Low Neurosteroids Identifies a Biological Subtype of Depression in People with HIV

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ABSTRACT

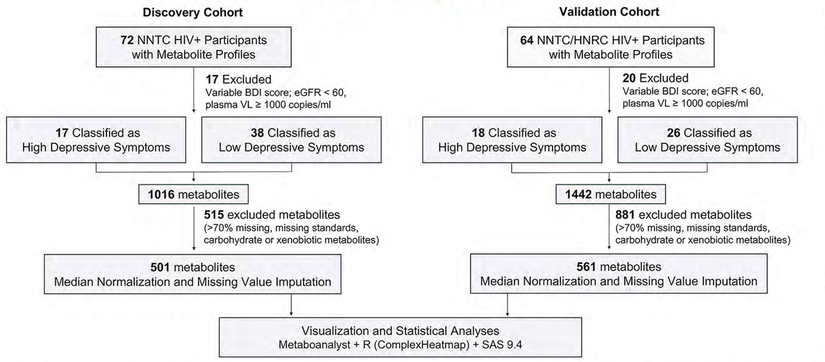
Background: The prevalence and mortality risk of depression in people with Human Immunodeficiency Virus (HIV) infection (PWH) on antiretroviral therapy (ART) is higher than in the general population, yet biomarkers for therapeutic targeting are unknown. Here, we aimed to identify plasma metabolites associated with depressive symptoms in PWH on ART.

Methods: This is a prospective study of 99 ART-treated HIV-infected adults (94% with plasma VL < 200 copies/ml) with or without depressive symptoms assessed using the Beck Depression Inventory (BDI) from the NNTC and HNRC cohorts. Participants with BDI scores > 20 were classified as having high depressive symptoms. Plasma metabolite profiles from 55 participants comprised the discovery set; profiles from 44 additional participants were used to validate the accuracy of models. Metabolite profiling was performed using ultra high-performance liquid chromatography and tandem mass spectrometry (UHLC/MS/MS2) and gas chromatography/MS.

Results: Median age, CD4+ T-cell count, and nadir CD4+ T-cell count were 50 years, 373 cells/µl, and 66 cells/µl in the discovery cohort and statistically similar to the validation set. Seventeen (31%; median BDI 32) and 18 (41%; median BDI 23) participants were classified as having high depressive symptoms in the discovery and validation cohort, respectively. Participants with depressive symptoms had lower neuroactive steroids (dehydroepiandrosterone sulfate (DHEA-S), androstenediols, pregnenolone sulfate) compared to those without depressive symptoms. Cortisol/DHEA-S ratio, an indicator of hypothalamic-pituitary-adrenal axis imbalance, was associated with depressive symptoms due to low DHEA-S, and discriminated between participants with high vs. low depressive symptoms with AUC of 0.70 (P=0.03, discovery) and 0.80 (P=0.02, validation). When cortisol/DHEA-S was coupled with androstenediol and pregnenolone sulfate, discrimination improved with AUC of 0.81 (discovery) and 0.85 (validation). The odds of having depressive symptoms increased with higher cortisol/DHEA-S ratios [odds 2.5 per z-score, 95% confidence interval 1.3-4.7], independent of age and gender. Kynurenine to tryptophan ratio showed no significant associations.

Conclusions: These findings suggest that altered neuroactive steroid metabolism may contribute to the pathophysiology of depression in ART-treated HIV infected adults, representing a potential biological pathway for therapeutic targeting.

METHODS



Workflow to identify metabolites altered in HIV-infected cohorts with and without high depressive symptoms. Abbreviations: BDI: Beck Depression Inventory; eGFR: estimated glomerular filtration rate; HNRC: HIV Neurobehavioral Research Consortium; NNTC: National NeuroAIDS Tissue Consortium; VL: Viral Load

Study population and depressive symptom classification: Longitudinal clinical data, BDI scores, and plasma samples from HIV infected adults were obtained from the NNTC and the HNRC. All study procedures were approved by each site's Institutional Review Board and all participants provided written informed consent. Inclusion criteria were HIV infected adults on ART and over age 35 (to reduce confounding by age). All participants had two or more BDI scores, including one or more assessments with metabolite profiling within 12 months. Participants with BDI scores ≤13 at all assessments were classified as having low depressive symptoms, while participants with BDI scores > 20 were classified as having high depressive symptoms. Participants whose BDI were not consistently ≤ 13 or > 20 were excluded. Additional exclusion criteria were plasma VL ≥ 1000 copies within 90 days of metabolite profile (93 participants (94%) had a viral load < 200 copies/ml) and estimated glomerular filtration rate < 60 mL/minute (calculated using Modification of Diet in Renal Disease (MDRD)).

