AIDS, Publish Ahead of Print

DOI: 10.1097/QAD.0000000000004482

Comprehensive study of cerebrospinal fluid β_2-microglobulin, a marker of central nervous system immune activation in individuals with HIV

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Disclosures

This work was supported by the Swedish state, under an agreement between the Swedish government and the county councils (ALF agreements ALFGBG-965885, ALFGBG-1005848 and ALFGBG-71320), the Swedish Research Council (#2023-00356; #2022-01018 and #2019-02397), and the European Union's Horizon Europe research and innovation programme under grant agreement No 101053962. Dr Henrik Zetterberg is a Wallenberg Scholar and a Distinguished Professor at the Swedish Research Council.

Dr Birgitta Anesten reports no disclosures.

Dr Henrik Zetterberg has served at scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, Alzinova, ALZPath, Amylyx, Annexon, Apellis, Artery Therapeutics, AZTherapies, Cognito Therapeutics, CogRx, Denali, Eisai, LabCorp, Merry Life, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Alzecure, Biogen, Cellectricon, Fujirebio, Lilly, Novo Nordisk, and Roche, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work).

Dr Staffan Nilsson reports no disclosures.

Dr Dietmar Fuchs reports no disclosures.

Dr Magnus Gisslén has received research grants from Gilead Sciences and honoraria as speaker, DSMB committee member and/or scientific advisor from Amgen, AstraZeneca, Biogen, Bristol-Myers Squibb, Gilead Sciences, GlaxoSmithKline/ViiV, Janssen-Cilag, MSD, Novocure, Novo Nordic, Pfizer and Sanofi. All mentioned engagements have concluded and are not ongoing.

Dr Aylin Yilmaz has received honorarium as a lecturer from Gilead and GSK/ViiV.

Title: 120 characters. Running head: 39 characters. Abstract: 248 words. Paper: 3268 words. References: 40. Tables: 2. Figures: 3.

ABSTRACT

Objective: Cerebrospinal fluid (CSF) β_2-microglobulin (β_2M) has not been fully characterized in people with HIV (PWH) in different categories of disease.

Design: Retrospectively, we determined levels of β_2M, neopterin, HIV RNA, albumin ratio, IgG index, $CD4^+$ T cell count, neurofilament light chain protein (NfL), and leukocyte counts (WBC), in CSF and blood in PWH with and without antiretroviral treatment (ART). Persons without HIV served as controls.

Methods: Participants were grouped as: 1) neuroasymptomatic HIV (NA), 2) NA CSF escape, 3) symptomatic CSF escape, 4) secondary CSF escape, 5) HIV-associated dementia (HAD), 6) opportunistic infections in the central nervous system (CNS) (OI), and 7) elite controllers.

Results: We included 638 individuals: NA (N = 556); NA CSF escape (N = 33); symptomatic CSF escape (N = 4); secondary CSF escape (N = 5); HAD (N =16); OI (N = 18); elite controllers (N = 6) and 59 controls. Highest CSF β_2M levels were found in HAD, OI and symptomatic CSF escape. In 112 longitudinally followed NA participants, elevated CSF β_2M levels (89%) were found at baseline, and (96%) for CSF neopterin. After ART initiation CSF β_2M levels decreased by 10% per month and CSF neopterin by 17%. After three years 39% had CSF β_2M and 44% had CSF neopterin levels above the reference values, similar to the controls (39%).

Conclusion: CSF β_2M levels were elevated in the majority of individuals across HIV stages. Suppressive ART decreases β_2M toward control levels, although neuroinflammation persists, most likely driven by non-HIV factors.

Keywords: HIV, cerebrospinal fluid, central nervous system, AIDS dementia complex, β_2-microglobulin, neopterin.

INTRODUCTION

HIV-1 enters the central nervous system (CNS) soon after transmission,[1] establishing a chronic infection and inflammation. Without antiretroviral treatment (ART) this can harm the brain and lead to a substantial risk of developing HIV-associated dementia (HAD).[2]

ART has drastically decreased the incidence of HAD, but some studies report the presence of minor neurocognitive impairment.[3, 4] Current classification based solely on neuropsychological testing can inaccurately categorize healthy individuals with neurocognitive impairment, neglecting educational and socioeconomic diversity.[5-7] Despite suppressive ART with undetectable viral levels in blood and cerebrospinal fluid (CSF), immune activation often persists, systemically and within the CNS,[8, 9] and may be linked to neurocognitive impairment.[10-12]

Neopterin, a 253 Da metabolite released mainly from monocytes, macrophages, and microglia, is a well-studied CSF biomarker of immune activation. In contrast, CSF β_2-microglobulin (β_2M) is less explored in HIV infection.[13] It is a 11.8 kDa component of the histocompatibility complex (MHC) class 1 molecule[14] expressed on the surface of all nucleated cells, except neurons. Elevated β_2M reflects cellular immune response to inflammation or lymphoproliferative diseases, via induced MHC expression on activated cells.[15]

Previous CSF β_2M studies were small, focusing mainly on neuroasymptomatic (NA) individuals and HAD.[15-20] This study examines CSF β_2M in a large cohort across HIV stages and assesses its association with other biomarkers of inflammation and neuronal injury.

