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Inflammation as a Contributor to
Depression in People Living with HIV

A DOCTORAL THESIS SUBMITTED BY

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Declaration of Authorship

I, Arish Mudra Rakshasa-Loots, declare that the work presented in this thesis titled “Inflammation as a contributor to depression in people living with HIV” is my own, except where explicitly stated otherwise. I confirm that:

- This thesis was composed by me while in candidature for the degree of Doctor of Philosophy at the University of Edinburgh.
- This work has not been submitted for any other degree or professional qualification.
- The work submitted is my own, except where work which has formed part of jointly-authored publications has been included.
- Where I have collaborated with others on jointly-authored publications, my contribution and those of other authors have been explicitly indicated and acknowledged.
- Where I have consulted the published work of others, this is always clearly attributed.

Signed:

Date: 31 August 2024

Through the white gaze we are strong and resilient - two things it can never get enough of. The need to be constantly strong and independent is a response to trauma. It is glorious to be soft and to experience and give care, especially in the face of such a harsh existence.

Jamil Farouk Khan, *Khamr: The Makings of a Waterslams*

“क्या बिगाड़ के डर से ईमान की बात न कहोगे?”

मुंशी प्रेमचंद, पंच परमेश्वर

To Mom,
UNBREAKABLE AND SOFT.

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None of the research in this thesis would have been possible without the participants who generously volunteered their time for these studies. I am also grateful to the people living with HIV and people living with depression who contributed to this work by sharing their lived experience. In my starry-eyed optimism, I hope that in due course we can move the needle on accessible and effective mental healthcare in return.

Funders and institutions who supported this research have been acknowledged in each chapter; nevertheless, I am particularly thankful for the support of the Wellcome Trust, the Harold Hyam Wingate Foundation, and the SAMRC/SU Extramural Unit on the Genomics of Brain Disorders. I could not have found a better home for my PhD than Edinburgh’s Translational Neuroscience PhD programme, whose leaders (in particular Marja Main, Jane Haley, and David Price) have always gone above and beyond to look out for us.

Successfully arranging for human beings - chaotic, vital, ever-changing - to come into a clinic and fill out a questionnaire or undergo a brain scan is no small feat. This is made doubly challenging when we are trying to reach those living with depression. I am grateful to the many collaborators and colleagues who have made these clinical research studies possible: Thandi Hamana, Shalena Naidoo, Kaylee van Wyhe, Kurt Smith, Thembi Boyana, the neurocognition team, and everyone else at FAMCRU; Goabaone Diteko, Itamar Ronen, Nick Dowell, Lisa Barbour, and the clinical and neuroimaging research teams at Brighton & Sussex Medical School. Thanks also to the HIV & Sexual Health Research Group in Brighton who have so warmly welcomed and supported me, despite (in many cases) only having met me on a screen.

Although this thesis is the culmination of a not insignificant number of years of my life, it did not - perhaps shockingly - consume the entirety of my life. The remainder of my time was made enjoyable by the company (virtual or otherwise) of friends. Thank you for boundless solidarity and confidence: Mila Redžić, Reesha Zahir, Sulagna Basu, Tammy Merrill, Melinda Sauls, Thobeka Mnisi. Thank you, as well, to friends and professors-turned-friends

from Earlham College, with special shout outs to Michelle Tong, Beth Mechlin, Michael Lerner, and Maxwell Paule. Each of you have tempered me in invaluable ways.

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Finally, thanks to the three pieces of my heart who are my greatest cheerleaders. Ayaan, my secret-keeper, who understands me - where I come from, how I got here - better than anyone else. Habibi, who chooses every day to be my human (even when it means listening to me rambling about statistical modelling), and who inspires me to take life less seriously. And Mom, who taught me to question authority and be unapologetically myself: I owe you everything.

INFLAMMATION AS A CONTRIBUTOR TO DEPRESSION IN PEOPLE LIVING WITH HIV

People with HIV are at significantly higher risk for depression, but possible neurobiological mechanisms underlying this risk remain unclear. Inflammation may play a role in the pathogenesis of a subtype of depression, since some people with depression have elevated concentrations of biomarkers of inflammation, while anti-inflammatory medications can alleviate symptoms of depression. Given that people with HIV exhibit chronic inflammation, I hypothesised that inflammation may contribute to the higher risk for depressive symptoms in this community.

This thesis makes several theoretical contributions to this area of research. Using a narrative literature review, I showed that HIV, (neuro)inflammation, and depression are independently linked, but few studies have investigated the relationship between all three conditions. Then, using a scoping review of 33 studies, I showed that significant associations have previously been reported between key inflammatory cytokines (IL-6 and TNF- α) and depressive symptoms in people with HIV. The majority (70%) of these studies were carried out in the US, whereas the majority of people with HIV are located in eastern and southern Africa. Thus, there is an urgent need to diversify participant samples in this field. I further explored the need for authentic equity and inclusion in this field by demonstrating that research on HIV and co-morbidities, including mental health issues, is mostly carried out in the Global North with non-diverse participant samples. Finally, as my work is situated within the broader context of HIV research, I carried out a research priority setting workshop with young adults with HIV and their caregivers, the outcomes of which may inform future research design and ensure that community priorities are acknowledged and centred in this field.

To test the central hypothesis of my research, I first investigated whether inflammatory biomarkers in the brain, blood plasma, and cerebrospinal fluid mediated the association between HIV status and depressive symptoms. In a sample of $N = 204$ older adults in the UK and the Netherlands, I showed that participants with HIV were at greater risk for depressive symptoms (odds ratio [95% confidence interval] 3.27 [1.46, 8.09]), and that controlling for the effects of four biomarkers resulted in a reduction of at least 10% in this odds ratio: MIG (-15.0%) and TNF- α (-11.4%) in plasma and MIP1- α (-21.0%) and IL-6 (-18.0%) in cerebrospinal fluid. Thus, these cytokines and chemokines were found to be potential mediators of the association between HIV status and depressive symptoms in people with HIV.

I then investigated this relationship using inflammatory biomarkers measured in the brain (using magnetic resonance spectroscopy) and blood serum (using immunoassays) in a demographically distinct sample of $N = 60$ young people in South Africa. I observed that young people with HIV showed greater depressive symptom severity ($B = 3.32, p = 0.022$) and

altered concentrations of certain inflammatory biomarkers (higher choline in the basal ganglia, higher MCP-1 and higher YKL-40 in blood serum, and lower IL-1 β in blood serum) compared to young people without HIV. However, none of these biomarkers mediated a significant proportion of the association between HIV status and depressive symptoms in this sample (all $\beta < |0.09|$, $p_{FDR} > 0.05$).

Finally, I explored the relationship between the severity of depressive symptoms and the intracellular diffusion of neurometabolites, measured using diffusion-weighted magnetic resonance spectroscopy, in $N = 20$ virally suppressed adults with HIV in the UK, $n = 11$ of whom were living with severe and persistent depressive symptoms (PHQ-9 score ≥ 15). Depressive symptom severity in these participants was significantly correlated with the diffusivity of creatine ($\rho = 0.48$, $p = 0.034$), though this significance did not survive correction for multiple comparisons ($p_{FDR} = 0.135$). Creatine is a neurometabolite involved in energy metabolism, whose expression is higher in glial cells and shown to be upregulated concurrently with neuroinflammation in animal models. Recent studies have also shown increased creatine diffusion in patients with multiple sclerosis and neuropsychiatric lupus, both conditions characterised by neuroinflammation. Findings from this study thus suggest that severe depressive symptoms in people with HIV may be linked to hypermetabolism and neuroinflammation.

This research offers early, but promising, evidence that inflammation may contribute significantly to depressive symptoms in people with HIV. Substantial further work is necessary to confirm these findings in well-powered and diverse cohorts, as well as to determine the direction and temporality of these relationships through longitudinal studies. Further characterising the relationship between inflammation and depression in people with HIV may pave the way for individualised anti-inflammatory interventions to reduce the burden of depression in this community.

Non-Technical Summary

INFLAMMATION AS A CONTRIBUTOR TO DEPRESSION IN PEOPLE LIVING WITH HIV

People with HIV are at greater risk for depression than people without HIV. People with HIV also show more inflammation (activation of the immune response) in the brain and the body. Inflammation is linked to greater risk for depression in the general population. My goal was to test whether greater levels of inflammation may be driving the greater risk for depression in people with HIV.

First, I systematically searched databases of scientific research and found 33 studies which tested whether certain markers of inflammation were linked to symptoms of depression in people with HIV. In these studies, the proteins IL-6 and TNF- α , which are well known to be involved in inflammation, were often (but not always) reported to be linked to depression in people with HIV. I also found that most studies in this field had been carried out in the US. We need to increase the diversity of research in this field to accurately understand the relationship between inflammation and depression in people with HIV.

Next, I analysed data from 125 people with HIV and 79 people without HIV from London and Amsterdam. I found that people with HIV in this study were at greater risk for experiencing symptoms of depression than people without HIV, even when accounting for the possible effects of sociodemographic factors such as age, sex, ethnicity, and years of education received. When calculating this risk for depression, if I also removed the effects of certain markers of inflammation measured in blood and cerebrospinal fluid, participants' risk for depression was decreased by at least 10%. This tells us that these inflammatory markers may be contributing to the risk for depression.

I then tested this relationship in a group of 42 young people with HIV and 18 young people without HIV in Cape Town, South Africa. Young people with HIV in this group were more likely to report symptoms of depression, even when accounting for the possible effects of age and gender, and also had different concentrations of some inflammatory markers on average than young people without HIV. However, concentrations of these markers were not linked to the severity of people's depressive symptoms. Therefore, I did not find evidence that markers of inflammation significantly contributed to the increased risk for depressive symptoms in young people with HIV.

Finally, I explored whether people with HIV who develop severe depression may show any changes in their brain chemistry. In this study, 20 adults with HIV in the UK (11 of whom were living with severe and persistent symptoms of depression) went through a type of brain scan, called diffusion-weighted MRS, which can estimate the rate of movement of molecules in the brain. I found that the severity of symptoms of depression in these participants was linked to the rate of movement of a molecule called creatine in one region of their brain. Creatine is thought to be involved in energy consumption in the brain, which may increase when

the brain's immune cells are activated. Higher movement of creatine has also been found in people with conditions such as multiple sclerosis that involve neuroinflammation, so creatine movement may be considered a marker of neuroinflammation. Thus, the results from this study suggest that severe depression in people with HIV may be connected to increased energy consumption and inflammation in the brain.

My research has found early, but promising, evidence that inflammation may play a role in depression in people with HIV. However, more research which involves large and diverse groups of people with HIV is needed to fully understand these relationships. If further research continues to show that inflammation drives the risk for depression in people with HIV, we may be able to treat depression in this community using interventions which reduce inflammation.

Statement of Work

The majority of research contained in this thesis was carried out collaboratively. My contributions to the work contained in each chapter, and the contributions of others, are thus delineated below.

Chapter 1: Introduction

I wrote this chapter as sole author. The first paragraph of this chapter was also used in the introduction to the GOLD Depression Study publication (Chapter 9).

Chapter 2: Conceptual Framework

I carried out the literature search and wrote and revised the article. Co-authors critically reviewed and revised the article.

Chapter 3: Biomarkers of Depressive Symptoms in People with HIV

I carried out the literature search, study screening, and data analysis, and wrote and revised the article as sole author.

Chapter 4: Equity in HIV Mental Health Research

I wrote and revised the article as sole author.

Chapter 5: Research Priorities of Young People Living with HIV

I designed and organised the priority setting workshop, trained the facilitators, and co-facilitated the parents and caregivers' group discussions. Co-author TB recruited attendees for the workshop and co-facilitated the parents and caregivers' discussions; other co-authors facilitated the young people's workshop. I wrote and revised the article with input from co-authors.

Chapter 6: Aims & Methodology

I wrote this chapter as sole author.

Chapter 7: COBRA-DI

I designed the sub-study and statistical approach, with input from co-authors JHV, NB, and CAS. I did not carry out data collection or data analysis for this study myself due to data sharing restrictions. Data had been collected previously for the COBRA cohort by co-authors. Co-authors NB and CAS carried out the statistical analyses. I wrote and revised the article with input from co-authors.

Chapter 8: isiXhosa Translation of the PHQ-9

I designed the study and pre-registered it with co-author BL. I led the transcultural translation process at every stage, obtained regulatory approvals, set up the validation study, trained research staff, and carried out statistical analyses. Co-authors recruited participants and collected the data. I wrote and revised the Stage 1 and Stage 2 articles with input from co-authors.

Chapter 9: GOLD Depression Study

I designed the study with co-authors BL and EM. I obtained regulatory approvals, set up the study, trained research staff, analysed blood samples using ELISA and Luminex assays (with co-author SN) to generate blood biomarker data, and carried out statistical analyses.

Co-authors recruited participants, collected the data, and analysed neuroimaging scans to generate brain biomarker data. I wrote and revised the article with input from co-authors.

Chapter 10: INSIDE-HIV

I designed the study with co-authors JHV and IR. Co-author JHV and I obtained regulatory approvals, set up the study, and trained research staff. Co-authors recruited participants, collected the data, and analysed neuroimaging scans to generate brain biomarker data. I processed blood samples using Luminex assays to generate blood biomarker data and carried out statistical analyses. I wrote and revised the draft article with input from co-authors.

Chapter 11: Discussion

I wrote this chapter as sole author.

Publications, Positions, and Awards

RESEARCH POSITIONS

My doctoral research was interdisciplinary and cross-border in nature. To achieve my research objectives, I sought and sustained collaborations with research groups in several institutions beyond the University of Edinburgh. My work with these research groups resulted in two honorary research appointments during my PhD:

- **Research Fellow (Honorary)**, 2023-2025: Family Centre for Research with Ubuntu (FAMCRU), Department of Paediatrics & Child Health, Stellenbosch University, Cape Town, South Africa
- **Research Fellow (Visiting)**, 2021-2024: Department of Global Health and Infection, Brighton & Sussex Medical School, Brighton, UK

AWARDS

Following is an abridged list of awards presented to me during the course of my PhD, and which were in direct relation to my PhD research:

- Best research presentation award at the UK HIV & CNS Meeting in 2024, for a presentation of the INSIDE-HIV study (Chapter 10) - London, UK
- Winner of the 3 Minute Thesis competition for the University of Edinburgh in 2023, for a three-minute pitch summarising my doctoral research - Edinburgh, UK
- Honorable Mention in the General Audience category of the International Neuroethics Essay competition in 2023, for an essay titled “A blood test to predict depression” - Baltimore, USA
- ALBA-FKNE Diversity Prize in 2023, awarded annually to a scientist based in Europe “that has made outstanding contributions to promoting equality and diversity in the brain sciences”, for initiatives including the development of an isiXhosa translation of the PHQ-9 (Chapter 8) and inclusive demographic questionnaires for clinical research in English and isiXhosa (Appendix C) - Algarve, Portugal
- Poster presentation award at the Southern African Neuroscience Society (SANS) Symposium in 2023, for a presentation of the isiXhosa PHQ-9 validation study (Chapter 8) - Johannesburg, South Africa

- New Investigator Scholarship in the field of HIV from the Conference on Retroviruses and Opportunistic Infections (CROI) in 2022, for an abstract based on the COBRA-DI study (Chapter 7) - Seattle, USA

LIST OF PUBLICATIONS

Some work included in this thesis has been published in peer-reviewed journals in the form of singly- or jointly-authored publications.

Chapter 2: Conceptual Framework

Citation: Mudra Rakshasa-Loots, A., Whalley, H.C., Vera, J.H., & Cox, S.R. (2022). Neuroinflammation in HIV-associated depression: evidence and future perspectives. *Molecular Psychiatry* 27, pages 3619–3632.

DOI: [10.1038/s41380-022-01619-2](https://doi.org/10.1038/s41380-022-01619-2).

Author contributions: AMRL conceptualised the review, performed the literature search, and wrote the manuscript. HCW, JHV, and SRC critically reviewed the manuscript.

Chapter 3: Biomarkers of Depressive Symptoms in People with HIV

Citation: Mudra Rakshasa-Loots, A. (2023). Depression and HIV: a scoping review in search of neuroimmune biomarkers. *Brain Communications*.

DOI: [10.1093/braincomms/fcad231](https://doi.org/10.1093/braincomms/fcad231).

Author contributions: AMRL conceptualised the review, performed the systematic search, and wrote the article.

Chapter 4: Equity in HIV Mental Health Research

Citation: Mudra Rakshasa-Loots, A. (2022). Equity in HIV mental health research: a call to action. *Molecular Psychiatry* 27, pages 4824–4828.

DOI: [10.1038/s41380-022-01748-8](https://doi.org/10.1038/s41380-022-01748-8).

Author contributions: AMRL conceptualised this article and wrote the manuscript.

Chapter 5: Research Priorities of Young People Living with HIV

Citation: Mudra Rakshasa-Loots, A., van Wyhe, K. S., Naidoo, S., Daizana, N., Laughton, B., & Boyana, T. (2023). Taking lead from the community: What do young people living with HIV want us to research?. *PLOS Global Public Health*, 3(12), e0002605.

DOI: [10.1371/journal.pgph.0002605](https://doi.org/10.1371/journal.pgph.0002605).

Author contributions: AMRL, KvW, SN, ND, and TB organised and facilitated the workshop, with supervision and support from BL. All authors critically reviewed this manuscript.

Chapter 7: COBRA-DI

Citation: Mudra Rakshasa-Loots, A., Bakewell, N., Sharp, D. J., Gisslén, M., Zetterberg,

H., Alagaratnam, J., Wit, F.W.N.M., Kootstra, N.A., Winston, A., Reiss, P., Sabin, C.A., & Vera, J.H., on behalf of the COMorBidity in Relation to AIDS (COBRA) cohort. (2023). Biomarkers of central and peripheral inflammation mediate the association between HIV and depressive symptoms. *Translational Psychiatry*, 13(1), 190.

DOI: [10.1038/s41398-023-02489-0](https://doi.org/10.1038/s41398-023-02489-0).

Author contributions: AMRL conceptualised this sub-study and wrote the manuscript with supervision from JHV. NB carried out the statistical analyses, with supervision from CAS. AMRL, JHV, NB, and CAS designed and revised the statistical analysis plan. All other authors were involved in COBRA study design, data collection, and analysis as described in the acknowledgements. All authors reviewed and approved the article.

Chapter 8: isiXhosa Translation of the PHQ-9

The Stage 1 Registered Report for this project was previously published in *Frontiers in Psychiatry* (DOI: [10.3389/fpsyt.2022.840912](https://doi.org/10.3389/fpsyt.2022.840912)), and has been provided in Appendix A. Following are the details of the Stage 2 Registered Report included in Chapter 8.

Citation: Mudra Rakshasa-Loots, A., Hamana, T., Fanqa, B., Lindani, F., van Wyhe, K., Kruger, S., & Laughton, B. (2023). isiXhosa translation of the Patient Health Questionnaire (PHQ-9) shows satisfactory psychometric properties for the measurement of depressive symptoms [Stage 2]. *Brain and Neuroscience Advances*, 7, 1-13.

DOI: [10.1177/23982128231194452](https://doi.org/10.1177/23982128231194452).

Author contributions: AMRL and BL conceptualised and designed the study. TH, BF, FL, KvW, and SK carried out informed consent procedures and data collection. AMRL designed the statistical approach and carried out statistical analyses, and wrote the initial draft of the article. BL supervised the design and conduct of the study. All authors critically reviewed and approved this article.

Chapter 9: GOLD Depression Study

Citation: Mudra Rakshasa-Loots, A., Naidoo, S., Hamana, T., Fanqa, B., van Wyhe, K.S., Lindani, F., van der Kouwe, A.J.W., Glashoff, R., Kruger, S., Robertson, F., Cox, S.R., Meintjes, E.M., & Laughton, B. (2024). Multi-modal analysis of inflammation as a potential mediator of depressive symptoms in young people with HIV: the GOLD Depression Study. *PLOS One*, 7, 1-13.

DOI: [10.1371/journal.pone.0298787](https://doi.org/10.1371/journal.pone.0298787).

Author contributions: AMRL and BL designed the study. TH, BF, KvW, FL, and SK carried out informed consent procedures and questionnaire administration. Imaging aspects of the study were designed by AvdK and EM. TH supported acquisition of neuroimaging data, which were processed by FR and EM. AMRL and SN generated blood biomarker data. BL, EM, AvdK, and RG supervised data collection, analysis, and interpretation. AMRL carried out statistical analyses with supervision from SRC. All authors critically reviewed this article.

OTHER PUBLICATIONS

Chapter 10: INSIDE-HIV (SADS Psychometrics)

Published

Citation: Mudra Rakshasa-Loots, A., Diteko, G., & Vera, J.H. (2024). Using the South African Depression Scale (SADS) to measure depressive symptoms in a UK sample. *BMJ Mental Health*, 27, 1-2.

DOI: [10.1136/bmjment-2024-301257](https://doi.org/10.1136/bmjment-2024-301257).

The following articles were also published or accepted for publication during the course of my PhD, but were not directly related to my doctoral research.

Research Article

Published

Citation: Mudra Rakshasa-Loots, A. (2023). Embracing mental health considerations for survivors in gender-based violence inquiries at UK universities. *Journal of Gender-Based Violence*, 7(3), 515-522.

DOI: [10.1332/239868022XI6694836447679](https://doi.org/10.1332/239868022XI6694836447679).

Invited Expert Review

Published

Citation: Mudra Rakshasa-Loots, A., Vera, J.H., & Laughton, B. (2024). Neuroinflammation and mental health outcomes in adolescents living with HIV. *Current Opinion in HIV and AIDS*, 196, 355-360.

DOI: [10.1097/COH.0000000000000877](https://doi.org/10.1097/COH.0000000000000877).

Research Article

Published

Abbreviated Citation: Mudra Rakshasa-Loots, A. (2024). The collective unconscious of Rome and India: Narrative archetypes in the abduction of Proserpina of Ovid's *Metamorphoses* and Sita of the Ramayan. *International Journal of Jungian Studies*,

DOI: [10.1163/19409060-bja10037](https://doi.org/10.1163/19409060-bja10037).

List of Abbreviations

ACC	anterior cingulate cortex
ADC	apparent diffusion coefficient
BAFF	B cell activating factor
BBB	blood-brain barrier
BDI	Beck depression inventory
BDNF	brain-derived neurotrophic factor
BG	basal ganglia
CNS	central nervous system
CRP	C reactive protein
CSF	cerebrospinal fluid
CVL	cervico-vaginal lavage
DCE-MRI	dynamic contrast-enhanced magnetic resonance imaging
DW-MRS	diffusion-weighted magnetic resonance spectroscopy
ELISA	enzyme-linked immunosorbent assay
FDR	false discovery rate
FWM	frontal white matter
G-CSF	granulocyte colony-stimulating factor
GlycA	glycoprotein acetylation marker
HAM-D	Hamilton depression rating scale
HIV	human immunodeficiency virus
HSCL-D	Hopkins symptom checklist for depression
IDO-1	indoleamine 2,3-dioxygenase
IFN-α	interferon alpha
IFN-γ	interferon gamma
IL-1α	interleukin-1 alpha
IL-1β	interleukin-1 beta
IL-6	interleukin-6
IP-10	interferon gamma-induced protein
IRIS	immune reconstitution inflammatory syndrome
Kyn	kynurenine
KynA	kynurenic acid
MCP-1	monocyte chemoattractant protein-1
MCP-4	monocyte chemoattractant protein-4
MFGM	mid-frontal gray matter
MIG	monokine induced by gamma interferon
MIP-1α	macrophage inflammatory protein-1 alpha

MIP-1β	macrophage inflammatory protein-1 beta
MIP-3α	macrophage inflammatory protein-3 alpha
MMP9	matrix metalloproteinase 9
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
NAA	N-acetyl aspartate
PET	positron emission tomography
Phe	phenylalanine
PHQ	patient health questionnaire
PTSD	post-traumatic stress disorder
Quin	quinolinic acid
RANTES	regulated upon activation, normal T cell expressed and presumably secreted
SADS	South African depression scale
sCD14	soluble cluster of differentiation 14
sCD163	soluble cluster of differentiation 163
sCD27	soluble cluster of differentiation 27
sCD40L	soluble cluster of differentiation 40 ligand
sGP130	soluble glycoprotein 130
sIL1RII	soluble interleukin-1 receptor II
sIL2Rα	soluble interleukin-2 receptor alpha
sIL6R	soluble interleukin-6 receptor
SIMOA	single molecular array
sTNFR-I	soluble tumor necrosis factor receptor I
sTNFR-II	soluble tumor necrosis factor receptor II
TGF-β	transforming growth factor beta
TNF-α	tumor necrosis factor alpha
Trp	tryptophan
TSPO	translocator protein 18 kDa
Tyr	tyrosine
uPAR	urokinase plasminogen activator surface receptor
VEGF	vascular endothelial growth factor
VOI	volume of interest
YKL-40	chitinase-3 like-1

1

Introduction

1.1 HIV: A CASE STUDY FOR INFLAMMATORY DEPRESSION

GLOBALLY, 39 MILLION PEOPLE ARE LIVING WITH HIV, of whom the majority are located in the Global South [1]. The burden of depression is three times higher in this community compared to people without HIV [2]. In African countries, where the global majority of people with HIV are located, 3.63 million people with HIV were estimated to have major depressive disorder (MDD), resulting in loss of 1.57 million disability-adjusted life years [3]. The prevalence of suicide deaths amongst people with HIV (10.2/1000 persons) is almost 100 times higher than the global incidence of suicide deaths in the general population (0.11/1000 persons) [4]. Young people (i.e. children and adolescents) with HIV are similarly at elevated risk for depressive symptoms compared to young people without HIV [5, 6]. This risk is greater amongst women and girls, as well as those who experience stigma, food or housing insecurity, or other forms of social deprivation [7, 8]. Depression thus represents a substantial challenge for people with HIV, especially for those who are socioeconomically marginalised.

