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Switching from a regimen containing abacavir/lamivudine or emtricitabine/tenofovir disoproxil fumarate to emtricitabine/tenofovir alafenamide fumarate does not affect central nervous system HIV-1 infection

Aylin Yilmaz[a,b] (ID), Åsa Mellgren[c] (ID), Dietmar Fuchs[d] (ID), Staffan Nilsson[e] (ID), Kaj Blennow[f,g] (ID), Henrik Zetterberg[f,g,h,i] (ID) and Magnus Gisslén[a,b] (ID)

[a]Department of Infectious Diseases, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden; [b]Department of Infectious Diseases, Region Västra Götaland, Sahlgrenska University Hospital, Gothenburg, Sweden; [c]Clinic of Infectious Diseases, Södra Älvsborg Hospital, Borås, Sweden; [d]Division of Biological Chemistry, Biocenter, Innsbruck Medical University, Innsbruck, Austria; [e]Mathematical Sciences, Chalmers University of Technology, Gothenburg, Sweden; [f]Institute of Neuroscience and Physiology, Department of Psychiatry and Neurochemistry, University of Gothenburg, Gothenburg, Sweden; [g]Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Molndal, Sweden; [h]Department of Neurodegenerative Disease, UCL Institute of Neurology, Queen Square, London, UK; [i]UK Dementia Research Institute at UCL, London, UK

ABSTRACT
Background: Despite suppressive antiretroviral therapy (ART), many HIV-infected individuals have low-level persistent immune activation in the central nervous system (CNS). There have been concerns regarding the CNS efficacy of tenofovir alafenamide fumarate (TAF) because of its low cerebrospinal fluid (CSF) concentrations and because it is a substrate of the active efflux transporter P-glycoprotein. Our aim was to investigate whether switching from emtricitabine (FTC)/tenofovir disoproxil fumarate (TDF) or abacavir (ABC)/lamivudine (3TC) to FTC/TAF would lead to changes in residual intrathecal immune activation, viral load, or neurocognitive function.

Methods: Twenty HIV-1-infected neuro-asymptomatic adults (11 on ABC/3TC and 9 on FTC/TDF) were included in this prospective study. At baseline, all participants changed their nucleoside analogues to FTC/TAF without any other changes in their ART regimen. We performed lumbar punctures, venipunctures, and neurocognitive testing at baseline and after three and 12 months.

Results: During follow-up, there were no significant changes in CSF or plasma HIV RNA, CSF neopterin, CSF β2-microglobulin, IgG index, albumin ratio, CSF NFL, or neurocognitive function in assessed by Cogstate in any of the groups.

Conclusion: This small pilot study indicates that switching to FTC/TAF from ABC/3TC or FTC/TDF has neither a positive, nor a negative effect on the HIV infection in the CNS.

CONTACT
Aylin Yilmaz
aylin.yilmaz@gu.se
Department of Infectious Diseases,
Sahlgrenska University Hospital, 416 50
Gothenburg, Sweden

Introduction

Shortly after transmission, HIV establishes a chronic infection in the central nervous system (CNS), mainly within macrophages and microglial cells, with accompanying immune activation [1–3]. Antiretroviral treatment (ART) effectively suppresses viral replication in blood as well as in cerebrospinal fluid (CSF) in most individuals [4]. The degree of cellular immune activation in the CNS can be determined by quantifying CSF biomarkers such as white blood cell count (WBC), neopterin (a marker of macrophage activation mainly), and β2-microglobulin (part of the major histocompatibility complex class I molecule). ART also reduces the immune activation and inflammatory response in CNS [5,6]. We have previously found an association between CNS inflammation and HIV-induced axonal injury as measured by CSF concentrations of the light subunit of neurofilament protein (NFL) [7]. CSF NFL concentrations are highest in individuals with HIV-associated dementia (HAD) where axonal damage and loss is prominent, but elevated levels can also be found in untreated individuals with low $CD4^+$ T-cell counts without neurocognitive symptoms [8–12].