STUDY PARTICIPANTS AND METHODS

Participants

Since 1985, individuals with HIV in Gothenburg, Sweden, have been invited to a longitudinal study with repeated CSF and blood sampling.[21] This retrospective study included all individuals ≥ 18 years old with archived samples and healthy volunteers without drug abuse as HIV-negative controls with or without pre-exposure prophylaxis (PrEP) for HIV.[22] Neurocognitive testing was performed in some individuals, and all underwent clinical evaluation for neurological or neurocognitive symptoms at each visit. Those with varicella zoster virus (VZV) infection within two years prior to lumbar puncture (LP) were excluded from cross-sectional analyses, except in symptomatic and secondary escape.[23] Data were analyzed cross-sectionally and longitudinally.

For cross-sectional analyses, untreated participants were required to be treatment-naïve or off ART for ≥ six months. Participants on ART had to maintain plasma HIV RNA < 50 copies/mL for ≥ six months; blips (HIV RNA < 500 copies/mL) were accepted. Three participants with HAD had slightly higher levels (56–95 copies/mL). If multiple samples were available, the most recent was used. Exclusion criteria were primary HIV, CNS neoplasms, Guillan-Barré syndrome, acute cranial nerve paresis, psychosis, neurological disease, and acute or symptomatic syphilis.

For longitudinal analyses, neuroasymptomatic (NA) participants were included between 1 January 2000 and 27 May 2021. They had to be treatment-naïve, or off ART for ≥ six months before the first LP, with ART initiated within three months. Viral suppression (plasma HIV RNA < 50 copies /mL) had to be achieved and maintained ≥ six months. Participants with HAD or opportunistic infections (OI) were included if they showed sustained viral response, even with HIV RNA > 50 copies/mL. LPs were performed at three and 12 months after ART initiation, and annually thereafter. All LPs conducted during viral suppression on ART were included.

Participants were divided into the following subgroups:

1. NA – neuroasymptomatic, untreated.
2. NA on ART – neuroasymptomatic, on antiretroviral treatment.
3. NA escape – neuroasymptomatic on ART, with detectable CSF HIV RNA but not plasma, or higher CSF than plasma HIV RNA.[24, 25]
4. Symptomatic escape – on ART, elevated CSF HIV RNA but not in plasma, or two times higher CSF HIV RNA compared with plasma, and new or progressive CNS symptoms.[26]
5. Secondary escape – on ART, higher CSF than plasma HIV RNA with a non-HIV related infection.[26]
6. CNS OI – untreated with CNS opportunistic infection.
7. CNS OI on ART – on ART, with CNS opportunistic infection.
8. HAD – untreated with HIV-associated dementia. The diagnosis was based on Centers for Disease Control and Prevention and American Academy of Neurology Task Force criteria.[3, 27]
9. HAD on ART – on ART, with HIV-associated dementia.
10. Elite controller – no ART, plasma HIV RNA below detection for years.[28]

Participants were also grouped by ART regimen: two nucleoside reverse transcriptase inhibitors plus either a boosted protease inhibitor, a non-nucleoside reverse transcriptase inhibitor, or an integrase inhibitor. Other regimens, including two-drug combinations, were categorized together.

Ethical approval

CSF samples were gathered in compliance with the Helsinki Declaration. All participants gave their informed consent. Ethical approval was obtained from the Human Research Ethics Committee of the Medical Faculty, University of Gothenburg (Dnr: Ö588-01).

Methods

β_2M concentrations were determined by nephelometry using the N Latex β_2M kit on the Atellica NEPH 630 System (Siemens Healthcare GmbH. Erlangen, Germany); earlier used manufacturers were Dade Behring Prospec and Beckman Immage. CSF normal reference values were < 1.2 mg/L (18–50 years) and < 1.8 mg/L (> 50 years); serum < 1.8 mg/L (< 50 years) and < 2.1 mg/L (≥ 50 years).

Neopterin was quantified with a commercial immunoassay (BRAHMS, Berlin, Germany) according to the manufacturer's instructions. Normal reference values < 8.8 nmol/L (serum) and < 5.8 nmol/L (CSF).[13]

CSF neurofilament light chain (NfL) was analyzed by sandwich ELISA (NF-light kit, UmanDiagnostics, Umeå, Sweden), with age-adjusted values; mean adjusted age was 44 years, upper normal reference 823 ng/L.[29]

Albumin ratio was calculated as CSF albumin (mg/L)/plasma albumin (g/L) measured by nephelometry (Behring Nephelometer, Marburg, Germany). Reference values < 6.8 (< 45 years) and < 10.2 (≥ 45 years).[30]

HIV RNA was determined by using Roche Amplicor Monitor version 1.5, Abbott RealTime HIV-1 assay, or Roche Taqman assays, version 1 or 2. Limits of quantification ranged 20 − 50 copies/mL, depending on assay. Viral suppression was defined as plasma HIV RNA below the detection limit of the assay used.

$CD4^+$ T-cell counts and CSF leukocytes (WBC) were measured in local clinical laboratories.

IgG index was calculated as [CSF IgG (mg/L) / serum IgG (g/L)] ÷ [CSF albumin (mg/L) / serum albumin (g/L)],[31] and quantified with nephelometry (Behring Nephelometer Analyser, Marburg, Germany). Reference value < 0.63.[30]

Assays were standardized across time and batches, to ensure reproducibility.

