonden slechts een bescheiden verbetering vergeleken met vooraf aan de ATI. Gedurende de ATI was bij 6 van de 20 deelnemers met een viremie sprake van een slechts lage concentratie hiv-1 RNA in de liquor, en werden op groepsniveau geen veranderingen gezien in markers van immuunactivatie in de liquor of bij DTI (diffusion tensor imaging)-metingen op de MRI van de hersenen. Er was post-ATI sprake van een beperkte verandering in de detectie van de hoeveelheid choline in de basale ganglia met behulp van MRS (magnetische resonantie spectroscopie), wat duidt op een verandering in neuronale membranen. De bevindingen ondersteunen de afwezigheid van grote nadelige effecten op het CZS tijdens een kortdurende en nauwlettend gemonitorde periode van ATI bij de RV254-deelnemers.

Algemene Discussie

Het laatste hoofdstuk, Hoofdstuk 13, combineert de bevindingen uit dit proefschrift en andere publicaties op basis van RV254-gegevens en plaatst ze in perspectief. Kort samengevat dringt hiv-1 het CZS binnen tijdens de acute infectie en is dan geassocieerd met afwijkende neurologische verschijnselen, neurocognitieve disfunctie en depressieve symptomen. Onmiddellijk starten van ART resulteert in herstel van deze afwijkingen en de ermee geassocieerde ontsteking van het CZS. Wat nog belangrijker is, is dat personen die ART starten tijdens AHI over het algemeen geen neuropsychiatrische complicaties vertonen zoals die vaak worden gezien bij andere hiv-positieve populaties. De goede neurologische resultaten suggereren dat mogelijke neurotoxiciteit van ART, indien überhaupt aanwezig, waarschijnlijk een beperktere rol speelt dan hiv-gerelateerde factoren bij de ontwikkeling van HAND. Toekomstige studies zouden zich moeten concentreren op de nadelige neurologische effecten die worden veroorzaakt door veel voorkomende co-infecties en middelengebruik, de frequentie waarvan wereldwijd een toename laat zien bij mensen met hiv.

List of Abbreviations and Acronyms

AAN	American Academy of Neurology
ADC	AIDS dementia complex
AHI	Acute HIV Infection
AIDS	Acquired immunodeficiency syndrome
ANI	Asymptomatic neurocognitive impairment
ART	Antiretroviral Therapy
CNS	Central nervous system
CSF	Cerebrospinal fluid
DTG	Dolutegravir
EFV	Efavirenz
GBTA	Group-based trajectory analysis
HAD	HIV-associated dementia
HAND	HIV-associated neurocognitive disorder
HCV	Viral hepatitis C
HIV	Human Immunodeficiency Virus type 1
HIVE	HIV-1 encephalitis
IHDS	International HIV Dementia Scale
LP	Lumbar puncture
MoCA	Montreal Cognitive Assessment
MND	Mild neurocognitive disorder
MSM	Men who have sex with men
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NP	Neuropsychological
NP-AEs	Neuropsychiatric adverse events
OI	Opportunistic infection
PET	Positron emission tomography

PHQ-9	Patient Health Questionnaire-9
PLPH	Post lumbar puncture headache
PLWH	People living with HIV
PreP	Pre-exposure prophylaxis
rsFC	Resting-state functional connectivity
SD	Standard deviation
SEARCH	South East Asia Research Collaboration on HIV
T/F	Transmitted/founder
TPHA	Treponema pallidum hemagglutination
TRCARC	Thai Red Cross AIDS Research Centre
UNAIDS	The Joint United Nations Programme on HIV/AIDS
VDRL	Venereal disease research laboratory

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1/2019	Cerebrospinal fluid compartmentalization of HIV-1 and correlation with plasma viral load and blood–brain barrier damage. Infection 2019
	Global and regional brain hypometabolism on FDG-PET in treated HIV-infected individuals. Neurology 2018
8/2019	HIV vasculopathy versus VZV vasculitis in an HIV patient with multiple brain ischaemic infarcts. BMJ case report 2019
	Targeting the HIV-infected brain to improve ischemic stroke outcome. Nature communication 2019
4/2020	Markers of CNS Injury in Adults Living With HIV With CSF HIV Not Detected vs Detected <20 Copies/mL. Open Forum Infectious Diseases 2019
8/2020	Cerebral Microstructural Changes in COVID-19 Patients: An MRI-based 3-month Follow-up Study. EClinicalMedicine 2020
12/2020	Higher levels of plasma inflammation biomarkers are associated with depressed mood and quality of life in aging, virally suppressed men, but not women, with HIV. Brain, Behaviour, & Immunity 2020