Emtricitabine/tenofovir disoproxil fumarate (FTC/TDF) and abacavir/lamivudine (ABC/3TC) have been the most widely used nucleoside reverse transcriptase inhibitor (NRTI) combinations for several years [13]. In 2015, when this study was initiated, a new prodrug of tenofovir, tenofovir alafenamide fumarate (TAF), was introduced. The older prodrug TDF is converted to tenofovir in blood, taken up intracellularly, and then phosphorylated to its active form tenofovir diphosphate (DP). TAF, however, is largely delivered unchanged into lymphocytes and macrophages and then metabolised to tenofovir and phosphorylated to active tenofovir DP. These differences allow a lower dose of TAF (25 mg) than TDF (300 mg) and lead to much lower plasma concentrations of tenofovir with TAF than with TDF and to much higher tenofovir DP levels in lymphocytes and macrophages [14,15]. This could be of importance since macrophages and microglia are the main targets for HIV in the CNS. One potential concern regarding TAF and its effect in CNS is that TAF is a substrate for P-glycoprotein (P-gp), which could theoretically decrease its CNS exposure since substrates for P-gp are subject to active blood-brain barrier efflux. TDF is also a substrate for P-gp, but as mentioned, TDF is present in the systemic circulation as tenofovir, which is not a P-gp substrate [16].

Treatment with ABC has been associated with higher risk of cardiovascular disease in several studies [17–20]. The mechanism for this cardiotoxicity has not been fully identified, but it has been shown that ABC can affect inflammatory pathways [21], for example by increasing neutrophil adhesion of endothelial cells, by interfering with purine signalling pathways, by upregulation of pro-inflammatory cytokine messenger RNA transcription, and by increasing platelet activation [22]. In serum, high sensitive CRP and IL-6 are markers that in some studies have been increased in individuals on ABC [23]. If ABC has the ability to drive inflammation in the periphery, it could be hypothesised that ABC could be a driver of CNS immune activation as well. ABC penetrates CSF in adequate concentrations with an average CSF/plasma ratio of 36% [24]. In a study investigating neurotoxic effects *in vitro* of different antiretroviral drugs, ABC was among the ones causing highest degree of neurotoxicity, together with efavirenz, etravirine, nevirapine, and atazanavir [25].

The main aim of the study was to investigate whether switching to FTC/TAF from either FTC/TDF or ABC/3TC would result in changes in CNS HIV infection as determined by CSF HIV RNA levels, degree of cellular and humoral CSF inflammation, neuronal injury, and neurocognitive performance.

Methods

Participants

For this small pilot study, participants were prospectively included from the Department of Infectious Diseases, Sahlgrenska University Hospital between May and December 2016. To be eligible for the study, participants were required to have had plasma HIV RNA less than 50 copies/mL for at least 12 months and to have been on continuous and stable ART for at least 18 months with

FTC/TDF or ABC/3TC and a third agent (protease inhibitor, non-nucleoside reverse transcriptase inhibitor, or integrase inhibitor). In addition, patients were required to have undergone a lumbar puncture in the context of the longitudinal Gothenburg HIV CSF study cohort [26,27] at least three months prior to baseline in the study. This was to determine if there were any significant changes in the variables we were interested in during unchanged ART. We did not include a control group, but by including the pre-study lumbar puncture, participants were used as their own controls. Individuals with conditions that could have an influence on CNS inflammation, such as prior or ongoing neurological symptoms, severe neurocognitive impairment, and/or CNS opportunistic infections or malignancies were excluded.

Study procedures

All participants underwent a lumbar puncture, venipuncture, and testing of four neurocognitive domains with the computerized CogState test at study entry (baseline). They then switched their NRTI combination to FTC/TAF and were followed with lumbar punctures, venipunctures, and neurocognitive testing after three and 12 months. FTC/TAF was dosed in accordance with the Summary of Product Characteristics; 200 mg/10 mg once daily when administered together with a ritonavir- or cobicistat-boosted regimen and 200 mg/25 mg otherwise. Ten patients received 200 mg/10 mg (8 on a boosted protease inhibitor and two on elvitegravir) and ten received 200 mg/25 mg (three on dolutegravir, three on nevirapine, two on efavirenz, one on etravirine, and one on rilpivirine). The study protocol was approved by the Research Ethics Committee of the University of Gothenburg (Dnr: 790-15) and the Swedish Medical Products Agency. Participants were included after written informed consent. The study is registered at ClinicalTrials.gov, identifier number NCT02771054.