Statistical analyses

Descriptive statistics were performed in Prism version 9.0 (GraphPad, La Jolla, CA) and SPSS version 28 (IBM, Armonk, NY). Biomarkers were $\log_{10}$-transformed to reduce skewness. Cross-sectional differences between $CD4^+$ T-cell stratified groups were tested with Analysis of Variance (ANOVA) and Fisher´s least significant (LSD); Student t test was used for two-group comparisons. Associations were assessed with Pearson correlation, and predictors of CSF β_2M were evaluated by multiple linear regression. Decline rate was defined by the slope on a log-scale at three months (early decline, per month), and at one, three, and five years (late decline, per year) with the closest surrounding measurements. Early decline was calculated only for individuals with a follow-up LP within six months of ART initiation, to avoid bias from long intervals. Longitudinal analyses were not adjusted for treatment changes, but confounders such as morbidity burden were addressed through inclusion

criteria. All presented p-values are nominal and only $p < 0.001$ can be considered significant in a multiple inference framework.

RESULTS

A total of 638 participants were included and categorized as follows (Table 1): untreated NA (N = 342), NA on ART (N = 214), NA CSF escape (N = 33), symptomatic escape (N = 4), secondary escape (N = 5), untreated HAD (N = 11), HAD on ART (N = 5), untreated with CNS OI (N =12), CNS OI on ART (N = 6), and elite controllers (N = 6).

In the symptomatic escape group, one presented with confusion and hydrocephalus, one with memory deficits, one with severe concentration deficits, and one with epileptic seizures. Four of five individuals with secondary escape had an ongoing or recent peripheral VZV infection, and one had herpes simplex-2 (HSV-2) meningoencephalitis.[23] Controls comprised 59 individuals without HIV, 33 on PrEP (32 men who have sex with men (MSM)) and 26 without PrEP (5 MSM).

Cross-sectional analyses of CSF and serum β_2M and neopterin

β_2M is the main focus of this study, as it parallels CSF neopterin in reflecting CNS immune activation. In CSF, individuals with symptomatic escape, CNS OI, and HAD had the highest β_2M levels (Figure 1). Untreated NA had significantly higher CSF β_2M levels than NA on ART ($p < 0.0001$), and levels were significantly increased with lower $CD4^+$ T-cell count ($p < 0.01$), except in the two groups with the lowest $CD4^+$ T-cell counts, where β_2M levels were similar. Untreated NA had significantly higher CSF β_2M levels than NA escape ($p < 0.0001$) but not compared with secondary escape. Those with symptomatic escape had significantly higher CSF β_2M ($p < 0.001$) than untreated NA. Compared with NA on ART, CSF β_2M was significantly elevated in all escape groups: symptomatic ($p < 0.0001$), secondary ($p < 0.0001$), and NA escape ($p < 0.01$). CSF β_2M levels in NA escape ($p < 0.0001$) and NA on ART ($p < 0.01$) were significantly higher compared with controls without HIV but did not differ from elite controllers.

Serum β_2M followed a similar pattern (Figure 1), with the highest levels in the CNS OI group. As for CSF, levels of serum β_2M were significantly higher in untreated NA compared with NA on ART ($p < 0.0001$) and significantly higher the lower the $CD4^+$ T-cell count ($p < 0.0001$), except for the two groups with the lowest values. In line with CSF β_2M, NA escape ($p < 0.01$) and NA on ART ($p < 0.05$) had significantly higher serum β_2M levels compared with controls but not compared with elite controllers. There was no significant difference between untreated NA and untreated HAD.

Neopterin was included since it is an established marker of immune activation, extensively used in studies of HIV infection and well characterized in this context. CSF and serum neopterin distributions closely paralleled those of β_2M across the different groups (Figure 1).

In untreated NA, 91% (310/342) had elevated CSF β_2M and 96% (325/340) had elevated neopterin levels (Table 2). Among controls, CSF β_2M and neopterin levels were above normal reference values in 39% (23/59) and 29% (17/59), respectively. Of these, 74% (17/23) and 59% (10/17) were on PrEP.

To assess CSF β_2M in relation to CNS inflammation and injury, we examined its associations with other known markers of immune activation, viral load, BBB integrity and neuronal injury. In untreated NA there was a significant correlation between CSF β_2M levels and CSF HIV RNA ($r = 0.42$), albumin ratio ($r = 0.47$), CSF neopterin ($r = 0.69$), CSF NfL ($r = 0.43$) (Figure 2), age ($r = 0.33$), plasma HIV RNA ($r = 0.39$), $CD4^+$ T-cell count ($r = -0.30$), IgG index ($r = 0.22$), serum neopterin ($r = 0.45$), WBC ($r = 0.23$), and serum β_2M ($r = 0.61$) (all $p < 0.0001$). Independent predictors of CSF β_2M were albumin ratio ($p < 0.001$), IgG index ($p < 0.001$), serum β_2M ($p < 0.001$), serum ($p < 0.05$) and CSF neopterin ($p < 0.001$), plasma HIV RNA ($p < 0.05$), and CSF NfL ($p < 0.001$).

In NA on ART, a significant correlation was found between CSF β_2M levels and albumin ratio ($r = 0.45$, $p < 0.0001$), CSF neopterin ($r = 0.41$, $p < 0.0001$) (Figure 2), CSF HIV RNA ($r = 0.15$, $p < 0.05$), age ($r = 0.47$, $p < 0.0001$), $CD4^+$ T-cell count ($r = -0.17$), $p < 0.05$), serum neopterin ($r = 0.16$, $p < 0.05$), and serum β_2M ($r = 0.41$, $p < 0.0001$). All, except CSF HIV RNA were independent predictors of CSF β_2M.

For individuals with HAD, CSF β_2M levels correlated significantly with albumin ratio ($r = 0.74$, $p < 0.01$), CSF neopterin ($r = 0.80$, $p < 0.01$), and age ($r = 0.65$, $p < 0.05$). In individuals with CNS OIs, there was a significant correlation between CSF β_2M levels and CSF neopterin ($r = 0.63$, $p < 0.05$) and WBC count ($r = 0.59$, $p < 0.05$).

