Tryptophan Metabolism and Its Relationship with Depression and Cognitive Impairment Among HIV-infected Individuals

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ABSTRACT

OBJECTIVE: Cognitive impairment (CI) and major depressive disorder (MDD) remain prevalent in treated HIV-1 disease; however, the pathogenesis remains elusive. A possible contributing mechanism is immune-mediated degradation of tryptophan (TRP) via the kynurenine (KYN) pathway, resulting in decreased production of serotonin and accumulation of TRP degradation products. We explored the association of these biochemical pathways and their relationship with CI and MDD in HIV-positive (HIV+) individuals.

METHODS: In a cross-sectional analysis, concentrations of neopterin (NEO), tumor necrosis factor-alpha, TRP, KYN, KYN/TRP ratio, phenylalanine (PHE), tyrosine (TYR), PHE/TYR ratio, and nitrite were assessed in the cerebrospinal fluid (CSF) and plasma of HIV+ ($n = 91$) and HIV-negative (HIV–) individuals ($n = 66$). CI and MDD were assessed via a comprehensive neuropsychological test battery. A Global Deficit Score ≥0.5 was defined as CI. Nonparametric statistical analyses included Kruskal–Wallis and Mann–Whitney *U* tests, and multivariate logistic regression.

RESULTS: Following Bonferroni correction, NEO concentrations were found to be greater in CSF and TRP concentration was found to be lower in the plasma of HIV+ versus HIV– individuals, including a subgroup of aviremic (defined as HIV-1 RNA <50 cps/mL) HIV+ participants receiving antiretroviral therapy ($n = 44$). There was a nonsignificant trend toward higher KYN/TRP ratios in plasma in the HIV+ group ($P = 0.027$; Bonferroni corrected $\alpha = 0.0027$). In a logistic regression model, lower KYN/TRP ratios in plasma were associated with CI and MDD in the overall HIV+ group ($P = 0.038$ and $P = 0.063$, respectively) and the aviremic subgroup ($P = 0.066$ and $P = 0.027$, respectively), though this observation was not statistically significant following Bonferroni correction (Bonferroni corrected $\alpha = 0.0031$).

CONCLUSIONS: We observed a trend toward lower KYN/TRP ratios in aviremic HIV+ patients with CI and MDD.

KEYWORDS: HIV, cognitive impairment, depression, tryptophan, kynurenine, IDO

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Introduction

HIV-1 enters the central nervous system (CNS) during the early stages of HIV infection[1] and has been associated with neurological and neuropsychiatric effects, including major depressive disorder (MDD) and cognitive impairment (CI). The reported rates of MDD in people living with HIV (PLWH) vary from 5% to almost 50%.[2] The relationship between HIV infection and MDD is complex with several factors likely contributing to its development and persistence.[3–8] Untreated depression in PLWH is associated with nonadherence to antiretroviral therapy (ART),[9] more rapid progression to AIDS,[10] and shorter survival.[11]

CI is highly prevalent among HIV-positive (HIV+) individuals. Following the advent of effective ART, the incidence of the most severe forms of HIV-related CI has decreased dramatically. In contrast, the prevalence rates of less severe forms remains high,[12–16] although in recent studies of patients well maintained on ART, rates are lower.[17]

Despite being of a milder form, HIV-related CI affects daily function, ART adherence, and quality of life.[18,19]

HIV-related CNS disease is characterized by neuronal loss, reactive astrogliosis, activated microglia, and leukocyte infiltration. HIV-infected cells in the CNS include perivascular macrophages and microglia, but not neurons,

implicating indirect pathways for neuronal injury and death.[20,21] This might be accomplished by the inherent toxicity of viral proteins and the release of inflammatory mediators and neurotoxins by infected or activated macrophages and microglia.[20,21]

The role of tryptophan (TRP), the effects exerted by its catabolites on neural tissue, and the consequences for mood and cognition in PLWH are areas of increasing interest. TRP acts as a substrate of the tetrahydrobiopterin (BH4)-dependent tryptophan-hydroxylase (TPH), leading to the production of 5-hydroxytryptamine (serotonin). TRP can also be catabolized by the heme-dependent enzymes, TRP 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO-1; Fig. 1), ultimately resulting in the production of kynurenine (KYN) and its derivatives.[22–24]

Under physiological conditions, TRP degradation occurs primarily in the liver, mediated by TDO, which is activated when the concentration of TRP exceeds the requirements for its metabolic needs. In contrast, IDO-1 is responsible for extrahepatic TRP degradation and is inducible by proinflammatory cytokines.[25,26]

In the brain, the cellular localization of the KYN pathway has been shown to be primarily in infiltrating macrophages and resident microglial cells.[23,27] Increasing TRP degradation via the IDO-1 pathway diverts it away from serotonin production and results in the production of several neuroactive intermediates, some of which can be either neurotoxic, such as 3-hydroxykynurenine (3-HK), 3-hydroxyanthranilic acid (3-HAA), and quinolinic acid (QUIN), or neuroprotective, such as kynurenic acid (KYNA) and picolinic acid (PIC).[22,23,27–29] The net result during HIV infection is, as yet, unknown, but there is potential for neural damage to occur.[30–34]