Methods

Blood $CD4^+$ T-cell count and CSF cell counts were performed in the local clinical laboratories using standard methods. Plasma and CSF HIV-1 RNA was quantified using RT-PCR (COBAS Taqman HIV-1 test, version 2, Roche Diagnostic Systems) with a lower limit of quantification of 20 copies/ml. Undetectable HIV RNA levels were set as 18 copies/mL and detectable levels but <20 copies/mL were set as 19 copies/mL. CSF neopterin was analysed with a commercially available immunoassay

(BRAHMS, Berlin, Germany). The upper normal reference value was 5.8 nmol/L [28]. CSF β2-microglobulin was measured by nephelometry (Dade-Behring Prospec). Reference values were age-dependent as follows: <1.2 mg/l for individuals ≤49 years old and <1.8 mg/L for individuals >49 years of age.

Quantification of immunoglobulin G (IgG) and albumin in CSF and serum was performed by nephelometry (Behring Nephelometer Analyser, Behringwerke AG, Marburg, Germany). IgG index was used for determination of intrathecal IgG synthesis, and was defined as (CSF IgG (mg/L)/serum IgG (g/L))/(CSF albumin (mg/L)/serum albumin (g/L)). Reference value for IgG index was <0.63. Albumin ratio was calculated as CSF albumin (mg/L)/plasma albumin (g/L). Reference values were <6.8 for individuals younger than 45 years and <10.2 for individuals >45 years of age.

CSF NFL was measured by a sandwich ELISA method with a lower limit of quantification of 50 ng/L (NF-light® ELISA kit, UmanDiagnostics AB, Umeå, Sweden) at the Clinical Neurochemistry Laboratory at the University of Gothenburg. CSF NFL concentrations have been shown to increase with normal ageing in healthy individuals [7,12]. NFL concentrations were age-adjusted to the median age of all participants of 54 years. Upper normal reference value was 1121 ng/L, based on the antilog of the log scale mean + 2SD in 359 healthy controls [12].

Neurocognitive testing was performed with a computerized cognitive test battery (CogState™, Melbourne, Australia) that has been validated for HIV-infected individuals and for screening of HIV associated neurocognitive disorder (HAND) [29–31]. Four different tests from the CogState Brief Battery were used to assess five cognitive domains: the detection test measured psychomotor function and attention, the identification test assesses speed of information processing and attention, the one-card learning test evaluated learning, and the one back card memory test assessed working memory. Estimated time for completing the test was 20–30 min. Scores were standardised to a z-score using a normative data set and then a combined cognition score was calculated in each individual by taking the average of the standardised scores across all tasks. Each participant served as their own control.

Statistical analysis

Continuous variables were log_{10} transformed wherever appropriate for the tests used. Parametric methods were used for statistical analysis. Differences between the two