Longitudinal follow-up of β_2M and neopterin after ART initiation

Of 342 untreated NA participants, 112 were followed before and after ART initiation. At baseline, 89% had elevated CSF β_2M and 96% had elevated CSF neopterin levels. After ART initiation, CSF β_2M levels declined by 10% per month during the first three months ($p < 0.0001$), 10% per year at one year ($p < 0.01$) and 5% per year at three years ($p < 0.05$), with no further decline thereafter (Figure 3). Three months after ART initiation, 78% (73/93) still had elevated CSF β_2M levels, 58% (49/85) at one year, 39% (22/56) at three years, 40% (17/42) at five years, and 57% (12/21) at ten years.

Serum β_2M declined by 8% per month in the early phase ($p < 0.0001$), 9% per year at one year ($p < 0.0001$), 4% per year at three years ($p < 0.05$), with no further significant decline thereafter.

CSF neopterin levels declined by 17% per month during the early phase ($p < 0.0001$), 27% per year at one year ($p < 0.0001$) and 2% per year at three years (not significant), with no further decline thereafter (Figure 3). After three months on ART 80% (75/94) had elevated CSF neopterin levels, 56% (48/85) at one year, 44% (25/57) at three years in, 27% (12/44) at five years, and 62% (13/21) at ten years.

Serum neopterin declined by 16% per month in the early phase ($p < 0.0001$) and by 17% per year at one year ($p < 0.001$), with no statistically significant changes thereafter.

All five participants with HAD had abnormal baseline CSF β_2M and neopterin levels. After ART initiation, CSF β_2M declined by 21% per month (not significant) and serum β_2M by 15% ($p < 0.05$)

during the early phase (Figure 3). At three months, 3/3 had CSF β_2M levels above the reference values, 2/3 after one year, and none at three (0/2) years. CSF neopterin levels declined by 32% per month (not significant) and serum neopterin by 29% ($p < 0.05$) in the early phase. At three months, 3/3 had elevated CSF neopterin, 2/3 at one year, and none at three (0/1) years.

All four participants with CNS OIs had elevated CSF β_2M levels at baseline. CSF β_2M levels declined by 10% per month in the early phase (not significant) (Figure 3), and serum β_2M by 13% ($p = 0.05$). At three months and one year, 3/4 had elevated CSF β_2M, but none after three (0/2) years. CSF neopterin levels were elevated in 3/4 at baseline and declined by 17% per month (not significant), while serum neopterin declined by 27% ($p < 0.01$). All (4/4) had elevated CSF neopterin at three months and one year, and 2/2 at three years.

Time to normalization of CSF β_2M and neopterin levels in NA is shown in Figure 3.

Neither early decline rates nor biomarker levels after three months differed by ART regimen for CSF β_2M or neopterin among NA individuals.

Longitudinal results for other inflammatory markers, viral load, and neuronal injury are presented in Supplementary Table 1, http://links.lww.com/QAD/D812, and Doc1, http://links.lww.com/QAD/D813.

DISCUSSION

This is the largest study to date on CSF β_2M levels in HIV-1 infection. We found that most participants without ART had elevated CSF β_2M levels, regardless of stage of infection. As expected, the highest levels occurred in advanced conditions such as HAD and CNS OIs, where elevation above reference values was universal. Importantly, this is the first investigation of CSF β_2M in different types of CSF escape. Participants with secondary and symptomatic escape showed particularly high levels. Secondary escape involved HSV-2 or VZV-infections, which are neurotropic viruses capable of triggering intrathecal inflammation.[23] In symptomatic escape, elevated CSF HIV RNA levels have previously been linked to CSF inflammation.[32-34]

In our previous work we have demonstrated that most untreated NA individuals have elevated CSF neopterin and although levels decline markedly after ART initiation, nearly half retain elevated CSF neopterin levels.[35, 36] We find the same pattern for CSF β_2M. More than 90% of untreated NA participants, especially those with low $CD4^+$ T-cell counts, had elevated levels. Among virally suppressed NA participants, 37% remained elevated. Half of the longitudinally followed NA group had low $CD4^+$ T-cell nadir counts (200 or below), which may contribute to the plateau of neuroinflammatory markers after ART initiation. Individuals with a low $CD4^+$ T-cell nadir prior to ART might not be expected to normalize inflammatory markers to the same extent as those with higher $CD4^+$ T-cell counts, underscoring the importance of early ART. Interestingly, one third of the controls also showed increased CSF β_2M and neopterin levels. This is consistent with a recent study reporting higher CSF β_2M levels in controls on PrEP compared with individuals without PrEP, suggesting that non-HIV factors, including lifestyle (e.g. drug use) and viral co-infections (e.g. cytomegalovirus), may

contribute to CSF inflammation.[22, 37] After three years on ART, the proportion of NA individuals with elevated CSF β_2M levels was similar to that of controls. Given that the majority of controls in our study were on PrEP and considering the similarities between NA individuals and those on PrEP, these findings further support the contributions of factors other than HIV to CSF inflammation.

CSF β_2M correlated with neopterin in the untreated NA, HAD, and CNS OI groups, consistent with previous studies.[16, 36] In addition, NA participants with increased CSF β_2M levels had higher albumin ratios and CSF NfL levels, markers of BBB integrity and axonal injury. In both NA and HAD, CSF β_2M correlated with albumin ratio. We have previously found an association between BBB damage and CSF neopterin, and a correlation between albumin ratio and CSF NfL in NA individuals.[38] This study shows a similar association between CSF inflammation, BBB permeability and neuronal injury, with CSF β_2M as the inflammatory marker.