International Conference Attendance

3/2019	The annual Conference on Retroviruses and Opportunistic Infections (CROI), Seattle, USA
6/2019	4th Asia Pacific AIDS & Co-infections Conference (APACC), Hong Kong,

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3/2020 The annual Conference on Retroviruses and Opportunistic Infections (CROI), Boston, USA (virtual)

10/2020 5th Asia Pacific AIDS & Co-infections Conference (APACC), Bangkok, Thailand (virtual)

Teaching / Conference Presentation

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3/2019 Abstract for Themed Discussion "Neuropsychiatric Outcomes Before and After Switching to Dolutegravir-based Therapy".

The annual Conference on Retroviruses and Opportunistic Infections (CROI) 2019, Seattle, USA

6/2019 Moderator of capacity-building workshop: Research statistics.
4th Asia Pacific AIDS & Co-infections Conference (APACC), Hong Kong, China

1/2020 Workshop Speaker, Neurosyphilis, Co-morbidities and Toxicities
22nd Bangkok International Symposium on HIV Medicine, Bangkok, Thailand

10/2020 Moderator of poster tour 2: Impact of COVID-19 Among People Living With HIV in Asia.

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Parameters of Esteem

Grants

2019-20 The Hong Kong Neurological Society Research Fund "Blood Transcriptomic Analysis of Parkinson's Disease in Han Chinese Population"

Awards and Prizes

2017 Young Investigator Scholarship, Conference on Retroviruses and Opportunistic Infections (CROI)

2019-20 Young Fellowship Program, Asia Pacific AIDS & Co-infections Conference (APACC)

PUBLICATIONS

Peer reviewed **Year**
Publications in Thesis

1. **Phillip Chan**, Stephen J Kerr, Eugène Kroon, Donn Colby, Carlo Sacdalan, Joanna Hellmuth, Peter Reiss, Sandhya Vasan, Jintanat Ananworanich, Victor Valcour, Serena Spudich, Robert Paul, RV254/SEARCH 010 Research Team. Cognitive trajectories after treatment in acute HIV infection. AIDS. 2021 Jan 29. 2021

2. **Phillip Chan**, Donn J. Colby, Eugène Kroon, Carlo Sacdalan, Suteeraporn Pinyakorn, Robert Paul, Merlin Robb, Victor Valcour, Jintanat Ananworanich, Christina Marra, Serena Spudich. Clinical and laboratory impact of concomitant syphilis during acute HIV. In press, HIV Medicine. 2021

3. Ryan Handoko, **Phillip Chan**, Linda Jagodzinski, Suteeraporn Pinyakorn, Sasiwimol Ubolyam, Nittaya Phanuphak, Carlo Sacdalan, Eugene Kroon, Netsiri Dumrongpisutikul, Robert Paul, Victor Valcour, Jintanat Ananworanich, Sandhya Vasan, Serena Spudich. Minimal detection of cerebrospinal fluid escape after initiation of antiretroviral therapy in acute HIV-1 infection. AIDS. 2020 Dec 9. 2020

4. Joanna Hellmuth, Camilla Muccini, Donn J Colby, Eugène Kroon, Mark de Souza, Trevor A Crowell, **Phillip Chan**, Carlo Sacdalan, Jintana Intasan, Khunthalee Benjapornpong, Somporn Tipsuk, Suwanna Puttamaswin, Nitiya Chomchey, Victor Valcour, Michal Sarnecki, Frank Tomaka, Shelly J Krebs, Bonnie M Slike, Linda L Jagodzinski, Netsiri Dumrongpisutikul, Napapon Sailasuta, Vishal Samboju, Nelson L Michael, Merlin L Robb, Sandhya Vasan, Jintanat Ananworanich, Praphan Phanuphak, Nittaya Phanuphak, Robert Paul, Serena Spudich. Central nervous system safety during brief analytic treatment interruption of antiretroviral therapy within four HIV remission trials: an observational study in acutely treated people living with HIV. CID 2020. 2020