Prevous studies have compared TRP metabolism to mood disturbance and CI in HIV+ individuals, but most of these were performed in small, heterogeneous samples and used plasma samples alone.[35–41] In a recent retrospective cross-sectional analysis of cerebrospinal fluid (CSF) samples from HIV+ individuals, Grill et al.[42] reported untreated HIV–infection to be associated with increased CNS IDO-1 activity compared to treated individuals, as reflected by elevated KYN-to-TRP (KYN/TRP) ratios. In patients with acute HIV infection, increased KYN/TRP ratios were associated

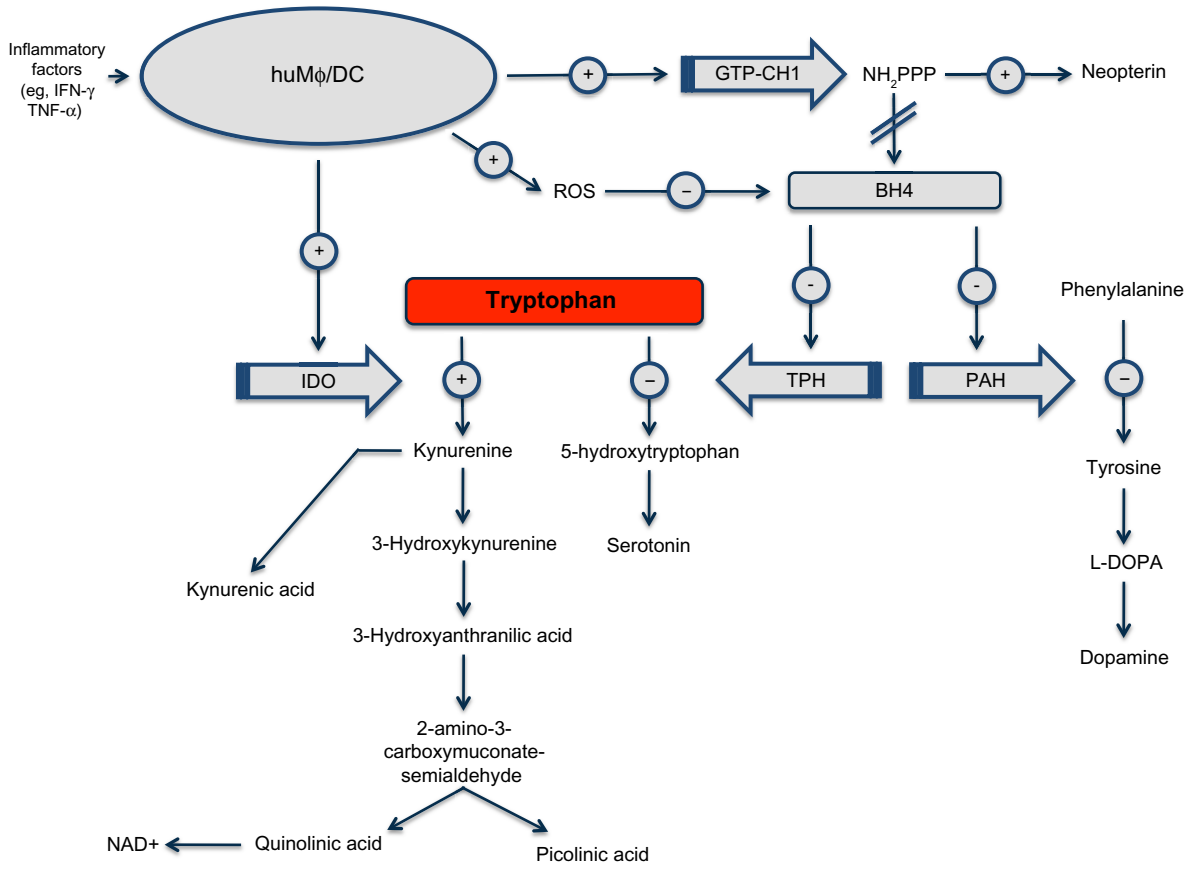

Figure 1. Effects of inflammatory factors on indoleamine 2,3-dioxygenase (IDO-1) and guanosine-triphosphate-cyclohydrolase-1 (GTP-CH1) pathways.[22,23,29,30]

Notes: Reprinted from Capuron et al[29], with permission from Elsevier. "+" indicates upregulated; "–" indicates downregulated.

Abbreviations: IFN-γ, interferon gamma; TNF-α, tumor necrosis factor-alpha; huMϕ/DC, human monocytes and dendritic cells; GTP-CH1, guanosiine-triphosphate-cyclohyrdolase-1; NH_2PPP, 7,8-dihydroneopterin triphosphate; BH4, tetrahydropbiopterin; PAH, phenylalanine-hydroxylase; TPH, tryptophan-hydroxylase; L-DOPA, L-3,4-dihydroxyphenylalanine; ROS, reactive oxygen species; NAD+, nicotinamide adenine dinucleotide.

with increased depressive symptoms. In addition, patients with more severe CI displayed a markedly elevated phenylalanine (PHE)/tyrosine (TYR) ratio.

The aim of our study was to determine if chronic immune activation in HIV+ individuals, as estimated by concentrations of inflammatory biomarkers (IFN-γ, tumor necrosis factor-alpha [TNF-α], NEO), is associated with increased TRP metabolism via the KYN pathway (as measured by the KYN/TRP ratio),[41,43,44] resulting in depressed mood and CI. We also explored the role of phenylalanine-hydroxylase (PAH) activity (as measured by the phenylalanine-to-tyrosine [PHE/TYR] ratio).