Inflammation is being recognised as a contributor to depression. People with depression exhibit increased concentrations of markers of inflammation, including cytokines such as interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF- α) [9]. Early-life increases in the inflammatory biomarker IL-6 can predict the total number of depressive episodes later in life, even when adjusting for gender, body mass index, and socioeconomic status [10]. Depressive symptoms may persist despite standard antidepressants, and those with evidence

of elevated inflammatory biomarkers are more likely to be resistant to these treatments [11]. Conversely, recent clinical trials have shown that anti-inflammatory medication can alleviate depressive symptoms, especially for those with elevated inflammation [12–14]. Together, this evidence strongly suggests that a subtype of depression is mediated by inflammation.

HIV offers a potentially impactful “case study” to investigate a possible inflammatory subtype of depression. *In vitro* modelling has shown that microglia – the resident macrophages of the brain – release cytokines such as TNF- α in response to HIV infection, leading to neuroinflammation [15]. People with HIV exhibit chronic inflammation, evidenced by increased cytokine release and monocyte activation, which persists despite antiretroviral therapy (ART) [16, 17]. Given this, it is possible that the greater risk for depressive symptoms amongst people with HIV may be driven, at least in part, by increased inflammation. Determining whether this is the case may enable more effective detection and treatment of depression for millions of people with HIV. Findings from this “case study” in HIV may also extend more widely to depression risk in people with elevated inflammation resulting from other sources, such as autoimmune diseases or early life adversity [18]. Therefore, characterising the contribution of inflammation to depression in people with HIV has the potential to positively impact mental healthcare for many individuals worldwide.

1.2 RESEARCH QUESTION AND APPROACH

The central question for this research was:

Does peripheral and central nervous system inflammation contribute significantly to symptoms of depression in people with HIV?

To investigate this question, I carried out a multi-country portfolio of cross-sectional, observational research studies involving people with HIV. Depressive symptoms were measured using standard validated self-report questionnaires, and inflammation was measured using key biomarkers in blood, cerebrospinal fluid (CSF), and the brain (via neuroimaging). I aimed to determine whether these biomarkers mediated the relationship between HIV status and depressive symptoms. Mediation represents “the process or mechanism by which one variable affects another” in a chain of relationships [19]. I hypothesised that inflammatory biomarkers may be mediating variables which at least partly explain why people with HIV

are at greater risk for experiencing depressive symptoms.

1.3 ROADMAP TO THIS THESIS

This *Introduction* provides a brief overview of the rationale behind my research exploring the contribution of inflammation to depression in people with HIV. *Chapter 2* summarises existing evidence supporting this conceptual framework in greater detail. In this chapter, I reviewed the literature on the relationships between HIV and depression, depression and inflammation, and HIV and inflammation, as well as the more limited body of evidence attempting to connect these three conditions. In *Chapter 3*, I adopted a systematic approach to evidence synthesis to carry out a scoping review of all studies published between 2013-2022 which tested for associations between depressive symptoms and biomarkers of inflammation in people with HIV. In doing so, I identified inflammatory biomarkers which have been frequently associated with depressive symptoms in this community.

I strive to centre issues of research culture, equity and inclusion, and meaningful engagement with people with lived experience in my work. *Chapter 4* presents a brief scientific commentary highlighting the lack of diversity in the field of HIV mental health research, both amongst researchers and research participants, and potential solutions to address these inequities. I implemented several of these suggestions in the studies described within this thesis to better integrate principles of equity and inclusion in this research. *Chapter 5* reports the outcomes of a priority setting workshop hosted in Cape Town. This workshop was attended by young people with HIV and their parents and caregivers, who identified key research priorities which can inform the direction of HIV research in future.

Chapter 6 describes the aims of this research and provides a high-level overview of the participant cohorts and methodologies which I used to achieve these aims. I investigated the central question for this research through three separate observational studies. The first was an analysis of data from the COMorBidity in Relation to AIDS (COBRA) cohort, which included older adults with and without HIV in the UK and the Netherlands. I designed a study leveraging the data on depressive symptoms and inflammatory biomarkers (blood plasma, cerebrospinal fluid, and neuroimaging) available for $N = 204$ participants within the COBRA cohort. Findings from this study, titled **COBRA-DI** (COBRA - Depression and Inflammation), are reported in *Chapter 7*.

Next, I tested these associations between HIV, inflammation, and depression in a demo-

graphically distinct sample in South Africa. When designing this study, I discovered that there was no freely-available version of the nine-item Patient Health Questionnaire (a widely-used tool to measure depressive symptoms) in isiXhosa, which is the first language for over 8 million people in South Africa. I therefore led the development of an isiXhosa translation of the PHQ-9 and validated this translation in a pre-registered pilot study, reported in *Chapter 8*. This translation, alongside the English version of the PHQ-9, was used in the **GOLD Depression Study**, which involved $N = 60$ young people with or without HIV recruited from the Adolescent Cognitive Brain Imaging (GOLD) cohort in Cape Town. This study, reported in *Chapter 9*, used similar measures of inflammation (blood serum and neuroimaging) and depression as COBRA-DI, but a more sophisticated statistical approach was used to directly quantify the mediation effects of inflammatory biomarkers on the association between HIV status and depressive symptoms.

Finally, I explored whether leading-edge neuroimaging techniques could detect differences in brain structure or chemistry between people with HIV who experience severe depression and those who do not. **INSIDE-HIV** (Imaging Severe, Persistent Depression in HIV) was an exploratory study which recruited $n = 11$ people with HIV and severe depressive symptoms and $n = 9$ people with HIV and no or mild depressive symptoms in Brighton, UK. In this study, participants underwent diffusion-weighted magnetic resonance spectroscopy, an emerging neuroimaging technique which was recently shown to be sensitive to experimentally-induced neuroinflammation in humans, as well as assessments of depressive symptoms, blood-brain barrier permeability, and blood biomarkers of inflammation. Results from this exploratory study are reported in *Chapter 10*.

Chapter 11 summarises my findings and highlights the contributions made by this project to knowledge generation in the field of HIV mental health. I also note the limitations and advantages of my research design, methodologies, and statistical approach and offer directions for future research. *Appendix A* includes the Stage 1 Registered Report protocol for the isiXhosa PHQ-9 validation study. *Appendix B* includes the brief report of the psychometric assessment of the South African Depression Scale in a UK-based sample. *Appendix C* provides the self-report questionnaires used in these studies. *Appendix D* provides supplementary materials for the INSIDE-HIV study (*Chapter 10*); supplementary materials for all other chapters (where applicable) are available alongside the corresponding publications.

1.4 A NOTE FOR NON-SPECIALIST READERS

The PhD thesis is generally written for specialist readers with expertise in a given research field. This thesis contains several articles which were published in scientific journals, and thus written in an academic style and assuming relevant expertise and knowledge of scientific terminology. However, I want this thesis to also be accessible to non-specialist readers who are interested in HIV, mental health, or biomedical research more broadly. **At the beginning of each chapter, I have provided a “Highlights” section consisting of a few short bullet points written in non-technical language.** Non-specialist readers of this thesis may use these highlights as summaries of the contents of each chapter. I have also provided below a plain-language glossary of key terms relevant to this research which are used throughout this thesis.

GLOSSARY OF KEY TERMS

Antiretroviral therapy, or ART: Medications which help to reduce the amount of HIV in a person’s body. Effective ART can successfully reduce the amount of HIV in the body to almost zero (although HIV still remains “dormant” in certain organs, including the brain) and help people with HIV to lead full lives.

Biomarker: A biological marker; something that can be measured in a clinical laboratory and which helps in predicting or diagnosing a disease or informing which treatment might be most suitable for it.

Depression: A complex mental health condition which includes symptoms such as low mood, feelings of worthlessness or guilt, changes in sleep and appetite, and (for some people) thoughts of suicide or death.

Global South: A term to describe a broad grouping of countries within Asia, Africa, Central and South America, and Oceania, many of which were formerly colonised and many of which continue to be economically deprived. Its opposite term is *Global North*, which refers loosely to the US, Canada, Europe, and Australia. These terms are similar to the previously used (and now considered outdated) terms “developed nations” (= Global North) and “developing/underdeveloped nations” (= Global South). Note that these terms do not refer to geographical location of these countries.

HIV (Human Immunodeficiency Virus): A virus which is transmitted via bodily fluids (including semen, blood, or breastmilk) and infects certain immune cells of the body. As

a result of HIV infection, a person's immune response is compromised, so that they may become more vulnerable to other infectious diseases. HIV is not the same as AIDS; HIV is a virus, and AIDS is a condition which is caused by HIV infection if it is left untreated over the course of several years.

Inflammation: Activation of the body's immune response; can include fever (in response to an infection) or a rash (in response to an allergy), but also includes many cellular processes which are not visible on the surface.

Mediation: A process by which X influences Y *via* a third factor M; changes in X cause a change in M, which then leads to change in Y. For instance, growing up in a wealthy neighbourhood might allow you to get higher-quality education, which then leads you to getting a better-paying job. In this case, the effect of your childhood neighbourhood on your income later in life is *mediated* by the quality of your education.

Neuroimaging: Brain scans; a set of techniques which allow a glimpse at brain structure or function without surgery; common neuroimaging techniques which readers may be familiar with include CT (which involves X-rays), MRI (which involves magnetic fields), and PET (which involves a radioactive molecule).

Neuroinflammation: Activation of the immune response in the brain or the spinal cord; also known as central inflammation.

2

Conceptual Framework

HIGHLIGHTS

- We can measure inflammation in humans indirectly, using levels of certain proteins in blood or cerebrospinal fluid (the liquid that flows around the brain and spinal cord) or using certain brain scanning techniques.
- People with HIV show more inflammation in the brain and the body, are and are at greater risk for depression, than people without HIV. Increased inflammation is linked to greater risk for depression in the general population, but this relationship has not been tested widely in people with HIV.
- In this article, I summarised some recent studies which have shown that greater levels of certain biomarkers of inflammation are linked to greater symptoms of depression in people with HIV.
- I suggested some potential research questions related to the role of inflammation in depression amongst people with HIV, such as figuring out whether people with HIV experience a subtype of depression linked to any particular biomarkers of inflammation. I also recommended exploring these questions across diverse populations.

2.1 INTRODUCTION

THE CENTRAL NERVOUS SYSTEM (CNS) IS ONE OF THE KEY RESERVOIRS for latent HIV infection [20]. Microglia and astrocytes, which are involved in the neuroinflammatory response, serve as persistent HIV reservoirs [21–23]. Chronic HIV infection in the CNS results in neuroinflammation, despite suppressive ART [17]. In turn, peripheral and central inflammation are associated with depression [24, 25]. Therefore, the increased risk for depression amongst people with HIV may be driven by increased inflammation.

In this literature review, I sought to summarise evidence which separately links HIV, depression, and neuroinflammation. A similar literature review had previously focused on connections between HIV, depression, and circulating markers of peripheral inflammation [26]. This article was intended to focus specifically on evidence of neuroinflammation as a contributor to depression in people with HIV.

2.2 LITERATURE REVIEW: *MOLECULAR PSYCHIATRY*

REVIEW ARTICLE OPEN



Neuroinflammation in HIV-associated depression: evidence and future perspectives

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People living with HIV face a high risk of mental illness, especially depression. We do not yet know the precise neurobiological mechanisms underlying HIV-associated depression. Depression severity in the general population has been linked to acute and chronic markers of systemic inflammation. Given the associations between depression and peripheral inflammation, and since HIV infection in the brain elicits a neuroinflammatory response, it is possible that neuroinflammation contributes to the high prevalence of depression amongst people living with HIV. The purpose of this review was to synthesise existing evidence for associations between inflammation, depression, and HIV. While there is strong evidence for independent associations between these three conditions, few preclinical or clinical studies have attempted to characterise their interrelationship, representing a major gap in the literature. This review identifies key areas of debate in the field and offers perspectives for future investigations of the pathophysiology of HIV-associated depression. Reproducing findings across diverse populations will be crucial in obtaining robust and generalisable results to elucidate the precise role of neuroinflammation in this pathophysiology.

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INTRODUCTION

We are living in a global moment of transition, as AIDS-related mortality declines globally and HIV becomes a manageable chronic infection. Due to advancements in combination antiretroviral therapy (cART), the life expectancy of people living with HIV has begun to approach that of the general population [1, 2], although considerable variability remains among key population subgroups [3, 4]. Of the 1.5 million people that were diagnosed with HIV in 2020, many can expect to lead a full life [5].

There are notable clinical concerns in the management of chronic HIV infection, chief among which is the high risk for a range of mental health issues and cognitive deficits [6, 7]. Long-term HIV infection leads to neurocognitive impairment beyond that which is associated with healthy ageing [8], and distinct from that seen in neurodegenerative disorders [9], which persists in mild forms despite cART [10]. Young people living with HIV, including perinatally infected children, are also vulnerable to these cognitive deficits [11].

People living with HIV also face an alarmingly high prevalence of depression compared to the general population, with 30–50% of HIV+ participants reporting depressive symptoms in some studies [12, 13]. The risk of depression in people living with HIV is estimated to be twice that of the general population [14]. Suicide ideation is also substantially higher in people living with HIV, consistent with the observed rates of depression [15, 16]. Therefore, depressive symptomatology is a widespread and serious concern among people living with HIV.

We do not yet know the precise neurobiological mechanisms underlying HIV-associated depression. Some theories suggest that

deficits in serotonergic [17] or dopaminergic [18] pathways might explain this risk of depression. Others argue that HIV-associated depression may be a consequence of inflammatory responses [19]. Inflammation, or activation of the body's immune responses, has been correlated with depression in the general population [20, 21]. This theory is supported by overlapping somatic features such as sleep disturbance and lethargy (collectively termed “sickness behaviour”) that are shared between inflammation and depression [22]. There is growing support for an inflammatory subtype of depression, since some people with depression experience alleviation of symptoms when administered anti-inflammatory medication [23]. Additionally, there is some evidence to suggest that neuroinflammation—a collection of immune responses in the central nervous system, characterised by microglial activation and release of inflammatory cytokines—might be involved in the aetiology of depression [24]. Given the associations between depression and inflammation, and since HIV infection in the brain elicits a neuroinflammatory response [25], it may be argued that neuroinflammation contributes to HIV-associated depression. However, literature in this field is extremely limited and few studies have attempted to elucidate the relationship between neuroinflammation, depression, and HIV.

The objective of this review is to synthesise and critically appraise existing evidence which suggests that neuroinflammation may be a significant contributor to the high risk of depression amongst people living with HIV. We begin with a brief discussion of the strengths and limitations of various techniques currently in use to measure neuroinflammation in humans. We then describe evidence in the literature that points to independent relationships

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between HIV, depression, and inflammation, and discuss recent preclinical and clinical studies that offer preliminary evidence for an association between neuroinflammation and depression in HIV. Finally, we propose future directions for research into the contribution of neuroinflammation in HIV-associated depression.

MEASURING NEUROINFLAMMATION IN HUMANS

In order to identify the potential contribution of neuroinflammation in HIV-associated depression, we must first be able to accurately assess degree of neuroinflammation in humans. The most direct way to achieve this would be to assess features such as level of activation of resident immune cells or concentration of neuroinflammatory cytokines in brain tissue using brain biopsies or histopathology [26]. However, brain biopsies are highly invasive and generally cannot be justified in research studies, whereas histopathological analyses in humans are limited by availability of post-mortem brain tissue samples [27, 28]. As a result, to assess neuroinflammation in vivo, indirect markers are generally used. Methodologies for in vivo measurement of neuroinflammation have been extensively reviewed elsewhere [29], and we summarise the most widespread techniques here to aid in the consequent discussion of neuroinflammation in HIV-associated depression.

CSF and blood biomarkers

In the absence of brain tissue to directly assess glial cell activation or cytokine elevations, cerebrospinal fluid (CSF) and blood are useful tissue samples for indirect measurement of neuroinflammation. Metabolic markers in CSF have been used as an indication of neuroinflammation, since CSF has a direct interface with the brain [30]. Inflammatory cytokines such as interleukin-15 (IL-15) and monocyte chemoattractant protein-1 (MCP-1) in CSF may in fact be useful to characterise neuroinflammatory disease progression [31, 32]. However, there is considerable variability in reported CSF biomarker concentrations even within specific neurodegenerative conditions [33], and CSF biomarkers may not accurately reflect the state of cells in the brain. In addition, lumbar puncture to obtain CSF samples is quite an invasive procedure and may deter participation in studies.

Blood assays are less invasive than CSF assays, but also represent a less direct measure of inflammatory processes in the brain as there is limited exchange of molecules across the blood–brain barrier (BBB). C-Reactive Protein (CRP) is a widely-studied inflammatory cytokine whose serum concentrations have been correlated with depression severity [21]. The cytoskeletal protein neurofilament light (NFL), which is released into CSF and blood as a consequence of neuroaxonal injury, has diagnostic value in many neurological diseases [34]. In addition to being a marker of neuroaxonal injury and neurodegeneration, NFL may be a promising proxy measure of neuroinflammation as well [35–37]. Recent studies suggest that NFL levels in both CSF and plasma correlate with levels of commonly-used biomarkers of neuroinflammation such as CXCL10 and neopterin [38]. Plasma NFL is also correlated with the kynurenine-to-tryptophan ratio and kynurenine metabolites, which are both traditional measures of neuroinflammation. Finally, there are correlations between CSF and serum concentrations of both CRP and NFL [39–41], indicating that blood assays may be a preferable method of measuring inflammation to avoid highly invasive and painful procedures for participants. Taken together, these findings indicate that NFL in blood serum or plasma may be used as an indirect biomarker of neuroinflammation.

In addition to serum levels of inflammatory markers such as CRP (which represent acute inflammation), recent advancements have also allowed us to probe DNA methylation signatures of many markers of peripheral inflammation [42, 43]. These epigenetic signatures represent more stable, longitudinal measures of chronic inflammatory markers and have been calculated using

data from epigenome-wide association studies (EWAS) for cytokines such as CRP and BDNF [21, 44–46]. However, DNA methylation signatures for NFL, which has only recently been recognised as a proxy measure of neuroinflammation, have not yet been studied. Calculating methylomic signatures for NFL and studying these in large cohort studies could thus be a meaningful opportunity to indirectly explore aspects of chronic neuroinflammation in humans.

Neuroimaging biomarkers

Neuroimaging techniques offer a more indirect methodology for assessment of neuroinflammation in human participants than blood or CSF assays [47]. The two most widely-used neuroimaging techniques to measure neuroinflammation are positron emission tomography (PET) and magnetic resonance spectroscopy (MRS). PET involves administration of a radioligand designed to bind to a target protein and emit radioactivity, which may be used to quantify a physiological function [48, 49]. In the context of neuroinflammation, this physiological function of interest is microglial activation, which is assessed using PET radioligands that bind specifically to the mitochondrial 18 kDa translocator protein (TSPO) [50, 51]. TSPO binding is considered a measure of microglial activation and is altered in various psychopathologies [52, 53] but not in healthy ageing [54], providing a sensitive biomarker that can be used to distinguish healthy ageing from neuropsychiatric and neurodegenerative disorders. Numerous TSPO-targeted PET radioligands have been developed in recent years, and the choice of radioligand when assessing neuroinflammation may introduce an additional confounding variable and limit comparisons across studies [55, 56]. Moreover, as PET involves injection of a radioactive tracer, use of this methodology to study neuroinflammation can be ethically contentious, especially for children or participants who are seriously ill [57, 58].

^{99m}Tc -tilmanocept is another neuroimaging radiotracer which may possibly be used to measure neuroinflammation via imaging. This radiotracer binds to the CD206 receptor, which is constitutively expressed on the surface membrane of microglia and macrophages, and thus may be used as a marker of microglial activation [59]. Early studies suggest that in vivo nuclear imaging of tilmanocept can serve as a measure of BBB dysfunction [60]. Since the use of tilmanocept as a potential imaging biomarker of inflammation is fairly new, the studies that have applied this radiotracer in samples of people living with HIV so far have focused on cardiovascular disease [61, 62]. However, as tilmanocept has the potential to serve as a measure of microglial activation and BBB disruption, it may in future be used to assess neuroinflammation and neuropathology amongst people living with HIV.

MRS allows us to non-invasively identify and quantify metabolites in the brain as an additional indirect measure of neuroinflammation [63]. MRS investigates the behaviour of atoms with non-zero spin under an external magnetic field to determine the chemical composition of a localised brain region [64, 65]. Proton MRS (or ^1H -MRS, targeting hydrogen atoms in brain tissue) is most commonly used in clinical studies to assess neurometabolite concentrations, which are often altered in disease states, including in HIV [66–69]. The neurometabolites myo-inositol (mI) and glutamate/glutamine (Glx) are upregulated in activated glial cells, whereas levels of N-acetyl-aspartate (NAA) and choline-containing metabolites such as glycerophosphocholine and phosphocholine (GPC + PCh) are altered when neuronal membrane integrity is compromised [70–73]. Thus, alterations in mI, Glx, NAA, or GPC + PCh concentrations may be indicative of neuroinflammation. Cerebral metabolite concentrations as measured via MRS also correlate with CSF concentrations of some chemokines amongst people living with HIV, which further supports the use of MRS as a non-invasive tool to measure neuroinflammation in this population [74]. However, due to the ubiquity of hydrogen atoms in the

Table 1. Strengths and limitations of commonly-used techniques to measure neuroinflammation in humans in the context of HIV.

Technique	Example studies	Strengths	Limitations
Neuropathology	Smith et al. [26] Anthony et al. [223]	Cellular and molecular features of neuroinflammation such as microglial ramification or activation may be observed and quantified directly	Limited availability of post-mortem human brain tissue
Cerebrospinal fluid markers	Cheng et al. [224] Guha et al. [225]	Concentrations of inflammatory cytokines circulating in the central nervous system may be determined directly	High variability; lumbar puncture is an invasive and painful procedure
Blood biomarkers	Lyons et al. [226] Wada et al. [227]	Venepuncture for sample collection is a routine procedure; levels of some blood biomarkers are correlated with CSF levels	Less direct measure of CNS inflammation as there is limited exchange of molecules across the blood brain barrier
PET Imaging	Garvey et al. [228] Vera et al. [97]	In vivo measurement of microglial and/or astroglial activation in the brain	Heterogeneity in choice of radioligands and analysis; invasive and with potential risks with injection of radioactive tracers
MRS Imaging	Graham et al. [69] Cysique et al. [229]	Non-invasive, relatively straightforward to administer; can detect changes in a range of neurometabolites in localised brain regions	Low signal-to-noise ratio; requires pre-specific Regions of Interest (ROIs)

Studies employing these techniques to assess neuroinflammation in HIV are included as examples.

brain, MRS struggles with a very low signal-to-noise ratio and requires substantial signal enhancement, and often requires pre-specified Regions of Interest (ROIs) [75].

The advantages and disadvantages of the most commonly-used methodologies to measure neuroinflammation in humans discussed here are summarised in Table 1.

PIECES OF THE PUZZLE

HIV and (neuro)inflammation

HIV is, at its core, an inflammatory condition [76]. When HIV is first detected in the body, a range of innate immune responses are activated to defend the body, including elevations in circulating cytokines and activation of Natural Killer (NK) cells [77]. These systemic changes represent features of HIV-induced inflammation [78, 79]. Since HIV targets CD4+ T cells, which are crucial for mounting an immune response in the human body, the inflammatory response to HIV is paradoxical in that level of immune activation is positively correlated with disease progression [80, 81]. This inflammation predisposes people living with HIV to a variety of physiological effects, such as cardiovascular disease [82], renal function alterations [83], increased gut permeability [84], and clusters of illnesses collectively termed “inflamm-aging” [85]. In addition to physical health effects, the inflammation elicited by chronic HIV infection has also been linked to cognitive impairment [86].

One of the key reservoirs for latent HIV infection is the brain [87], so it is unsurprising that HIV infection also elicits a neuroinflammatory response [88, 89]. Even though HIV cannot directly infect neurons, its viral proteins (especially gp120 and tat [90, 91]) are known to induce neuronal cell death. Even non-replicating, latent proviral HIV DNA can be transcribed and translated into these neurotoxic viral proteins, which trigger inflammatory responses in the brain [92, 93]. Activation of the NLRP3 inflammasome, triggered by HIV infection and leading to the production of pro-inflammatory cytokines IL-1 β and IL-18, is implicated in HIV disease progression and depletion of CD4+ T cells [94, 95]. This inflammasome activation in microglia has been shown to constitute an early and integral aspect of HIV-associated neuropathology, suggesting that HIV induces a neuroinflammatory response that may be detrimental to the central nervous system (CNS) [96].