5. **Phillip Chan**, Orlanda Goh, Eugène Kroon, Donn Colby, Carlo Sacdalan, Suteeraporn Pinyakorn, Peeriya Prueksakaew, Peter Reiss, Jintanat Ananworanich, Victor Valcour, Serena Spudich, Robert Paul. Neuropsychiatric outcomes before and after switching to dolutegravir-based therapy in an acute HIV cohort. AIDS Res Ther. 2020; 17: 1. 2020

6. **Chan P**, Dumrongpisutikul N, Subra C, Colby DJ, Kroon E, Fletcher J, Sacdalan C, Phanuphak N, Valcour V, Ananworanich J, Trautmann L, Spudich S. Neurosyphilis during Acute HIV Infection: A CNS Immunologic and Virologic Characterization. J Acquir Immune Defic Syndr. 2019 May 28. doi: 10.1097/QAI.0000000000002114. 2019

7. **Chan P**, Ananworanich J. Perspective on potential impact of HIV CNS latency on eradication. AIDS. 2019 May 13. doi:10.1097/QAD.0000000000002264. 2019

8. Chan FCC, **Chan P**, Chan I, Chan A, Tang THC, Lam W, Fong WC, Lee MP, Li P, Chan GHF. Cognitive screening in treatment-naïve HIV-infected individuals in Hong Kong - a single center study. BMC Infect Dis. 2019 Feb 13;19(1):156. doi: 10.1186/s12879-019-3784-y. 2019

9. **Chan P**, Patel P, Hellmuth J, Colby DJ, Kroon E, Sacdalan C, Pinyakorn S, Jagodzinski L, Krebs S, Ananworanich J, Valcour V, Spudich S; RV254/SEARCH 010 Study Team. Distribution of HIV RNA in CSF and Blood is linked to CD4/CD8 Ratio During Acute HIV. J Infect Dis. 2018 May 7. 2018

10. **Chan P**, Hellmuth J, Colby D, Kroon E, Sacdalan C, Fletcher J, Patel P, Pinyakorn S, Valcour V, Ananworanich J, Spudich S. Safety of lumbar puncture procedure in an international research setting during acute HIV infection. J Virus Eradication. 2018; 4:16–20. 2018

11. **Chan P**, Hellmuth J, Spudich S, Valcour V. Cognitive Impairment and Persistent CNS Injury in Treated HIV. Curr HIV/AIDS Rep. 2016 Aug;13(4):209-17. 2016

Other peer reviewed publications

1. Carissa L Philippi, Leah Reyna, Laura Nedderman, **Phillip Chan**, Vishal Samboju, Kevin Chang, Nittaya Phanuphak, Nisakorn Ratnaratorn, Joanna Hellmuth, Khunthalee Benjapornpong, Netsiri Dumrongpisutikul, Mantana Pothisri, Merlin L Robb, Jintanat Ananworanich, Serena Spudich, Victor Valcour, Robert Paul. Resting-state neural signatures of depressive symptoms in acute HIV. J Neurovirol. 2020 Jan 27. doi: 10.1007/s13365-020-00826-3. 2020

2. Kallianpur KJ, Jahanshad N, Sailasuta N, Benjapornpong K, **Chan P**, Pothisri M, Dumrongpisutikul N, Laws E, Ndhlovu LC, Clifford KM, Paul R, Jagodzinski L, Krebs S, Ananworanich J, Spudich S, Valcour V; SEARCH010/RV254 Study Group. Regional Brain Volumetric Changes Despite Two Years of Treatment Initiated During Acute HIV Infection. AIDS. 2019 Nov 12. doi: 10.1097/QAD.0000000000002436. 2019

3. Tovanabutra S, Sirijatuphat R, Pham PT, Bonar L, Harbolick EA, Bose M, Song H, Chang D, Oropeza C, O'Sullivan AM, Balinang J, Kroon E, Colby DJ, Sacdalan C, Hellmuth J, **Chan P**, Prueksakaew P, Pinyakorn S, Jagodzinski LL, Sutthichom D, Pattamaswin S, de Souza M, Gramzinski RA, Kim JH, Michael NL, Robb ML, Phanuphak N, Ananworanich J, Valcour V, Kijak GH, Sanders-Buell E, Spudich S; MHRP Viral Sequencing Core; RV254/SEARCH 010 Study Team. Deep Sequencing Reveals Central Nervous System Compartmentalization in Multiple Transmitted/Founder Virus Acute HIV-1 Infection. Cells. 2019 Aug 15; 8(8). pii: E902. 2019