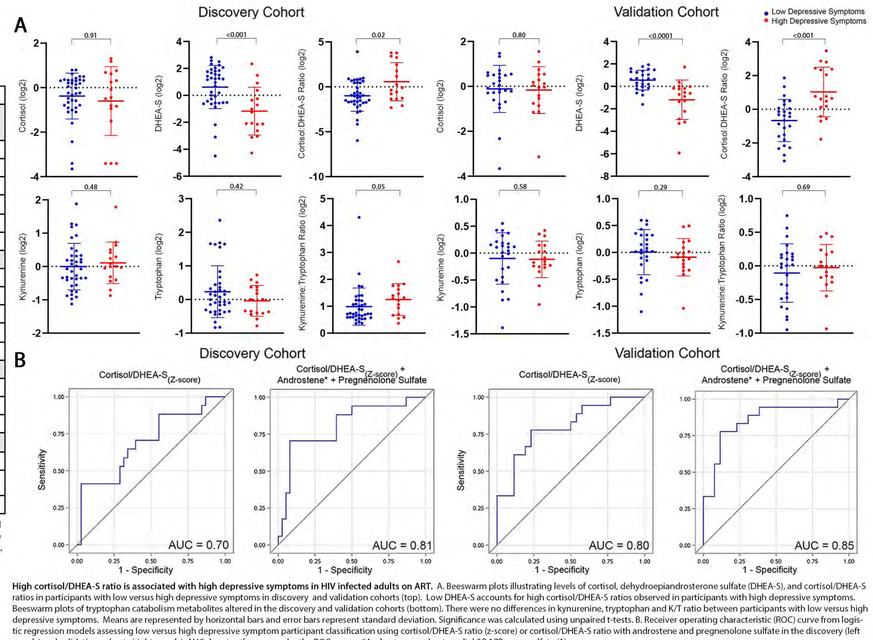
Metabolite profiling, data processing, and statistical analysis: Plasma metabolite profiles obtained from 55 NNTC participants comprised the discovery set (calendar period 2000-2013), while profiles from an 44 NNTC or HNRC participants (calendar period 2006-2016) were used to validate the accuracy of models. Plasma metabolite profiling was performed by Metabolon, Inc (Durham, NC) using ultra high-performance liquid chromatography and tandem mass spectrometry (UHLC/MS/MS2) and gas chromatography/MS. A total of 1016 and 1442 metabolites were identified in the discovery and validation cohorts, respectively. After quality control, data extraction, and normalization steps, a total of 501 (discovery) and 561 (validation) metabolites were considered appropriate for further analyses. Of these, 447 metabolites were identified in both the discovery and validation set and analyzed in models. To standardize values between cohorts, a z-score for metabolite ratios was calculated using means and pooled standard deviations (SD). Spearman correlations, Mann Whitney tests and logistic regression analyses were performed in R 3.5.1; area under the curve (AUC) for receiver-operator characteristic (ROC) curve analysis was performed using SAS 9.4. Fold changes and t-tests were calculated in the Metaboanalyst web portal (http://www.metaboanalyst.ca). Correction for multiple hypotheses testing was performed for fold change analyses by calculating the false discovery rate (FDR) using the 'fdrtool' package. Beeswarm plots were created in Prism (Version 8.0.2, GraphPad Software). A heatmap showing the relative abundance of plasma metabolites in participants with or without depressive symptoms, and forest plot depicting the odds ratio and confidence intervals from logistic regression analyses were created in R.