Neopterin has been considered more sensitive than CSF β_2M for detecting intrathecal immune activation, based on an earlier study reporting full normalization of CSF β_2M after two years on ART, whereas only 55% of CSF neopterin levels normalized.[19] Those participants were slightly older than ours (median 42 vs 38 years), but gender distribution and disease severity were comparable. Our study is larger with longer follow-up, and 39% of NA participants still had elevated CSF β_2M levels even after three years on ART, similar to CSF neopterin. Although the decline rate of CSF neopterin was slightly faster during the first three months of ART compared with CSF β_2M, the proportion of participants with persistent elevation of these markers was nearly identical after one and three years of treatment.

Normally, MHC 1 expression in the brain is low, but it can be upregulated under certain conditions, such as CNS lymphomas.[39] In older studies, increased CSF β_2M was found in individuals with HAD correlating with dementia severity,[15] and levels were even used to predict dementia in PWH before quantification of plasma HIV RNA was available.[18, 40] As MHC-1 is upregulated during inflammatory response, increased CSF β_2M levels likely originate from shedding of proliferating inflammatory cells within the CNS, such as infiltrating lymphocytes and macrophages. Higher CSF than serum β_2M in CNS engaging conditions, and lack of correlation between CSF and serum β_2M in participants with HAD and CNS OIs, support a CNS origin of CSF β_2M. Also, individuals with CSF escape showed higher CSF β_2M levels than controls, consistent with findings for CSF neopterin in NA with escape. [25]

In conclusion CSF β_2M shows decay characteristics similar to neopterin, with substantial reduction after ART and normalization in many PWH. The association with BBB disruption and neuronal injury supports its value as a marker of HIV-related CNS involvement and it may serve as a clinical alternative to neopterin, used together with other inflammatory or dementia-related markers, when evaluating cognitive difficulties in PWH.

Declarations

Ethics approval and consent to participate:
This study was approved by the Human Research Ethics Committee of the Medical Faculty, University of Gothenburg, (Sweden) (Dnr: Ö588-01). All participant information was confidentially treated.

Anonymous, coded identification was used. Written informed consent was obtained from all participants or, if their capacity to provide consent was questioned, consent was also obtained from those with power of attorney.

Acknowledgements

This study was supported by the Swedish state under an agreement between the Swedish government and the county councils (ALF agreements ALFGBG-965885, ALFGBG-1005848 and ALFGBG-71320), the Swedish Research Council (#2023-00356; #2022-01018 and #2019-02397), and the European Union's Horizon Europe research and innovation programme under grant agreement No 101053962). M.G. originated the idea. M.G., A.Y. and B.A. designed the study and A.Y. supervised the study. D.F. and H.Z. performed the biochemical analyses. B.A., A.Y., M.G. and S.N. performed acquisition, analysis, and interpretation of data. B.A. and S.N. performed the statistics. B.A. wrote the article. All authors contributed to manuscript preparation.

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Figure 1. Levels of β_2-microglobulin in cerebrospinal fluid (CSF) (A), serum (B) and neopterin in CSF (C) and serum (D) in individuals with HIV and in controls without HIV. Participants were divided into: neuroasymptomatic (NA) without antiretroviral treatment (ART) and stratified by $CD4^+$ T-cell count (cells/μL); NA on ART; NA escape (neuroasymptomatic on ART, with detectable CSF HIV RNA but not plasma, or higher CSF than plasma HIV RNA); symptomatic escape (on ART, elevated CSF HIV RNA but not in plasma, or two times higher CSF HIV RNA compared with plasma, and new or progressive central nervous system (CNS) symptoms); secondary escape (on ART, higher CSF than plasma HIV RNA with a non-HIV related infection); untreated with opportunistic infection in CNS (CNS OI); CNS OI on ART; untreated HIV-associated dementia (HAD); HAD on ART; and elite controllers (no ART, plasma HIV RNA below detection for years). Controls were individuals without HIV (HIV−). Boxes encompass interquartile range with median (line); whiskers indicate 10th – 90th percentiles.