There are three lines of evidence supporting the theory that HIV infection is linked to neuroinflammation. First, *in vitro* studies indicate that exposure to HIV viral proteins leads to microglial activation, a hallmark feature of neuroinflammation [25]. These findings are corroborated by clinical investigations which found that people living with HIV show global increases in microglial activation compared to negative controls [97]. Second, HIV infection induces changes in neurometabolite concentrations consistent with inflammation in several brain regions [68, 69, 98]. Finally, concentrations of NFL in both blood and CSF have been shown to be elevated in people with HIV [99–101], indicating neuronal damage, and these levels are reduced when participants start receiving cART [102]. Together, these results strongly suggest that HIV induces a neuroinflammatory response in humans.

HIV is a genetically diverse pathogen, with several subtypes and recombinant variants, including Subtype B (most common in Europe and Americas) and Subtype C (prevalent in southern Africa and India) [103]. Subtype C contains a “defective” Tat protein and has thus been hypothesised to be less neuropathogenic than Subtype B [104]. However, recent studies have shown that the (neuro)inflammatory responses elicited by these two subtypes of HIV do not differ significantly, with a wide range of CSF and serum inflammatory chemokines and cytokines being significantly elevated in individuals with Subtype B or Subtype C HIV compared to HIV-negative controls, but with no difference in the biomarker levels between the two subtypes [105]. On the contrary, an *in vitro* study testing inflammation and BBB dysfunction in response to Tat proteins from the HIV Subtype B and recombinant HIV CRF02_AG (prevalent in West Africa) found that Tat.B elicited a greater inflammatory response in comparison to Tat.AG [106]. Together, these results suggest that while Subtype C may not be less neuropathogenic than other subtypes as previously hypothesised, there may still be subtype-dependent variability in neuroinflammatory responses which warrant further investigation.

HIV and depression

Depression has long been known to be the most common neuropsychiatric condition associated with HIV infection [107]. Studies consistently find prevalence rates of about 30% for depression amongst people living with HIV [108], and large-scale meta-analyses estimate that this prevalence rate is twice that in

the general population [14]. Amongst people living with HIV, this risk for depression seems to be somewhat dependent on sex [109], such that women are at higher risk for severe or moderate depression whereas men are at higher risk for moderate depression, and on time passed since diagnosis [110], such that individuals who have been diagnosed more recently are at higher risk for depression. Additionally, prevalence rates vary by geography and are substantially higher in the Global South than in the Global North [111], with estimated rates of 40% or higher for countries such as South Africa [112]. Combined with the fact that healthcare and research funding is concentrated in the Global North [113], this severely exacerbates the burden of depression amongst people living with HIV in the Global South.

The potential mechanisms underlying HIV-associated depression have been extensively reviewed elsewhere [19]. Briefly, possible ways in which HIV might render people susceptible to depression may include neurotoxicity leading to neurotransmitter dysfunction, metabolic dysfunction, and chronic elevations in inflammatory cytokines. Some studies have found that changes in immunometabolism, such as the ratio of kynurenine-to-tryptophan (KYN/TRP), are linked to depression amongst people living with HIV [114]. This is particularly important as ART-induced improvements in depressive symptoms seem to be mediated in part by the reversal of tryptophan catabolism [115]. There are significant overlaps between these various hypothesised mechanisms behind HIV-associated depression, for instance, as inflammatory cytokines may induce glial cell activation and an increase in tryptophan metabolism, which may further cause serotonin depletion and elicit overexpression of metabolites such as quinolinic acid [116]. Therefore, the high risk of depression amongst people living with HIV may stem from a combination of dysfunction in immunometabolism, inflammation, and neurotransmitter cascades.

Interestingly, concentrations of CSF HIV RNA, but not plasma HIV RNA, seem to predict onset of depressive symptoms [117]. This suggests that it may be necessary for HIV to interact directly with aspects of the central nervous system—whether this is metabolism, inflammation, or neurotransmitters—in order to elicit depressive behaviours, and that viral load outside the CNS may not be sufficient to explain HIV-associated depression. However, the directionality of this relationship is unclear: depressive symptoms may conversely increase susceptibility to HIV (by increasing risky behaviours) and are known to predict worse clinical outcomes in people living with HIV [118, 119]. Moreover, this relationship is further complicated when considering that antiretroviral medication may affect metabolism and inflammation [120]. Latest-generation ART, while less neurotoxic, is still associated with metabolic alterations that may contribute to chronic inflammation and immune dysfunction, which ultimately may lead to depression [121]. Conversely, while ART normalises the levels of some inflammatory cytokines in people living with HIV, concentrations of other cytokines such as IL-6 and CRP (which are strongly linked to depression) remain elevated [122, 123]. In all, the relationship between HIV and depression is multi-faceted, but seems to be dependent (at least in part) on the direct interaction of HIV with the CNS and independent of ART-induced normalisations in metabolism or inflammation.

It is important to note here that a purely neurobiological explanation will likely not be able to account for the alarmingly high risk of depression in HIV, as the aetiology of depression is complex and multi-faceted. The role of psychosocial and individual factors cannot be neglected, especially as people living with HIV are likely to experience housing or financial insecurity, discrimination on the basis of gender or sexual identity, intimate partner violence, or external and internalised stigma, all of which can contribute to depression [124, 125]. People living with HIV may also receive poor social support and experience social isolation, which may further contribute to increased inflammation

and behavioural deficits [126, 127]. Moreover, some people with depression (including people living with both HIV and depression) do experience symptom relief in response to non-pharmacological interventions such as psychotherapy and social support [128], further indicating that psychosocial factors play a critical role in the progression of HIV-associated depression. Nevertheless, given that depressive symptoms do have strong biological correlates and predict negative clinical outcomes with regards to HIV, investigating the neurobiological pathogenesis of HIV-associated depression is essential in improving quality of life for people living with HIV.

Depression and (neuro)inflammation

Identifying the pathophysiology of depression is crucial to designing effective, targeted therapeutic interventions for treatment. For several decades, the prevailing theory in biological psychiatry was that depression stems from a dysfunction in serotonergic pathways [129]. This theory was supported by serendipitous clinical findings that pharmacological interventions such as monoamine oxidase inhibitors could alleviate the symptoms of depression in some patients [130]. Dysregulation of serotonin and other neurotransmitters has been strongly implicated in depression over the years [131, 132]. However, the neurotransmitter theory of depression struggles with certain key challenges, including that a third of patients do not respond to treatment with antidepressants such as selective serotonin reuptake inhibitors (SSRIs) [133, 134]. Given this, and since there is increasing evidence that depression may in fact be a constellation of symptoms, efforts have been made to classify depression into subtypes of varying aetiology, so that interventions may be precisely targeted to individuals instead of a “one-size-fits-all” approach to antidepressant treatment [135, 136].

Since there is considerable overlap between the somatic features of inflammation and depression, there is rising support for the theory that depression (or at least a subtype of depression) may be associated with inflammation [137]. In particular, behavioural changes such as fatigue, motivational anhedonia, and sleep disturbances (“sickness behaviours”) can be observed in both depression and inflammation [138], and depressive symptoms can be alleviated with anti-inflammatory medication [139]. Significant elevations in concentrations of inflammatory cytokines such as IL-1, IL-6, CRP, and Tumour Necrosis Factor (TNF- α) have consistently been observed in people with depressive symptoms [140–143]. A large meta-analysis also found that inflammation is a significant predictor of depression [144]. CRP, especially, has been the subject of much attention as a peripheral marker of inflammation that seems to be correlated with depression incidence and severity [41, 145]. Associations between CRP levels and depression remain significant even after controlling for some clinical and psychosocial factors [146], and elevations in CRP levels predict decreased functional connectivity in key brain regions [147]. While the precise nature of this association remains unknown, it is theorised that this may be a bidirectional relationship [148]. Evidence from a longitudinal twin-study suggests that different inflammatory cytokines might interact with depression differently: elevations in CRP may perhaps be a consequence of depression, while elevations in IL-6 act as a risk factor for depression [149]. Later studies have linked CRP and IL-6 to specific symptoms of depression and identified a potential causal link between IL-6 and depression [150]. NFL, which has recently gained attention as a useful biomarker of neuronal injury and neuroinflammation, has also been associated with depressive symptom severity [151]. Further genetic and immunological investigation is necessary to determine whether CRP, IL-6, NFL, or other inflammatory cytokines may have a causal relationship with depression.

The gut microbiome also interacts significantly with inflammation and immunometabolism to influence depression incidence

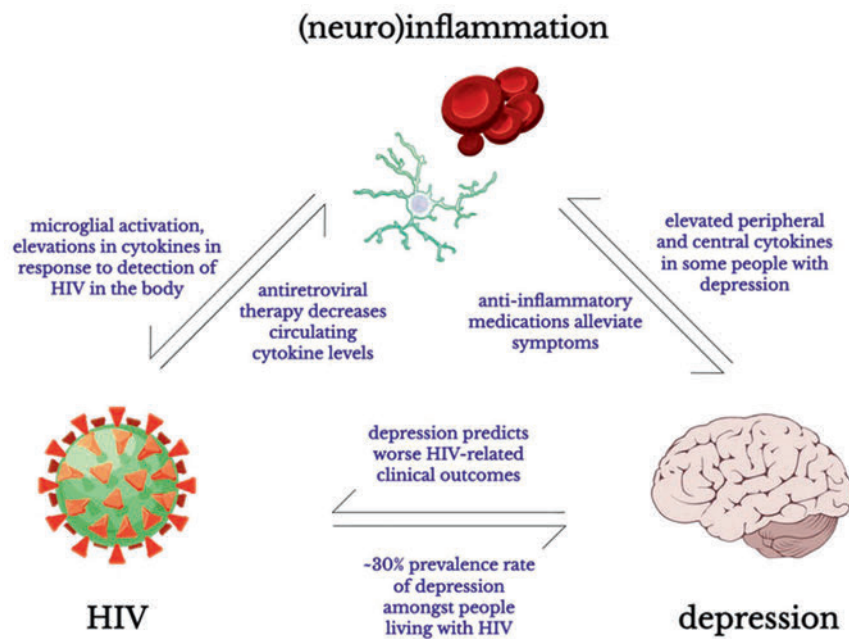


Fig. 1 Schematic representation of the relationship between HIV, depression, and (neuro)inflammation. Key pieces of evidence pointing to independent associations between these conditions are summarised. Open source (CC-BY) schematic drawings via SciDraw. Microglia: <https://doi.org/10.5281/zenodo.3926033>; erythrocytes: <https://doi.org/10.5281/zenodo.3926235>; human brain: <https://doi.org/10.5281/zenodo.3925925>; virus: <https://doi.org/10.5281/zenodo.3926017>.

and severity [152]. Manipulation of the gut microbiome has shown some promise in the treatment of depression, although the mechanisms underlying this “microbiota-gut-brain axis” remain unclear [153]. Recent evidence has shown that gut microbiome dysbiosis is linked to worse psychological well-being amongst people living with HIV and depression in individuals co-infected with HIV and HCV [154, 155]. While further work is necessary, these studies suggest that the gut microbiome may be an important influence in regulating the relationship between HIV and depression.

Strong evidence has been identified in recent years pointing to a link between depression and neuroinflammation [156, 157]. Elevations in neurometabolites such as ml in the brain are associated with depression incidence and severity [158]. CSF concentrations of markers such as TNF- α and IL-1 β are also correlated with depression severity [159, 160], and exogenous induction of inflammation can produce some core features of depression [161]. Conversely, administration of minocycline (an anti-inflammatory drug) attenuates depressive symptoms [162], and animal models indicate that minocycline achieves this by inhibiting microglial activation [163, 164]. Indeed, Richards et al. [165] found that TSPO binding (recall that this is a marker of microglial activation) is highest in patients with depression who are *not* on antidepressant medication, whereas medicated participants show comparable TSPO binding to undepressed controls. These results lend support to the theory that microglial activation (a key feature of neuroinflammation) may be targeted by antidepressant therapies for treatment-resistant depression [24]. Taken together, these findings suggest that inflammation, and neuroinflammation specifically, may play a critical role in the psychopathology of depression.

PUTTING THE PIECES TOGETHER

A substantial body of evidence has shown that independent associations exist between depression, neuroinflammation, and HIV (Fig. 1). However, very few studies have attempted to explore the relationship between all three conditions. The relationship

between (neuro)inflammation and depression has been described in great detail in an excellent systematic review by Toenders et al. recently [148]. We point readers particularly to Fig. 3 in the review article by Toenders et al. [148] [p.321] which summarises various proposed mechanisms connecting inflammation and depression, such as metabolic dysfunction including leptin and insulin resistance and hypothalamic-pituitary axis hyperactivity. These physiological alterations have been observed with HIV infection [166, 167], and thus the molecular pathways discussed by Toenders et al. may be highly relevant to HIV-associated depression as well. Therefore, we will not discuss specific molecular or physiological pathways connecting HIV, inflammation, and depression here, and will instead focus on highlighting recent studies that have demonstrated preliminary evidence for such an association amongst people living with HIV specifically.

Preclinical and post-mortem evidence

Preclinical studies of neuroinflammation in HIV-associated depression present a unique set of strengths and challenges. On the one hand, features of neuroinflammation such as microgliosis may be directly measured in animals using immunostaining of tissue samples [168] or in vivo imaging through cortical windows [169], which is seldom possible in humans as access to primary human brain tissue is not often feasible. On the other, due to the narrow host range of HIV, animal models of HIV infection cannot accurately replicate the progression of HIV infection in humans [170, 171]. Moreover, although animal models of depression have been developed to exhibit many somatic features of depression [172, 173], it remains difficult to study higher-order cognitive and emotional components of depression (such as loneliness or suicidality) in animals. For these reasons, neuroinflammation in HIV-associated depression has not been widely studied in preclinical models.

Advancements in animal models for HIV-associated depression were reviewed by Barreto et al. who suggested that it may be worthwhile to investigate the role of neuroinflammation in these animal models [174]. Since publication of their review, there have been very few attempts to explore this line of enquiry. Nemeth

et al. [175] tested whether developmental expression of HIV-related proteins in transgenic rats may be linked to depressive-like behaviours and elevations in neuroinflammatory responses. They found that adolescent HIV Tg rats did exhibit depressive-like behaviours and increased concentrations of MCP-1 (a key neuroinflammatory chemokine) in the hippocampus. Treatment with meloxicam (a selective COX-2 inhibitor) successfully reduced *Mcp-1* gene expression in the hippocampus, but failed to rescue depressive-like behavioural deficits in the HIV Tg rats. Their findings suggest that HIV-related proteins can trigger depressive behaviours in rats, but the expression of *Mcp-1* in the hippocampus may not contribute to HIV-associated depression. The same group later found that the expression of HIV-related proteins during development induced anxiety-like behaviours in Tg rats and hyper-ramification of microglia (theorised to be involved in microglial surveillance and phagocytosis of apoptotic cells [176, 177]), but did not test for depressive-like behaviours in this instance [178]. Taken together, their results suggest that the presence of HIV viral proteins is associated with depressive-like behaviours and neuroinflammatory responses in rats, but whether HIV-induced neuroinflammation elicits these depressive-like behaviours remains unclear. Since research in this area is sparse, it is crucial for future studies to explore the role of neuroinflammation (especially biomarkers other than MCP-1) in animal models of HIV-associated depression and clarify whether blocking specific neuroinflammatory pathways may rescue HIV-related depressive-like behaviours.

Few post-mortem studies have specifically investigated the relationship between neuroinflammatory markers and depressive symptomatology in people living with HIV. One autopsy study did not find any pathological features in post-mortem brain tissues of people with HIV that could predict depression [179], whereas another found that ante-mortem plasma levels of the inflammatory biomarker sCD163 predicted post-mortem observations of microglial activation, but only included one participant with depression in their study sample and thus could not test for associations with depression [180].

Clinical studies: peripheral inflammatory biomarkers

Clinical investigations of neuroinflammation in HIV-associated depression are limited. The most closely-related studies look at markers such as CRP that may be more closely linked to peripheral inflammation (instead of neuroinflammation), but these are worthy of discussion as they provide some insights into the kind of relationship we may expect between neuroinflammation, HIV, and depression.

An early study of 23 people living with HIV measured plasma concentrations of several inflammatory cytokines (IL-15, IP-10, IL-12, and G-CSF) and found that these cytokines were significantly elevated in HIV+ individuals with depression compared to HIV+ individuals without depression [119]. Plasma cytokine levels and depressive symptom severity were significantly correlated, suggesting that peripheral inflammatory responses may predict depression severity in people living with HIV. Further studies, including a large multi-centre cohort study of 1,727 participants [181], have also observed this correlation between depression and blood biomarkers of systemic inflammation amongst people living with HIV [182, 183]. This evidence supports the hypothesis that people living with HIV may be susceptible to an inflammatory subtype of depression. Since recent evidence suggests that there is significant overlap between systemic inflammation and neuroinflammation in many disease states (contrary to our previous understanding of the CNS as isolated from the peripheral immune system) [184, 185], these trends may perhaps be reproduced when studying neuroinflammation in HIV-associated depression.

While studies generally agree that cytokines such as IL-6 and TNF- α are correlated with depression, the role of CRP (a cytokine

that generally exhibits robust correlations with depression [141, 186]) specifically in HIV-associated depression is a major area of debate. Poudel-Tandukar et al. [187] saw a linear relationship between serum CRP levels and depression severity, and Memiah et al. [188] also observed significant differences in CRP levels between HIV+ participants with depressive symptoms and those without. However, several other studies have found no significant association between CRP concentrations and depression amongst people living with HIV [189–192]. (Notably, Ellis et al. [192] did not observe such a correlation for MCP-1 either, which lends support to the preclinical findings discussed previously [175].) In fact, these studies saw significant correlations between depression and other inflammatory markers such as IL-6 [189, 192] and TNF- α [190], but not CRP. Perhaps this indicates that the subtype of depression to which people living with HIV are most susceptible is mediated by other inflammatory cytokines, but not CRP? Inflammation is not the only physiological state in which cytokines such as CRP and IL-6 serve a function, which complicates any inferences we may make about their role in HIV-associated depression [193]. Further studies are necessary to resolve this debate and determine what role, if any, is played by CRP in HIV-associated depression, as this understanding may aid in developing translational tools for subtype-specific screening of depression in people living with HIV.

The inflammatory biomarkers assayed in studies of HIV-associated depression vary considerably, which limits our ability to identify strong candidates with diagnostic or therapeutic value. A recent study [194] investigated associations between levels of six cytokines (IL-1 β , eotaxin, IL-15, IL-6, TNF- α , and leptin) and chronic multisite pain (CMP) in a sample of people living with HIV, but also incorporated PHQ-9 scores as a measure of depression in their regression models. They found that only one cytokine—IL-1 β —out of the six analytes tested was significantly associated with CMP, and notably, exploratory analyses showed that accounting for total PHQ-9 score reduced the odds ratio of this model. Depression severity and concentrations of the biomarker IL-1 β may thus be interlinked amongst people living with HIV. Memiah et al. [188] tested whether “mental health symptoms” correlated with five inflammatory biomarkers: CRP, IL-6, IL-18, soluble tumour necrosis factor receptor-I (sTNFR-I) and soluble TNFR-II. While their primary outcome measure was not specifically depression, the “self-reported mental health symptoms” in this study included many key features of depression. The authors reported that CRP and soluble TNFR-II were significantly associated with self-reported mental health issues amongst people living with HIV. Pala et al. [195] similarly sought to identify biomarkers associated with depressive symptoms amongst people living with HIV, and found that IL-6 concentrations and monocytes correlated significantly with a severe subtype of depression in their participant sample. Notably, Memiah et al. did not find a significant association between IL-6 and symptoms of depression, whereas Pala et al. did observe such a correlation. Another study found that depression, but not HIV status, was associated with the release of IL-6 [196] which aligns with the findings of Memiah et al. and suggests that the relationship between IL-6 and depressive symptoms may be independent of HIV status. Taken together, these initial findings implicate several central and peripheral inflammatory markers in depression amongst people living with HIV, but heterogeneity in results limits synthesis across studies.

Clinical studies: neuroinflammatory biomarkers

While most studies discussed in the previous section involve a broad panel of central and peripheral inflammatory biomarkers, few published studies have attempted to expressly investigate a relationship between a specific measure of neuroinflammation and HIV-associated depression. Saloner et al. [197] recently compared levels of blood biomarkers of neuroinflammation between people living with HIV and negative controls. In this

study, the authors examined the associations of CSF dopaminergic biomarkers with depression severity (measured as Beck Depression Inventory [BDI-II] scores) and neuroinflammation (measured as a composite score of CSF concentrations of sCD14, MCP-1, IP-10, and neopterin) in a sample of HIV+ and HIV- participants [197]. Although their primary research question concerned the relationship between depressive symptoms and CSF dopaminergic markers, they did report a statistically non-significant trend between higher BDI-II scores and higher composite CSF neuroinflammation score [p.4] [197]. They also saw a significant correlation between BDI-II scores and CSF IP-10 concentrations specifically, but this statistical significance did not persist after FDR correction. Crucially, no such correlations were observed in the HIV- group, offering preliminary evidence that the relationship between neuroinflammation and depression may be dependent on HIV infection status.

Woods et al. [198] present the most promising results to date that implicate neuroinflammatory markers in depressive symptoms amongst people living with HIV. In this study of older adults with and without HIV, the authors found that plasma concentrations of brain-derived neurotrophic factor (BDNF) were significantly associated with depression, such that lower levels of plasma BDNF were linked to higher levels of depressive symptoms. These findings are encouraging, but isolated: substantial further work is necessary to determine the association between various neuroinflammatory markers and depression severity in HIV, and to utilise other measures of neuroinflammation (such as neuroimaging) in this direction. It will also be crucial to replicate these findings in samples with distinct demographic profiles, as no effort has yet been directed at determining the interactions, if any, of psychosocial factors with neuroinflammation in HIV-associated depression.

THE WAY FORWARD

Clinical studies of neuroinflammation and depression in HIV

Given the strong body of evidence linking neuroinflammation, depression, and HIV, and yet the dearth of studies investigating the relationship between the three, the vital first step towards the future is to conduct clinical studies of neuroinflammation in HIV-associated depression. Through these clinical studies, we may begin to resolve the major areas of debate in the field that have been identified through this critical review:

- Is there a reliable association between peripheral concentrations of CRP and depression amongst people living with HIV?
- Are people living with HIV susceptible to a subtype of depression mediated by specific cytokines (such as IL-6) and not others (CRP), for which we can develop subtype-specific therapeutic interventions?
- Is the relationship between neuroinflammation and depression dependent on HIV infection status, and is there subgroup variability or differences in brain structure and function within this population?

Although research in this field is quite sparse, some efforts are underway. Notably, an ongoing clinical trial recruiting HIV+ participants in Uganda is investigating the potential for group psychotherapy to improve the severity of depression and whether depression is linked to inflammation in this group [199]. Results from this clinical trial will shed further light on the complex interactions between neuroinflammation, depression, and HIV.

A range of research methodologies are available to neurovirologists to measure neuroinflammation in people living with HIV, and these were summarised in this review. We particularly draw the readers' attention to neurofilament light (NFL) as a blood biomarker of great potential in assessing degree of neuroinflammation. Although NFL is a marker of neuroaxonal injury, given

the evidence discussed here which points to strong correlations between NFL and "traditional" biomarkers of neuroinflammation, and since there is considerable overlap between neurodegeneration and neuroinflammation, plasma or serum NFL may in future prove to be a useful indirect measure of neuroinflammation for which sensitive assays are already available. NFL concentrations in blood correlate with CSF levels, indicating a reliable measure of CNS inflammatory responses, and can be measured with an assay that is considerably less painful for participants than lumbar puncture, which may help boost participant willingness to contribute to these studies. Furthermore, calculating methylomic signatures for NFL using EWAS data (as has been done previously for CRP [42, 44]) might allow us to assess chronic elevations in NFL and offer a longitudinal measure of neuroinflammation, provided that we obtain relevant and sufficiently-powered discovery data. These serum and epigenetic NFL markers may allow us to study short- and long-term changes in NFL levels, which, combined with longitudinal data on depression severity and HIV viral loads, may elucidate the relationship between neuroinflammation and depression through the course of chronic HIV infection.

There are considerable differences in methodology, statistical approach, and reporting of effect sizes in the literature in the field. Consider three representative studies of inflammation and cognition amongst people living with HIV. Anderson et al. [102] report a slope of -11.5 for their multivariate mixed-effects model between plasma NFL concentrations and composite cognitive scores, indicating that cognition worsened by 11.5 points for each logarithmic unit increase in plasma NFL. Saloner et al. [183] report an unstandardised regression estimate of -0.025 for their cross-level interaction between plasma CRP and average depression scores on global cognition, which suggests that global cognition worsened by 0.025 points for each unit increase in CRP for participants with a specific average depression score. Imp et al. [200] report a standardised beta weight of -0.30 for their regression model testing associations between plasma sCD163 levels and global cognitive scores, indicating that global cognition worsened by 0.30 points for each unit increase in plasma sCD163 concentration. This heterogeneity in methodology and statistical reporting limits synthesis of results across studies. Variability in reporting effect sizes also restricts our ability to carry out appropriate sample size estimations for future studies. Many studies in the field, especially those involving PET, struggle with small sample sizes due to resource constraints, and accurate power analyses (involving standardised effect sizes) are crucial in ensuring that the conclusions drawn from these studies are placed in the appropriate statistical context. Thus, there is a need to standardise statistical approaches in the field to allow for robust experimental design and meta-analysis of findings.