4. Goh OQ, Colby DJ, Pinyakorn S, Sacdalan C, Kroon E, **Chan P**, Chomchey N, Kanaprach R, Prueksakaew P, Suttichom D, Trichavaroj R, Spudich S, Robb ML, Phanuphak P, Phanuphak N, Ananworanich J; RV254/SEARCH 010 Study Group. Switch to dolutegravir is well tolerated in Thais with HIV infection. J Int AIDS Soc. 2019 Jul;22(7):e25324. doi: 10.1002/jia2.25324. 2019

5. Crowell TA, Colby DJ, Pinyakorn S, Sacdalan C, Pagliuzza A, Intasan J, Benjapornpong K, Tangnaree K, Chomchey N, Kroon E, de Souza MS, Tovanabutra S, Rolland M, Eller MA, Paquin-Proulx D, Bolton DL, Tokarev A, Thomas R, Takata H, Trautmann L, Krebs SJ, Modjarrad K, McDermott AB, Bailer RT, Doria-Rose N, Patel B, Gorelick RJ, Fullmer BA, Schuetz A, Grandin PV, O'Connell RJ, Ledgerwood JE, Graham BS, Tressler R, Mascola JR, Chomont N, Michael NL, Robb ML, Phanuphak N, Ananworanich J; **RV397 Study Group**. Safety and efficacy of VRC01 broadly neutralising antibodies in adults with acutely treated HIV (RV397): a phase 2, randomised, double-blind, placebo-controlled trial. Lancet HIV. 2019 Apr 15. pii: S2352-3018(19)30053-0. 2019

6. Samboju V, Philippi CL, **Chan P**, Cobigo Y, Fletcher JLK, Robb M, Hellmuth J, Benjapornpong K, Dumrongpisutikul N, Pothisri M, Paul R, Ananworanich J, Spudich S, Valcour V; SEARCH 010/RV254; RV304 protocol teams. Structural and functional brain imaging in acute HIV. Neuroimage Clin. 2018 Jul 27;20:327-335. 2018

7. D'Antoni ML, Byron MM, **Chan P**, Sailasuta N, Sacdalan C, Sithinamsuwan P, Tipsuk S, Pinyakorn S, Kroon E, Slike BM, Krebs SJ, Khadka VS, Chalermchai T, Kallianpur KJ, Robb M, Spudich S, Valcour V, Ananworanich J, Ndhlovu LC; RV254/SEARCH010, SEARCH011, and RV304/SEARCH013 Study Groups. Normalization of Soluble CD163 after Institution of Antiretroviral Therapy During Acute HIV Infection Tracks with Fewer Neurological Abnormalities. J Infect Dis. 2018 Jun 2. 2018

8. Sacdalan C, Crowell T, Colby D, Kroon E, **Chan P**, Pinyakorn S, Chomchey N, Prueksakaew P, Puttamaswin S, Chintanaphol M, Cheng T, Phanuphak N, Ananworanich J. Brief Report: Safety of Frequent Blood Sampling in Research Participants in an Acute HIV Infection Cohort in Thailand. J Acquir Immune Defic Syndr. 2017 Sep 1;76(1):98-101. 2017

9. Erdem H, Inan A, Guven E, Hargreaves S, Larsen L, Shehata G, Pernicova E, Khan E, Bastakova L, Namani S, Harxhi A, Roganovic T, Lakatos B, Uysal S, Sipahi OR, Crisan A, Miftode E, Stebel R, Jegorovic B, Fehér Z, Jekkel C, Pandak N, Moravveji A, Yilmaz H, Khalifa A, Musabak U, Yilmaz S, Jouhar A, Oztoprak N, Argemi X, Baldeyrou M, Bellaud G, Moroti RV, Hasbun R, Salazar L, Tekin R, Canestri A, Čalkić L, Praticò L, Yilmaz-Karadag F, Santos L, Pinto A, Kaptan F, Bossi P, Aron J, Duissenova 2017

A, Shopayeva G, Utaganov B, Grgic S, Ersoz G, Wu AKL, Lung KC, Bruzsa A, Radic LB, Kahraman H, Momen-Heravi M, Kulzhanova S, Rigo F, Konkayeva M, Smagulova Z, Tang T, **Chan P**, Ahmetagic S, Porobic-Jahic H, Moradi F, Kaya S, Cag Y, Bohr A, Artuk C, Celik I, Amsilli M, Gul HC, Cascio A, Lanzafame M, Nassar M. The burden and epidemiology of community-acquired central nervous system infections: a multinational study. Eur J Clin Microbiol Infect Dis. 2017 Apr 10.

