Persistent central nervous system immune activation following more than 10 years of effective HIV antiretroviral treatment.

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ABSTRACT

OBJECTIVE:

Low-grade immune activation is common in people living with HIV (PLHIV), despite long-term viral suppression by antiretroviral therapy (ART). The clinical significance of this activation remains unclear. The aim of this study was to examine residual intrathecal immune activation in relation to signs of neuronal injury and neurocognitive impairment in PLHIV who had been virally suppressed on ART for more than 10 years.

DESIGN/METHODS:

Twenty neuroasymptomatic PLHIV on suppressive ART for a median of 13.2 years were retrospectively identified from the longitudinal prospective Gothenburg HIV cerebrospinal fluid (CSF) study. HIV-RNA, neopterin, and neurofilament light protein (NFL) levels were measured in paired plasma and CSF samples. Pretreatment samples were available for 14 patients. Cognitive function was assessed by CogState at follow-up.

RESULTS:

CSF neopterin decreased from a median (IQR) of 17.8 (10.6-29.7) to 6.1 (4.6-8.0) nmol/l during treatment (P < 0.001). In 11 out of 20 participants (55%), CSF neopterin levels were above the upper normal reference limit (5.8 nmol/l) at follow-up. Age-adjusted CSF NFL decreased to within-normal levels from a median of (IQR) 1179 (557-2707) to 415 (292-610) ng/l (P < 0.001). No significant correlations were found between CSF neopterin and CSF NFL or neurocognitive performance.

CONCLUSION:

Although CSF neopterin decreased significantly, more than 50% of the patients had CSF concentrations above the upper normal reference value despite more than 10 years of suppressive ART. We found no correlation between CSF neopterin, CSF NFL or neurocognitive performance at follow-up, indicating that low-grade immune activation during suppressive ART may be clinically benign.

Introduction

HIV-1 (hereafter referred to as 'HIV') infection is established in the central nervous system (CNS) shortly after disease transmission. In the CNS, the virus initiates a local immune response leading to intrathecal immune activation that may lead to neuronal damage if the infection is left untreated [1,2]. Although direct sampling of the CNS is usually not possible, the infectious process can be monitored using various cerebrospinal fluid (CSF) biomarkers [3,4].

In advanced HIV disease, many patients develop HIV- associated dementia (HAD), characterized by debilitating motor and/or cognitive impairments [5,6]. As the introduction of combined antiretroviral treatment (ART), the number of HAD cases has been markedly reduced. However, milder forms of HIV-associated neurocognitive disorders (HAND) remain prevalent despite long-term suppressive therapy [7–9]. It has not been established whether these milder forms of neurocognitive impairments reflect ongoing brain injury or residual damage caused by prior events.

Although substantially reduced by virally suppressive ART, residual CNS immune activation has been found to be present even in patients with durably suppressed CSF virus (HIV RNA below the detection limits of sensitive PCR assays) [10–13]. Whether the residual immune activation is a result of ongoing persistent viral replication within the CNS or is generated by other processes remains unclear [14–17]. Moreover, it is not known if low-grade CNS immune activation during long-term effective ART increases the risk of developing HAND or enhances its severity [10,18].

CSF neopterin is a marker of macrophage and microglial activation that decreases in response to ART [19]. However, CSF neopterin is often not normalized, despite what appears to be effective therapy. We have previously shown that a majority of patients have residual immune activation in the CNS even after more than 4 years of effective viral suppression with ART [10,13]. Further- more, by using nonlinear models, it has been estimated that only about 40% of neuroasymptomatic people living with HIV (PLHIV) on ART will achieve normal CSF neopterin levels [20]. However, the duration and stability of the residual CNS immune

activation during virally suppressive ART is not fully known. An additional marker of low-grade immune activation is intrathecal immunoglobulin production, that has also been shown to be increased in a substantial proportion of virally suppressed patients [10].

The light subunit of neurofilament (NFL), a major structural component of myelinated axons, is a well established indicator of axonal injury in a variety of neurodegenerative disorders [21]. CSF NFL has been shown to be a sensitive and useful marker for the study of CNS injury associated with HIV infection although its global use has been limited by assay availability and it is currently not implemented in any clinical guidelines for monitoring patients [1,22,23].