Therefore, methodological approaches to measuring (neuro) inflammation and reporting of effect sizes in these studies are quite variable. Nevertheless, it is possible to make some estimation of the effect size for this relationship with two principles in mind: (1) our measures of neuroinflammation in humans are indirect and imprecise; and (2) neuroinflammation is only one of several biological and psychosocial factors that may be relevant to HIV and depression. In that light, we would reasonably expect neuroinflammation to have a small contribution to the pathogenesis of HIV-associated depression when correctly estimated. Therefore, going forward, researchers should clearly report standardised estimates of effect sizes and exercise caution when reviewing reports of large effects in this context [201].

Once a link between neuroinflammation and HIV-associated depression has been established through future studies, the exact nature of this relationship may be investigated. It is probable that HIV and depression interact actively with each other and comorbidity of the two conditions would necessitate more concerted effort than treating each condition [202]. If

neuroinflammation is indeed linked with depression in HIV, what is the direction and causality of this relationship? It is possible that HIV elicits a neuroinflammatory response, which then leads to depressive symptoms. However, it is equally likely that an HIV infection (through biological or psychosocial pathways) causes depression, which then leads to neuroinflammation. Which pathway represents the true mechanism underlying this association will impact the development of targeted therapeutics: if HIV-associated neuroinflammation causes depression, then we may use anti-inflammatory medication to treat this depression, but if HIV-associated depression manifests as neuroinflammation, then anti-inflammatory medication might not attenuate depressive symptoms. Therefore, it is crucial to determine the precise relationship between neuroinflammation, HIV, and depression.

Reproducing findings across diverse populations

Some of the incongruity in evidence relating to neuroinflammation, depression and HIV may be attributed to experimental variability, but the role of participant profiles must also be taken into account when interpreting these findings [203]. Social, economic, cultural, and structural factors can confound results and contribute to heterogeneity in findings across studies [204]. Moreover, the burden of depression is not universal, and there is substantial variability in the prevalence of depressive symptoms and accessibility of mental healthcare for certain population subgroups [205, 206]. Finally, replication of findings instills greater confidence in the generalisability of results and aids in effective translation to clinical interventions [207, 208]. For these reasons, future work into the role of neuroinflammation in HIV-associated depression must attempt to replicate findings across diverse populations.

In particular, we recommend that researchers and funding bodies prioritise these investigations in three key populations. First, there is a meaningful opportunity to focus on HIV-associated depression amongst children and adolescents. Not only is there strong evidence for the burden of mental health challenges faced by this population, but exploring neuroinflammation as a specific contributor to HIV-associated depression in longitudinal studies might help us develop distinct neuroinflammatory profiles for healthy development and HIV-induced developmental changes [209, 210]. Indeed, such investigations offer a unique opportunity to determine what effects, if any, perinatal HIV infection has on

neuroinflammation, whether these early neuroinflammatory responses can predict risk for depression later in life, and whether there may be a critical neurodevelopmental window for interventions to minimise long-term depression trajectories [40, 69]. Second, participants who use psychoactive drugs should be included in studies of neuroinflammation and HIV. Drugs such as methamphetamine or cocaine may elicit inflammatory responses in the brain [211], and there is overlap between depression risk, drug use, and HIV [212]. However, well-designed preclinical and clinical experiments may distinguish between HIV-associated neuroinflammation and drug-associated neuroinflammation, while helping us understand the interactions between drug use, HIV, neuroinflammation, and risk for depression. Such studies may improve our ability to screen for and treat depression for HIV+ individuals who use drugs, but this is not possible if people who use drugs continue to be automatically excluded from participating.

Finally, the greatest burden of HIV infection lies in the Global South, where access to latest-generation cART and psychological healthcare is also limited [213, 214]. However, a majority of neurovirology research is carried out in the Global North, with over 80% of research outputs concerning HIV/AIDS being produced by researchers from North America and Europe (González-Alcaide et al. provide an excellent summary of these concerns [215]). These trends are reflected in research specific to HIV-associated depression, as well. We ran a systematic search for all clinical trials in the 11 registries catalogued in the new open source database ScanMedicine using the keywords “HIV+ depression” (<https://www.scanmedicine.com/>) and examined the distribution of country of recruitment for these trials (Fig. 2). 4471 clinical trials met the search criteria. These trials recruited participants across 5619 total recruitment sites, of which 1287 (22.9%) were located in the US. The top five countries by number of registered clinical trials investigating HIV and depression, representing nearly half ($n = 2518$, 44.7%) of all trials, were the US, Germany, the UK, Spain, and France. In fact, only two countries (India and China) outside of North America, Europe, or Australia were in the top 20 countries by number of trials (see Supplementary Materials). This preliminary data is presented here to illustrate the fact that, given the overwhelming concentration of research funding in affluent nations, these studies often involve participant groups whose physical and mental health, socioeconomic



Fig. 2 Global distribution of recruitment sites by country for clinical trials investigating HIV and depression. We ran a search for clinical trials catalogued in 11 registries using the keywords “HIV+ depression” in the open source database ScanMedicine. **A** Map of the world with number of clinical trial recruitment sites for HIV-associated depression. Darker shading represents greater number of recruitment sites in that country. **B** Distribution of recruitment sites in the top 10 countries. The 4471 clinical trials that met the search criteria recruited participants across 5619 total recruitment sites, of which 1287 (22.9%) were in the US. Nearly half ($n = 2518$, 44.7%) of all recruitment sites were located in the US, Germany, the UK, Spain, and France. The global distribution of clinical trials of depression and HIV is skewed, with few studies involving participants from the Global South, where there is a substantial burden of HIV-associated depression. World map data visualisation adapted from: ScanMedicine, NIHR Innovation Observatory (NIHRI), Newcastle University (scanmedicine.com).

backgrounds, and institutional access may not reflect those of the majority of people who bear the burden of HIV-associated depression (for instance, see ref. [216]).

Moreover, the majority of these studies involve HIV+ participants who are virally-suppressed and receiving cART [181, 217]. Only a few - those primarily recruiting participants in the Global South - include ART-naïve participants [189]. Antiretroviral drugs are known to contribute to neuropsychiatric effects such as sleep disturbances and depressive symptoms [218]. This makes it difficult to ascertain whether inflammatory responses or depressive symptoms observed in these studies arose as a result of HIV infection or ART. Studies involving HIV + individuals who are not yet receiving cART will be essential in delineating the individual contributions of HIV itself and of cART to these neuropsychiatric complications. Since it is difficult to recruit ART-naïve participants in the cART era in the Global North, such studies are more feasible in the Global South. For these reasons, investigations of HIV-associated depression involving participant cohorts in the Global South - especially southern Africa, which is fast becoming a hub for HIV/AIDS research [219]—must remain a focus for funding bodies in order to better understand confounding influences on findings, prioritise global health equity, and improve the generalisability of our findings. Involving participants from these underserved research populations using co-production or participatory research methods may also provide insight into which research questions and outcomes should be a focus for future work and ways to engage stakeholders more broadly [220].

Attempting to reproduce findings across diverse populations will allow us to parse out the complex interactions between neurobiological, psychosocial, and institutional factors that give rise to HIV-associated depression [221]. No single mechanism can sufficiently explain the aetiology of HIV-associated depression, but if we are intentional in our efforts to study these phenomena in populations from distinct clinical, socioeconomic, and cultural backgrounds, we may better understand the individual contributions of each of these factors to this overarching burden of depression. By leveraging this understanding to develop targeted mental health interventions, we may be able to reduce transmission of HIV via “positive prevention” [222]. Therefore, by investigating the risk of depression faced by people living with HIV, we may make substantive progress towards the global goal of HIV/AIDS eradication.

CONCLUSION

In this critical review, we synthesised evidence for independent associations between HIV, depression, and (neuro)inflammation. Preliminary preclinical and clinical evidence lends support to the hypothesis that neuroinflammation may play an important role in HIV-associated depression, although data on this relationship is extremely sparse. We also identified some key areas of debate where further work is necessary to reconcile contradictory findings. Future investigations should focus on further characterising this relationship. Studies with robust experimental design that attempt to reproduce findings in key populations must be a priority in order to approach an accurate and generalisable understanding of the contribution of neuroinflammation to HIV-associated depression.

DATA AVAILABILITY

Data from the ScanMedicine clinical trial registry search is available in Supplementary Materials for this paper. No other new data was created or analysed in this project.

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AUTHOR CONTRIBUTIONS

AMRL conceptualised the review, performed the literature search, and wrote the paper. HCW, JHV, and SRC critically reviewed the paper.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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2.3 CONCLUSIONS

In this review, I briefly discussed techniques which allow us to indirectly quantify neuroinflammation in humans, namely neuropathology using human brain tissue, CSF and blood measures, and neuroimaging (positron emission tomography [PET] and magnetic resonance spectroscopy [MRS]). I then synthesised evidence supporting independent links between HIV, depression, and (neuro)inflammation: HIV infection leads to peripheral and CNS inflammation, people with HIV are at increased risk for depression, and inflammation is in turn associated with depression (though the directionality of this relationship remains to be resolved).

Finally, I summarised findings from some recent studies which investigated the associations between inflammatory biomarkers and depression in people with HIV. These studies reported significant associations between depressive symptoms and concentrations of cytokines such as interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF- α) in people with HIV. Saloner *et al.* [27] observed a trend towards higher neuroinflammation (measured in this study as a composite score of CSF concentrations of sCD14, MCP-1, IP-10, and neopterin) correlated with worse depressive symptoms in people with HIV. This study in particular offered early evidence that neuroinflammatory biomarkers may be associated with depressive symptoms in people with HIV.

The aim of this review was to summarise evidence connecting neuroinflammatory biomarkers with depressive symptoms in people with HIV. However, the final version of our published review included discussions of several biomarkers which are implicated in peripheral as well as CNS inflammation (e.g. IL-6 and TNF- α), exclusively involved in peripheral inflammation (e.g. CRP), or markers not of inflammation but of related physiological processes (e.g. BDNF and NFL). This widened focus was, in part, because investigations involving biomarkers of peripheral inflammation and related processes may still provide useful evidence to inform hypotheses about the relationship between neuroinflammation, depression, and HIV. In addition, discussion of these other biomarkers was motivated by peer reviewers. For instance, the inclusion of Woods *et al.*'s 2021 study [28] exploring BDNF and depressive symptoms in people with HIV was directly requested by a peer reviewer, even though BDNF is not a neuroinflammatory biomarker (although its concentration may be regulated by neuroinflammatory biomarkers such as TNF- α [29]) and was thus outside the direct scope of the review. Finally, no studies had previously used brain tissue biopsies or neuroimaging

biomarkers to assess the relationship between neuroinflammation and depression in people with HIV; thus, evidence relating to biomarkers which may more directly indicate neuroinflammation in this context was virtually non-existent. For these reasons, this review included discussions of biomarkers of peripheral inflammation and related processes in addition to neuroinflammatory biomarkers.

This review highlighted certain outstanding questions in the field which I have attempted to address in this thesis. For instance, I found conflicting reports about whether circulating CRP is associated with depressive symptoms in people with HIV. I directly investigated this relationship in COBRA-DI (Chapter 7) and the GOLD Depression Study (Chapter 9). I also highlighted that the majority of studies of HIV and depression are carried out in the Global North, so the GOLD Depression Study (conducted in South Africa) thus adds much-needed geographic diversity to this field. Finally, through INSIDE-HIV (Chapter 10), I report an investigation of depression in people with HIV involving neuroimaging biomarkers of inflammation, thus addressing a gap in the literature identified in this review.

3

Biomarkers of Depressive Symptoms in People with HIV

HIGHLIGHTS

- I systematically searched databases of scientific research to find all research articles published between 2013 and 2022 which tested whether biomarkers of inflammation were linked to symptoms of depression in people with HIV. I found 33 studies which had explored this question, and 55 different biomarkers had been tested across these studies.
- The biomarkers IL-6 and TNF- α , which are well known to be involved in inflammation, were often (but not always) reported to be associated with depression in people with HIV. On the other hand, biomarkers such as MCP-1 and IL-8 (involved in other aspects of inflammation) had been tested in multiple studies but almost never found to be linked to depression.
- I also found that most studies in this field had been carried out in the US, and many of them involved participants who were older men. We need to increase the diversity of people included in research in this field to really understand the relationship between inflammation and depression in people with HIV.
- This review can help other researchers working on similar questions decide on which biomarkers may be most promising for further research.

3.1 INTRODUCTION

IN CHAPTER 2, I QUALITATIVELY SYNTHESISED EVIDENCE supporting the hypothesis that peripheral and CNS inflammation may play a role in HIV-associated depression. I next wanted to summarise existing research on whether individual biomarkers of inflammation (and related physiological processes, such as neurogenesis and neuroimmunometabolism) are associated with depressive symptoms in people with HIV. To do this, I carried out a systematic search to capture all English-language research articles published between 2013 and 2022 which recruited people with HIV and which tested for an association of at least one biomarker of interest with depression or depressive symptoms. Using this search, I aimed to identify biomarkers which have been previously and frequently associated with depressive symptoms in this population.

3.2 SCOPING REVIEW: *BRAIN COMMUNICATIONS*

BRAIN COMMUNICATIONS

REVIEW ARTICLE

Depression and HIV: a scoping review in search of neuroimmune biomarkers

Arish Mudra Rakshasa-Loots^{1,2,3}

People with HIV are at increased risk for depression, though the neurobiological mechanisms underlying this are unclear. In the last decade, there has been a substantial rise in interest in the contribution of (neuro)inflammation to depression, coupled with rapid advancements in the resolution and sensitivity of biomarker assays such as Luminex, single molecular array and newly developed positron emission tomography radioligands. Numerous pre-clinical and clinical studies have recently leveraged these next-generation immunoassays to identify biomarkers that may be associated with HIV and depression (separately), though few studies have explored these biomarkers in co-occurring HIV and depression. Using a systematic search, we detected 33 publications involving a cumulative $N = 10\,590$ participants which tested for associations between depressive symptoms and 55 biomarkers of inflammation and related processes in participants living with HIV. Formal meta-analyses were not possible as statistical reporting in the field was highly variable; future studies must fully report test statistics and effect size estimates. The majority of included studies were carried out in the United States, with samples that were primarily older and primarily men. Substantial further work is necessary to diversify the geographical, age, and sex distribution of samples in the field. This review finds that alterations in concentrations of certain biomarkers of neuroinflammation (interleukin-6, tumour necrosis factor- α , neopterin) may influence the association between HIV and depression. Equally, the chemokines monocyte chemoattractant protein-1 (MCP-1) and interleukin-8 (IL-8) or the metabolic index kynurenine:tryptophan (Kyn:Trp), which have been the focus of several studies, do not appear to be associated with depressive symptoms amongst people living with HIV, as all (MCP-1) or most (IL-8 and Kyn:Trp) available studies of these biomarkers reported non-significant associations. We propose a biomarker-driven hypothesis of the neuroimmunometabolic mechanisms that may precipitate the increased risk of depression among people with HIV. Chronically activated microglia, which trigger key neuroinflammatory cascades shown to be upregulated in people with HIV, may be the central link connecting HIV infection in the central nervous system with depressive symptoms. Findings from this review may inform research design in future studies of HIV-associated depression and enable concerted efforts towards biomarker discovery.

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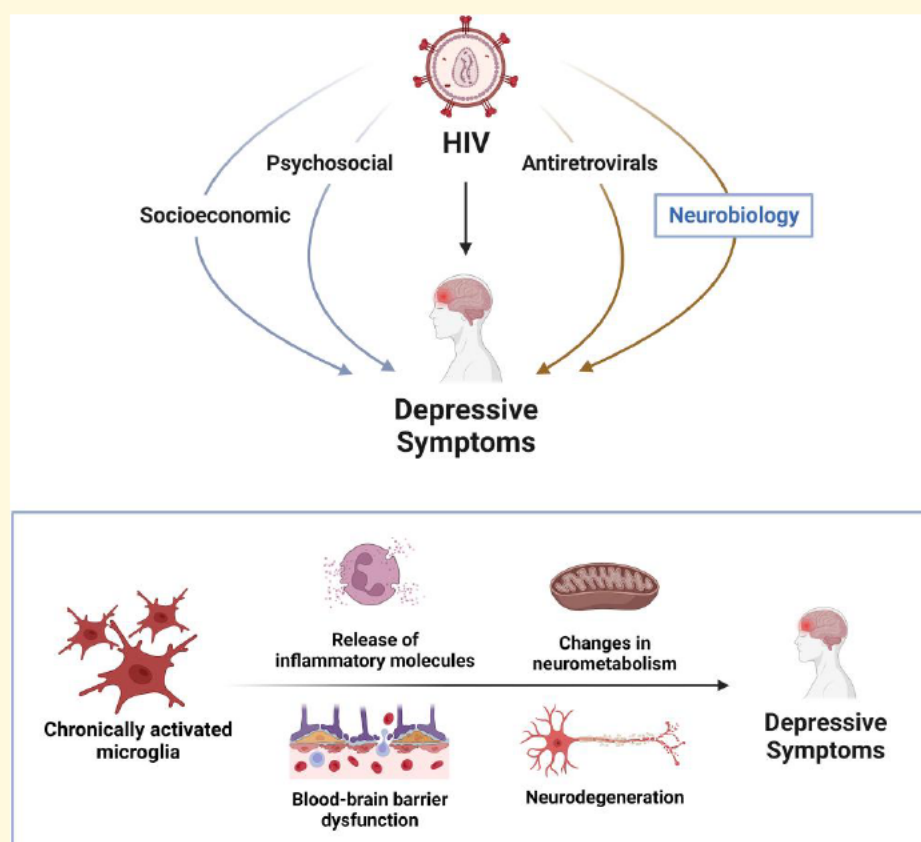
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Graphical Abstract



Neuroinflammation in HIV-associated depression

'No one cares about me, no one cares about my living or death. [...] I cannot count on anyone, alone, lonely ... Not counting how many days, it is in my mind all the time, always thinking about it, always thinking, 'How do I live now?' No housing, everyone spurns me, no money, unstable job, with illness, no one accepts me to work.'¹

People with HIV experience depression at a rate at least twice as high as the general population.^{2,3} As the participant quoted above suggests, HIV status can impact an individual's ability to access social support and employment. In addition, needing regular medical check-ups, adhering to drug regimens (with varied side-effects), and managing comorbid conditions represent heavy personal and economic burdens for many people with HIV. Together with widespread stigma and marginalization, these factors contribute to the high risk for depression amongst people with HIV, especially for women with HIV and those in the Global South.^{4,5} However, as the brain is a key reservoir of HIV infection, it is likely that neurobiological processes also

contribute to this high risk for depression. Identifying the precise mechanisms by which HIV leads to depression is crucial as it may enable us to identify individuals at greatest risk for developing depression, tailor the HIV care continuum, and deliver effective pharmacological or psychosocial interventions.

The last substantial review of the possible neurobiological mechanisms underlying HIV-associated depression was published by Del Guerra and colleagues in 2013.⁶ In this review, the authors hypothesized that HIV infection interacts with neurobiological and psychosocial factors to give rise to depression. Specifically, HIV infection in the brain results in sustained microglial activation, elevations in circulating cytokines, depletion of monoamines, and neurotoxicity (especially in dopaminergic neurons). Cerebral elevations in inflammatory neurometabolites and white matter abnormalities arising due to HIV infection persist despite viral suppression.⁷ These neurobiological consequences of central nervous system (CNS) infection may result in specific components of depression, such as cognitive loss (neuronal death), chronic fatigue (TNF- α elevations), and sickness behaviour (cytokine storms). HIV infection in the CNS may elicit a chronic neuroinflammatory response. Neuroinflammation is, in turn, associated with depressive symptoms in the

general population. Thus, it is possible that neuroinflammation contributes to the increased risk for depressive symptoms among people living with HIV.⁸

In the intervening decade since the publication of Del Guerra *et al.*'s review, there has been continued interest in the contribution of (neuro)inflammation to depression, coupled with rapid advancements in the resolution and sensitivity of biomarker assays. Luminex, single molecular array, and similar next-generation multiplex assays have enabled the quantification of tens of biomarkers from single samples with enhanced sensitivity compared to enzyme-linked immunosorbent assays.^{9,10} These technologies have been leveraged in several recent investigations of biomarkers that may influence the association between HIV and depression.

To translate these findings into clinical practice, we must identify biomarkers that are reliably associated with depressive symptoms in people living with HIV. Depending on the study design and statistical methods, at least three questions may thus be investigated:

1. Is the onset of depressive symptoms in people living with HIV preceded by elevations in concentrations of certain biomarkers (*predictive biomarkers*);
2. Are depressive symptoms associated with certain biomarkers *only* in people living with HIV, and not in demographically comparable people without HIV, thus, helping identify an HIV-specific subtype of depression if one exists (*diagnostic biomarkers*); and
3. Is the severity of depressive symptoms in people living with HIV mediated by concentrations of certain biomarkers; that is, are these biomarkers at least partly mechanistically involved in the pathogenesis of symptoms and can therefore be targeted by antidepressant interventions (*therapeutic targets*).

By answering these questions, we may identify biomarkers that enable early risk stratification, phenotyping, and individualized interventions for depression in people living with HIV. Crucially, the utility of these biomarkers may extend beyond the context of HIV. Many inflammatory insults, including clinical conditions such as rheumatoid arthritis and psychosocial stressors such as early life adversity, result in an increased risk for depression.^{11,12} The connection between neuroinflammation and depression is therefore not unique to HIV. As a result, identifying biomarkers associated with HIV-associated depression may offer the 2-fold advantage of highlighting potential targets to reduce the prevalence of depression amongst people living with HIV (a community that remains globally under-served), and facilitating the translation of new and improved tools for stratification in the treatment of depression (a major global mental health concern).

The biomarkers tested in existing studies of HIV-associated depression are highly variable, in part due to the diversity and customizability of available biomarker panels. Recent reviews have narratively surveyed the literature on the potential role of

inflammatory biomarkers in HIV-associated depression, but no attempt has been made to systematically capture and synthesize these findings.^{8,13} This review aims to address this gap in the literature. Our objective was to synthesize recent evidence on associations between depressive symptoms and biomarkers of neuroinflammation or related biological processes in people living with HIV. Notably, in this scoping exercise, we did not seek to elucidate the mechanisms behind these associations but synthesize the reported associations themselves.

Search methods

Using the Systematic Review Facility (<https://syrf.org.uk/>), we carried out a systematic search aimed at capturing all recent studies of people living with HIV which reported a measure of depression and at least one inflammatory biomarker. PubMed and Web of Science (Core Collection) were searched using detailed search terms, which are available in [Supplementary Files 2–5](#). Studies eligible for inclusion in the review were those which: (i) were conducted in a human cohort including people with HIV, (ii) reported original data, (iii) were in English, (iv) were published between 1 January 2013 and 10 November 2022, (v) measured at least one biomarker of inflammation or related processes (as defined by a pre-determined list), and (vi) tested for an association between a biomarker of interest and depression or depressive symptoms. Studies were excluded if they involved participants with any other co-infections with HIV, such as tuberculosis or meningitis. Studies not reporting original data (such as reviews, meta-analyses, editorials, and case reports), animal studies, *in vitro* studies, and genetic studies were also excluded. The review protocol was not pre-registered. Study screening was carried out by a single reviewer.

In order to assess the clinical utility and strength of evidence for each inflammatory biomarker, available evidence from each included study was scored against the validation criteria in [Table 1](#), modelled on criteria used by Oestreich and O'Sullivan.¹⁴ Data collection was carried out by a single reviewer. We first recorded whether each study reported a significant association between a biomarker of interest and depression or depressive symptoms (+1 score), which was our primary outcome of interest. Notably, we included studies that assessed depression as a clinical diagnosis (using, for instance, structured psychiatric interviews) and those that measured depressive symptoms using screening tools such as the Beck Depression Inventory. Diagnostic or screening tools used in each included study are listed in [Supplementary Table 1](#).

For studies reporting a significant association, we recorded the direction of association (positive/negative) and whether this association was significant only amongst participants living with HIV (+1 score). An HIV status-dependent association would be important for a truly useful predictive biomarker of HIV-associated depression. Although neuroinflammation is linked to depressive symptoms in the general population as well, it may be possible that certain

Table 1 Criteria for validation of inflammatory biomarkers for HIV-associated depression

Grade	Definition	Rationale
0	No statistically significant differences in biomarker concentration between depressed and non-depressed people living with HIV	When assessed directly in a sample of people with HIV, suggests that the biomarker likely is not associated with depression in this population.
1	Biomarker concentration altered with depression amongst people living with HIV	Suggests a role for the biomarker in depression amongst people living with HIV, but whether this effect is HIV-specific cannot be inferred without direct comparisons to people without HIV.
2	Biomarker concentration altered with depression amongst people living with HIV but not among controls	Suggests strongly that the biomarker may be associated with an HIV-specific subtype of depression, thus, potentially a valid inflammatory biomarker of HIV-associated depression.
3	Biomarker concentration is associated with depressive symptom severity among people living with HIV but not amongst controls	As above; correlations with depressive symptom severity may suggest a dose response and thus, greater utility and sensitivity of the biomarker in community-based samples.

inflammatory processes that are heightened during HIV infection (but not other immune challenges) may exacerbate the risk for depressive symptoms amongst people living with HIV. Biomarkers which are found to be associated with depressive symptoms in people living with HIV, but not in those *without* HIV, may thus represent an HIV-specific risk factor for depressive symptoms and thus at least partly explain the greater prevalence of depression in this population.