10. Fong WC, Ismail M, Lo JW, Li JT, Wong AH, Ng YW, **Chan PY**, Chan AL, Chan GH, Fong KW, Cheung NY, Wong GC, Ho HF, Chan ST, Kwok VW, Yuen BM, Chan JH, Li PC. Telephone and Teleradiology-Guided Thrombolysis Can Achieve Similar Outcome as Thrombolysis by Neurologist On-site. J Stroke Cerebrovasc Dis. 2015 Jun;24(6):1223-8. 2015

11. Cysique LA, Hey-Cunningham WJ, Dermody N, **Chan P**, Brew BJ, Koelsch KK. Peripheral blood mononuclear cells HIV DNA levels impact intermittently on neurocognition. PLoS One. 2015 Apr 8; 10(4):e0120488. 2015

12. **Chan P**, Brew BJ. HIV associated neurocognitive disorders in the modern antiviral treatment era: prevalence, characteristics, biomarkers, and effects of treatment. Curr HIV/AIDS Rep. 2014;11.3:317-24. 2014

13. Brew BJ, **Chan P**. Update on HIV dementia and HIV-associated neurocognitive disorders. Curr Neurol Neurosci Rep. 2014;14.8:468. 2014

14. Chan CH, Leung AK, Cheung YF, **Chan PY**, Yeung KW, Lai KY. A rare neurological complication due to lithium poisoning. Hong Kong Med J. 2012;18:343-5. 2012

15. YF Cheung, KW Tang, **Phillip YC Chan**, YW Wong, FC Cheung, John HM Chan, Samuel CL Leung, Patrick CK Li. Spontaneous intracranial hemorrhage: uncommon but important. Hong Kong Med J. 2010;16:71-2 2010

Other
Book Chapters (Co-authorship)

1. Brew, Bruce J; Wright, Edwina J; **Chan, Phillip**; Post, Jeffrey J. Approach to neurological symptoms. HIV management in Australia: Supporting the HIV, Viral Hepatitis and Sexual Health Workforce. Australasian Society for HIV Medicine, 2016. 2016

2. Wright, Edwina J; Cherry, Catherine; **Chan, Phillip**; Brew, Bruce J. Neurological disorders in HIV infection. HIV management in Australia: Supporting the HIV, Viral Hepatitis and Sexual Health Workforce. Australasian Society for HIV Medicine, 2016. 2016

ACKNOWLEDGEMENT

I am indebted to the support and guidance of my mentors, Prof Jintanat Ananworanich, Prof Serena Spudich, Prof Peter Reiss and Prof Victor Valcour, to refine the quality of this thesis. First, the research works in the thesis are primarily based on the RV254 cohort study in Bangkok established by Prof Ananworanich and Prof Valcour. It provides a comprehensive and systematic platform not only for immunology study but also neurology research. Second, I am also thankful to the mentors, Prof Valcour and Prof Spudich, for their contributions to widen my horizons about neuro-infection and cognitive health. By working with Prof Reiss, his expertise in the field of infectious disease has benefited me enormously. Over the years, they all have been providing me with invaluable advice and support to pave my way as clinical researcher and neurologist.

There are innumerable people at SEARCH whose assistance is influential to my study. I hereby would like to send my heartfelt thanks to Dr Eugene Kroon, who is always eager to share his experience in running clinical research effectively. What is more, Dr Donn Colby and Dr Carlo Sacdalan have played essential and indispensable roles in fine-tuning the analyses in the manuscripts. As for data collection, Ms Nitiya Chomchey and Ms Jintana Intasan, the gatekeepers of RV254 study, help me get access to operational information with ease, and Mr Ratchapong Kanaprach, our data manager, is devoted to the building up of all the dataset in the listed studies. Last, my gratitude is extended to all RV254 participants who spent their precious time to take assessments and optional procedures, but also to research nurses who help consolidate mutual trust between participants and the research team. Finally yet importantly, the collaborative work at SEARCH is run smoothly under the leadership of Prof Praphan Phanuphak and Dr Nittaya Phanuphak.

The publications in the thesis fundamentally hinge on the teamwork amongst collaborators of different academic disciplines. In particular, Prof Robert Paul provided unlimited support devotedly in the manuscripts related to neuropsychological testing. Dr Stephen Kerr also created the wonderful trajectory model for the RV254 longitudinal cognitive performance analysis. At the meantime, Ms Suteera Pinyakorn reviewed all my statistical works in the manuscripts in due course.