Methods

Study design. We performed a cross-sectional analysis of archived CSF and plasma samples collected from HIV+ and HIV– individuals. Because this study analyzed de-identified, archived samples, it was exempt from seeking ethics committee approval.

Patient selection. Study participants with MDD and CI, as well as comparable unaffected comparison groups, were selected from the University of California, San Diego's (UCSD) HIV Neurobehavioral Research Center-based projects, which had already been reviewed and approved by the UCSD's Human Subjects Protection Program.[16,45]

Eligible participants were adults who were tested as either positive or negative for HIV-1 antibody, with or without CI and/or MDD. HIV+ individuals and HIV– controls were matched for gender only. Specific exclusion criteria included a history of MDD, acute HIV infection, active opportunistic infection, history of head trauma associated with neurological complications, and those older than 50 years (older age is associated with lower TRP levels).

Study procedures. All participants received a comprehensive medical examination, which included an assessment of HIV disease (eg, current and nadir CD4+ cell count, and HIV-1 RNA in CSF and plasma) and ART characteristics, in addition to a comprehensive psychiatric and neuropsychological evaluation. Diagnostic and Statistical Manual, Fourth Edition (DSM-IV)[46] diagnoses for current and lifetime MDD were determined using the Composite International Diagnostic Interview (CIDI).[47] Cognitive performance was determined with a comprehensive battery of tests covering seven ability domains (learning, memory, attention/working memory, verbal fluency, processing speed, executive functioning, and motor speed). Raw test scores were converted into demographically adjusted *T*-scores and used to derive domain and global deficit scores (GDS), ranging from 0 (no impairment) to 5 (severe impairment).[48] A GDS greater than or equal to 0.5 was used to classify CI.[12,49] All staff administering these tests were certified according to standardized procedures at their entry into the workforce and periodically recertified. No deviations were detected in testing for MDD or CI. Whilst conducting this research, the study team has complied with the ethical principles for medical research involving human subjects as defined by the World Medical Associations' Declaration of Helsinki.

Laboratory procedures. Plasma and CSF concentrations of TRP, KYN, PHE, and TYR were measured by high-performance liquid chromatography (HPLC) by two methods using the ProStar 210 solvent delivery system (Agilent Technologies Inc.). Sample injection was controlled by a ProStar 400 autosampler, a ProStar 360 fluorescence detector, and a ProStar 325 ultraviolet detector (Agilent). Separation was accomplished at room temperature using a reversed-phase LiChroCART 55-4 mm cartridge (Merck), filled with Purospher STAR RP-18 (3 µm grain size; Merck) together with a reversed-phase C_{18} precolumn (Merck). Before HPLC, serum protein was precipitated with 0.015 mM trichloroacetic acid. For both measurements, L-nitro-tyrosine is used as an internal standard and monitored at the 360 nm wavelength.

TRP and KYN concentrations were measured in one chromatographic run using dihydrogen phosphate solution for separation on reversed-phase C18 material with mobile phase 0.015 M sodium acetate/acetic acid (pH = 4) + 5% methanol and with the fluorescence detector set at 285 nm excitation and 360 nm emission wavelengths. Ultraviolet (UV) absorption to detect KYN and L-nitro-tyrosine concentrations was measured at the 360 nm wavelength.[50,51] For PHE and TYR measurements, the mobile phase was aqueous 15 mM KH_2PO_4, at a flow rate of 0.9 mL/minute, with the fluorescence detector set at 210 nm excitation and 302 emission wavelengths.[52] UV absorption to detect L-nitro-tyrosine concentrations was measured at the 360 nm wavelength (see above). Within-run and between-run coefficients of variance (CVs) are all <5% for all analytes.

The ratios of KYN/TRP and PHE/TYR were calculated as indexes of IDO-1 and PAH activity, respectively.

Neopterin (NEO) concentrations were measured by enzyme-linked immunosorbent assay (BRAHMS Diagnostics) following the manufacturer's protocol (sensitivity, 2 nmol/L).

To estimate the production of nitrous oxide (NO), the stable NO metabolite nitrite (NO_2^-) was determined in the cell-free culture supernatants using the Griess reaction assay (Promega),[53,54] where sulfanilamide was quantitatively converted to a diazonium salt on reaction with NO_2^- in the presence of phosphoric acid. The diazonium salt was then coupled to *N*(1-naphthyl)ethylenediamine dihydrochloride, forming an azo dye that was read at 540 nm in a spectrophotometer and calculated by external standardization.

IFN-γ and TNF-α were quantified using the commercially available Human TNF-alpha Quantikine ELISA Kit (DTA00C; sensitivity, 5.5 pg/mL) and the Human IFN-gamma Quantikine ELISA (DIF50; sensitivity, 8 pg/mL), respectively (both from R&D Systems), using the manufacturer's protocol. The majority of IFN-γ samples obtained were below the detection limit, and so, this parameter was excluded from further analyses.