groups (ABC/3TC vs FTC/TDF) at baseline were compared with unpaired t-tests. Longitudinal changes were analysed with paired t-tests, $p < .05$ was considered statistically significant. Correlations were determined with Pearson correlation.
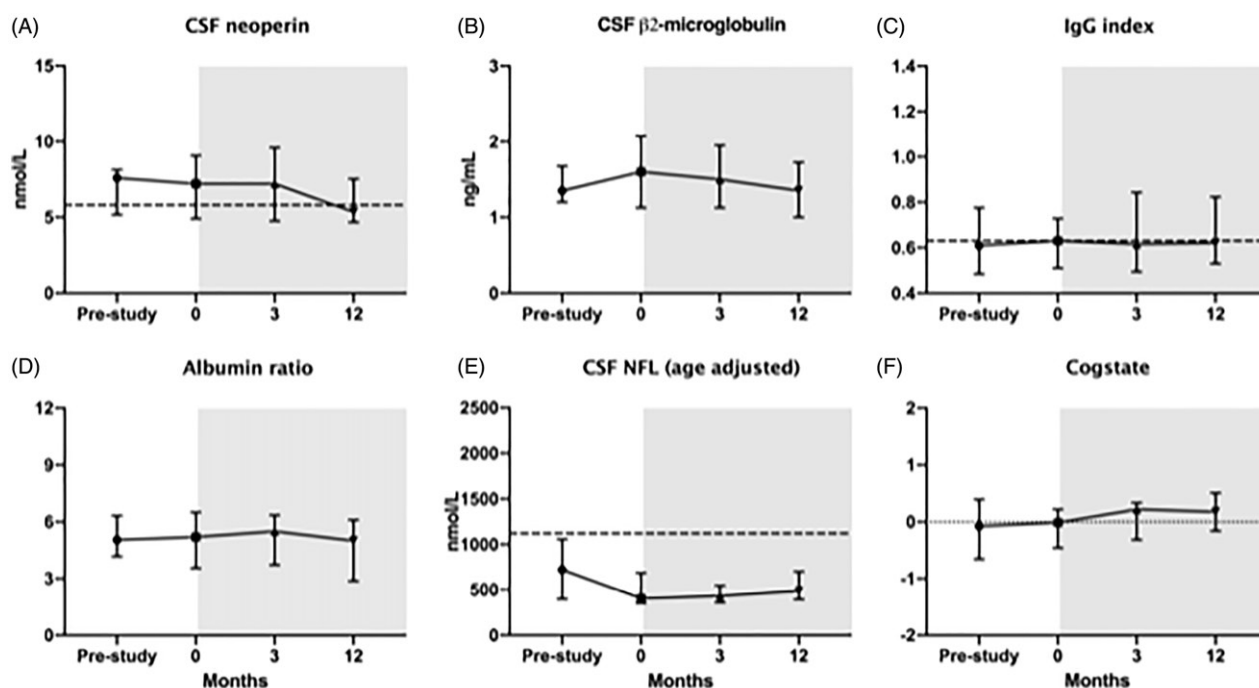
Results

A total of 20 participants (16 men and 4 women) were included, 11 on ABC/3TC and nine on FTC/TDF. One participant with a history of multidrug resistance was also taking darunavir/ritonavir, raltegravir, and etravirine. All other participants took only one additional antiretroviral

Table 1. Characteristics of participants.

	ABC/3TC	FTC/TDF	Total
Number	11	9	20
Sex (male:female)	8:3	8:1	16:4
Age in years	50 (30–69)	55 (27–67)	54 (27–69)
CD4 nadir (cells/mm^3)	190 (40–357)	80 (10–780)	170 (10–780)
CD4 baseline (cells/mm^3)	630 (170–1100)	610 (330–1000)	620 (170–1100)
Years since HIV-diagnosis	15 (3–21)	12 (5–27)	14 (3–27)
Treatment duration (years)	11 (3–20)	6 (1–20)	11 (1–20)
Additional antiretroviral drugs			
-Protease inhibitor (number)	5	3	8
-NNRTI (number)	4	4	8
-Integrase inhibitor (number)	2	4	6

ABC/3TC: abacavir/lamivudine; FTC/TDF: emtricitabine/tenofovir disoproxil fumarate; NNRTI: non-nucleoside reverse transcriptase inhibitor. Values presented as median (range).

drug besides the two NRTIs. Patient characteristics are presented in Table 1. Median (range) age for all participants was 54 (27–69) years. CD4$^+$ T-cell count nadir and at baseline were median (range) 170 (10–780) cells/mm^3 and 620 (170–1100) cells/mm^3, respectively. Participants had been diagnosed with HIV for a median of 13.9 (range, 3.4–26.6) years ago and had been on ART for a median of 10.4 (range, 2.3–19.9) years. There was a significant reduction of age-adjusted CSF NFL between the pre-study and baseline lumbar punctures, from median 721 (range 170–2135) ng/L to median 405 (range 246–2276) ng/L ($p = .012$, 95% confidence interval (CI) reduction 8–46%) (Figure 1). At baseline, median treatment duration in the FTC/TDF group was 6 (range 1–20) years and 11 (range 3–20) years in the ABC/3TC group (Table 1). At the time of the pre-study lumbar puncture, 2/9 in the FTC/TDF group had been on ART for less than two years and 4/9 for less than five years compared to 0/11 and 2/11 in the ABC/3TC group. Otherwise there were no significant changes in any of the other included CSF biomarkers between the pre-study lumbar puncture performed in median 12.6 (range 3.5–56.0) months before initiation of the study and the baseline lumbar puncture (Figure 1).