Finally, for studies that reported a significant association only amongst people living with HIV, we recorded whether there was a reported correlation between the biomarker concentration and a continuous scale of depressive symptom severity (+1 score). Correlations with depressive symptom severity may suggest a dose response and thus, indicate greater utility and sensitivity of the biomarker in community-based samples.

For all studies, we recorded all biomarkers assessed (including those which were reported only as part of a cluster of biomarkers), biofluids in which biomarkers were assessed, total number of participants (*N*), number of participants with HIV, mean [standard deviation (SD)] age of participants, % men in studies, recruitment country, % participants on ART, and % participants reported as virally suppressed. Where variables were reported as medians and interquartile ranges (IQR), means and SDs were imputed using equations (14) and (17) in Wan *et al.*¹⁵ Where variables

were reported only for subgroups, mean (SD) for the full sample was imputed using equations in Table 6.5.a of the Cochrane Handbook.¹⁶ For biomarkers that were assessed in at least five publications, we extracted the type of screening or diagnostic tool used to measure depression or depressive symptoms, type of test statistic, and value of the effect size estimate. Data collection was carried out by a single reviewer.

Search results and discussion

Of 165 records identified from the two databases, three records identified from manual citation searching, and one record identified based on reviewer recommendation, a total of 33 studies (representing 28 unique samples and a cumulative 10 590 participants) were eligible for inclusion in this review (Fig. 1).^{17–49} Key characteristics of participants in these studies are summarised in Fig. 2. Overall, mean (SD) age of participants was 46.6 (8.8) years, with two studies not reporting the age of participants. Median (IQR) % of participants in the included studies who were men was 75.5% (56%, 90%). The overwhelming majority of studies (*n* = 23, 70%) recruited participants from the US. Five studies (two in the US, two in Uganda, and one in Thailand) involved participants who were not virally suppressed, whereas six studies did not report viral suppression status for their samples. At least a proportion of participants in all remaining studies were virally suppressed; median (IQR) % of virally suppressed participants overall was 79% (37%, 91%). Active HIV replication results in elevated inflammation, which may in turn worsen depressive symptoms.⁵⁰ However, given that many participants in the included studies were on ART and virally suppressed, inferences about the impact of untreated HIV infection on inflammation and depression cannot be made.

Collectively, the included studies tested associations of depression or depressive symptoms with 55 biomarkers of inflammation and related processes, of which IL-6, TNF- α , C-reactive protein (CRP) and MCP-1/CCL2 were assessed most frequently (Fig. 3). Biomarkers were assessed in a wide range of biofluids, including blood, cerebrospinal fluid, urine and saliva. A recent meta-analysis has revealed poor correlations between blood and cerebrospinal fluid (CSF) measures of inflammatory biomarkers,⁵¹ which suggests that care must be taken to choose a biofluid that is appropriate for the study design and with consideration for acceptability and scalability. Seven studies measured a single biomarker;^{29,37,38,41,42,45,52} all other included studies measured more than one biomarker. Three studies only reported associations involving a cluster or composite score of multiple biomarkers, such as effects for individual biomarkers could not be parsed out.^{22,31,40} Included biomarkers were broadly categorized based on their primary putative function in inflammation (or related processes) as follows: neuroinflammation, chemotaxis, systemic inflammation, monocyte activation, immunometabolism, coagulation, and neurogenesis. Note that biomarkers may be involved

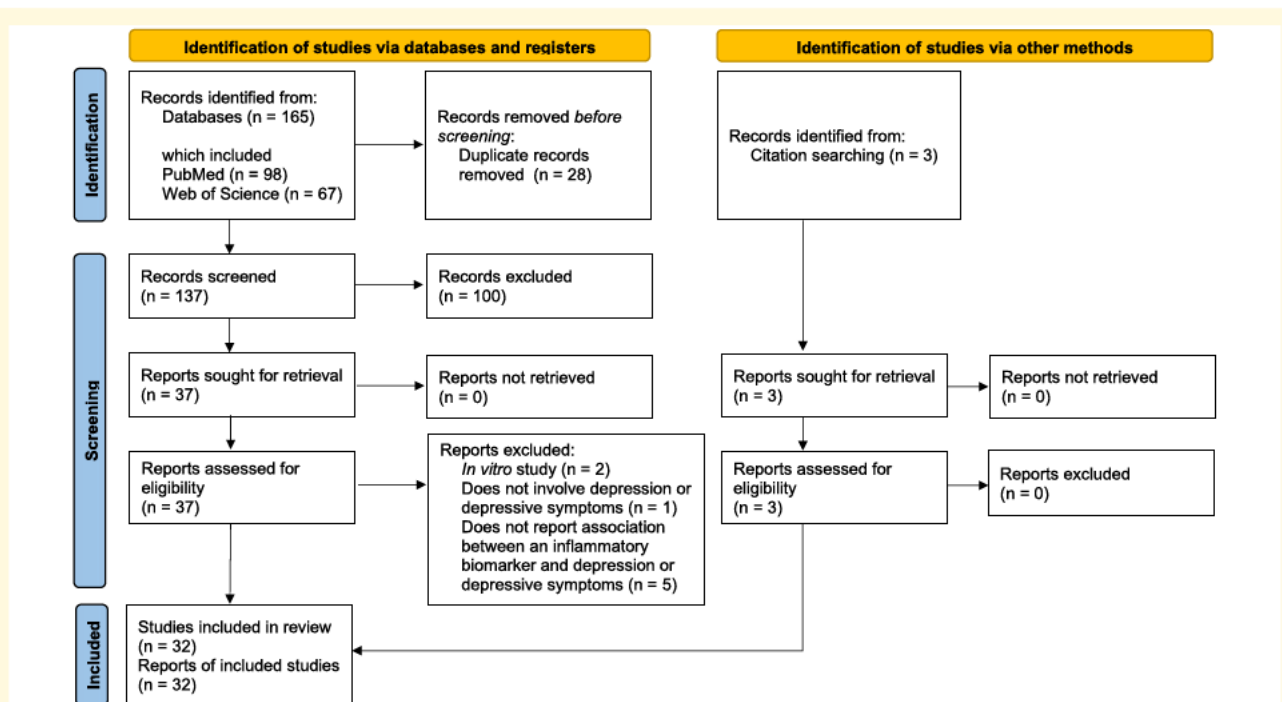


Figure 1 PRISMA flowchart depicting the number of studies included in the review from database and manual citation searches. Data from three unique studies was analysed and reported in two publications each; data from one unique study was analysed and reported in three publications.

in multiple processes; for instance, many biomarkers that were categorized as neuroinflammatory in this review likely also play a role in systemic inflammation.

The majority of included studies used a screening tool to measure depressive symptoms, rather than a diagnostic interview to establish a clinical diagnosis of depression (Supplementary Table 1). Three studies reported non-specific associations between biomarkers and depressive symptoms: one as ‘mental health symptoms’,³³ another as a composite of ‘depression or PTSD’,^{41,52} and another as an interaction of biomarkers with depression and neurocognition.

Formal meta-analyses were not possible in this review for a number of reasons. First, there was substantial heterogeneity in the scales used to measure depressive symptoms and the biofluids in which biomarkers were measured. Of 55 included biomarkers, 30 (54.5%) were assessed in only one publication, and a further 9 (16.4%) were assessed in two publications. We extracted effect size estimates for biomarkers which were assessed in at least five publications (Supplementary Table 1). Reporting of effect sizes in a substantial proportion of these studies was poor: of 115 reported associations, 29 (25.2%) did not include any test statistics or measure of effect size at all, whereas a further 10 (8.7%) were only reported collectively for a cluster of biomarkers, such that effect sizes for individual biomarkers could not be parsed out. Other associations were reported using estimates such as % feature importance ($n = 6$, 5.2%) or scaled intensity ($n = 3$, 2.6%) which could not reliably be converted to a meta-analysable statistic. Therefore, at least 41.7% of

reported associations were not meta-analysable. Given these issues, an assessment of publication bias or a meta-analysis of the strength of the associations between biomarkers and depressive symptoms was not possible.

Included studies adopted varied approaches to accounting for confounding demographic or clinical variables. Some studies included demographically and clinically comparable controls,¹⁸ some adjusted for confounding variables statistically,^{19,21} while others combined both approaches.^{24,44} Where available, we extracted data for associations from statistical models adjusted for relevant covariates.⁵³ Four included studies reported an active hepatitis C co-infection in at least a proportion of their participant samples,^{17,18,40,49} whereas one study specifically excluded participants with co-infection during recruitment.³⁶ Active co-infections were not reported in the remaining studies. Several studies also reported psychosocial factors that may influence inflammation. In particular, a number of included studies focused on participants living with HIV who had experienced sexual violence in their lifetime.^{20,26,46} Jones *et al.*²⁹ specifically attempted to assess depression as one component of ‘syndemic burden’, which was defined as incidence of depression, cigarette use, low education, or obesity. They found that syndemic burden was associated with increased viral load, but not inflammatory biomarkers, in women living with HIV. Overall, only a proportion of included studies reported measurement of and statistical adjustment for potential clinical or psychosocial confounders.

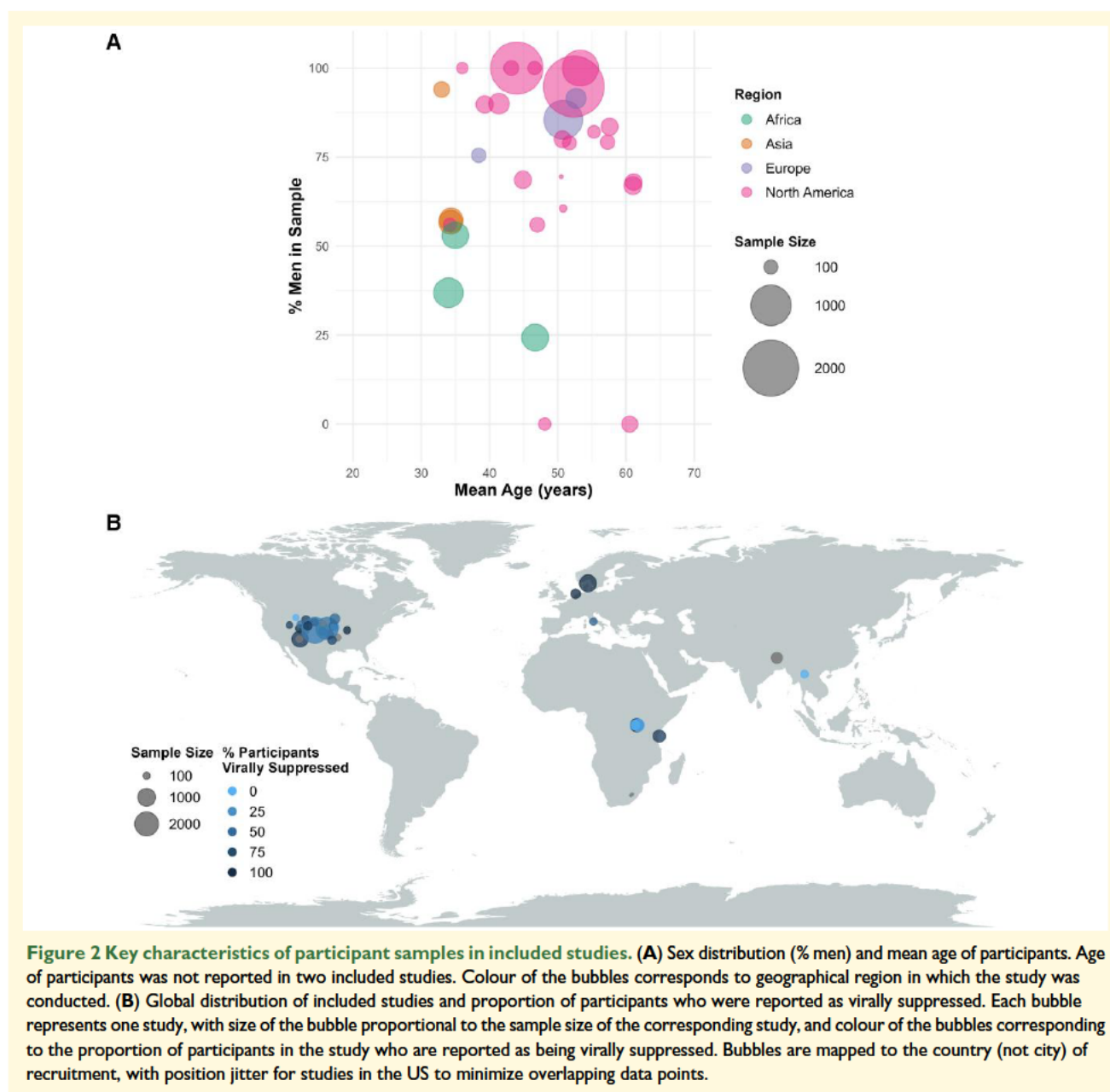


Figure 2 Key characteristics of participant samples in included studies. (A) Sex distribution (% men) and mean age of participants. Age of participants was not reported in two included studies. Colour of the bubbles corresponds to geographical region in which the study was conducted. (B) Global distribution of included studies and proportion of participants who were reported as virally suppressed. Each bubble represents one study, with size of the bubble proportional to the sample size of the corresponding study, and colour of the bubbles corresponding to the proportion of participants in the study who are reported as being virally suppressed. Bubbles are mapped to the country (not city) of recruitment, with position jitter for studies in the US to minimize overlapping data points.

Proportion of studies reporting a significant association and direction of those associations are summarized in Fig. 4. Validation scores for biomarkers assessed in included studies are summarized in Table 2, and validation scoring for each included study is available in Supplementary Tables 2–4. The following sections report and discuss findings for broad categories of biomarkers across included studies.

Cytokines

Cytokines are biomolecules produced by immune cells to activate, promote, or regulate the (neuro)inflammatory response. Concentrations of these biomarkers can be measured in biofluids including CSF, blood plasma or

serum, saliva, or cervico-vaginal lavage. Included studies that assessed the relationship between neuroinflammatory cytokines and depressive symptoms are summarized in Supplementary Table 2.

The most frequently assessed neuroinflammatory cytokines in included studies were interleukin-6 (IL-6), tumour necrosis factor alpha (TNF- α), neopterin, interleukin-1 beta (IL-1 β) and soluble tumor necrosis factor receptor-II (sTNFR-II). Numerous studies have found an association between circulating IL-6 or TNF- α concentrations and depressive symptoms in the general population, albeit with varied strengths.^{54–61} A recently published meta-analysis involving a cumulative 3075 participants also indicated that blood neopterin (regardless of type of blood sample) is significantly

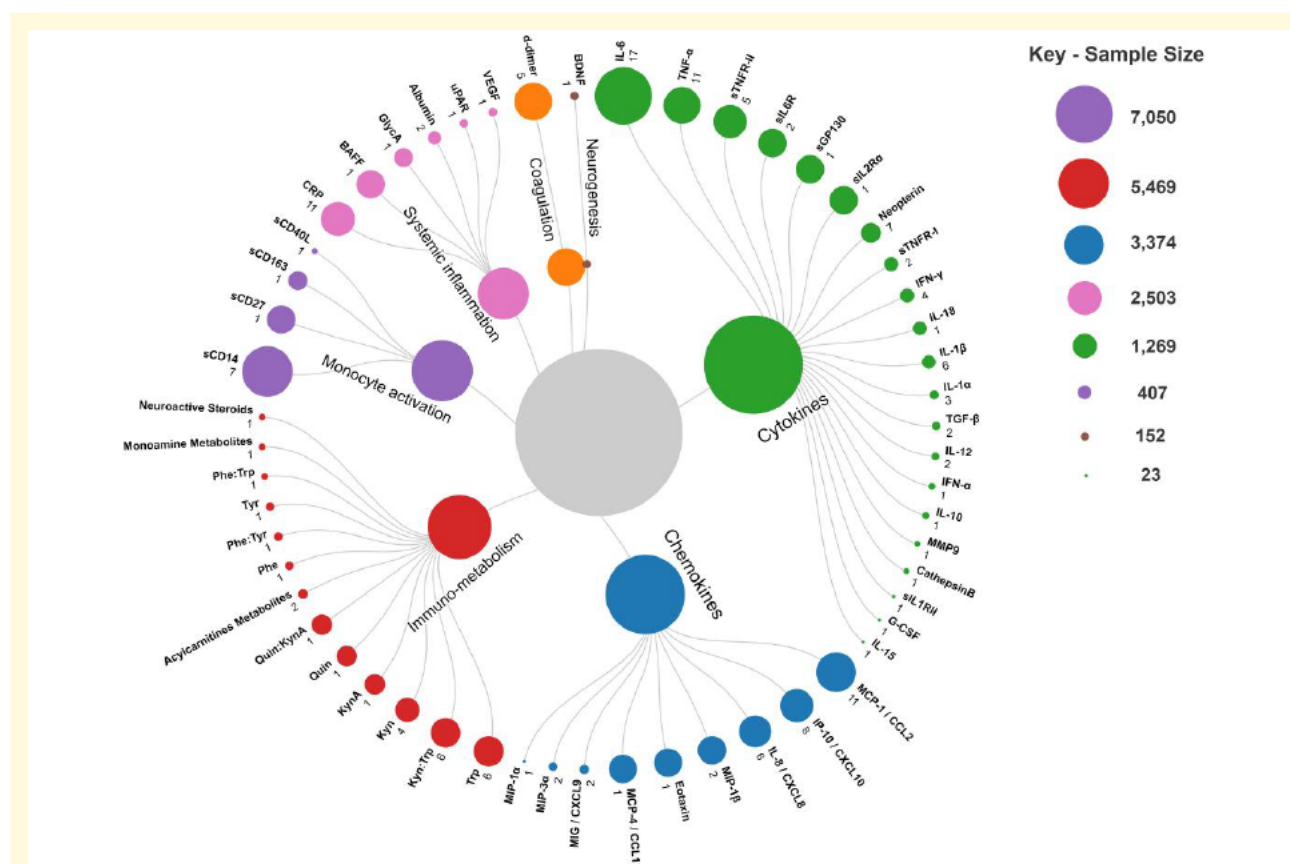


Figure 3 Biomarkers of inflammation and related processes assessed in the included studies. Included biomarkers were classified broadly into categories based on their primary putative function. Size of each circle is proportional to the cumulative sample size of publications involving that category or biomarker. Note that some publications leveraged data from overlapping samples. Each data label indicates the biomarker (in bold) and the number of publications in which the biomarker was assessed (in plain text). A list of abbreviations is available in [Supplementary Table 5](#).

higher in participants with depression.⁶² The current review detected 17 publications that tested the relationship between IL-6 and depression or depressive symptoms, of which seven reported a significant positive association such that increased IL-6 was associated with greater risk for depression or depressive symptoms.^{21,22,25,35,36,39} Similarly, a proportion of studies exploring the relationship between TNF- α ^{21,22,35,39,48} or neopterin^{28,42,46} and depressive symptoms observed significant positive associations. None of the studies reporting a significant association for IL-6 or TNF- α included participants without HIV. Saloner *et al.*⁴² observed a correlation between neopterin and depressive symptoms, which was significant only amongst people living with HIV, indicating that neopterin may be a valuable biomarker for HIV-associated depression. Together, these findings suggest that increased IL-6, TNF- α and neopterin may be associated with increased depression, including in people living with HIV. However, further studies that include appropriate controls are necessary to determine whether these cytokines (especially IL-6 and TNF- α) mediate the risk for depressive symptoms in a manner explicitly dependent on HIV status:

that is, whether these associations are more pronounced in people living with HIV compared to those without HIV.

In line with findings related to TNF- α , two studies reported a significant association between increased serum concentrations of sTNFR-II and greater depressive symptoms,^{31,33} though Lu *et al.* only reported this association for sTNFR-II as part of a cluster of biomarkers [‘exploratory factor analysis-identified inflammatory processes’ (EIP)]. Similarly, Lu *et al.*³¹ also reported a significant association with depressive symptoms for soluble interleukin-2 receptor alpha (sIL2R α), soluble interleukin-6 receptor (sIL6R) and soluble glycoprotein 130 (sGP130) but only as part of an EIP. Thus, no inferences can yet be drawn regarding the contributions of these individual biomarkers to HIV-associated depression.

Other neuroinflammatory cytokines for which a significant association with depressive symptoms was reported were interferon gamma (IFN- γ , two publications from an overlapping participant sample) and interleukin-12 (IL-12), interleukin-15 (IL-15) and interleukin-18 (IL-18), though these studies did not include participants without HIV and

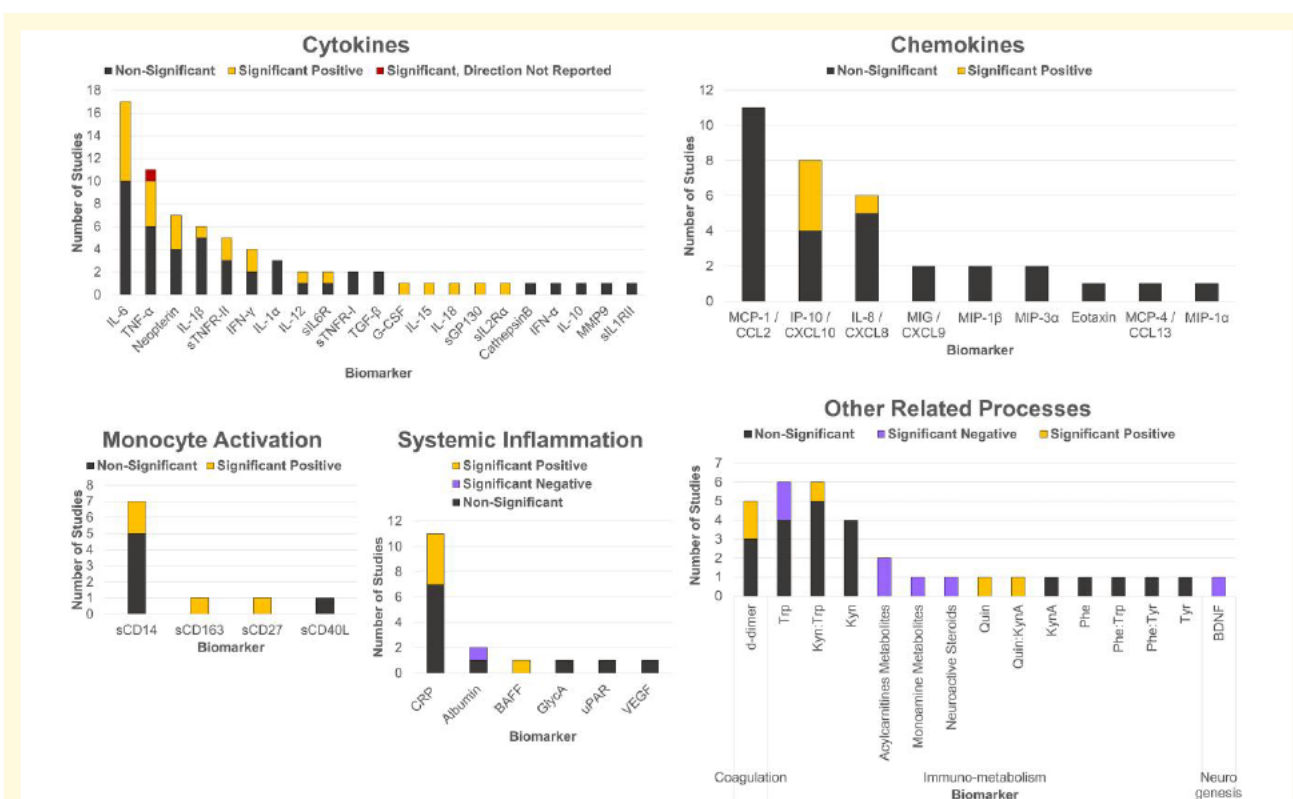


Figure 4 Summary of findings from included studies on the association between inflammatory biomarkers and depression or depressive symptoms in people living with HIV. Each column indicates the proportion of publications for that biomarker reporting a non-significant (in black), significant positive (in yellow), or significant negative (in purple) association. Note that some publications leveraged data from overlapping samples. A significant positive association implies that higher biomarker concentrations are linked to greater depression or depressive symptoms, whereas a significant negative association implies that lower biomarker concentrations are linked to greater depression or depressive symptoms. A list of abbreviations is available in [Supplementary Table 5](#).

the findings from the overlapping sample in particular warrant further replication in independent samples.^{21,22,33,49} No significant associations with depression or depressive symptoms were observed in the included studies for the cytokines interleukin-1 alpha (IL-1 α), soluble tumor necrosis factor receptor I (sTNFR-I), transforming growth factor beta (TGF- β), cathepsinB, interferon alpha (IFN- α), interleukin 10 or 12 (IL-10, IL-12), matrix metalloproteinase 9 (MMP9) or soluble interleukin-1 receptor II.

Chemokines

Chemokines are biomolecules primarily involved in the recruitment of immune cells to locations of immunological challenges. Expression of these biomarkers is often regulated by neuroinflammatory cytokines such as TNF- α and IL-1 β . Studies that assessed relationships between chemokines and depression in people living with HIV are summarized in [Supplementary Table 3](#).