The aims of this study were to explore to what extent intrathecal immune activation persists after long-term durable viral suppression by ARTand to determine whether residual intrathecal immune activation is correlated to signs of axonal damage and neurocognitive performance.

Methods

Study design and participants

Since 1985, PLHIV monitored at the Sahlgrenska University Hospital, Gothenburg, Sweden, have been continuously included in a longitudinal study including serial sampling of CSF, plasma, and serum. Lumbar punctures are performed in a standardized manner at least annually, and more frequently on initiation or cessation of ART. Asymptomatic as well as symptomatic patients are included, and as of December 2017, the cohort included 590 patients who have undergone 2201 lumbar punctures. Details of this cohort have been described previously [23]. Computerized neurocognitive perfor- mance testing by CogState has been included in the protocol since 2011. The study was approved by the institutional review board of the Sahlgrenska Academy (Regional Ethics Review Board in Gothenburg, O€ 588–01) and was performed in accordance with the Helsinki Declaration. All blood and CSF samples were obtained after written informed consent from patients under the institutional review board-approved protocol.

From this cohort, we retrospectively identified patients who had been continuously treated

with ART and had plasma HIV-1 RNA less than 50 copies/ml for more than 9.5 years. Transient increases in plasma viral load ('blips'), defined as a single plasma

HIV-1 RNA less than 250 copies/ml preceded and followed by a plasma HIV-1 RNA

less than 50 copies/ml, were allowed during the study period. However, ART treatment

interrup- tions, or plasma HIV-1 RNA values at least 250 copies/ ml, disqualified individuals from

inclusion. Patients with CNS disease, including cerebrovascular disease, CNS malignancies, or

CNS infections, were also excluded.

Cerebrospinal fluid and blood measurements Stored paired samples of cell-free plasma and CSF were

used in the current analysis. HIV-1 RNA levels in plasma and CSF were quantified using the

Roche Amplicor Monitor version 1.5, or the Roche Taqman assay version 1 or 2 (Hoffman La-Roche,

Basel, Switzerland). These produced PCR assays with a lower limit of quantification (LLQ) of 50, 40, and 20 copies/ml for Roche Amplicor Monitor version 1.5, Roche Taqman assay

version 1, and Roche Taqman assay version 2, respectively. Blood CD4þ T-cell count and CSF

white blood cell count (WBC) measurements were performed in the local clinical

laboratory using routine methods.

Neopterin concentrations in plasma and CSF were measured using a commercially

available immunoassay (NEOPT-SCR.EIA 384 Det., Thermo Fisher Scientific

– BRAHMS GmbH, Henningsdorf, Germany) with an upper normal reference value of 8.8 nmol/l in plasma and 5.8 nmol/l in CSF [19].

Quantification of immunoglobulin G (IgG), and albumin in serum and CSF, were performed by

nephelometry (Behring Nephelometer Analyser, Behringwerke AG, Marburg, Germany). IgG index was used for determination of intrathecal IgG synthesis and defined as [CSF IgG (mg/l)/serum IgG

(g/l)]/[CSF albumin (mg/l)/serum albumin (g/l)]. The reference value for the IgG index was less

than 0.63 [24,25]. Albumin ratio was calculated as CSF albumin (mg/l)/plasma albumin

(g/l). Albumin ratios were age-adjusted to 50 years, the median age of patients after all

samples had been considered. Normal age-adjusted reference value was less than 10.4, based on

the antilog of the log scale mean 2 standard deviations (SD) in 273 healthy HIV-negative

controls.

CSF NFL concentrations were measured using the commercial NF-light ELISA kit (UmanDiagnostics AV, Umea˚, Sweden). As in the case of albumin ratios, all NFL values were age-adjusted to the study population median of 50 years. Normal reference value was less than 990 ng/ l, based on the antilog of the log scale mean 2SD in 359 healthy controls [26].