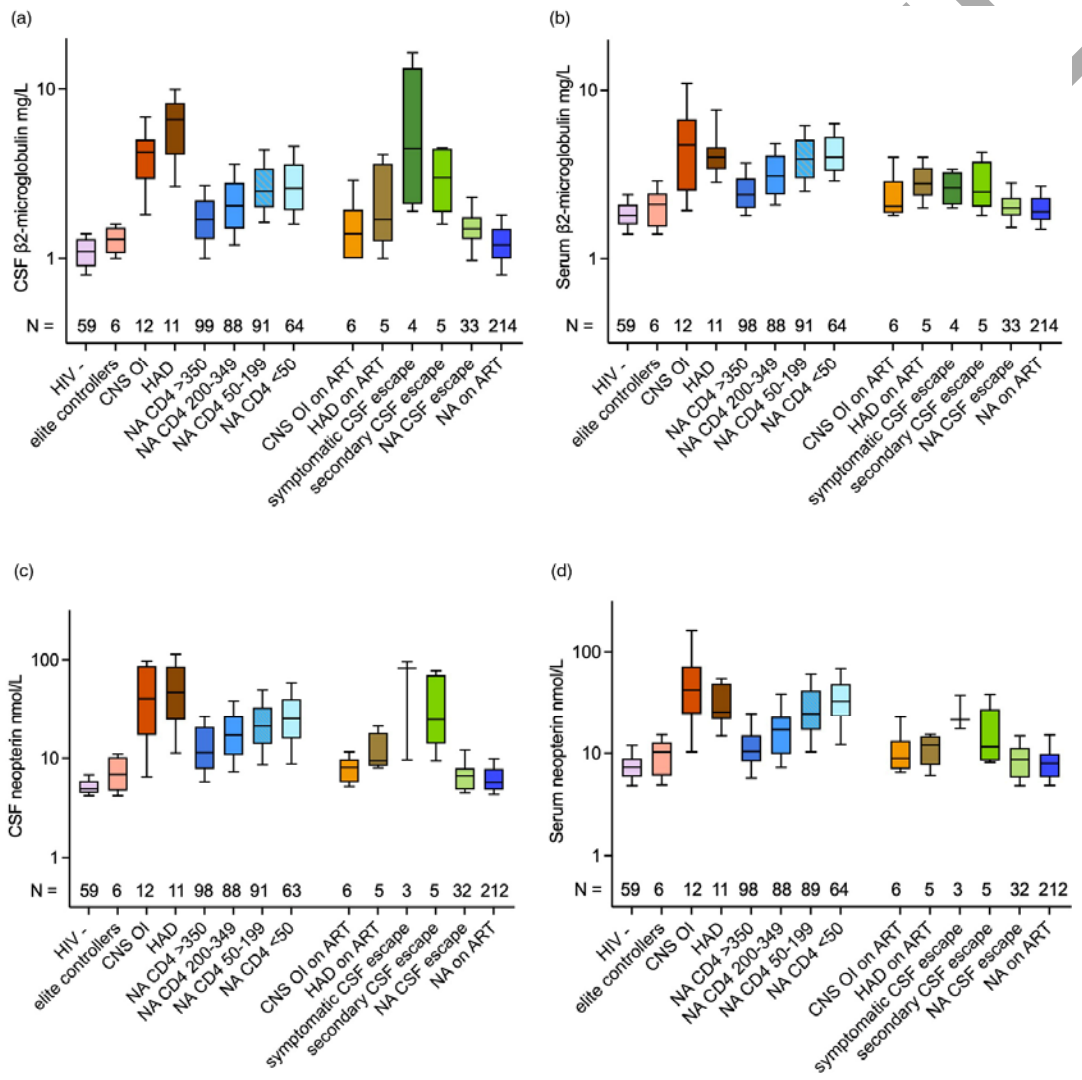

Figure 2. Correlations of cerebrospinal fluid (CSF) β_2-microglobulin with other biomarkers in neuroasymptomatic HIV (NA): Untreated (A) CSF HIV RNA; (B) albumin CSF/plasma ratio; (C) CSF neopterin; (D) CSF NfL, **and on antiretroviral treatment (ART):** E) albumin CSF/plasma ratio; F) CSF neopterin.