RESULTS

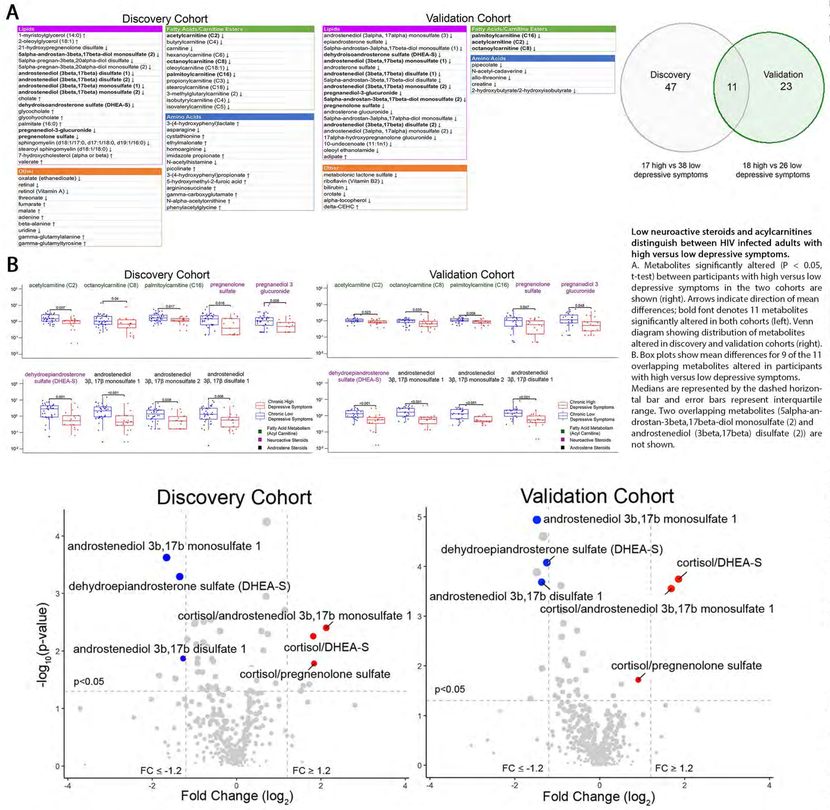
Clinical characteristics stratified by depressive symptoms

	Discovery Cohort		Validation Cohort	
Demographics (median [IQR])	**Low Depressive Symptoms (n=38)**	**High Depressive Symptoms (n=17)**	**Low Depressive Symptoms (n=26)**	**High Depressive Symptoms (n=18)**
Age, years	52.2 [48.2, 60.6]	52.6 [46.5, 60.0]	49.3 [45.9, 56.6]	49.0 [46.8, 55.5]
Race (%)				
Black	9 (23.7)	5 (29.4)	7 (26.9)	6 (33.3)
White	17 (44.7)	9 (52.9)	15 (57.7)	10 (55.6)
Male Gender (%)	28 (73.7)	13 (76.5)	23 (88.5)	14 (77.8)
Education (years)	12.0 [11.0, 13.0]	13.0 [12.0, 16.0]	12.0 [12.0, 14.0]	13.0 [12.2, 14.0]
HIV Characteristics (median [IQR])				
CD4+ T-cell Count, cells/µl	359.50 [218.50, 524.50]	399.00 [168.00, 603.00]	361.5 [217.2, 653.2]	617.0 [383.8, 726.2]
CD4+ T-cell Nadir, cells/µl	74.00 [21.50, 153.75]	66.00 [24.00, 114.00]	**14.0 [6.0, 44.5]**	**142.0 [33.2, 215.0]**
Plasma Viral Load, copies/ml	40.00 [40.00, 40.00]	40.00 [40.00, 103.00]	40.0 [40.0, 40.0]	40.0 [40.0, 40.0]
Efavirenz Use (%)	8 (21.1)	2 (11.8)	**3 (11.5)**	**8 (44.4)**
Duration of HIV (mean (sd), years)	15.65 (6.66)	16.76 (6.3)	16.57 (5.58)	17.3 (8.10)
Neurological Characteristics				
BDI Score (median [IQR])	**2.50 [0.25, 5.75]**	**32.00 [25.00, 35.00]**	**1.0 [0.0, 4.5]**	**23.0 [22.0, 30.5]**
Antidepressant Use, current (%)	9 (23.7)	8 (47.1)	4 (15.4)	8 (44.4)
HAND Diagnosis, current (%)	10 (26.3)	6 (35.3)	6 (23.1)	7 (38.9)
Renal Characteristics and Drug Use				
eGFR (mean (sd))	80.04 (22.71)	76.49 (21.94)	86.31 (26.67)	87.50 (19.47)
Cocaine Use, current (%)	5 (13.2)	4 (23.5)	5 (19.2)	2 (11.1)
Smoker, current (%)	22 (57.9)	9 (52.9)	13 (50.0)	12 (66.7)