Monocyte chemoattractant protein-1 (MCP-1/CCL2) was the most frequently assessed chemokine in included studies. Of 11 studies, none reported a significant association between MCP-1 concentration and depression or depressive

symptoms among people living with HIV. These findings are compatible with pre-clinical studies using a transgenic rat model of HIV, where Nemeth *et al.*⁶³ found that animals who exhibited depression-like behaviour had increased concentration of MCP-1 in the hippocampus, but treatment with meloxicam did not rescue depression-like behaviour in these animals, despite successfully inhibiting *Mcp-1* gene expression. Therefore, although MCP-1 has been implicated in the risk for depression in the general population,^{64,65} the evidence suggests that this chemokine may not play a significant role in depression among people living with HIV specifically.

Interferon gamma-induced protein 10 (IP-10/CXCL10) and interleukin-8 (IL-8/CXCL8) are closely related chemokines belonging to the C-X-C family. Cassol *et al.*¹⁸ reported that increased plasma IP-10 was linked to depressive symptoms amongst people living with HIV but not controls. Three other studies also observed a significant positive association between IP-10 and depressive symptoms, but findings were limited only to men,³⁹ in a small sample ($n = 23$),⁴⁹ or reported only as part of a cluster of biomarkers.⁶⁶ One study found that IL-8 concentrations were significantly higher amongst people with HIV and remitted depression compared

Table 2 Summary of validation grading for included studies investigating associations between inflammatory biomarkers and depressive symptoms in people living with HIV

Category	Biomarker	Number of studies	Total validation grade	Maximum validation grade	Mean validation grade
Cytokines	IL-6	17	16	2	0.94
	TNF- α	12	12	2	1.00
	Neopterin	10	10	3	1.00
	IL-1 β	6	3	1	0.50
	sTNFR-II	5	5	3	1.00
	IFN- γ	4	5	2	1.25
	IL-1 α	3	1	1	0.33
	IL-12	2	3	2	1.50
	sIL6R	2	3	3	1.50
	sTNFR-I	2	0	0	0.00
	TGF- β	2	1	1	0.50
	CathepsinB	1	0	0	0.00
	G-CSF	1	2	2	2.00
	IFN- α	1	1	1	1.00
	IL-10	1	1	1	1.00
	IL-15	1	2	2	2.00
	IL-18	1	1	1	1.00
	MMP9	1	0	0	0.00
	sGPI30	1	3	3	3.00
	sIL1RII	1	0	0	0.00
Chemokines	sIL2R α	1	3	3	3.00
	MCP-1/CCL2	12	7	1	0.58
	IP-10/CXCL10	8	10	3	1.25
	IL-8/CXCL8	6	5	1	0.83
	MIG/CXCL9	2	1	1	0.50
	MIP-1 β	2	1	1	0.50
	MIP-3 α	2	1	1	0.50
	Eotaxin	1	1	1	1.00
	MCP-4/CCL13	1	1	1	1.00
	MIP-1 α	1	0	0	0.00
Monocyte activation	sCD14	7	9	3	1.29
	sCD163	1	2	2	2.00
	sCD27	1	3	3	3.00
	sCD40L	1	0	0	0.00
Systemic inflammation	CRP	11	9	2	0.82
	Albumin	3	2	2	0.67
	B cell activating factor	1	3	3	3.00
	GlycA	1	1	1	1.00
	uPAR	1	1	1	1.00
Coagulation immunometabolism	VEGF	1	1	1	1.00
	d-dimer	5	6	3	1.20
	Kyn:Trp	7	5	2	0.71
	Trp	7	7	2	1.00
	Kyn	5	3	1	0.60
	Acylcarnitines	2	4	3	2.00
	Metabolites				
	Phe	2	2	1	1.00
	Phe:Tyr	2	2	1	1.00
	Tyr	2	2	1	1.00
	KynA	1	0	0	0.00
	Monoamine Metabolites	1	3	3	3.00
	Neuroactive Steroids	1	1	1	1.00
	Phe:Trp	1	0	0	0.00
	Quin	1	1	1	1.00
	Quin:KynA	1	1	1	1.00
Neurogenesis	BDNF	1	3	3	3.00

to people with HIV and no depression,³⁹ though several other studies with larger sample sizes did not find a significant association for IL-8. No notable associations were

reported between depression or depressive symptoms in people living with HIV and the chemokines monokine induced by gamma interferon (MIG/CXCL9), macrophage

inflammatory protein-1 alpha (MIP-1 α /CCL3) macrophage inflammatory protein-3 alpha (MIP-3 α /CCL20), macrophage inflammatory protein-1 beta (MIP-1 β), eotaxin, or monocyte chemoattractant protein-4 (MCP-4/CCL13).

Other inflammatory markers

Evidence for associations between depression or depressive symptoms in people living with HIV and biomarkers of systemic inflammation or monocyte activation are summarized in [Supplementary Table 3](#).

Systemic inflammation markers

C-reactive protein is an acute phase reactant which promotes phagocytosis and macrophage proliferation.⁶⁷ During an inflammatory response, CRP is induced by inflammatory cytokines such as IL-6, TNF- α and IL-1 β , which promote its production in the liver and secretion into plasma.⁶⁸ CRP is elevated in people with HIV^{69,70} and people with depression,⁵⁴ although the role of CRP in depression is unresolved due to contradictory findings. This review detected 11 studies which assessed CRP concentrations in people living with HIV, of which only four reported a significant association with depression or depressive symptoms.^{33,37,39,41} Notably, Rubin *et al.*³⁹ only saw this association in women living with HIV and Saloner *et al.*⁴¹ only reported this association as an interaction between HIV, depressive symptoms, and neurocognition. None of these four studies reported that the association between CRP and depression was only significant for participants with HIV and not for those without HIV. Together with the existing contradictions in the literature on the role of CRP in depression, these findings suggest that CRP may not reliably influence HIV-associated depression.

Serum albumin was assessed in two studies, of which one study reported a negative association with depressive symptoms.³⁸ The authors of this study speculate that albumin may be protective against depression, though such a claim would require substantial experimental work to verify. Furthermore, this study did not include participants without HIV and thus no conclusions can be drawn whether this effect may be influenced by HIV status. Other biomarkers of systemic inflammation assessed in the included studies were B cell activating factor (BAFF, for which Lu *et al.*³¹ reported a significant positive association with depressive symptoms, but only as part of a cluster of biomarkers), glycoprotein acetylation marker (GlycA), urokinase plasminogen activator surface receptor (uPAR/CD87), and vascular endothelial growth factor (VEGF), though no significant associations were reported for any except BAFF.

Monocyte activation markers

Biomarkers of monocyte activation are expressed on the surfaces of monocytes and released in soluble form when these cells are activated. Neuroinflammatory cytokines such as

IL-6 or IL-1 β are involved in the induction of these markers.⁷¹ Included studies assessed four different biomarkers which are released by monocytes when they are activated: soluble cluster of differentiation 14, 163, 27, and 40 Ligand (sCD14, sCD163, sCD27 and sCD40L, respectively). Of the studies Investigating sCD14, Stewart *et al.*⁴⁴ reported a significant correlation between somatic depressive symptoms and serum sCD14 concentrations amongst people living with HIV but not in people without HIV, which offers support for the validity of sCD14 as a biomarker for HIV-associated depression, though the difference in effect size for both groups was small. Similarly, Anderson *et al.*¹⁷ observed that higher plasma sCD163 was associated with increased odds for severe depressive symptoms only amongst people living with HIV. Lu *et al.*⁶⁶ reported a significant association for sCD14 and sCD27 only as part of an EIP, whereas other studies captured in this review did not report a significant association between these biomarkers and depression. In all, sCD14 and sCD163 may drive the risk for depressive symptoms in people living with HIV, though further work is necessary to confirm these results.

Astrocyte activation marker: glial fibrillary acidic protein

Glial fibrillary acidic protein (GFAP) is an intermediate filament protein which is constitutively expressed in astrocytes, and thus, serves as a marker of astrocyte activation and CNS pathologies.⁷² Ellis *et al.*⁷³ recently investigated associations between CSF concentrations of GFAP with depressive symptoms in a sample of $n = 212$ people living with HIV in the US, with the majority (82.1%) of participants being men. They observed a statistically significant association between GFAP concentrations and total BDI-II scores with higher CSF GFAP correlating with higher BDI-II scores, suggesting that astrocytic activation may be associated with depressive symptoms in people living with HIV. These early findings indicate that GFAP may be a useful biomarker for depressive symptoms in this population. Further research into the function of astrocytes in neuroinflammation and depression in the context of HIV is warranted. Notably, this study was published in December 2022, outside the stated date range for our systematic searches, and was, therefore, not included in reported analyses in the rest of this review.

Markers of related processes

Several included studies measured biomarkers of physiological processes that overlap with neuroinflammation. Findings from these studies are summarized in [Supplementary Table 4](#).

Immunometabolism markers

Kynurenine pathway metabolites

The kynurenine pathway of tryptophan metabolism sits at the intersection of neuroinflammation and metabolism. Typically, tryptophan (Trp) is catabolized by tryptophan

hydroxylase into 5-hydroxytryptophan, the neurotransmitter otherwise known as serotonin and heavily implicated in the pathogenesis of depression.⁷⁴ Under certain conditions, such as an inflammatory response, immune cells (including microglia and astrocytes) can instead metabolize tryptophan into kynurenine [Kyn, regulated by indoleamine 2,3-dioxygenase (IDO-1)], kynurenic acid (KynA) and quinolinic acid (Quin), all metabolites which form part of the kynurenine pathway.^{75,76} The ratio of kynurenine to tryptophan (Kyn:Trp) is an index of IDO-1 activity, which is triggered by key neuroinflammatory cytokines such as IL-6, TNF- α , IL-1 β and IFN- α .^{77,78}

Activation of the kynurenine pathway depletes available tryptophan for serotonin production and thus is considered a possible mechanism by which immune responses interact with neurotransmitter dysfunction to give rise to depressive symptoms. The kynurenine pathway has particular clinical relevance to HIV infection, which has been reviewed elsewhere.⁷⁹ HIV viral proteins can activate the kynurenine pathway, in addition to inducing the release of pro-inflammatory cytokines which further promote this activation, leading to a feedforward loop of sustained tryptophan depletion.^{80,81}

Several included studies tested for associations between Kyn or KynA and depressive symptoms, with none reporting a significant association. Five studies investigated associations between Trp in plasma or CSF with depressive symptoms. Of these, Martinez *et al.*³² and Vadaq *et al.*⁴⁵ both observed that lower plasma tryptophan was significantly associated with greater depressive symptoms, though their samples did not include people without HIV. Finally, although the Kyn:Trp ratio was assessed in several studies, only Martinez *et al.*³² observed a statistically significant association between higher plasma Kyn:Trp ratio and greater depressive symptoms in people living with HIV, and the effect size of this association was small (+0.01 HSCL-D score for each 10 nM/ μ M increase in Kyn:Trp ratio). Conversely, Keegan *et al.*³⁰ saw a trend towards lower plasma Kyn:Trp weakly associating with depression in aviremic people with HIV (compared to viremic people with HIV or people without HIV), though this difference was not statistically significant after Bonferroni correction. Taken together, these findings suggest that lower plasma Trp may be a useful biomarker of the risk for depression in HIV, but further work is necessary to resolve contradictory findings on the association between Kyn:Trp ratio and depression.

Quinolinic acid is considered a neurotoxic metabolite, leading to the hypothesis that increased production of Quin may be a driver of an inflammatory subtype of depression.⁷⁷ Absolute concentrations of Quin are increased in untreated depression and correlate with depression severity.⁸² Furthermore, antidepressant behavioural activation therapy leading to reduction in depressive symptoms is significantly associated with decreased serum Quin.⁸³ Crucially, this relationship has also been observed in the specific context of HIV. Drivsholm *et al.*²³ saw that higher Quin:KynA ratio (but not absolute KynA concentrations) and higher absolute

concentrations of Quin were both significantly associated with depression in a large sample of people with HIV. Given these findings, Quin may be a reliable biomarker of depression in people living with HIV.

Other neuroimmunometabolites

Studies included in this review did not find significant associations of tyrosine (Tyr), phenylalanine (Phe), Phe:Tyr ratio, or Phe:Trp ratio with depressive symptoms.^{19,30} Two publications from an overlapping participant sample^{18,34} found that monoamine metabolites (phenylacetate and 4-hydroxyphenylacetate), acylcarnitine metabolites (isovalerylcarnitine, isobutyrylcarnitine, and propionylcarnitine), and neuroactive steroids (pregnenolone sulphate, pregnanediol-3-glucuronide, and DHEA sulphate) were decreased in people living with HIV who experience depression. These findings suggest that these metabolites may be protective against depressive symptoms, though replication of these results in independent samples is warranted. Notably, Cassol *et al.*¹⁸ observed the effect of monoamines and acylcarnitines amongst participants *without* HIV as well, suggesting that these relationships are not dependent on HIV status.

Coagulation marker: D-dimer

D-dimer is a biomarker of coagulation which is also elevated in systemic inflammation.⁸⁴ Increased d-dimer concentration is independently linked to serious HIV-related conditions such as cardiovascular disease or immune reconstitution inflammatory syndrome (IRIS) and with depressive symptoms.^{85–87} The current review detected five studies that tested for associations between d-dimer and depressive symptoms, of which two reported a significant association.^{25,44} Notably, Stewart *et al.*⁴⁴ observed that this association was only significant for people with HIV (and not for those without HIV), and that serum concentration of d-dimer was positively correlated with somatic symptoms of depression. Each SD increase in PHQ-9 somatic score was associated with a 6% increase in d-dimer concentration in people living with HIV, but no increase in people without HIV. These studies offer early evidence that d-dimer may be associated with depressive symptoms in a manner dependent on HIV status.

Neurogenesis marker: brain-derived neurotrophic factor

Brain-derived neurotrophic factor (BDNF) is a regulator of neurogenesis and synaptic plasticity, with additional functions in apoptosis and T cell survival.⁸⁸ BDNF binds to the receptor tropomyosin receptor kinase B (TrkB) to promote neuronal and synaptic growth and survival. Given this, reduction in BDNF is associated with neuronal death and synaptodendritic injury. Decreased BDNF has been linked with depression in rigorous meta-analyses, though the effect size of this association is relatively small.⁸⁹

BDNF is not a marker of neuroinflammation, but it does interact with HIV-induced neuroinflammatory processes in ways which are relevant to the current discussion. Notably, HIV infection in the brain leads to production of neuroinflammatory cytokines such as TNF- α and IL-1 β , which have recently been shown to inhibit expression (TNF- α), retrograde transport (IL-1 β) and anterograde transport (TNF- α) of BDNF.⁹⁰ Concentrations of BDNF are elevated in CSF following CNS infections, suggesting that neuroinflammation influences BDNF expression.^{91,92} Furthermore, there is some evidence that BDNF signalling may exhibit cross-talk with the kynurenine pathway and associated immuno-metabolic dysfunctions.⁹³ Pre-clinical studies show that induction of depression-like behaviours in rats was accompanied by decreased BDNF expression and increased IDO-1, Kyn, and Trp production, whereas inhibition of the kynurenine pathway significantly increased cortical BDNF levels.^{94,95} Therefore, BDNF signalling is influenced by neuroimmunometabolic responses known to be induced by HIV infection.

Given this evidence, it is possible that BDNF may be associated with the risk for depression in HIV. Woods *et al.*⁴⁷ recently explored this question in a sample of older adults with and without HIV. In this study, the authors found that lower plasma BDNF concentrations were associated with higher scores on specific dimensions of mood disorders, notably Depression-Dejection (as measured by the Profile of Mood States, POMS). This association was only significant for participants with HIV, crucially demonstrating that the effect of BDNF on depressive symptoms may be dependent on HIV status. This study offers promising (though early) evidence that BDNF may be a useful biomarker of HIV-associated depression, with further work necessary to replicate this finding in diverse cohorts.

Insights in absence

As the resident immune cells in the CNS, microglia are essential to the neuroinflammatory response. Under homeostatic conditions, microglia are highly ramified, whereas ‘activated’ microglia reacting to immunological challenges are amoeboid. These activated microglia—and, to some extent, astrocytes—perform key immune functions in the brain, such as phagocytosing cellular debris and triggering immunological cascades.⁹⁶ For this reason, glial cell activation is widely considered to be the key characteristic of neuroinflammation. However, measuring glial cell activation directly in humans is challenging, as this requires access to brain tissue via biopsies or autopsies.

Positron emission tomography (PET) and magnetic resonance spectroscopy (MRS) allow for non-invasive *in vivo* assessment of certain biomarkers which are considered indirect measures of glial cell activation, namely, 18 kDa translocator protein, myo-inositol and choline. However, despite explicitly including search terms for TSPO, myo-inositol and choline, our systematic search did not detect any studies published since 2013 which examined

changes in these neuroimaging biomarkers directly in relation to depression or depressive symptoms among people living with HIV. Newly developed radiotracers for PET ligands offer greater capacity to measure microglial activation *in vivo*.⁹⁷ The leading-edge imaging modality diffusion-weighted MRS has recently been shown to be sensitive to an experimental model of neuroinflammation in humans.⁹⁸ Thus, it will be useful for future research to test the associations of these rapidly developing neuroimaging biomarkers with depressive symptoms amongst people living with HIV.

In a scoping review of the field, it is useful to consider which communities and lived experiences are not yet represented in the literature. This review found that the majority (70%) of all eligible studies investigating associations between inflammatory markers and depression in people living with HIV were carried out in the US. There was also a clear skew towards a greater proportion of men in participant samples (Fig. 2). Studies that included a greater proportion of women were conducted in Africa or Asia, with the exception of publications reporting findings from the Women’s Interagency HIV Study in the US, which intentionally recruited only women participants. No studies included participants who were transgender or gender non-conforming, despite the rates of HIV and depression being elevated in these groups.^{99,100} Participants in included studies also tended to be older, with none of the included studies investigating biomarkers of depression in children or adolescents living with HIV. More people live with HIV in the Global South, and the majority of these are women.^{101,102} Biomarkers of depression have been shown to significantly differ based on sex or gender,¹⁰³ with different biomarkers found to be elevated in men with depression compared to women with depression.¹⁰⁴ Additionally, women living with HIV exhibit greater systemic inflammation compared to men living with HIV.¹⁰⁵ These demographic factors—age, sex and gender, and geography—interact significantly such that the prevalence of depression is higher in young women in the Global South.¹⁰⁶ Therefore, the participant samples in included studies do not reflect the global epidemiology of HIV or depression. Effective translation of biomarker discovery into predictive or therapeutic tools will require careful consideration of differences in immune activation by factors such as age, sex, gender, and geography. Equitable cross-border collaborations are necessary to ensure greater inclusion in future research, as recommended elsewhere.¹⁰⁷

Conclusions and hypothesized mechanisms

This review aimed to identify studies investigating associations between depression or depressive symptoms and biomarkers of neuroinflammation and related processes in people living with HIV. Eligible studies utilized a variety of depressive symptom scales and assessed 55 biomarkers across neuroinflammation, chemotaxis, systemic

inflammation, monocyte activation, immunometabolism, coagulation and neurogenesis. A key limitation of this review was that statistical reporting was highly variable, with many studies not reporting any measures of effect size at all. As a result, we could not conduct a meta-analysis of effect size estimates or assess whether studies in the field showed evidence of publication bias. Few included studies met our validation criteria for a grade of 3, which represented a biomarker which was significantly associated with depression only in people living with HIV (not in controls) and correlated with depressive symptom severity. Given this, for many biomarkers in this review, it is difficult to ascertain whether the significant associations observed in included studies are reliably dependent on HIV status and offer clinical utility. Finally, study screening and data extraction were carried out by a single reviewer, and the search was restricted to studies published in English, which are notable limitations to the methodology of this review.

Regardless of these limitations, this review identified promising evidence that increased concentrations of IL-6, TNF- α , neopterin, IP-10, sCD14, sCD163 and d-dimer may be linked to greater depressive symptoms among people living with HIV. Conversely, higher concentrations of tryptophan and BDNF may be protective, since early evidence indicates significant negative associations between these biomarkers and depressive symptoms in people living with HIV. The biomarkers MCP-1 and IL-8 as well as the Kyn: Trp ratio were assessed in several studies, but no significant associations with depression were observed in the majority of these studies, indicating that these biomarkers may not substantially influence HIV-associated depression. The remaining biomarkers in the included studies were only assessed in a few studies, with little evidence for a significant association with depression in people living with HIV.

These biomarkers are known to interact with each other as part of the (neuro)inflammatory response. For instance, TNF- α regulates the release of chemokines such as MCP-1 and IP-10.¹⁰⁸ The cytokines TNF- α and IL-1 β regulate the production of IL-6, all of which in turn induce the release of CRP.¹⁰⁹ Soluble monocyte activation markers such as sCD14 can induce the production of these cytokines.¹¹⁰ These inflammatory biomarkers may also trigger the kynurenine pathway of tryptophan metabolism, thus contributing to immuno-metabolic dysfunction, and inhibit the production of BDNF, thus impairing neurogenesis and synaptic plasticity. Kynurenine and other immunometabolites may similarly contribute to the release of chemokines such as MCP-1 and IL-8.¹¹¹ Concentrations of MCP-1 and IP-10 are correlated with lower homovanillic acid, a dopamine metabolite, which in turn is correlated with depressive symptoms.⁴⁰ Alterations in inflammatory biomarkers may thus also interact with dopaminergic signalling deficits to produce depressive symptoms.¹¹² Together, these biomarkers thus represent feed-forward loops of neuroinflammation and related physiological processes which may drive the pathogenesis of depressive symptoms as a result of CNS HIV infection.

Our findings support the mechanisms underlying HIV-associated depression which were previously proposed by Del Guerra *et al.*⁶ The evidence identified in this review implicates neuroinflammatory and metabolic biomarkers in HIV-associated depression. Given this, we hypothesize that chronically activated microglia, which trigger these neuroimmunometabolic cascades, are the central link connecting HIV infection in the CNS with the elevated risk for depressive symptoms (Fig. 5). In response to HIV infection, activated microglia stimulates the release of various cytokines and chemokines, which elicit sickness behaviours (such as sleep and appetite issues) associated with depression. HIV infection also leads to the inhibition of the production and cellular transport of BDNF via the pro-inflammatory cytokines TNF- α and IL-1 β , as well as increases in blood-brain barrier permeability. The inhibition of BDNF in the CNS leads to synaptodendritic injury and neurodegeneration, which contributes to the development of certain depressive symptoms. Elevations in circulating cytokines such as IL-6 and TNF- α and the upregulation of the kynurenine metabolic pathway, which are induced by HIV infection, are also linked to depressive symptoms. Thus, HIV may drive the pathogenesis and severity of depressive symptoms by inducing neuroinflammatory cascades and immunometabolic dysfunction via activated microglia.

In a recent study involving 204 participants with and without HIV, we sought to directly assess potential mediation of the relationship between HIV status and depressive symptoms by inflammatory biomarkers. We showed that statistically adjusting for four biomarkers of inflammation—MIG and TNF- α in plasma and MIP1- α and IL-6 in CSF—attenuated the odds for depressive symptoms in people living with HIV, suggesting that these biomarkers are involved in mediating this risk.¹¹³ This recent study offers promising evidence that biomarkers of inflammation may be mechanistically involved in the increased risk for depression in people living with HIV and could thus be targeted when developing new or improved therapeutic interventions.

Microglial activation due to inflammatory challenges such as infections is involved in the pathogenesis of depression. Evidence supporting the role of microglia in inflammation and depression has been extensively reviewed elsewhere.^{114,115} Mouse models have shown that inflammatory challenges increased microglial activation in the anterior cingulate cortex (ACC) and induced depressive-like behaviour, whereas mice with deleted ACC microglia did not display depressive-like behaviour in response to experimentally induced inflammation.¹¹⁶ Crucially, the microglial activation marker TSPO is elevated in the ACC of people experiencing moderate or severe depressive episodes as well.¹¹⁷ Furthermore, novel human brain organoid models with co-cultured microglia have directly demonstrated that microglia release TNF- α and IL-1 β in response to HIV infection, which are key cytokines frequently associated with depression.¹¹⁸ Taken together, these results strongly suggest that microglia may play a critical role in the pathogenesis of HIV-associated depression.