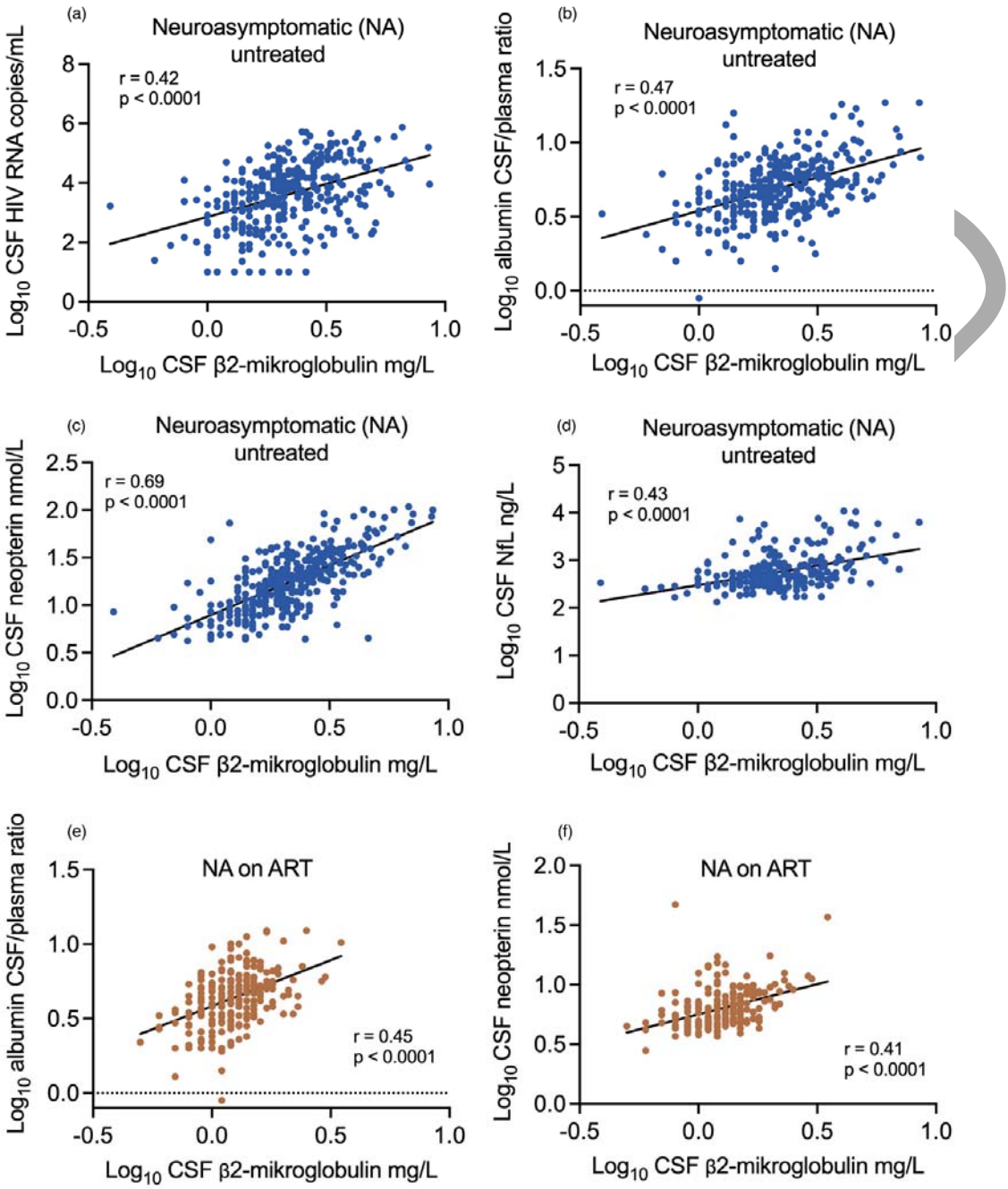

Figure 3. Cerebrospinal fluid (CSF) levels of β_2-microglobulin (A) and neopterin (B) measured over time on antiretroviral treatment (ART) in participants with neuroasymptomatic HIV (NA), HIV-associated dementia (HAD), and opportunistic infection in the central nervous system (CNS) (OI). Decline rate for each biomarker from baseline was calculated at three months and after one, three, and five years after starting ART. Dashed line indicates normal reference value for participants 18–50 years of age (< 1.2 mg/L) and the dotted line for participants > 50 years of age (< 1.8 mg/L). Normal reference value for CSF neopterin was < 5.8 nmol/L (B; dotted line). Time to normalization in NA **(C)** is shown in pink for CSF β_2-microglobulin and in blue for CSF neopterin.

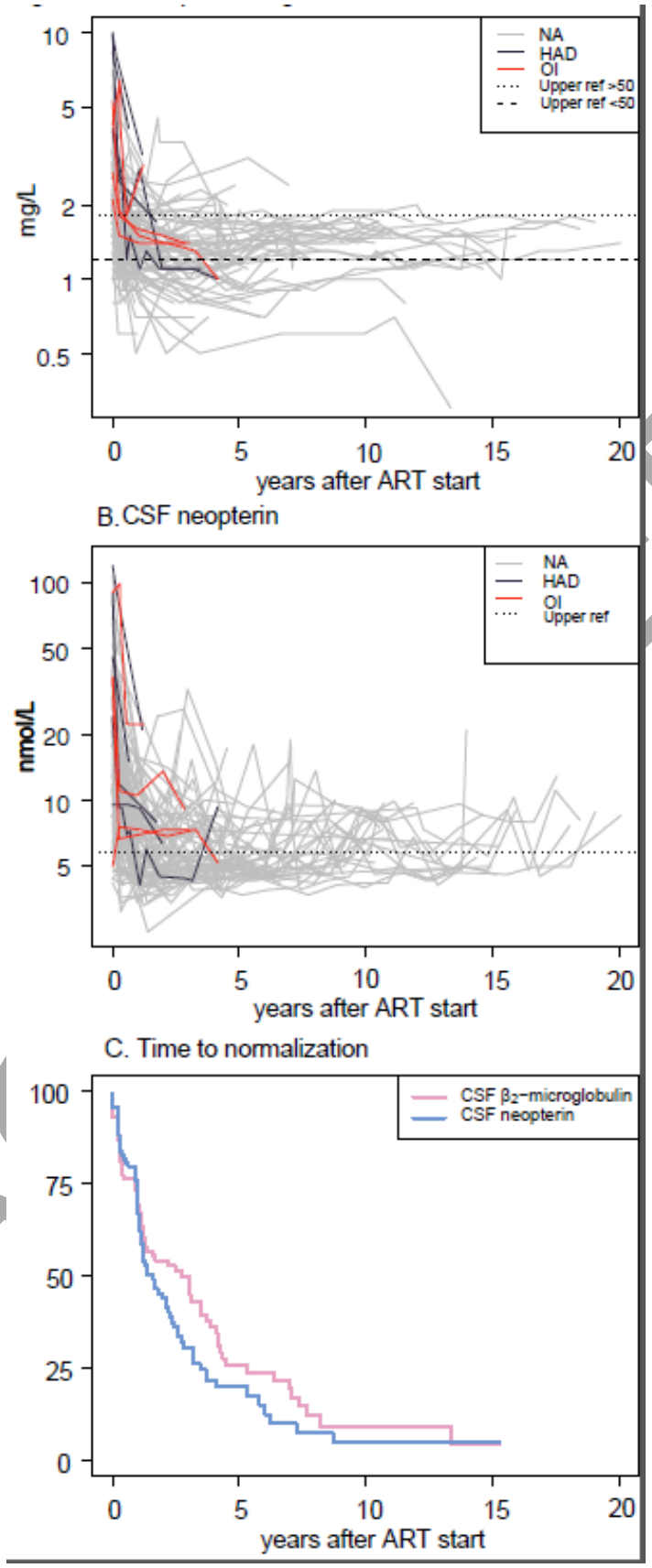

Table 1. Baseline characteristics in the different categories of participants.