Neurocognitive testing

Neurocognitive function was measured using a comput- erized cognitive test battery (CogState, Melbourne, Australia) that has been validated for PLHIV [27–29] and has been previously described in detail [27,30]. We used four tests from the CogStateBrief Battery to assess five cognitive domains: detection (DET), measuring psychomotor function and attention; identification (ID), assessing speed of information processing and attention; one-card learning (OCL), a learning test; one back (OB), a test to assess working memory. The time estimated for completing the test was 20–30 min. Control data used for comparison in the current study was obtained from the CogState normative data set that includes 13 290 age- group stratified normative data for adults 18–89 years old. No adjustment was made regarding education years, gender and sex. CogState results performed after 18 months, along with follow-up laboratory testing, were included in our analysis. Functional status was assessed in each patient by three self-reported standardized questions adapted from the EACS guidelines [31]: Do you

experience frequent memory loss (e.g. do you forget the occurrence of special events, appointments etc.)? Do you feel that you are slower when reasoning, planning activities, or solving problems? Do you have difficulties paying attention (e.g. to a conversation, a book, or a movie)? Additionally, all patients were asked if any knowledgeable others had noticed cognitive decline.

Statistical methods

Descriptive statistics were performed using SPSS (IBM SPSS version 22 software, Armonk,

New York, USA). Continuous variables were log10 transformed wherever appropriate for the tests used. Paired pretreatment and on-treatment values were compared by paired samples t-test, with $P < 0.05$ considered as statistically significant. Correlations were explored using Pearson correlation.

Predictors of CSF neopterin and CSF NFL levels were analysed by multiple regression analysis. CogState test results were age-adjusted and analysed according to the guidelines provided by the manufacturer. The results of each test were standardized to a z-score, with 0 representing no deviation from the age-stratified controls. For each patient, a composite z-score was calculated as the mean of four z-scores.

Results

Study population

Twenty patients (80% men) fulfilled the inclusion criteria and were included in the analysis. Pretreatment samples were available for 14 of the 20. Median [interquartile range (IQR)] age at follow-up was 52 (50–59) years, and median (IQR) interval with plasma HIV-1 RNA less than 50 copies/ml was 149 (127–171) months. Samples included in the analysis were collected between 1996 and 2015. Treatment history and laboratory results on plasma covering the entire treatment period were available for all patients. Characteristics of the study patients are summarized in Table 1. Three and five of the patients were also included in previous reports by Eden et al. [10] and Abdulle et al. [13], respectively.

Cerebrospinal fluid and blood measurements Treatment effects on HIV RNA in plasma and CSF, and CD4þ
T-cell count is shown in Fig. 1. Figure 2 shows the treatment effect on CSF neopterin, CSF NFL, IgG index, and albumin ratio.

Median (IQR) CD4þ T-cell count after more than 10 years of treatment was 580 (393–735)

cells/ml compared with 145 (40–264) cells/ml before ART initiation. Mean number of blips in plasma was 0.9 (range 0–4) corresponding to 0.07 (range 0–0.27) blips per treatment year. The median blip amplitude was 104 (range 50–245) copies/ml. Levels of HIV RNA before treatment were median (IQR) 3.76 (3.16–4.11) log10 copies/ml in CSF

Neopterin levels decreased significantly in both plasma and CSF (P < 0.001). The median (IQR) level of neopterin in CSF after more than 10 years of treatment (n 20) was 6.1 nmol/l (4.6–8.0), compared with

17.8 nmol/l (10.6–29.7) at baseline. After more than 10 years of treatment, 11 of 20 (55%) of the patients had CSF neopterin concentrations above the upper normal reference value (5.8 nmol/l). In comparison, above normal-CSF neopterin concentrations were found in all (14/14) of the patients where pretreatment samples were available. In plasma, the number of patients with neopterin concentrations above the upper normal reference value (8.8 nmol/l) was seven of 20 (35%) during therapy, and 13 of 14 (93%) pretreatment.

No significant correlations were found between CSF neopterin and CSF NFL, plasma or CSF viral load, CD4þ nadir, CSF WBC or albumin ratio either during suppressive ART or before treatment initiation. Addi- tionally, no correlation was found between on-treatment CSF neopterin and neurocognitive performance (Cog- State; r ¼ -0.19, P ¼ 0.51).

IgG index decreased from median (IQR) 0.68 (0.48– 0.95) to 0.57 (0.54–0.70) during treatment (P 0.12). Seven of 20 (35%) patients had elevated index values (0.63) at follow-up, whereas seven out of 14 (50%) had elevated values at baseline. IgG index after more than 10 years of treatment was not significantly correlated to pretreatment or follow-up albumin ratios or CSF NFL.