Although we focus on chronically activated microglia for this discussion, the role of astrocytes in HIV-associated

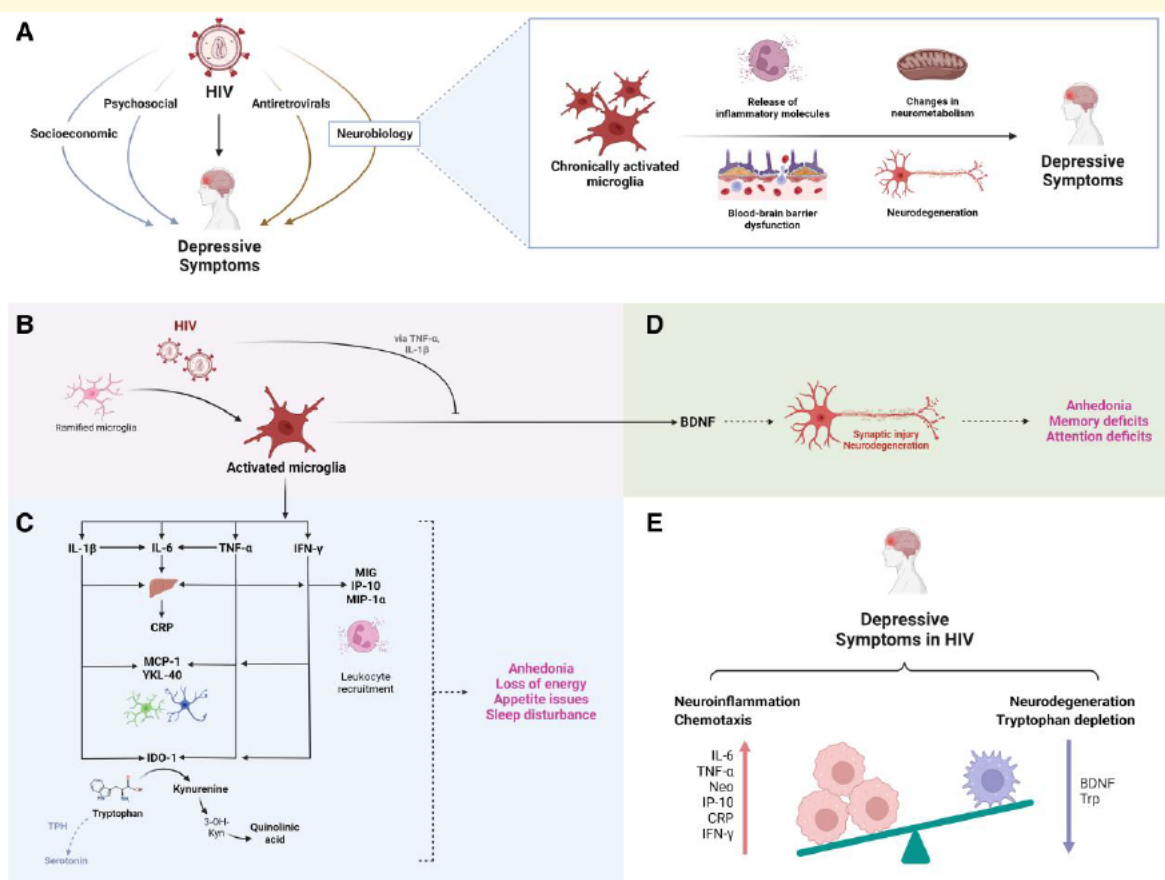


Figure 5 Visual summary of the hypothesized mechanisms driving the interrelationship between HIV, depression, and neuroinflammation. (A) High rates of depressive symptoms amongst people living with HIV may arise from a syndemic of psychosocial factors (such as social isolation or discrimination), socioeconomic stressors (such as unemployment or food insecurity), side-effects from certain antiretroviral medications, and possible neurobiological disruptions. (B) HIV infection in the CNS leads to the activation of resident immune cells, particularly microglia. (C) Activated microglia trigger a host of overlapping processes constituting a (neuro)inflammatory response, including the release of various cytokines and chemokines, recruitment of leukocytes, and upregulation of the kynurenine pathway of tryptophan catabolism. This inflammatory response is linked to sickness behaviours, such as loss of energy and sleep or appetite issues, which overlap with depressive symptoms. (D) HIV infection also inhibits the production and cellular transport of BDNF via the pro-inflammatory cytokines TNF- α and IL-1 β . The inhibition of BDNF in the CNS leads to synaptodendritic injury and neurodegeneration, which in turn contributes to the development of certain depressive symptoms such as memory and attention deficits. (E) In summary, HIV may drive the pathogenesis and severity of depressive symptoms by inducing neuroinflammatory cascades, neurodegeneration, and immuno-metabolic dysfunction via chronically activated microglia. A list of abbreviations is available in [Supplementary Table 5](#). Illustration created with BioRender.com.

depression also merits investigation. Pre-clinical evidence suggests that astrocytes are important regulators of neuroinflammation and may produce neuroprotective responses to cytokines.¹¹⁹ One recent study⁷³ specifically explored associations between depressive symptoms and an astrocytic activation marker in people living with HIV. Replication and further investigation of such associations are necessary to determine whether astrocytic markers may be valuable predictive biomarkers or therapeutic targets for HIV-associated depression. Glial cell functions in neuroinflammation do overlap: in an experimental model, both microglia and astrocytes express inflammatory cytokines corresponding to sickness behaviours, but microglial responses precede astrocytic responses.¹²⁰ This temporal distinction may implicate

microglia as ‘first responders’ to inflammatory challenges, and therefore, an important target for anti-inflammatory interventions to treat depression.

It is crucial to recognize that neurobiological mechanisms such as those outlined above can only explain in part, if at all, the elevated risk for depression amongst people living with HIV. The pathogenesis of depression in this population is likely driven by multiple contributing factors, including:

- psychosocial and structural factors such as social isolation, racism, or transphobia, which impact many of the communities disproportionately affected by HIV;¹²¹
- socioeconomic stressors, including unemployment and food or housing insecurity;¹²²

- antiretroviral medication, some of which lead to depressive symptoms as a side-effect and may thus confound the measurement of depression in virally suppressed people living with HIV;⁵² or
- managing other co-morbid chronic health conditions unrelated to HIV, such as diabetes.¹²³

Tackling the challenge of depression within this population will therefore require concerted efforts to systemically improve quality of life and social support, combat discrimination and stigma, and expand access to psychosocial services alongside biomedical interventions.

Future directions

This review revealed evidence from the past decade identifying associations of key neuroinflammatory cytokines, chemokines, and monocyte activation markers with depressive symptoms among people living with HIV. Future research may focus on testing the association between biomarkers such as IL-6 and TNF- α in samples that include participants without HIV, in order to establish whether the effects of these biomarkers are reliably influenced by HIV status. Leveraging developing techniques, especially neuroimaging biomarkers, may also support further investigation of neuroinflammation in HIV-associated depression. Although discussion of the gut microbiome was outside the scope of the present review, it must be noted that the composition of the gut microbiome may also modulate the impact of inflammation on depression.¹²⁴ Notably, depression has been linked to gut microbiome dysbiosis in people living with HIV.¹²⁵ These associations may be explored further in future evidence-synthesis exercises.

Future studies must endeavour to fully report test statistics and effect size estimates, not just *P* values, for all biomarkers, including those which do not show statistically significant associations. Reporting effect size estimates such as Odds Ratios (for dichotomous depression/no depression) or correlation coefficients (for continuous depressive symptom scales) will enable a robust meta-analysis of the strength of associations between various biomarkers and depressive symptoms.

Findings from this scoping review may support the translation of existing findings into clinical care for depression in people living with HIV. Biomarkers highlighted in this review may be further investigated in the following ways for appropriate translation:

1. Validating predictive biomarkers, by employing machine learning prediction techniques to large, diverse cohorts of people living with HIV for whom inflammatory biomarkers and depressive symptoms have been measured at multiple time-points.
2. Validating diagnostic biomarkers, by comparing concentrations of biomarkers in large, diverse cohorts of people living with HIV and demographically comparable people without HIV;
3. Validating potential therapeutic interventions, by directly assessing mediating effects of biomarkers (thus identifying biomarkers that are mechanistically involved in the pathogenesis of depression) and developing new therapies or re-purposing existing therapies which target these biomarkers.

Going forward, a strategic balance will be necessary between exploring biomarkers which have shown promise in several studies (as highlighted in this review) and employing broad panels of biomarkers which have not yet been widely assessed. In doing so, future studies can consolidate efforts for biomarker discovery while remaining receptive to serendipitous discoveries. Identifying robust and reliable biomarkers which are correlated with depressive symptoms in people living with HIV may enable the application of immunotherapies to target specific features of depression within this population. These interventions may then be delivered using a precision medicine approach to people living with HIV who exhibit an elevated immune profile alongside depressive symptoms. Together with socioeconomic and psychosocial support interventions, these immunotherapies may thus help reduce the burden of depression among people living with HIV.

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Supplementary material

Supplementary material is available at *Brain Communications* online.

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Competing interests

The author(s) declare no competing financial interests.

Data availability

No primary research data was generated for this article. Systematic search results (initial results, deduplicated results, studies screened using full text, and final included studies) are provided as CSV files in [Supplementary Materials](#).

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3.3 CONCLUSIONS

This scoping review extended the work described in Chapter 2 by adopting a systematic evidence synthesis approach to capture all recent studies exploring the role of inflammation in depression amongst people with HIV. I found that the biomarkers IL-6 and TNF- α , in particular, have been frequently associated with depressive symptoms in previous studies. Importantly, the direction of reported associations (where significant) was consistent for each biomarker, suggesting a degree of agreement in findings across studies. Based on this evidence, I proposed a mechanistic hypothesis involving chronically-activated microglia, which trigger the release of several of the biomarkers identified in this review, as the central link connecting HIV infection in the CNS with the increased risk for depressive symptoms in people with HIV.

These findings offer some support for the overarching hypothesis of the current research. However, at least a proportion of studies captured by this review did not find significant associations between inflammatory biomarkers and depressive symptoms in people with HIV. These mixed findings may, in part, be due to heterogeneity in biofluids (blood serum or plasma, CSF, saliva, or cervico-vaginal lavage) analysed in the included studies. Concentrations of inflammatory biomarkers across biofluids are poorly correlated, thus choice of biofluid may impact the ability to detect a true underlying association between biomarkers and depressive symptoms [30]. Another possible explanation for these mixed findings may be the sensitivity of immunoassays used in each study. Enzyme-linked immunosorbent assays (ELISAs), for instance, may detect inflammatory biomarkers with lower sensitivity or accuracy than Luminex, Meso Scale Discovery (MSD) or single molecular array (SIMOA) assays [31–33]. Differences in choice of immunoassays between studies may therefore also have contributed to the mixed findings.

Identifying and highlighting this heterogeneity in the field is a strength of this article, and arguably one of the core objectives of such a systematic evidence synthesis exercise. Findings from this scoping review provide a birds-eye view of research on inflammation and depression in people with HIV in the last 10 years and thus inform future investigations and biomarker discovery. Given the mixed findings surrounding IL-6 and TNF- α , this review identifies a need for harmonising research protocols across studies and further investigation of these biomarkers in larger cohorts to resolve these contradictions. Many studies in the field did not recruit participants without HIV. Including these participants as well-matched con-

trols may help compare the risk for depressive symptoms, and corresponding contributions of inflammatory biomarkers, between these groups. In COBRA-DI (Chapter 7) and the GOLD Depression Study (Chapter 9), I included data from demographically comparable people without HIV to allow for this comparison. Finally, no studies captured in this review had examined associations between inflammation and depression in children or adolescents with HIV; I have begun to address this critical gap in the literature through the GOLD Depression Study.

4

Equity in HIV Mental Health Research

HIGHLIGHTS

- Research on HIV and mental health conditions related to HIV are mostly carried out in the Global North, and often involve older White men. However, the majority of people with HIV are women and are located in southern and eastern Africa.
- To improve the chances of our research results being widely applicable, we need to diversify the participant samples in our studies. We also need to support researchers from a diverse range of backgrounds to thrive in HIV mental health research.
- In this article, I identified some ways to achieve this goal:
 - Funders can invest in setting up research cohorts of people with HIV in the Global South.
 - Scientific publishers can provide greater financial support and English language editing services to researchers.
 - Academic research centres can reward researchers who spend the time and energy required to set up studies involving people from diverse backgrounds.
 - Finally, individual scientists can educate themselves on building equitable partnerships and citing research from the Global South.

4.1 INTRODUCTION

CHAPTERS 2 AND 3 HAVE ALLUDED TO THE LACK OF DIVERSITY in participant samples in research on HIV and depression. This lack of diversity also extends to researchers (in addition to research participants) in the field. For instance, authorship of HIV research is overwhelmingly concentrated in high-income countries in the Global North [34], and even within specific countries such as the US, ethnic minorities are disproportionately underrepresented in HIV research [35, 36]. In this chapter, I highlighted issues of inequity in participant samples and researcher demographics within studies investigating mental health co-morbidities in people with HIV, and proposed potential approaches to improving equity and diversity in the field.

4.2 COMMENTARY: *MOLECULAR PSYCHIATRY*

COMMENT OPEN



Equity in HIV mental health research: a call to action

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The brain remains a key reservoir of latent HIV infection, and people living with HIV (PLWH) face a high risk for cognitive impairment and psychiatric disorders. Although the burden of HIV infection and co-morbidities is greatest in the Global South, a large proportion of HIV mental health research is carried out in the Global North. Large, well-funded observational cohort studies exploring HIV-associated psychopathology generally involve participant groups from WEIRD (Western, educated, industrialised, rich and democratic) settings. The socioeconomic status and institutional access afforded to these participant groups on average does not reflect those of the majority of beneficiaries of HIV mental health research. This misalignment may lead to limitations in generalising findings and developing effective interventions to improve the mental health of PLWH. Here, I offer recommendations to actively cultivate authentic diversity and inclusion in the field, with four focus points: (1) for funding bodies, to actively invest in neuroscientists in the Global South for investigations of HIV-related psychopathology; (2) for scientific publishers, to fund professional support services for researchers in the Global South; (3) for academic institutions, to facilitate meaningful, equitable collaborations with researchers in the Global South and incentivise studies with diverse participant groups; and (4) for individual neuroscientists, to actively cite and converse with colleagues in the Global South, tackle personal biases in those conversations, and avoid overgeneralising findings from primarily WEIRD participant groups.

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INTRODUCTION

The mental health of people living with HIV (PLWH) represents an important yet challenging frontier in neurovirology. Advancements in immunology and drug development have made viral suppression possible for PLWH who have access to combination antiretroviral therapy (cART). However, the brain remains a key reservoir of latent HIV infection even in virally-suppressed patients, and HIV-related neurotoxicity leads to substantial neuronal damage in PLWH [1]. Chronic HIV infection leads to a cluster of symptoms associated with neurocognitive impairment, notably memory issues and slowness, which have been collectively termed HIV-Associated Neurocognitive Disorders [2]. PLWH also face a remarkably high risk of depression, with studies estimating the prevalence of HIV-associated depression to be over 50% [3]. Together, these neuropathologies significantly reduce the quality of life for a large proportion of PLWH.

INEQUITY IN HIV MENTAL HEALTH RESEARCH

An estimated 5.8% of PLWH are in western/central Europe or North America, whereas 67.7% of PLWH are in Africa or the Middle East [4]. Despite this, a review of 68,808 studies found that over 80% of research into HIV published between 2010–2017 was produced in North America and Europe [5]. Authorship of HIV research is clustered in high-income countries in North America and Europe [6], whereas the greatest burden of HIV infection lies in the Global South, where access to latest-generation cART and psychological healthcare is also limited. Similarly, although the

global proportion of PLWH who are women is estimated to be 53% [4], only 19.17% of participants in studies of mental health issues amongst PLWH are women [7]. This divide represents a major gap in the field: studies of HIV-associated psychopathology continue to be carried out in settings that do not accurately capture the complex interrelationships between health, society, and culture that drive HIV mental health co-morbidities.

Not all clinical studies are created equal—and in HIV neurovirology, especially, studies often involve participant groups from Western, educated, industrialised, rich, and democratic (WEIRD) backgrounds. The socioeconomic status and institutional access afforded to many of these participant groups in large, well-funded observational cohort studies do not reflect those of the majority of people who are affected by HIV-associated psychopathologies and would thus be the beneficiaries of this research. Figure 1 highlights the distribution of sex and ethnicity in ten large observational cohort studies of HIV in Europe. (These studies were selected as examples since they each included more than 1000 participants and assessed HIV co-morbidities. Many of these cohorts included specific measurements of neuropsychiatric co-morbidities.) The total sample size for these ten cohorts was $N = 125,107$. Within this total sample, a remarkable 78.9% of participants were male, and 74.8% were White/European.

It may be argued that this skew in participant profiles may be because these studies are set in countries with predominantly White populations. That would not, however, explain why such a substantial proportion of study participants are male. This is particularly concerning since gender is a significant factor

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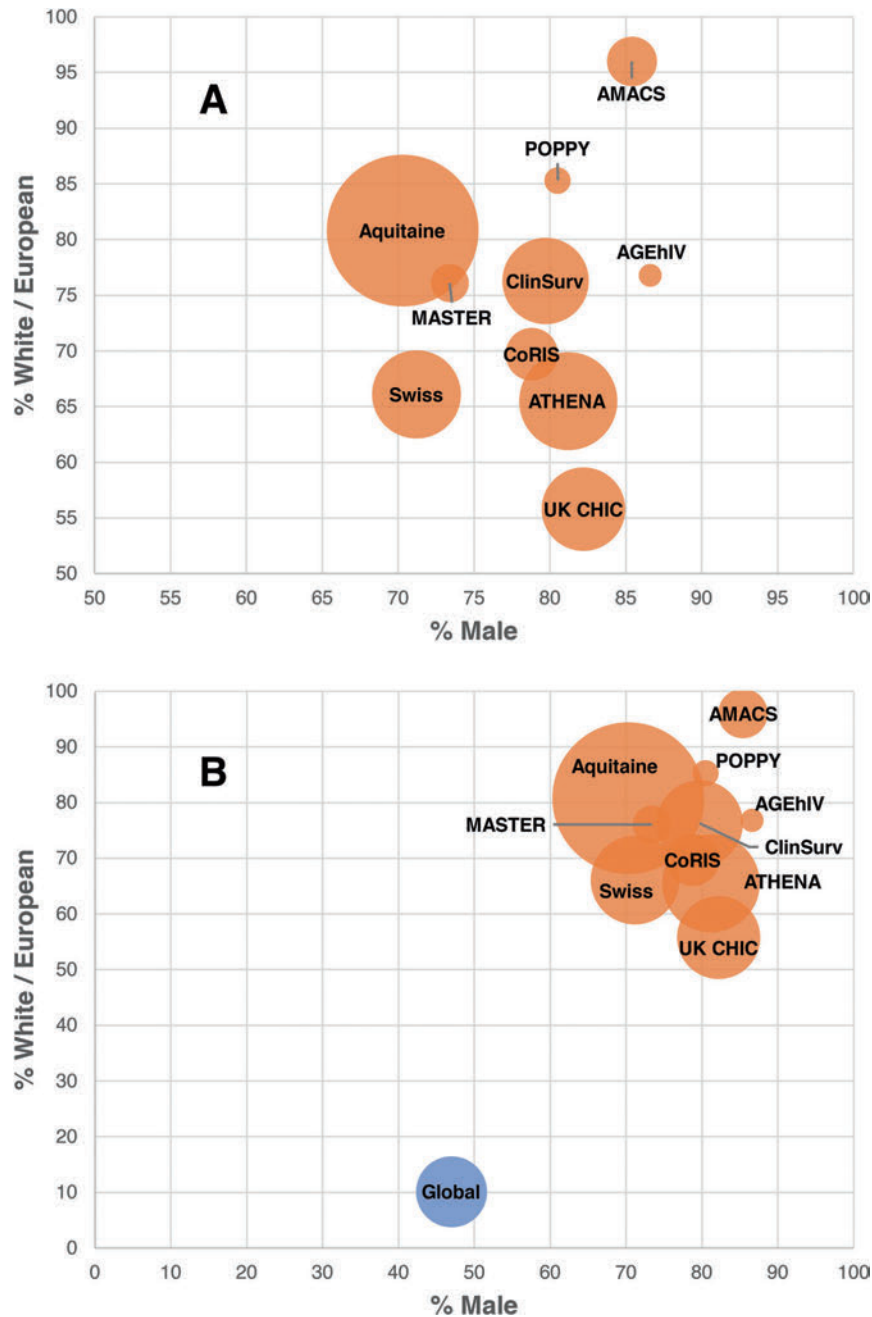


Fig. 1 Sex and ethnicity distribution in samples of ten European HIV cohort studies with $N > 1000$ each. A For cohort studies alone, and **B** alongside global estimates of % PLWH who are White and male. Size of each bubble is proportional to the total sample size of each study. For reference, global estimates of % White and % male PLWH using UNAIDS data are also shown. Data used to produce this visualisation, along with references for each cohort study, may be found in Supplementary Table 1.

predicting psychiatric morbidity amongst PLWH, such that women living with HIV are more likely to experience severe depression than men with HIV [8, 9].

The impact of this lack of gender and ethnic diversity cannot be understated. Follow-up studies rely on data obtained from these large cohorts to investigate new questions, and ultimately reproduce the same limitations in participant demographic distribution as the original studies. For instance, the AGE_hIV (Netherlands) and Pharmacokinetic and clinical Observations in PeoPle over fifty (POPPY, UK) cohorts were established to explore HIV co-morbidities. Both cohorts included assessments of mental health and neuropsychiatric outcomes. The COMorBidity in Relation to AIDS (COBRA) study utilised data from these parent

studies to further explore age-associated co-morbidities with HIV such as cognitive impairment. Reflecting the lack of diversity in participant groups within the parent studies, the participant sample included in the COBRA cohort was 92.7% White and 92.8% male [10]. Large cohort studies with non-diverse participant groups may thus encourage citation of, and secondary analyses based on, data from homogeneous samples with limited generalisability.

Crucially, findings from these studies may be used to inform policy worldwide, especially as studies with sample sizes in the thousands may be perceived as inherently more reliable. Together with the lack of investment in HIV cohort studies in the Global South, this means that any evidence-based public health policies

related to HIV, even in countries where most PLWH are not White or male, must rely on findings from these European cohort studies. Key sociocultural factors such as access to latest generation cART, degree of social stigma associated with HIV, and lifestyle influence progression of neuropsychiatric co-morbidities and differ considerably across the globe. Studies in the Global North leverage participant groups with a narrow sociocultural profile, which limits the application and relevance of these findings to policymaking in the Global South.

Moreover, prevalence rates of psychiatric co-morbidities can vary drastically by sex, ethnicity, income, and geography [11], with studies in WEIRD settings often reporting lower rates of mental health issues. Without rigorous comparative research in diverse world regions and conscious investment in improving the sex distribution in participant samples, findings from primarily male samples and WEIRD settings may be the only evidence available to inform public health policy. Given varying prevalence rates, this evidence may therefore drive public sector support away from critical psychosocial services for PLWH, and women with HIV specifically, in regions where prevalence rates—and thus need for support and public health investment—may in fact be much higher.

Finally, a lack of representation and local capacity-building may encourage “helicopter” or neo-colonial research, which can open the door for unethical or potentially harmful clinical trials being carried out with populations in the Global South. This is not unprecedented [12]. Improving representation within the field would allow for the involvement of researchers with cultural or historical understanding, thus facilitating better monitoring of global trials and ensuring equitable study design. Similarly, improving diversity of participant groups has the potential to enhance our understanding of mental health disparities, better track psychiatric co-morbidities that disproportionately affect certain groups of PLWH, and more equitably disseminate findings from research [13]. Therefore, there is much to be gained from addressing the lack of diversity within studies of HIV mental health.

WHERE DO WE GO FROM HERE?

Given that much of HIV neurovirology research is carried out in the Global North, and since participant groups in many of these large HIV cohort studies are overwhelmingly White and male, there is an urgent need to pursue authentic diversity and inclusion in the field. As a growing number of institutions across the world have committed to enhancing diversity and inclusion in science, following are some recommendations to actualise these commitments in the context of HIV and mental health research.

Funding bodies must actively invest in scientists in the Global South to investigate HIV-related psychopathology. This is increasingly urgent given recent UK funding cuts to foreign research that have severely impacted researchers in the Global South [14]. Setting up HIV cohort studies, particularly those with large (>1000) sample sizes, is an expensive affair anywhere, and doing so is especially challenging in formerly-colonised nations where resources have been drained over several generations. Being at the favourable end of currency exchange rates, funding bodies in the Global North are well-positioned to create significant impact with relatively small investments. Therefore, these agencies must prioritise funding for research and administrative costs associated with establishing longitudinal HIV cohorts and developing language- and culture-specific research tools in the Global South.

An initiative funded by the National Institutes of Health placed US scientists from minority ethnic backgrounds into resource-limited settings through the HIV Vaccine Trials Network at domestic and international placement sites [15]. The rationale behind this project was that scientists from minority ethnic backgrounds may better understand the issues affecting underserved communities and be able to build relationships with community members. This initiative is an example of successful

investment in HIV research that generated tangible professional developments for the scientists who completed these placements.

However, going forward, the training and professional development of scientists from local communities must be prioritised. It is important to avoid homogenising individuals across artificial categories of race or ethnicity in such cross-border initiatives. For instance, it is unfair to assume that a Black scientist from the US can automatically relate to and engage with Black research participants in a placement in South Africa. Instead, investing in empowering researchers from indigenous backgrounds to conduct and disseminate research in their communities is necessary to truly build capacity in the Global South and move away from a “top-down” power dynamic.

Funding decisions can be shaped by the ability to navigate complex and often unwritten rules of grant writing, which are inaccessible to researchers from minority backgrounds without significant mentorship or generational links. Investing in local capacity-building and mentorship will allow researchers from the Global South to gain access to these funding mechanisms and help create a more equitable funding landscape. In turn, this will empower these researchers to take the lead in driving the research agenda.