Bold indicates a p value < 0.05 within the cohort; GFR: Calculated using the Modification of Diet in Renal Disease Study equation. The number (%) of participants with viral load < 200 copies/ml was 51 (93%) and 42 (95%) in the discovery and validation cohort, respectively. HAND diagnosis includes asymptomatic neurocognitive impairment (ANI), mild neurocognitive disorder (MND) or HIV-associated dementia HAD). The number (%) of participants with and without depressive symptoms diagnosed with MND or HAD were 5 (29%) and 5 (13.1%) in the discovery cohort, respectively and 3 (16%) and 1 (3.8%) in the validation cohort, respectively. Abbreviations: ART, antiretroviral therapy; BDI, Beck Depression Inventory, eGFR, estimated glomerular filtration rate, HAND, HIV-Associated Neurocognitive Disorders, SSRI, selective serotonin reuptake inhibitor; SNRI, serotonin and norepinephrine reuptake inhibitor.

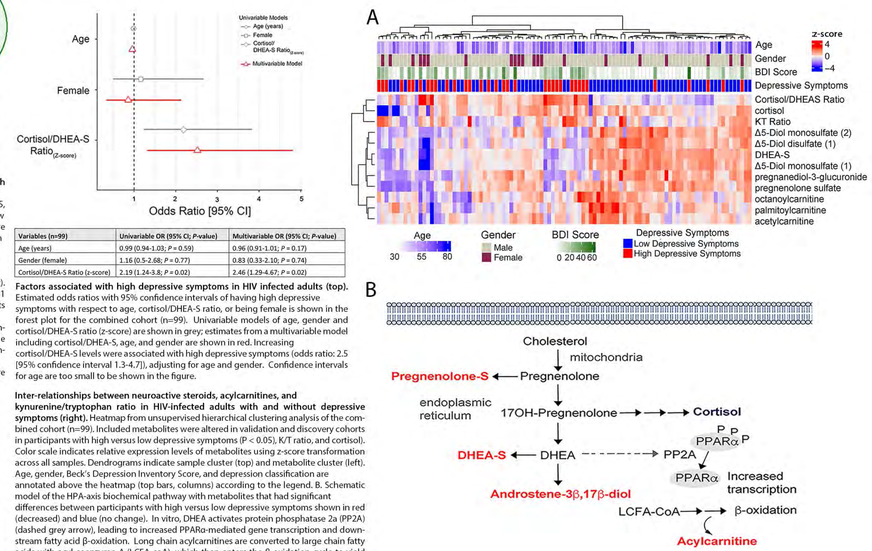


High cortisol/DHEA-S ratio is associated with high depressive symptoms in HIV infected adults on ART. A. Beeswarm plots illustrating levels of cortisol, dehydroepiandrosterone sulfate (DHEA-S), and cortisol/DHEA-S ratios in participants with low versus high depressive symptoms in discovery and validation cohorts (top). Low DHEA-S accounts for high cortisol/DHEA-S ratios observed in participants with high depressive symptoms. Beeswarm plots of tryptophan catabolism metabolites altered in the discovery and validation cohorts (bottom). There were no differences in kynurenine, tryptophan and K/T ratio between participants with low versus high depressive symptoms. Means are represented by horizontal bars and error bars represent standard deviation. Significance was calculated using unpaired t-tests. B. Receiver operating characteristic (ROC) curve from logistic regression models assessing low versus high depressive symptom participant classification using cortisol/DHEA-S ratio (z-score) or cortisol/DHEA-S ratio with androstene and pregnenolone sulfate in the discovery (left panels) and validation cohorts (right panels). AUC denotes the area under the ROC curve. *Androstene: androstenediol 3β,17β monosulfate (1)



Low neuroactive steroids and acylcarnitines distinguish between HIV infected adults with high versus low depressive symptoms. A. Metabolites significantly altered ($P < 0.05$, t-test) between participants with high versus low depressive symptoms in the two cohorts are shown (right). Arrows indicate direction of mean differences; bold font denotes 11 metabolites significantly altered in both cohorts (left). Venn diagram showing distribution of metabolites altered in discovery and validation cohorts (right). B. Box plots show mean differences for 9 of the 11 overlapping metabolites altered in participants with high versus low depressive symptoms. Medians are represented by the dashed horizontal bar and error bars represent interquartile range. Two overlapping metabolites (5alpha-androstan-3beta,17beta-diol monosulfate (2) and androstenediol (3beta,17beta) disulfate (2)) are not shown.