Statistical methods. Statistical analyses were performed with IBM's SPSS Software Version 21. The results were subjected to tests of the normal distribution. Nonparametric tests were selected for use due to skewed distributions in the data. Univariate analyses were conducted using Mann–Whitney *U* tests and chi-square tests for independence to look for differences in baseline characteristics between the HIV+ and HIV– groups, and between the HIV+ individuals receiving ART and those not receiving ART. Comparisons of the biochemical and immunological markers in CSF and plasma between the HIV+ and HIV– groups, and between the subgroup of HIV+ individuals who were taking virologically suppressive ART ($n = 44$) and the HIV– group were made using Mann–Whitney *U* tests. Initially, the alpha for the Mann–Whitney *U* tests was set at 0.05 as these were exploratory analyses. Bonferroni adjusted alpha values of 0.0027 per test (0.05/18) were also applied.

Multivariate linear regression analysis explores the relationship between TNF-α and NEO with the clinically relevant covariates such as HIV-1 RNA in CSF and plasma (≤ or >50 cps/mL), current CD4+ cell count, and ART-use. In order to understand whether an association exists between the inflammatory biomarkers and TRP metabolism, the relationship between TNF-α and NEO and the KYN/TRP ratio in CSF and plasma from HIV+ individuals was analyzed in a second multivariate model.

To compare TRP metabolism via the KYN pathway with neuropsychiatric outcomes, logistic regression analyses were performed for the KYN/TRP ratio in both CSF and plasma and either binary MDD or CI status. Due to the number of comparisons performed, Bonferroni adjusted alpha values of 0.0031 per test (0.05/16) were applied to the final multivariate analyses exploring the depressive and cognitive parameters.

In order to account for the potential confounding effects of the use of antidepressant agents in some patients, Mann–Whitney *U* tests were performed to determine whether there was a correlation between the use of antidepressant medication and TRP, KYN, and the KYN/TRP ratio. These were performed for the overall HIV+ group, as well as for patients with HIV-1 RNA >50 cps/mL or <50 cps/mL in both CSF and plasma.

Results

Baseline characteristics. The baseline characteristics of the 157 participants (HIV+ = 91 and HIV– = 66) are listed in Table 1. Of the 91 HIV+ patients, 65 were receiving ART (information on the regimens used was available for 44 of 65 patients). Those on ART had lower nadir CD4+ cell counts (144 vs. 330 cells/μL). In the HIV+ group, the median current CD4+ count was 421 cells, reflecting substantial immune recovery on ART. HIV+ participants had higher rates of CI (39% vs. 14%), MDD (46% vs. 15%), and current antidepressant use (serotonin reuptake inhibitors, including both tricyclic antidepressants and selective serotonin reuptake inhibitors; 30% vs. 3%) compared to HIV– participants. The CSF and plasma samples were collected from patients between 1991 and 2009 and subsequently stored at −80 °C.

Univariate analysis of biochemical and immunological markers. The concentrations of each of the biochemical and immunological markers in both plasma and CSF are presented in Table 2.

Significantly higher concentrations of TNF-α were observed in the CSF of both the overall HIV+ group ($P < 0.001$; $\alpha = 0.0027$) and the ART-treated aviremic (<50 cps/mL HIV-1 RNA) subgroup ($P = 0.008$; $\alpha = 0.0027$) compared with HIV– controls, though the subgroup comparison did not remain a significant post-Bonferroni correction. No differences were observed in plasma concentrations of TNF-α in either group.

Higher concentrations of NEO were observed in both the CSF and plasma of the HIV+ group ($P < 0.001$ and $P = 0.02$, respectively; $\alpha = 0.0027$) and in the CSF of the aviremic subgroup ($P = 0.002$; $\alpha = 0.0027$) compared with controls. Again, the observed difference in plasma was not statistically significant after Bonferroni correction.

Higher concentrations of nitrite were observed in the plasma, but not CSF, of both the overall HIV+ group ($P = 0.010$; $\alpha = 0.0027$) and the aviremic subgroup ($P = 0.036$; $\alpha = 0.0027$) compared with controls, though neither observation remained significant following the Bonferroni correction.

Lower concentrations of TRP were observed in both the CSF and plasma of the HIV+ group ($P = 0.012$ and $P < 0.001$, respectively; $\alpha = 0.0027$) and in the plasma of the aviremic subgroup ($P = 0.004$; $\alpha = 0.0027$) compared with controls. No significant differences were observed for KYN. HIV+ participants had greater KYN/TRP ratios in plasma than HIV– controls ($P = 0.027$; $\alpha = 0.0027$). Only the difference in TRP concentration between the HIV+ and HIV– groups in plasma remained significant following the Bonferroni correction.

Concentrations of either PHE or TYR did not differ between any of the groups. A significantly elevated PHE/TYR ratio was observed in the CSF of the HIV+ group compared with controls ($P = 0.038$; $\alpha = 0.0027$), though this was not significant following Bonferroni correction. There were no significant differences observed in plasma or in the CSF and plasma of the aviremic subgroup.

Multivariate analyses. In the first model, viremia and ART use positively correlated with TNF-α and NEO concentrations in CSF, while lower current CD4+ cell counts were associated with higher concentrations of these markers. Viremia and current CD4+ cell count were the strongest predictors of TNF-α and NEO. Nadir CD4+ cell count was also included as a covariate in the model but was not found to be significantly correlated with either marker. The models evaluating TNF-α and NEO in plasma were not statistically significant.

Table 1. Demographic and disease characteristics.