Figure 1. Longitudinal follow-up of cerebrospinal fluid (CSF) biomarkers. Results of all four lumbar punctures, from pre-study to 12 months of follow up, for participants switching from abacavir/lamivudine or emtricitabine/tenofovir disoproxil fumarate to emtricitabine/tenofovir alafenamide fumarate (FTC/TAF). Lumbar punctures performed after participants switched to FTC/TAF are shaded with a light-grey background. (A) CSF neoperin, (B) CSF ß2-microglobulin, (C) IgG index, (D) albumin ratio, (E) age adjusted CSF NFL, and (F) results from the neuropsychological testing with CogState. Dots depict median values and whiskers the interquartile ranges. Dashed lines indicate upper normal reference values and the dotted line for CogState results indicates zero standard deviations.
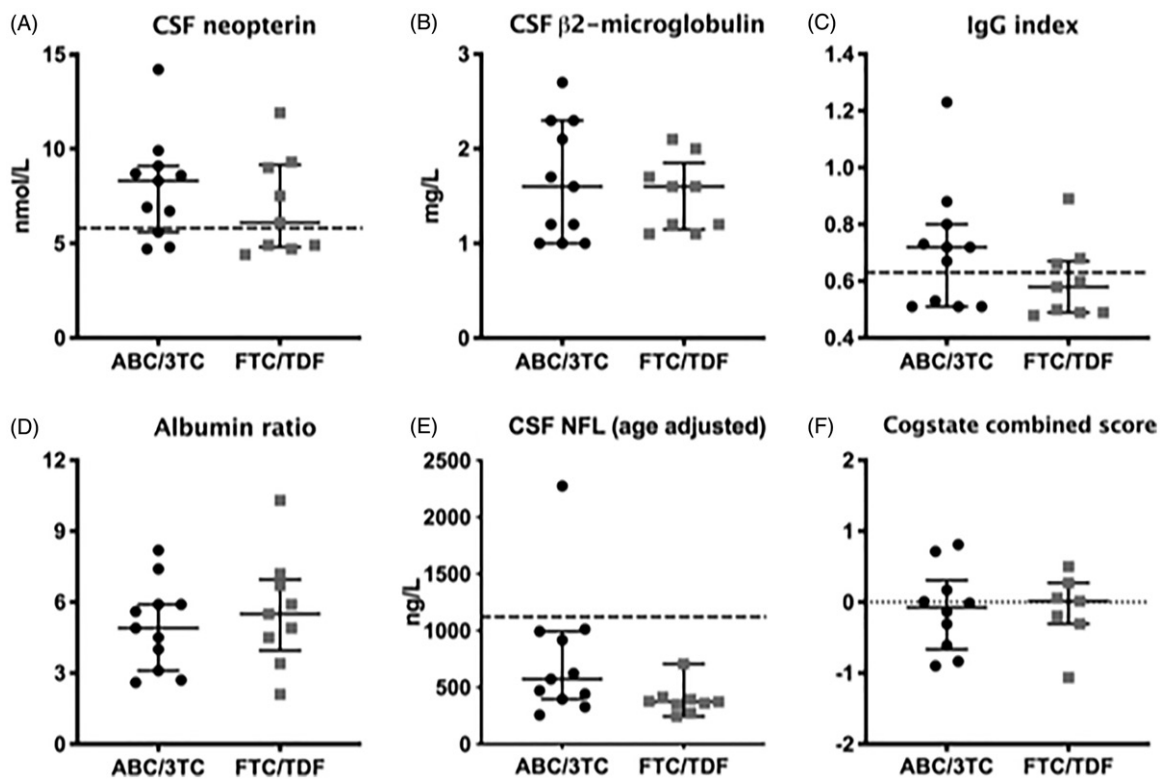
Figure 2. Baseline concentrations of (A) cerebrospinal fluid (CSF) neopterin, (B) CSF ß2-microglobulin, (C) IgG index, (D) albumin ratio, (E) CSF NFL (neurofilament light chain protein), and (E) results from the neuropsychological testing with CogState for the eleven participants on abacavir/lamivudine (ABC/3TC) (black dots) and the nine on emtricitabine/tenofovir disoproxil fumarate (FTC/TDF) (grey squares). There were no significant differences between the groups. Bars indicate median and interquartile range. The dashed lines indicate upper normal reference values for CSF neopterin, CSF ß2-microglobulin, IgG index, and CSF NFL, and the dotted line for CogState results indicates zero standard deviations.