High cortisol/DHEA-S ratio is associated with high depressive symptoms in HIV infected adults on ART. Volcano plots in the discovery (left, n=55) and validation set (right, n=44) illustrating statistical significance of the fold change for neuroactive steroid metabolites (blue data points) and cortisol/steroid ratios (red data points) in participants with high versus low depressive symptoms.



Variables (n=99)	Univariable OR (95% CI; *P*-value)	Multivariable OR (95% CI; *P*-value)
Age (years)	0.99 (0.94-1.03; *P* = 0.59)	0.96 (0.91-1.01; *P* = 0.17)
Gender (female)	1.16 (0.5-2.68; *P* = 0.77)	0.83 (0.33-2.10; *P* = 0.74)
Cortisol/DHEA-S Ratio (z-score)	2.19 (1.24-3.8; *P* = 0.02)	2.46 (1.29-4.67; *P* = 0.02)

Factors associated with high depressive symptoms in HIV infected adults (top). Estimated odds ratios with 95% confidence intervals of having high depressive symptoms with respect to age, cortisol/DHEA-S ratio, or being female is shown in the forest plot for the combined cohort (n=99). Univariable models of age, gender and cortisol/DHEA-S ratio (z-score) are shown in grey; estimates from a multivariable model including cortisol/DHEA-S, age, and gender are shown in red. Increasing cortisol/DHEA-S levels were associated with high depressive symptoms (odds ratio: 2.5 [95% confidence interval 1.3-4.7]), adjusting for age and gender. Confidence intervals for age are too small to be shown in the figure.

Inter-relationships between neuroactive steroids, acylcarnitines, and kynurenine/tryptophan ratio in HIV-infected adults with and without depressive symptoms (right). Heatmap from unsupervised hierarchical clustering analysis of the combined cohort (n=99). Included metabolites were altered in validation and discovery cohorts in participants with high versus low depressive symptoms ($P < 0.05$), K/T ratio, and cortisol). Color scale indicates relative expression levels of metabolites using z-score transformation across all samples. Dendrograms indicate sample cluster (top) and metabolite cluster (left). Age, gender, Beck's Depression Inventory Score, and depression classification are annotated above the heatmap (top bars, columns) according to the legend. B. Schematic model of the HPA-axis biochemical pathway with metabolites that had significant differences between participants with high versus low depressive symptoms shown in red (decreased) and blue (no change). In vitro, DHEA activates protein phosphatase 2a (PP2A) (dashed grey arrow), leading to increased PPARα-mediated gene transcription and downstream fatty acid β-oxidation. Long chain acylcarnitines are converted to large chain fatty acids with acyl coenzyme A (LCFA-coA), which then enters the β-oxidation cycle to yield free carnitine and acylcarnitines

SUMMARY AND CONCLUSIONS

- **Neuroactive steroids that include DHEA-S and androstenediols were reduced in participants with high depressive symptoms, while there were no significant differences in kynurenine, tryptophan, or K/T ratio.**
- **Cortisol/DHEA-S ratio, in combination with other neurosteroid metabolites, distinguished between participants with high versus low depressive symptoms with good accuracy (AUC 0.81 (discovery) and 0.85 (validation)), and high cortisol/DHEA-S ratio was independently associated with depressive symptoms after adjusting for age and gender.**
- **These data suggest that HPA-axis imbalance contributes to chronic depressive symptoms in ART-treated HIV infection and may represent a biological subtype ('biotype') of depression.**
- **Integrated approaches targeting neurosteroids and HPA-axis dysregulation may help to alleviate chronic depressive symptoms in a subset of people with HIV on ART.**

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