	HIV+	HIV–	*P* VALUE	HIV+ ON ART	HIV+ NOT ON ART	*P* VALUE
n	91	66	–	65	26	–
Age, yrs, Median (IQR)	40 (34,44)	35 (28,44)	0.027	40 (36,44)	37 (32,42)	0.085
Gender, Male	80 (88%)	61 (92%)	0.512	56 (86%)	24 (92%)	0.647
Ethnicity						
White	47 (52%)	36 (55%)	0.352	33 (51%)	14 (54%)	0.810
Black	21 (23%)	13 (20%)		14 (22%)	7 (27%)	
Asian	1 (1%)	2 (3%)		1 (2%)	0 (0%)	
Hispanic	20 (22%)	10 (15%)		15 (23%)	5 (19%)	
Other	2 (2%)	5 (8%)		2 (3%)	0 (0%)	
Current CD4 cells/uL, Median (IQR)[a]	421 (251,594)	820 (670,989)	<0.001	435 (219,609)	402 (263,510)	0.667
Nadir CD4 cells/uL, Median (IQR)	191 (35,300)	–	–	144 (23,222)	330 (200,450)	<0.001
HCV+[b]	5 (6%)	9 (17%)	0.102	3 (5%)	2 (3%)	0.979
GDS ≥0.5[c]	35 (39%)	9 (14%)	0.002	28 (44%)	7 (28%)	0.260
MDD	42 (46%)	10 (15%)	<0.001	27 (42%)	15 (58%)	0.163
Antidepressant use						
Ever[d]	25 (32%)	12 (21%)	0.240	15 (27%)	10 (43)	0.237
Current[e]	27 (30%)	2 (3%)	<0.001	21 (32%)	6 (23%)	0.537
Current ARV regimen[f]	–	–	–		–	–
NRTI(s) in regimen				40 (91%)		
NNRTI-based				14 (32%)		
PI-based				20 (45%)		
NRTI-based				5 (11%)		
Other				5 (11%)		
HIV-1-RNA in plasma Median HIV-1-RNA (IQR)[h]	–	–	–	<50 (0,393)	18,350 (7293,66600)	<0.001
HIV-1-RNA[g]	–	–	–			
≤50 cps/mL				n = 40	n = 0	–
>50 cps/mL				n = 22	n = 25	–
HIV-1- RNA in CSF Median HIV-1-RNA (IQR)[h]	–	–	–	<50 (0,6)	315 (73,1220)	<0.001
HIV-1-RNA[h]						
<50 cps/ml				n = 44	n = 5	–
>50 cps/mL				n = 6	n = 16	

Notes: [a]HIV+, *n* = 88; HIV–, *n* = 64; On ART, *n* = 63; not on ART, *n* = 25.[b]HIV+, *n* = 78; HIV–, *n* = 53; On ART, *n* = 55; not on ART, *n* = 23. [c]HIV+, *n* = 89; HIV–, *n* = 63; On ART, *n* = 64; not on ART, *n* = 25.[d]HIV+, *n* = 79; HIV–, *n* = 57; On ART, *n* = 56; not on ART, *n* = 23. [e]HIV+, *n* = 91; HIV–, *n* = 65; On ART, *n* = 65; not on ART, *n* = 26. [f]ARV regimen information only available for *n* = 44. [g]On ART, *n* = 62; not on ART, *n* = 25.[h]On ART, *n* = 50; not on ART, *n* = 21.
Abbreviations: GDS, global deficit score; ART, antiretroviral therapy; CSF, cerebrospinal fluid; IQR, interquartile range.

In the second model investigating the relationship between the inflammatory markers and the KYN/TRP ratio, levels of TNF-α and NEO positively correlated with the KYN/TRP ratio in both CSF ($R^2 = 0.277$; analysis of variance [ANOVA], $P = 0.008$) and plasma ($R^2 = 0.674$; ANOVA, $P < 0.001$) outcomes. There were no significant correlations for any of the groups in CSF for either MDD or CI status (data not shown). However, in plasma, a lower KYN/TRP ratio was weakly associated with MDD in the HIV+ group, although this was not statistically significant ($\chi^2 = 3.458$ [1, $n = 84$], $B = -1.643$, $P = 0.063$; $\alpha = 0.0031$). Stratification by binary viremia status identified that this trend was driven by the aviremic subgroup ($\chi^2 = 4.874$ [1, $n = 32$], $B = -4.054$, $P = 0.027$; $\alpha = 0.0031$; Fig. 2A), though this was not significant following Bonferroni correction.

A similar association was observed for the KYN/TRP ratio in plasma and CI. Following Bonferroni correction, nonsignificant negative correlation trends were observed in

Table 2. Univariate analysis – biochemical and immunological parameters in CSF and plasma, HIV+ individuals and HIV+ individuals with HIV-1 RNA <50 cps/mL vs. HIV-controls.