At baseline, all but two participants (22 and 27 copies/mL) had plasma HIV RNA levels <20 copies/mL and all but one had CSF HIV RNA levels <20 copies/mL. The individual with elevated CSF viral load was on ABC/3TC and the viral load was 55 (1.74 $\log_{10}$) copies/mL. Five participants (25%) (four on ABC/3TC and one on FTC/TDF) had minor CSF pleocytosis in the range 4–5 cells/μL. CSF neopterin and CSF ß2-microglobulin were elevated in 13 (65%) (8 on ABC/3TC and 5 on FTC/TDF) and in seven (35%) (six on ABC/3TC and one on FTC/TDF), respectively. IgG index was above normal reference value in nine participants (45%) (six on ABC/3TC and three on FTC/TDF). Albumin ratio was elevated in one individual (5%) on TDF/FTC. Median baseline values are presented in Figure 2.

There were correlations between baseline CSF HIV RNA and CSF neopterin ($r = 0.593$, $p = .006$), CSF neopterin and ß2-microglobulin ($r = 0.770$, $p = .001$), and CSF ß2-microglobulin and CSF NFL ($r = 0.446$, $p = .05$). Regarding the correlation between CSF HIV RNA and CSF neopterin this is driven by one individual with very high CSF neopterin and CSF HIV RNA.

All participants came to the visits at baseline and at three months, but two participants did not complete the 12 month follow up visit; one had died from heart failure secondary to coronary artery disease and the second one was lost to follow-up (moved abroad). Irrespective of treatment group at baseline, no significant changes were found after switching to FTC/TAF in any of the analysed biomarkers: CSF and plasma HIV RNA levels, CSF cell count, CSF neopterin, CSF ß2-microglobulin, IgG index, albumin ratio, or age-adjusted CSF NFL (Figure 1). Individual changes from baseline to the 12 month follow up are presented in Figure 3. One participant in the FTC/TDF-group had a substantial increase in CSF NFL at the last follow-up, from 297 at the prior lumbar puncture to 6485 ng/L. The patient was asymptomatic throughout the study period and he has been adherent to his medication for many years. A new lumbar puncture performed outside the study protocol one year later showed that CSF NFL concentrations had returned to normal. No significant changes were found in neurocognitive performance between baseline and follow-up for any of the groups.