Groups	N	Sex N m/f	Age years (IQR)	HIV RNA log_{10} copies/mL (IQR)		$CD4^+$ T cell count cells/μL (IQR)	$CD4^+$ T cell count nadir cells/μL (IQR)
				Plasma	CSF		
persons without HIV	59	47/12	36 (29–41)	–	–	810 (635–1100)	–
elite controllers[1]	6	2/4	47 (40–48)	<1.7 (1.0–2.2)	< 1.7	805 (575–1028)	587 (499–682)
NA	342	223/119	38 (31–48)	4.8 (4.2–5.4)	3.7 (3.0–4.4)	220 (84–382)	140 (45–250)
> 350[2]	99	66/33	35 (29–46)	4.2 (3.5–4.7)	3.4 (2.4–4.0)	510 (403–660)	320 (250–420)
200–349	88	53/35	36 (29–47)	4.8 (4.2–5.3)	4.0 (3.4–4.5)	251 (228–290)	200 (160–233)
50–199	91	61/30	38 (32–49)	5.2 (4.5–5.6)	4.0 (3.4–4.8)	120 (87–155)	90 (60–125)
< 50	64	43/21	42 (34–49)	5.4 (5.0–5.9)	3.1 (2.4–3.9)	20 (10–37)	20 (10–30)
NA, on ART[3]	214	139/75	45 (37–55)	< 1.7	< 1.7	600 (413–800)	220 (100–320)
NA, escape[4]	33	22/11	49 (39–60)	< 1.7	1.7 (1.5–1.9)	520 (400–740)	180 (100–220)
symptomatic escape[5]	4	2/2	43 (43–44)	2.0 (1.6–2.3)	3.3 (2.9–3.6)	480 (278–823)	25 (10–63)
secondary escape[6]	5	4/1	47 (45–48)	< 1.7	2.9 (2.7–3.0)	320 (300–470)	40 10–160
CNS OI	12	11/1	38 (33–41)	4.9 (4.6–5.3)	4.1 (3.3–5.0)	25 (15–46)	60 (30–80)
CNS OI, on ART	6	3/3	43 (41–44)	< 1.7	< 1.7	645 (243–965)	25 (15–33)
HAD	11	10/1	43 (37–47)	5.4 (4.7–6.0)	5.0 (3.4–5.5)	70 (56–131)	45 (15–98)

HAD, on ART	5	5/0	51 (44–57)	< 1.7	< 1.7	660 (350–660)	85 (48–118)

Abbreviations: IQR, interquartile range; CSF, cerebrospinal fluid; CNS central nervous system; NA, neuroasymptomatic HIV without neurological symptoms (untreated); ART: antiretroviral therapy; CNS OI, opportunistic CNS infection (in untreated HIV); HAD, HIV-associated dementia (untreated); m, male; f, female.

All data except numbers (N) are presented as Median (IQR).

[1] elite controllers, persons with HIV with plasma HIV RNA <50 copies/mL without ART,

[2] NA stratified for systemic $CD4^+$ T cell counts,

[3] ART with plasma HIV RNA <50 copies/mL,

[4] NA escape, on ART with detectable HIV RNA in CSF but not in plasma or higher HIV RNA in CSF than in plasma,

[5] symptomatic escape, on ART with detectable HIV RNA levels in CSF and new or progressive CNS symptoms,

[6] secondary escape, on ART with detectable HIV RNA levels in CSF and a non-HIV-associated concomitant infection.

Table 2. Number of participants with CSF levels of β2-microglobulin and neopterin above normal reference values (above ref) in various groups with HIV infection, and controls.

	β2-microglobulin		Neopterin	
Groups	Total N	Above ref N (%)	Total N	Above ref N (%)
persons without HIV	59	23 (39)	59	17 (29)
elite controllers[1]	6	3 (50)	6	4 (67)
NA[2]	342	310 (91)	340	325 (96)
> 350	99	80 (81)	98	89 (91)
200–349	88	80 (91)	88	85 (97)
50–199	91	87 (96)	91	90 (99)
< 50	64	63 (99)	63	61 (97)
NA, on ART[3]	214	80 (37)	212	105 (50)

NA, escape[4]	33	19 (58)	32	19 (59)
symptomatic escape[5]	4	4 (100)	3	3 (100)
secondary escape[6]	5	4 (80)	5	5 (100)
CNS OI	12	12 (100)	12	11 (92)
CNS OI, on ART	6	4 (67)	6	5 (83)
HAD	11	11 (100)	11	11 (100)
HAD, on ART	5	4 (80)	5	5 (100)

Abbreviations: IQR, interquartile range; CSF, cerebrospinal fluid; NA, neuroasymptomatic HIV without neurological symptoms (untreated); ART: antiretroviral therapy; CNS OI, opportunistic CNS infection (in untreated HIV); HAD, HIV-associated dementia (untreated).

[1] elite controllers, persons with HIV with plasma HIV RNA <50 copies/mL without ART,

[2] NA stratified for systemic $CD4^+$ T cell counts,

[3] ART, on antiretroviral therapy, with plasma HIV RNA <50 copies/mL,

[4] NA escape, on ART with detectable HIV RNA in CSF but not in plasma or higher HIV RNA in CSF than in plasma,

[5] symptomatic escape, on ART with detectable HIV RNA levels in CSF and new or progressive CNS symptoms,

[6] secondary escape, on ART with detectable HIV RNA levels in CSF and a non-HIV-associated concomitant infection.