	n	HIV-(TOTAL n = 66) MEDIAN (IQR)	n	HIV+ (TOTAL n = 91) MEDIAN (IQR)	HIV+ VS HIV– *P*-VALUE	n	HIV+, <50 cps/ml) (TOTAL n = 44) MEDIAN (IQR)	HIV+, <50 cps/mL VS HIV– *P*-VALUE
CSF								
TNF-α (µmol/mmol)	19	3.84 (3.59,4.47)	33	6.40 (4.92,7.18)	<0.001	21	5.43 (3.85,6.71)	0.008
Neopterin (µmol/mmol)	43	4.30 (3.77,4.65)	65	5.15 (4.41,9.23)	<0.001	42	4.90 (4.26,6.26)	0.002
Tryptophan (µmol/mmol)	43	1.98 (1.58,2.52)	63	1.66 (1.21,2.05)	0.012	41	1.72 (1.29,2.09)	0.076
Kynurenine (µmol/mmol)	43	0.24 (0.09,0.27)	63	0.13 (0.09,0.27)	0.621	41	0.10 (0.09,0.27)	0.536
Kynurenine-to-tryptophan ratio (µmol/mmol/µmol/mmol)	43	76.72 (44.20,151.17)	63	101.77 (54.19,165.21)	0.262	41	90.80 (51.92,151.90)	0.516
Tyrosine (µmol/mmol)	24	9.20 (7.77,13.32)	28	9.05 (6.80,11.14)	0.229	18	9.05 (7.01,11.41)	0.315
Phenylalanine (µmol/mmol)	24	8.68 (6.61,10.16)	28	9.17 (7.68,13.08)	0.174	18	9.17 (7.64,13.89)	0.195
Phenylalanine-to-tyrosine ratio (µmol/mmol/µmol/mmol)	24	0.77 (0.66,1.09)	28	0.96 (0.78,1.29)	0.038	18	0.95 (0.74,1.31)	0.089
Nitrite (µmol/mmol)	13	0.40 (0.10,1.50)	11	1.00(0.40,1.20)	0.393	7	1.00 (0.40,1.20)	0.420
Plasma								
TNF-α (µmol/mmol)	19	7.77 (6.16,10.41)	28	7.75 (4.72,10.39)	0.948	12	5.40 (3.49,8.13)	0.057
Neopterin (µmol/mmol)	66	5.65 (4.76,8.60)	84	9.27 (4.98,15.64)	0.020	32	7.60 (4.62,14.30)	0.265
Tryptophan (µmol/mmol)	66	55.39 (46.04,66.08)	84	45.85 (38.38,54.32)	<0.001	32	47.06 (40.41,55.28)	0.004
Kynurenine (µmol/mmol)	66	2.08 (1.52,2.53)	84	2.07 (1.61,2.66)	0.791	32	1.88 (1.32,2.55)	0.625
Kynurenine-to-tryptophan ratio (µmol/mmol/µmol/mmol)	66	37.56 (26.36,53.34)	84	47.49 (30.51,61.42)	0.027	32	41.37 (28.24,53.59)	0.413
Tyrosine (µmol/mmol)	47	49.15 (42.76,64.72)	55	46.80 (38.99,58.18)	0.200	20	49.07 (42.56,61.56)	0.902
Phenylalanine (µmol/mmol)	47	66.94 (56.42,85.87)	55	65.49 (56.52,88.95)	0.928	20	73.82 (54.11,92.53)	0.468
Nitrite (µmol/mmol)	47	16.86 (12.09,28.24)	55	25.30 (16.68,35.58)	0.010	20	26.49 (20.55,33.89)	0.036

Notes: Mann–Whitney *U* test was performed. Values are represented as median (IQR) unless otherwise noted.
Abbreviations: IFN-γ, interferon gamma; TNF-α, tumor necrosis factor-alpha; IQR, interquartile range.

the overall HIV+ group ($\chi^2 = 4.312$ [1, $n = 82$], $B = -1.926$, $P = 0.038$; $\alpha = 0.0031$) and the aviremic subgroup ($\chi^2 = 3.388$ [1, $n = 32$], $B = -3.078$, $P = 0.066$; $\alpha = 0.0031$).

Significant correlations were not observed for either the viremic subgroup or the HIV– controls (Fig. 2).

The logistic regression analyses described above were also performed using the PHE/TYR ratio in CSF and plasma as the predictor variable. No significant associations were observed (for all comparisons, $P > 0.05$ before Bonferroni correction).

In the models evaluating the effects of antidepressant drugs on TRP, KYN, and the KYN/TRP ratios, lower KYN concentrations ($Z = -2.645$; $P = 0.008$; $r = 0.289$; $n = 84$) and KYN/TRP ratios ($Z = 1.984$; $P = 0.047$; $r = 0.216$; $n = 84$) in plasma were significantly associated with the use of antidepressants in the overall HIV+ group. This association was not observed in either of the subgroups in plasma or in any of the groups in CSF. Multivariate analyses were also performed to explore the relationship between the KYN/TRP ratio in both CSF and plasma and clinically relevant covariates (viremia in plasma and CSF, current and nadir CD4 count, ART use and current antidepressant use). Neither of these models were found to be statistically significant overall, though there was a trend toward significance for the model investigating KYN/TRP in plasma ($R^2 = 0.190$; ANOVA, $P = 0.058$). In this model, antidepressant use was statistically significantly associated with lower KYN/TRP ratios (Correlation = −0.259; $P = 0.009$).

Discussion

In this study, PLWH had greater concentrations of TNF-α and NEO in CSF and had lower concentrations of TRP in plasma compared to HIV– controls. The changes observed in NEO and TRP persisted in virologically suppressed patients receiving ART. We observed a trend toward lower KYN/TRP ratios in patients with CI and MDD in plasma in both the overall HIV+ group and an aviremic subgroup; however, these findings were not statistically significant. This trend was not observed in HIV+ participants with detectable viral loads.