Additionally, we found no significant correlations between IgG index after more than 10 years of treatment and pretreatment viral loads (plasma and CSF HIV RNA), age, CD4þ cell count nadir, or CogState result at follow-up.

Pretreatment CSF NFL concentrations were available in

12 patients. Age-adjusted CSF NFL concentrations decreased significantly during ART (P < 0.001) to within normal range in all patients. At baseline, six of 12 (50%) had elevated NFL concentrations. A decrease was even seen in patients with normal CSF NFL at baseline

(P ¼ 0.055).

Age-adjusted CSF NFL concentrations were strongly correlated to age-adjusted albumin ratios at follow-up (r 0.79, P < 0.001). No significant association was found between on-treatment CSF NFL and CogState result (r ¼ 0.32, P ¼ 0.26).

No significant change was seen in age-adjusted albumin ratio in response to ART (P 0.72, nbaseline 14, nfollow- up 20). None of the patients had above-normal values during ART, compared with one of 14 (7%) at baseline.

Neurocognitive testing

At follow-up, none of the patients reported a cognitive impairment affecting their everyday functioning. Four- teen of 20 (70%) patients underwent neurocognitive evaluation by CogState in conjunction with follow-up laboratory testing. Mean (95% confidence interval) composite z-score was 0.36 (0.77 to 0.01), or slightly below the age appropriate norms.

Fig. 1. HIV-1 RNA and CD4R T-cell count. HIV RNA and CD4þ T-cell count in 20 PLHIV after more than 10 years of suppressive antiretroviral treatment (ART). Pretreatment status shown for 14 patients. Dotted lines represent upper normal reference values. (a) Plasma viral load decreased from median 5.41 (4.73– 5.86) log copies/ml before ART to less than 1.7 log copies/ml in all patients at follow-up. (b) CSF viral load decreased from median 3.76 (3.16– 4.11) to less than 1.7 log copies/ml in all patients at follow-up. (c) Blood CD4þ T-cell count increased from median 145 (IQR

40–264) to median 580 (IQR 393–735) cells/ml at follow-up. PLHIV, people living with HIV.

Discussion

Although CSF neopterin concentrations substantially decreased during suppressive ART, we found that more than half of the included patients had signs of low-grade residual CNS immune activation even after more than 10 years of virally suppressive ART. Additionally, 35% of the studied patients had an elevated IgG index, reflecting ongoing humoral immune activation within the CNS. These findings are in agreement with previous studies with a shorter follow-up period [10,13,32].

The cause of residual intrathecal immune activation during ART is not fully understood. Despite effective therapy, HIV-1 RNA levels below the quantification limits of standard clinical assays can be detected in plasma as well as CSF in many patients if more sensitive assays are used [14,33]. Detectable low-level CSF HIV-1 RNA has been linked to higher CSF neopterin [14,34,35]. It has

also been suggested that, similar to other CNS infections, once an inflammatory response is established, it may lead to a self-sustaining state of cellular activation [36,37]. Persistent intrathecal immunoglobulin produc- tion may be the result of a nonspecific immunological reaction [38,39]. It is, however, unlikely that such residual immune activation would remain for more than

10 years without the continuous presence of HIV virus [11,14,18], this being the most plausible explana- tion for the persistent low-grade intrathecal immune activation.

The clinical consequences of this 'chronic' CNS inflammation is not clear. Further studies will be needed to establish whether a causal link exists between neuroinflammation, brain injury, and cognitive perfor- mance in treated HIV. There is a known correlation between CSF neopterin and CSF NFL in untreated HIV [1,40,41]. In treated HIV, an association between CSF

Fig. 2. Biomarkers of central nervous system immune activation, central nervous system inflammation, and brain injury. Biomarkers measured in 20 PLHIV after more than 10 years of suppressive antiretroviral treatment (ART). Pretreatment status shown for 14 patients. Dotted lines represent upper normal reference values. (a) CSF neopterin decreased from median (IQR) 17.8 (10.6–29.7) to 6.1 (4.6–8.0). (b) Age-adjusted CSF NFL decreased to normal levels in all patients from median (IQR) 1179 (557– 2707) to 415 (292–610) ng/l at follow-up. (c) IgG index decreased from median (IQR) 0.68 (0.48–0.95) to 0.57 (0.54–0.70) at follow-up. (d) Age-adjusted albumin ratio decreased from median (IQR) 5.79 (3.77–7.49) to 4.43 (3.66–5.66) at follow-up. Before treatment 1/14 (7%) had above-normal values compared to normal values at follow-up. CSF, cerebrospinal fluid; NFL, neurofilament light protein.

neopterin and CSF NFL was only found in neurocogni- tively impaired – but not in neuroasymptomatic – patients [18], and a large cross-sectional study found no correlation between CSF neopterin and CSF NFL in patients on suppressive ART [1].