Discussion

In this small prospective study we found no significant changes in intrathecal immune activation, CSF HIV RNA levels, albumin ratios, degree of axonal injury, or
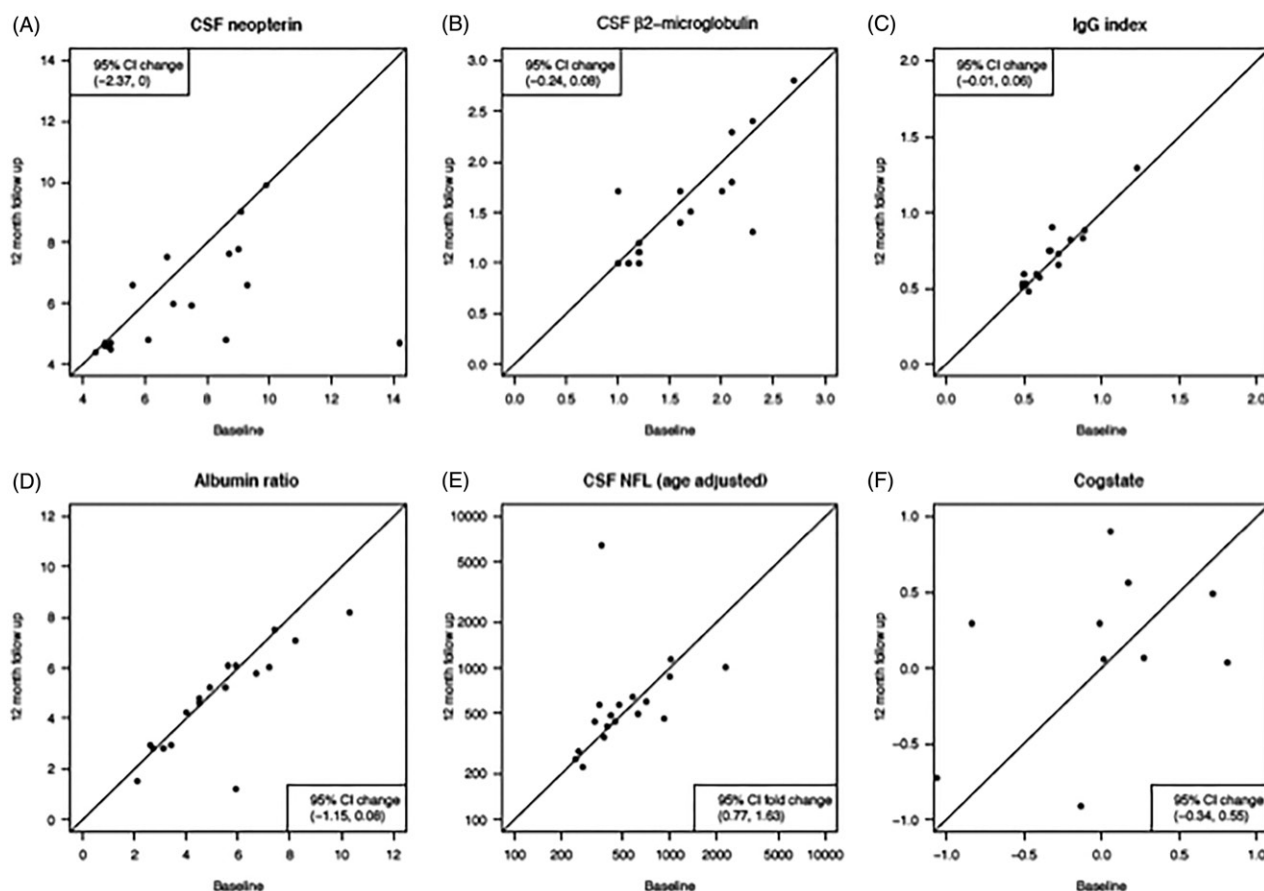
Figure 3. Individual changes with the 95% confidence interval (CI) of cerebrospinal fluid (CSF) biomarkers from baseline to the 12 month follow-up: (A) CSF neopterin, (B) CSF ß2-microglobulin, (C) IgG index, (D) albumin ratio, (E) CSF NFL (neurofilament light chain protein), and (E) results from the neuropsychological testing with CogState.

neurocognitive function within 12 months after switching from either FTC/TDF or ABC/3TC to FTC/TAF as part of a combination regimen. Theoretically, the effect of TAF in the CNS could have been poor based on the fact that CSF concentrations of tenofovir when administered as TDF are low in most, and undetectable in some individuals [32,33], and when administered as TAF, CSF (and plasma) tenofovir concentrations are even lower [34]. The lower tenofovir plasma concentrations and lower CSF concentrations are a consequence of TAF not being converted to tenofovir until after entry into the target cells. On the other hand, concentrations are much higher inside macrophages [14,15], where the drug is activated and exerts its effect. Another important explanation for low/undetectable CSF concentrations of tenofovir when given as TAF is that TAF is a substrate of P-gp and breast cancer resistance protein [16]. These efflux proteins are localized not only in the intestines but also in the blood-brain barrier, leading to decreased CSF exposure. Despite low CSF concentrations and active efflux out of the CNS, the results from our small study suggest that TAF, as part of a combination

regimen, is as efficacious as ABC/3TC and FTC/TDF in the CNS.