The increases in TNF-α and NEO were expected, which support the observations made in previous studies, indicating increased ongoing monocyte, macrophage and microglial activation, and correlating positively with IDO-1 activity.[41,55–63]

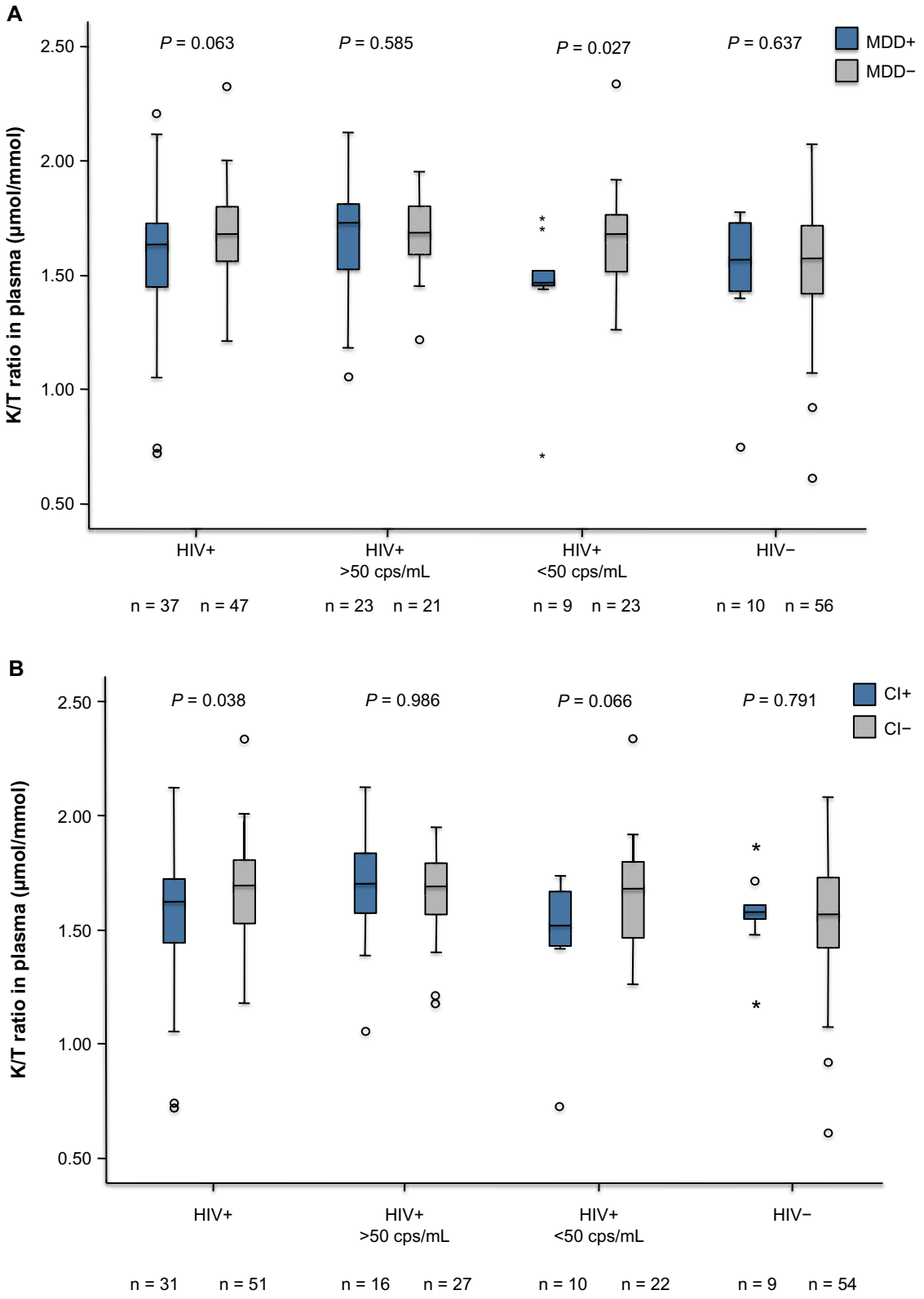

Figure 2. The K/T ratio in plasma and its relationship with MDD (**A**) and CI (**B**).
Abbreviations: K/T, kynurenine/tryptophan; MDD, major depressive disorder; CI, cognitive impairment.

In the study by Grill et al.[42], only untreated acute HIV infection was associated with an increase in the KYN/TRP ratio, which was associated with increased depressive symptoms. Treated HIV infection was not found to be associated with an increase in the KYN/TRP ratio, or with CI or MDD. In contrast, our observations do not support this and, instead, suggest that there may be a subpopulation of aviremic ART-treated patients who have low KYN/TRP

ratios and are experiencing CI and MDD. Our data suggest that the KYN pathway may protect against CI and MDD in aviremic patients. The reasons for this are, as yet, unclear. Interestingly, differences in the KYN/TRP ratio were not observed in HIV– subjects suffering from MDD or CI compared to those without symptoms, indicating that other factors are involved in psychiatric disease in these subjects.