In the present study, age-adjusted CSF NFL was normalized in all patients with elevated levels at baseline, as found previously [1,42]. It is not fully known whether this reflects that already affected neurons get better or if the HIV-driven neuronal injury is halted. Notably, age- adjusted CSF NFL has also decreased in the majority (5/

6) of patients with normal CSF NFL at baseline. This suggests that a low-grade axonal injury may also occur in many clinically cognitively asymptomatic patients with

untreated HIV, despite their having a CSF NFL within normal reference range.

Similar to a previous larger study [43], we found a clear correlation between age-adjusted CSF NFL and age- adjusted albumin-ratio. Although the albumin ratio did not change significantly after treatment initiation, all patients had albumin ratios within the normal range during ART, and only one patient had an abnormal pretreatment albumin

ratio, suggesting that only small changes in blood–brain barrier (BBB) integrity occurs during therapy. However, the association found between CSF NFL and albumin ratio suggests that even a small change in BBB integrity can facilitate neuronal injury. Moreover, the lack of association between albumin ratio

and CSF neopterin indicates that neuronal injury may not be exclusively linked to immune activation but may also be caused by other mechanisms such as influx of neurotoxic substances from outside the CNS [44].

A majority of the included patients had a low (median

145) pretreatment CD4þ T-cell count, reflecting advanced immunosuppression, which is known to increase the probability of having an established compartmentalized CNS [45]. Additionally, drug regi- mens used by patients have evolved over time, as newer drugs have become available. Although all included patients have changed ART combinations during the study period, no changes were made because of treatment failure. However, the changes in therapy made by patients prohibits any analysis of potential differences in CNS responses to individual drugs. Adverse events such as neurotoxicity have been demonstrated for several antiretrovirals, and the long- term effects of ART are still not fully known [46,47]. Differences have been shown in CNS penetration for individual drugs, but have not been clearly correlated to neurocognitive function [48].

It is possible that contemporary treatment strategies with earlier ART initiation, as well as the use of modern and possibly more potent drug regimens may have an impact on the frequency of persistent intrathecal immune activation in well treated patients. Although all patients included in the current analysis were durably virally suppressed with the ART regimens used,

the impact of newer treatment strategies and drugs will need to be investigated closely in future studies.

Twenty PLHIV evaluated with serial lumbar punctures performed after being closely monitored for a median of

13.2 years represents a unique cohort. Moreover, pretreatment CSF and plasma were available in 14 out of the 20 patients. By design, the study included only patients with a successful treatment history and excluded patients with confounding concomitant CNS conditions, less than perfect antiretroviral therapy and comorbidities potentially affecting either CSF biomarkers or neuro- cognitive performance. As a consequence, survivor bias and other factors needs to be considered when interpreting results in a clinical situation.

We found no association between neurocognitive performance and CSF neopterin or CSF NFL concen- trations during ART. Although our cohort performed slightly below the norm, the lack of baseline data prohibits any evaluation of treatment effects on neurocognitive performance. With an aging population of PLHIV, non- HIV-related dementia (such as Alzheimer's disease) and cerebrovascular disease will become increasingly preva- lent. Although it has been hypothesized that CNS inflammation seen in treated (as well as untreated) HIV- infection may increase the risk of non-HIV-associated

cognitive impairment, additional longitudinal studies are needed to examine this further.

In conclusion, intrathecal immune activation is signifi- cantly decreased by ART. However, despite effective treatment for more than 10 years, more than half of PLHIV continue to show signs of macrophage/microglia activation in the CNS. Additionally, we found no association between CSF neopterin and CSF NFL or neurocognitive performance, and all patients had CSF NFL concentrations within the normal range during therapy, indicating that ongoing low-grade immune activation is likely clinically benign in most cases.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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