Last but not least, I would like to thank my wife, my daughter and friends, for their tremendous support in my pursuit of academic work.

Curriculum Vitae

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WORKING EXPERIENCE

07/2005 - 06/2008	Basic Physician Training, Kowloon Central Cluster, Hospital Authority, Hong Kong
07/2008 - 12/2011	High Physician Training in Neurology, Queen Elizabeth Hospital, Hong Kong
01/2012 - 09/2015	Resident Specialist in Neurology, Queen Elizabeth Hospital, Hong Kong
10/2015 - present	Research Fellow, SEARCH, Thai Red Cross AIDS Research Centre, Thailand
11/2018 - present	Research Scientist, Hong Kong University of Science and Technology (HKUST)
03/2019 - present	Locum Specialist, Queen Elizabeth Hospital, HK

PARTICIPATION IN HIV RESEARCH, THAILAND (2015 -)

Co-Investigator/Site Neurologist of Clinical Trials:

NCT00796146	Establish and characterize an acute HIV infection Cohort in a high risk population
NCT00796263	Antiretroviral therapy for acute and chronic HIV infection
NCT01397669	Characteristics of immune cells in gut mucosa of HIV negative and HIV positive Thais
NCT02750059	Using Telmisartan with ART during acute HIV infection to reduce CNS reservoirs of HIV and lymph nodes fibrosis
NCT02614950	Viral suppression after analytic treatment interruption in Thai patients who initiated highly active antiretroviral therapy during acute HIV infection
NCT02919306	Safety and efficacy study of vaccine schedule with Ad26.Mos.HIV and MVA-Mosiac in HIV-infected adults
NCT02664415	Safety and therapeutic efficacy of the VRC01 antibody in patients who initiated antiretroviral therapy during early HIV infection
NCT02761200	Post analytic treatment interruption study
NCT02591420	Safety and Virologic Effect of a Human Monoclonal Antibody (VRC01) Administered Intravenously to Adults During Early Acute HIV Infection

PARTICIPATION IN NEURODEGENERATIVE DISEASE RESEARCH, HONG KONG (2018 -)

Co-Investigator/Project Neurologist:

HKUST Genetic screening of dementia in Chinese population

HKUST Blood transcriptomic analysis of Parkinson's disease in a Han Chinese population

PROFESSIONAL MEMBERSHIPS AND HONORS

2008-	Member, Hong Kong Neurological Society
2017 CROI	Young Investigator Scholarship, Conference on Retroviruses and Opportunistic Infections (CROI) 2017
2018 CROI	Oral Abstract "Longitudinal Cognitive Outcomes After Treatment in Acute HIV Infection"
2019 CROI	Abstract for Themed Discussion "Neuropsychiatric Outcomes Before and After Switching to Dolutegravir-based Therapy"
2019-2020	Young Fellowship Program, Asia Pacific AIDS & Co-infections Conference (APACC)
2019 & 2020	Abstract Reviewer, Asia Pacific AIDS & Co-infections Conference (APACC)
2019	The Hong Kong Neurological Society Research Fund 2019-20 "Blood Transcriptomic Analysis of Parkinson's Disease in Han Chinese Population"
Jan 2020	Workshop Speaker, Neurosyphilis, Co-morbidities and Toxicities, 22nd Bangkok International Symposium on HIV Medicine
2020	Abstract Reviewer, AIDS 2020 Conference.
Oct 2020	Workshop Moderator, Capacity-building session 4: Grant workshop 101, Asia Pacific AIDS & Co-infections Conference (APACC)

CLINICAL EXPERIENCE IN HONG KONG (2005-2015)

Neurology:	Stroke bed, 24-hours intravenous thrombolytic service of stroke, electro physiological investigation, neurology specialty clinics
Internal Medicine:	General internal medicine ward duties and clinics

OVERSEAS ATTACHMENT DURING SPECIALIST TRAINING

2014	Professor Bruce J Brew, Neurology Department, St. Vincent's Hospital, Sydney, Australia

EDUCATION AND QUALIFICATIONS

06/2004	MBChB (HK), Chinese University Hong Kong
06/2007	MRCP (UK)
12/2011	FHKCP (Neurology)
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ISBN:978-94-6421-470-3