Another theoretical outcome could have been that participants on a regimen containing ABC/3TC would have an improvement of CSF inflammatory biomarkers, and perhaps also of CSF NFL and neurocognitive function, but no change in CSF HIV RNA levels after switching to FTC/TAF, whereas participants on FTC/TDF would not. This possibility is based on a number of studies demonstrating an association between ABC use and cardiovascular events. ABC has been associated with impaired endothelial function, increased levels of circulating endothelial cells, increased oxidative stress, augmented leukocyte–endothelial cell interactions, and adherence of platelets to endothelial cells or to leukocytes [35–38]. To our knowledge, there are no published data on ABC and inflammation in CSF/CNS. If ABC/3TC was more prone to cause CNS inflammation than FTC/TDF, perhaps we would have seen a significant difference for the biomarkers of immune activation at baseline between the groups.

The only significant change was a reduction of median age-adjusted CSF NFL in individuals on FTC/TDF

from the pre-study to the baseline lumbar puncture, from 721 ng/L to 374 ng/L ($p = .02$). One explanation for this could be that participants on FTC/TDF had been on their regimen for a shorter period of time compared with those on ABC/3TC. Two out of nine and 4/9 in the FTC/TDF group had been on ART shorter than two and five years respectively, and for participants in the ABC/3TC group the corresponding numbers were 0/11 and 2/11.

Despite the fact that most participants had been on suppressive ART for many years, many had abnormal CSF biomarkers of inflammation and neuronal injury. The frequency we found is in the same range as previous studies. CSF neopterin levels are elevated in virtually all untreated HIV-infected individuals, and even though they decrease markedly after initiation of ART, approximately half will have slightly elevated CSF neopterin even after several years [6,27]. The same pattern is seen for the humoral immune system, with persistent mildly increased IgG index [27]. CSF biomarkers of immune activation strongly correlate with the axonal injury marker CSF NFL; in this small study we also found a correlation between CSF β2-microglobulin and CSF NFL. Even if the majority of individuals on suppressive ART have normal CSF NFL levels, concentrations are still slightly higher than in HIV-negative controls [7], including lifestyle-matched HIV-negative controls [39]. In addition to the lumbar punctures and CSF biomarkers, participants also underwent neurocognitive testing. No significant changes were found in the test performances during the study.

Strengths of this study are the prospective design, with both short term and long term follow-up, and that participants served as their own controls by inclusion of a previous research lumbar puncture. One weakness is the relatively small number of participants. This was a pilot study with the aim of exploring whether we would find any changes after switching to TAF; however, it is unlikely that any differences of substantial clinical significance would have been found in a larger study. Another weakness is that we did not perform any ultrasensitive quantification of CSF residual viral load, using, for example, the single copy assay (SCA). Since CSF neopterin concentrations, however, did not change, it is unlikely that we would find changes in low-level HIV RNA after switching regimens. It has previously been shown that the presence of HIV RNA at very low levels (detected with the SCA) is associated with higher CSF neopterin levels [40] and that patients with CSF HIV RNA levels above 2 copies/mL had higher levels of CSF neopterin than those with undetectable CSF HIV RNA (assay with lower quantification limit of 2 HIV RNA copies/mL) [41].

In conclusion, we did not find any significant changes in CSF viral loads, CSF biomarkers of inflammation or axonal injury, or neurocognitive function when switching to FTC/TAF from ABC/3TC or FTC/TDF in this small pilot study. This is reassuring from a CNS perspective since FTC/TAF is recommended in most treatment guidelines.

Disclosure statement

Funding

ORCID

Aylin Yilmaz http://orcid.org/0000-0001-7612-5828
Åsa Mellgren http://orcid.org/0000-0002-1202-3504
Dietmar Fuchs http://orcid.org/0000-0003-1627-9563
Staffan Nilsson http://orcid.org/0000-0003-4748-0446
Kaj Blennow http://orcid.org/0000-0002-1890-4193
Henrik Zetterberg http://orcid.org/0000-0003-3930-4354
Magnus Gisslén http://orcid.org/0000-0002-2357-1020

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