TRP degradation along the KYN pathway results in the synthesis of neuroactive metabolites—3-HK with neurotoxic, QUIN with excitatory, and KYNA with inhibitory properties.[64–68]

Greater CSF QUIN concentrations correlate with the severity of CI in PLWH.[66,69–71] CSF QUIN levels have been shown to fall rapidly with ART,[59,67,70–72] probably as a result of a reduction in virus-related immune activation and a reduction in the activity of IDO-1.[73]

KYNA is known to be neuroprotective due to its ability to block excitotoxic neuronal damage[74,75] and has been shown to be elevated in the brains of HIV-1 patients.[64] In our study, we observed that the aviremic patients with CI and MDD had lower KYN/TRP ratios from those not suffering from these conditions, suggesting that higher KYN/TRP ratios may potentially have a protective effect. It is tempting to speculate that this phenomenon is due to an increase in either KYNA concentration or activity; a hypothesis that should be further investigated.

We also observed a trend toward increases in the PHE/TYR ratio in CSF, indicating a reduction in the production of dopamine and its downstream catabolites. However, in contrast to the observations made by Grill et al.[42], the differences in the PHE/TYR ratio did not correlate with CI or MDD in our study population.

An important limitation of our analyses is that they did not account for serotonin production. Serotonin can be unstable and prone to oxidation, which can contribute to preanalytical errors. However, lowered serotonin concentrations have been described in the blood and CSF of patients with HIV-1 infection.[76,77] In support of this, many HIV+ patients with depression respond well to treatment with SSRIs, indicating that, in some patients, low serotonin levels may be the primary cause of depression. This is consistent with the hypothesis that low serotonin production could be due to immune-mediated TRP depletion.[78] In this study, antidepressant use was significantly greater in HIV+ patients compared with HIV– controls. We found that antidepressant use was associated with both lower KYN concentrations and KYN/TRP ratios in plasma in the overall HIV+ group. In contrast to this, in a previous study by Schroecksnadel et al and in the previous work by our group (unpublished data), no differences were observed in either TRP or KYN concentrations in depressed HIV+ individuals who were treated with antidepressants compared with those not treated.[39] However, in light of the findings from this study, our results should be interpreted with caution as antidepressant use could be a significant confounder.

Our study has several other important limitations. First, the HIV+ group was heterogeneous in terms of virological and immunological status, as well as ART use and the types of treatment used. ART can induce declines in CSF NEO and KYN/TRP ratio levels.[73] However, the impact, if any, of differences between different treatments on TRP metabolism has yet to be explored.

Second, the study population is derived from a diverse mix of ethnicities. There are six reported IDO-1 genetic transcript variants, the frequencies of which have been assessed in European, Chinese, Japanese, and African cohorts.[79] Genetic variations in one or more of these genes, or other genes in the KYN pathway, could account for variable susceptibility to disease or disease outcomes. However, these have not yet been fully examined in the scientific literature and so their effect, if any, is as yet unknown.[79] Also, the potential contribution of dietary differences to TRP levels could not be assessed in our study.[80]

The observed results should be interpreted with caution due to the cross-sectional study design. IDO-1 activity is not consistent over time, and so the observed values and associations only provide a snapshot. Additional studies, which include longitudinal follow-up, may be required to further elucidate the role of TRP catabolism and associated metabolic pathways in HIV-1-associated neurological complications.[81]

Given the exploratory nature of this pilot study, we initially chose not to correct for multiple comparisons in the statistical analysis so that possible associations were not missed due to conservative multiplicity corrections. We did, however, perform Bonferroni corrections resulting in a number of initially significant correlations no longer being significant. The Bonferroni correction is a conservative method when there are a large number of comparisons to account for, as in this case. Therefore, we need to acknowledge the possibility of type II statistical errors when evaluating these data.

While we excluded patients with active opportunistic infections and a history of head trauma associated with neurological complications, and collected information on the rates of HCV, MDD, and methadone use in our study population, we did not collect information on other comorbidities, such as metabolic disorders and vascular disease, which are associated with CI. Our observations could be confounded by the effects of other unknown comorbidities.

The severity of CI among HIV+ individuals was mostly mild. As a result, our findings may not generalize to more severely affected patients. Few patients met the criteria for HIV-associated dementia in our sample, which is consistent with the current rates of HIV-associated neurocognitive disorder (HAND) diagnoses.

In summary, we observed a trend toward lower KYN/TRP ratios in patients with CI and MDD in plasma in both the overall HIV+ group and an aviremic subgroup; however, these findings were not statistically significant. One may hypothesize that the net result of the KYN/TRP ratio in ART-treated, virologically suppressed patients may be neuroprotective in some cases. The causes of CI and MDD in

HIV+ individuals are likely to be multifactorial, and these findings warrant further exploration.

Further work is required in less heterogeneous groups with longitudinal follow-up. The effect, if any, of ART drugs, diet, and lifestyle on the KYN pathway needs to be characterized in HIV patients. Additional work should include measurement of serotonin and a more complete analysis of KYN catabolites.

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Author Contributions

Conceived and designed the experiments: SC, SLL, RJE. Analyzed the data: MRK, SC, SLL, RJE, AW, DF, AB, JI. Wrote the first draft of the manuscript: MRK. Contributed to the writing of the manuscript: SC, SLL, RJE, MRK, AW, DF, AB, JI. Agree with the manuscript results and conclusions: SC, SLL, RJE, MRK, AW, DF, AB, JI. Jointly developed the structure and arguments for the paper: SC, SLL, RJE, MRK, AW, DF, AB, JI. Made critical revisions and approved final version: SC, SLL, RJE, MRK, AW, DF, AB, JI. All authors reviewed and approved of the final manuscript.

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