Funding agencies are well-situated to foster a culture of substantive self-critique. It cannot simply be enough to say that 90% of participants in a study were White or male; authors must justify their participant profiles or offer comparisons with local or regional demographics. In doing so, researchers in the Global North can avoid overgeneralising findings from primarily WEIRD participant groups or overlooking consistent and systematic barriers that may prevent certain groups from participating in research. Instead, researchers can be empowered to embrace honest critiques of their own work when discussing their findings in publications. However, this is only possible when funding bodies encourage such reflections and commit to not penalising researchers for these self-critiques in future applications. This would especially allow early career researchers who may wish to seek funding to establish more diverse HIV cohorts in future to still critique their existing work honestly without fear of losing funding.

Scientific publishers must increase financial and professional support for researchers from the Global South. Currently, article processing fees charged to publish in open access journals represent a substantial hurdle for authors from the Global South. While some publishers offer open access waivers, researchers from the Global South are often ineligible for these if they take lead on projects which involve collaborators in the Global North. Publishers must continue to expand the open access waiver programmes for researchers from low- and middle-income countries in ways that allow for cross-border collaborations.

Another relatively straightforward way in which publishers can demonstrate their commitment to promoting diversity and inclusion in research is by funding English language editing for scientists in the Global South. Researchers who are non-native English speakers face a dual challenge: having to pay for English language editing services adds expenses to already stretched research budgets, but not paying for such services (or relying on unpaid labour of colleagues) runs the risk of robust, well-designed research being dismissed or perceived as “less than” amongst peers in the Global North due to superficial grammatical flaws. Providing free access to English language editing services before, during, and after the manuscript review process is thus a concrete way in which publishers can increase their support of researchers in the Global South. In turn, publishers who use a small proportion of their profits to offer funding for English language editing will gain a competitive edge over others, as authors will view such material support favourably and will be more likely to submit to, and publish with, publishers who offer these services.

Academic institutions, particularly those in the Global North, must actively facilitate collaborations with researchers in the

Global South and incentivise studies with diverse participant groups. Fostering collaborations with colleagues in different countries takes substantial work for scientists. Research institutions can offer meaningful support in turn by simplifying the process of establishing contracts to achieve these collaborations and giving researchers accessible resources on the relevant legal and administrative requirements.

Institutions can additionally promote capacity-building through strategic collaborations that create opportunities for researchers in the Global South to access high-quality professional development opportunities, training, and specialised equipment. Development and execution of studies involving diverse participant samples may also be prioritised in key academic processes such as tenure or institutional funding support. Improving diversity and inclusion in participant samples should not become an obligation or a chore that scientists in the Global North dread; that would be deeply counter-productive. Rather, funding bodies and academic institutions are uniquely well-placed to incentivise this pursuit and cultivate a positive ideal towards which researchers may strive.

Finally, **individual scientists** must actively cite and converse with colleagues in the Global South. Robust, reproducible research using gender- and ethnically-diverse participant samples must be highlighted at HIV and neurovirology conferences in the Global North, so that new collaborations and follow-up studies may emerge from this work. It is not sufficient to merely interact with researchers in the Global South; researchers must actively develop respectful and culturally-sensitive communication skills so as to tackle their own personal biases in these collaborations. Perhaps most crucially, individual scientists must listen to colleagues and, more broadly, people with lived experience of HIV and psychiatric co-morbidities from the Global South. By taking the lead from voices in the Global South, researchers in the Global North can participate in research that is inclusive and impactful.

When interviewing for a highly prestigious immunology PhD programme in the US a few years ago, I explained to one of the faculty that I planned to study HIV, who responded: "Oh, so you're gay?" That response is less unusual than one would hope, and it reflects a common assumption that HIV research is only important to the LGBTQ+ community or to people in sub-Saharan Africa. We cannot allow these stereotypes to persist in the field. As we work to establish large cohort studies with more diverse participant groups, it is important for individual researchers to challenge these assumptions and be respectful in their collaborations with colleagues in the Global South.

CONCLUSION

Sociocultural aspects play an important role in HIV-associated psychopathologies, and there is compelling evidence that socio-economic factors interact significantly with biology to predict disease progression [16]. Therefore, it is critical to replicate findings in diverse populations. Improving mental healthcare available to PLWH requires concerted, global interventions, which must necessarily be founded on robust and inclusive research. Diversifying participation in research in this field will require commitment from stakeholders across sectors. Furthermore, research on HIV mental health co-morbidities must include and empower the voices of PLWH themselves to shape the research agenda. The pursuit of authentic diversity and inclusion in HIV mental health research will enhance the reproducibility and generalisability of findings and ultimately benefit researchers, funders, and public health alike.

DATA AVAILABILITY

Study sample characteristics associated with Fig. 1 may be found in Supplementary Table 1. No other data were generated for this article.

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AUTHOR CONTRIBUTIONS

AMRL conceptualised this article and wrote the manuscript.

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4.3 CONCLUSIONS

In this article, I demonstrated some of the inequities in HIV mental health research by examining the demographic characteristics of 10 large and highly-cited European cohorts of people with HIV. The majority of participants in these cohorts are White/European men, despite the majority of people with HIV globally being women and located in southern or eastern Africa. Lack of ethnic and gender diversity in these large cohorts may perpetuate further inequities in health outcomes for people with HIV. Evidence from these cohorts – which may be perceived as inherently more reliable due to their size, and may sometimes be the only sources of data on certain health outcomes in people with HIV – can be used to drive health policy worldwide, but their findings may not be reproducible in diverse populations. It is therefore crucial to diversify participant samples through greater investment in HIV cohorts in the Global South, equitable cross-border research partnerships, and incentivising research involving gender- and ethnically-diverse samples. In parallel, we must empower and support researchers from marginalised backgrounds in the Global South to succeed in HIV mental health research.

5

Research Priorities of Young People with HIV

HIGHLIGHTS

- It is important that our research addresses questions that are truly important to whichever community we hope to serve. One way of ensuring this is by talking to people in this community and asking them which questions are important to them.
- For our research clinic in Cape Town, South Africa, the community we serve is young people (children and teenagers) with HIV. Some of these young people (and their parents or caregivers) were invited to share their priorities in a facilitated workshop.
- The most important priority for young people with HIV in this workshop was finding a cure for HIV. They were also interested in research on the effects of ART medications on the brain, body, and social relationships, causes of mental health issues, and ways of reducing stigma about HIV in schools.
- Parents and caregivers were interested in research on ways to make sure their children take their ART medication regularly as prescribed, mental health impacts of someone disclosing their child's HIV status without their permission, and improving support from local community clinics.

5.1 INTRODUCTION

TO ENSURE THAT OUR RESEARCH AUTHENTICALLY RESPONDS to community needs, we must actively listen to and take lead from people with HIV. Priority setting workshops are a form of community engagement which enable people with relevant lived experience to set the research agenda. Using such a workshop, I aimed to understand the research priorities of young people with HIV and their parents or caregivers. During the day-long workshop hosted in Cape Town, attendees collectively identified research questions concerning three themes (HIV-related physical health, mental health, and psychosocial support) and voted on the order of importance for these questions.

5.2 ARTICLE: *PLOS GLOBAL PUBLIC HEALTH*

RESEARCH ARTICLE

Taking lead from the community: What do young people living with HIV want us to research?

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*



Abstract

Priority setting workshops enable researchers to take the lead from people with relevant lived experience, and design research which authentically responds to community needs. Large-scale global priority setting exercises have previously identified key research questions related to paediatric and adolescent HIV treatment, prevention, and service delivery. However, priority setting workshops focused on the needs of young people living with HIV are lacking in southern Africa. Here, we report the outcome of a priority setting workshop organised in Cape Town, South Africa with 19 young people living with HIV and their parents and caregivers. Workshops were facilitated by trained research and clinical staff, who provided a plain-language introduction to research questions for the attendees. During the day-long workshop, attendees developed a list of research questions concerning HIV-related physical health, mental health, and psychosocial support and later voted on the order of importance for the questions which they had collectively identified. Facilitators did not prompt any questions or amend the phrasing of questions generated by the attendees. A cure for HIV was highlighted as the most important research priority for young people living with HIV. Other priorities for young people included the effects of antiretroviral therapy on the body, the brain, and their social relationships, causes of emotional issues such as depression and mood swings, and potential interventions to reduce HIV-related stigma in schools through positive education for teachers and students. Research priorities for parents and caregivers included improving antiretroviral adherence through long-acting injections, mental health impacts of HIV status disclosure without consent, and improving support provided by local community clinics. The research questions identified through this workshop may be used by researchers to develop future studies which truly benefit young people living with HIV in South Africa and beyond.

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Introduction

The importance of taking lead from the community when designing, conducting, and disseminating biomedical research is increasingly being recognised [1]. Many institutions and funders now require that biomedical research should meaningfully involve people with relevant lived experience as early as possible, including when designing the study. This represents a paradigm shift away from *researcher-led research design*, where researchers identify questions and design studies with little to no input from people with lived experience, towards *community-led research design*, where community members take the lead on identifying questions they want to be answered and working with technical experts to develop research studies that address these priorities [2].

Priority setting workshops provide a space for community members to collectively identify these priorities and determine their order of importance, so that research may be best placed to truly benefit the community [3]. Large-scale priority setting workshops have previously been carried out to define priorities for HIV-related biomedical research for children and adolescents. These workshops, led by the World Health Organization (WHO) and the Collaborative Initiative for Paediatric HIV Education and Research (CIPHER) of the International AIDS Society (IAS), identified key research questions for HIV testing, treatment, and service delivery from respondents in over 60 countries [4, 5].

Although these large-scale prioritisation exercises offer value in identifying global trends in community priorities, understanding local community needs is equally important. Few published priority setting workshops have focused exclusively on people living with HIV in southern Africa—and none, to our knowledge, focusing on young people living with HIV—in part because these formal approaches to community engagement are relatively new in this region. Clarke and colleagues [6] recently reported on their priority setting exercise for older adults living with HIV in Tanzania, in which they noted that basic needs (income, nutrition, shelter) and removal of stigma were highly important to these individuals. The authors suggest that these priorities are aligned with the national context, including vulnerability to financial, food, and housing insecurity and increasing costs of healthcare visits. Therefore, priority setting workshops can offer crucial context for researchers who serve people living with HIV in resource-limited settings, but this context is currently missing as very few such workshops have been reported in southern Africa.

The Family Centre for Research with Ubuntu (FAMCRU) is a clinical research centre established in 2002 at Tygerberg Hospital in Cape Town, South Africa, which focuses on infectious disease research. We regularly recruit children and young people living with HIV for large-scale research studies and clinical trials, focused on HIV treatment and prevention, mental health issues, and psychosocial support for young people living with HIV and their caregivers. We also host monthly peer support workshops for young people who attend our clinic (regardless of HIV status) led by an experienced social worker (co-author TB). Here, we report on a priority setting workshop which we organised at FAMCRU, with the dual aim of enhancing trust and transparency with the community and taking lead from young people living with HIV on the research questions which we should pursue in the future.

Methods

Workshop attendees

We invited young people (aged 14 to 18 years old) living with perinatally-acquired HIV to this workshop, along with one parent or caregiver for each young person. Potential attendees were invited from amongst young people who lived in Cape Town and surrounding areas (primarily

Khayelitsha) who had previously participated in research studies at FAMCRU. Attendees were invited to join the workshop regardless of their home language (English or isiXhosa). As this was a community engagement event and not a research study visit, we did not record any sociodemographic information for those who attended the workshop.

Workshop structure

Young people living with HIV and their parents or caregivers attended separate, parallel workshops in different rooms so that all attendees could speak freely. The structure for both parallel workshops was harmonised. Both workshops were facilitated by research and clinical staff from FAMCRU, and there was at least one facilitator fluent in isiXhosa in both rooms. Instructions provided to workshop facilitators are available in [S1 File](#) for any researchers seeking to conduct similar workshops in future.

Workshops began with personal introductions and ground rules established by attendees and facilitators in each room. The facilitators provided a plain-language introduction to research questions, with some examples of questions which are currently being investigated at FAMCRU. The goal of the workshops was for attendees to identify research questions which matter to them around three themes: HIV-related *physical health*, *mental health*, and *psychosocial support*. Attendees in each room were split into three smaller discussion groups, in which they discussed each theme (in rotation) and developed research questions for it.

Following the small-group discussions, questions for each theme developed by all smaller groups were compiled. Attendees in each room then had an opportunity to revise or clarify the phrasing of any questions, after which all questions for each room were compiled into a ballot box. All attendees were then given a printed ballot paper and an equal number of stickers, which they could “spend” on questions according to the importance which they ascribed to each question, with a greater number of stickers assigned to a question indicating greater perceived importance. Votes for each question were then tallied from all attendees to develop a ranked list of research priorities. Throughout this process, questions developed by young people living with HIV and their parents or caregivers were kept separate.

Before adding questions to the ballot box, facilitators merged any questions from smaller groups which were identical or closely matched. Apart from this, the facilitators did not suggest any questions to attendees, nor did they amend or delete any questions identified by attendees.

Ethical considerations

Community engagement exercises in the Global North are considered distinct from research. Participating in a research study, where a participant is told what to do by a researcher (passive participation) and is often exposed to novel or experimental procedures, is substantially different from participating in a community engagement event, where attendees (ideally) hold equal power to shape the direction of the event (active engagement) and risk of exposure to hazards is negligible. For these reasons, community engagement exercises in the Global North are often not subject to ethical review.

In southern Africa, where formal approaches to community engagement are newer, no standard guidance yet exists to inform ethical review for community engagement. Clarke et al. (2023) report, for instance, that their priority setting workshop underwent formal ethical review as a precautionary measure [6]. This lack of standard guidance may hinder good-faith efforts to create more opportunities for formal community engagement in the region, especially in comparison to the Global North. A summary of plans for our workshop were reviewed by leadership of the Stellenbosch University Health Research Ethics Committee (HREC), who

confirmed that this event was distinct from research and thus did not require formal ethical review or informed consent from attendees.

We organised this workshop with several ethical considerations in mind. As we only invited young people living with HIV to the workshop, all potential attendees were informed that their participation in the workshop would indirectly disclose their HIV status to other attendees. To preserve attendees' privacy, we did not request or record any personal identifiers or demographic information. No audio, photos, or video were recorded during the workshop. All attendees (young people and their parents or carers) were reimbursed for their time and their travel expenses to attend the workshop and lunch was provided.

Results

The workshop was attended by 19 young people living with HIV, and their parents or caregivers. Each small discussion group thus had 6–7 attendees. Attendees were actively engaged throughout the day-long workshop and developed many potential research questions collectively. The full list of questions generated by young people and their parents and caregivers is available in [S2 File](#). Note that we report questions generated by attendees verbatim, without editing any phrasing once these questions were approved by the attendees.

Research priorities for young people living with HIV

The top five research priorities identified by young people living with HIV are shown in [Table 1](#). These attendees collectively indicated that a cure for HIV remains the most important priority for young people living with HIV. Attendees also want to know effective ways of communicating one's HIV status and whether an undetectable viral load allows them to engage in condom-less sex without the risk of HIV transmission to their partners. Possible links between antiretroviral therapy (ARVs) and depression were also identified. Finally, young people living with HIV in our workshop wanted to know whether their friends and family perceive them differently because of their HIV status (i.e. HIV-related stigma or discrimination).

Research priorities for parents and caregivers

Many parents were themselves living with HIV, so attendees in the parents and carers' workshop also raised research questions that were relevant to their own experiences of living with HIV. The top five research priorities identified by parents and caregivers of young people living with HIV are shown in [Table 2](#). These attendees want to know whether long-acting injectable ARVs can help improve their children's adherence to ARV regimens. Attendees also expressed concerns about stigma at local community clinics, and wanted to know how these clinics can better support children living with HIV who have previously received their clinical care at specialist research clinics. Parents also identified gender-specific questions around their

Table 1. Top five research priorities identified by young people living with HIV.

Rank	Research Question
1	Can HIV be cured?
2	How can we communicate and disclose our HIV status to friends, family, and partners?
3	Is it safe to have unprotected sex when you are undetectable and your partner is HIV negative?
4	Do ARVs cause depression?
5	Do friends and family see us differently because of our HIV status?

ARV: antiretroviral therapy.

<https://doi.org/10.1371/journal.pgph.0002605.t001>

Table 2. Top five research priorities identified by parents and caregivers of young people living with HIV.

Rank	Research Question
1	Can ARV injections help improve adherence?
2	How can local clinics better support children who are referred from research clinics?
3	How can we empower girls living with HIV to make responsible sexual decisions?
4	How can we improve relationships between mothers and sons?
5	How do we encourage family members to accept someone's status?

ARV: antiretroviral therapy.

<https://doi.org/10.1371/journal.pgph.0002605.t002>

relationships with their children, including ways of empowering girls to make responsible sexual decisions as they approach young adulthood and improving communication between mothers and sons. Finally, parents and caregivers were interested in ways of encouraging family members to accept their children's HIV status, "as much as [their child] has accepted this already." (quote from anonymous workshop attendee)

Research priorities by theme

The top five research priorities identified by young people living with HIV, and top three research priorities identified by their parents and caregivers, ranked within each theme discussed in the workshop are shown in Fig 1. Parents and caregivers identified fewer questions (5–6 questions per theme) than young people living with HIV (10–12 questions per theme), so we highlight only the top three priorities for parents and caregivers in this figure, but the full list of questions identified by each group is available in S2 File.

For young people living with HIV, in addition to questions about a cure for HIV and risk of transmitting HIV when undetectable, the most important research priorities in HIV-related physical health revolved around ARVs. Attendees wanted to know how ARVs affect their bodies in positive or negative ways, the consequences of ARV interruption, and why ARVs must be taken daily. Potential consequences of taking ARVs were also highlighted in questions around mental health, such as why young people may not want to take ARVs and whether these medications affect their emotions, cognitive skills (e.g. exam-taking), relationships, or sex drive. Depression emerged as the top mental health research priority for young people living with HIV, with several highly-ranked questions revolving around the causes of depression, mood swings, and changes in emotional health. Notably, some (but not all) attendees had participated in depression-related research in 2022, so they may have been primed to thinking about depression as a primary concern. With regards to psychosocial support, young people living with HIV were most concerned with HIV status disclosure, perceived stigma or discrimination, and ways of positively educating both students and teachers at schools to reduce stigma.

For parents and caregivers of young people living with HIV, in addition to the potential benefits of long-acting injectable ARVs on treatment adherence, the top physical health-related priorities were potential side effects of ARVs, including changes in sex drive, body weight, and appetite (for their children as well as for themselves, in the case of parents who were also living with HIV). Parents and caregivers also prioritised research which may explain the mental health impacts of disclosure of one's HIV status without one's consent. In terms of psychosocial support, HIV status disclosure was highly ranked as a priority, with questions around encouraging others to accept someone's status while also equipping them to treat such a disclosure with discretion and confidentiality.



Fig 1. Top research priorities for young people living with HIV and their parents and caregivers for HIV-related physical health, mental health, and psychosocial support. The figure displays the top 5 ranked list of priorities for young people living with HIV, and the top 3 ranked list for their parents and caregivers; number of votes were tied for some questions identified by young people living with HIV. Size of each circle is proportional to the number of votes received for each question. Questions are displayed in descending order by number of votes within each category.

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Discussion

We organised a priority setting workshop involving young people living with HIV, and their parents and caregivers, who reside in Cape Town, South Africa. Attendees at this workshop collectively identified a set of research priorities and voted to decide on their order of importance. Perhaps unsurprisingly, the research priority highlighted as the most important for young people in our workshop was a cure for HIV. These research priorities identified by attendees can inform future directions of research for biomedical scientists and clinicians serving young people with HIV. Many questions posed by young people and their carers may lead directly into future research, and indeed, several are already under investigation: for instance, reducing the side effects of ARVs on the body, health-related consequences of ARV interruptions, and possible differences in cognitive skills or brain function between people living with and without HIV. The research questions that emerged from our workshop, provided in

Tables 1 and 2 and Fig 1, and S2 File, may be of broad interest to HIV researchers in South Africa and similar settings with high HIV prevalence.

Young people living with HIV and their parents and caregivers were particularly interested in how ARVs affect their health and their relationships. There were also overlapping interests in research on HIV status disclosure and stigma reduction between the two groups. Both groups also identified improved support from local community clinics for young people referred from research clinics as a priority. Given that the workshop attendees had previously received their HIV-related clinical care at FAMCRU, a specialist research clinic with staff who are trained to care appropriately for young people living with HIV, this research priority may indicate a gap in HIV-related sensitivity training in community clinics.

Notably, all young people who attended our workshop were living with perinatally-acquired HIV. While many of the questions identified by them could be relevant to other groups of young people living with HIV, those young people who acquire HIV later in life may have different priorities or may rank the importance of these priorities differently. Similarly, many parents of young people in our workshop were also living with HIV, whereas parents or caregivers of young people who acquire HIV later in life may not be living with HIV themselves and may propose different research priorities entirely. It will therefore be important to carry out similar workshops in future with other groups of young people living with HIV and their caregivers.

Notable differences between research priorities identified by young people and their carers included an emphasis amongst young people living with HIV on stigma reduction in school settings. These attendees spend a substantial proportion of their time in school, and many of their social relationships are also formed in this setting; thus, these young people highly prioritised research on positive education about HIV to help reduce stigma amongst both teachers and other students. Parents and caregivers were concerned about the mental health impacts of HIV status disclosure without consent on their children, as well as being interested in research on how they might emotionally prepare themselves for instances when their child's status is disclosed without consent.

These priorities align with the local and national context, as young people living with HIV in South Africa continue to face substantial stigma and educational delays [7]. Discrimination due to HIV status is also a common experience amongst young people with HIV in South Africa [8]. For these reasons, young people living with HIV may emphasise the development of interventions to positively educate communities about HIV at school, whereas their parents and caregivers may prioritise research around HIV status disclosure.

The outcome of our priority setting workshop makes a compelling case for greater and more effective science communication by researchers serving young people living with HIV in South Africa. Certain research questions identified by young people in our workshop have already been investigated; in particular, there is robust evidence that people living with HIV who have an undetectable viral load cannot transmit the virus to their partner through condom-less sex [9]. Nevertheless, this was one of the top five research questions for young people in our workshop, which suggests that findings from emerging HIV-related biomedical research are not successfully reaching these young people (and, by extension, health educators). Thus, research findings must be shared by researchers and science communicators with these young people living with HIV more intentionally and effectively. Successful approaches may involve appropriate transcultural translation of dissemination materials and accessible social media formats which are most likely to reach young people living with HIV in resource-limited settings. Dissemination plans must meaningfully involve young people living with HIV in the respective local contexts to maximise the reach and impact of research findings.

Our priority setting workshop may offer a helpful model for other researchers serving people living with HIV in South Africa and beyond. Our event was well-attended and all attendees were actively engaged; this likely stemmed from existing positive relationships between the attendees and staff at FAMCRU who facilitated these workshops. All facilitators must be appropriately qualified to work with young people living with HIV and trained in the necessary facilitation skills. This is particularly important to avoid leading prompts and ensure that all questions are developed organically by the attendees. While we were successful in using stickers as votes which each attendee could “spend” to indicate their preferences, other researchers may try different ways of conducting voting to determine the order of importance for research questions. Activities within these workshops must be tailored to the language, literacy, and health literacy levels of all attendees. We found that inviting groups to designate one note-taker amongst themselves was a suitable way of ensuring that all groups recorded the questions they discussed, but that any attendees who were not comfortable with reading or writing were not excluded.

Finally, although similar priority setting workshops in southern Africa have undergone formal ethical review, we were successful in making a case to the relevant ethics review board that this activity did not require formal review. We encourage other researchers in southern Africa who wish to carry out similar priority setting workshops (while, of course, taking into consideration all notable ethical concerns for involving vulnerable individuals such as young people and those living with HIV) to use our work as an example when communicating with ethics review boards to determine eligibility for ethical review. This form of community engagement can be carried out ethically and responsibly without necessarily requiring formal ethical approval. By standardising this approach to the ethics surrounding community engagement, we may level the playing field for researchers in the Global South who are interested in community engagement practices but often have to complete additional administrative requirements compared to their colleagues in high-income countries.

We have shown here the feasibility of organising a small-scale priority setting workshop involving young people living with HIV and their parents and caregivers. Through this workshop, attendees developed a ranked list of research priorities which can inform future directions of inquiry for biomedical researchers serving young people living with HIV in South Africa and beyond. This workshop provides a model for meaningful engagement with people with relevant lived experience in a resource-limited setting, so that researchers take lead from the community—rather than assuming community needs—to inform future research design.

Supporting information

S1 File. Instructions provided to workshop facilitators.
(DOCX)

S2 File. Research priorities identified by young people living with HIV and their parents and caregivers. Number of votes received for each priority are provided next to each research question, along with a section rank (i.e. rank within the category in which the question falls) and an overall rank (i.e. rank across all three categories).
(XLSX)

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5.3 CONCLUSIONS

This chapter reported the outcomes of a priority setting workshop hosted in Cape Town, South Africa. This workshop was attended by 19 young people with HIV and their parents or caregivers, who identified and ranked the research questions which were important to them. Of particular relevance to this thesis, the causes behind depression and other psychological concerns (such as mood swings and “brain fog”) were voted as highly important by the young people with HIV who attended our workshop. The research in this thesis therefore responds to a community need by exploring whether depression in people with HIV may be driven by inflammation.

No published priority setting workshops had previously sought to identify the research priorities of young people with HIV in southern Africa, and I organised this workshop to address this critical gap. This workshop was hosted in July 2023, well after I had developed my PhD studies – and, indeed, after several of these studies had already been completed. Although it would have been useful for me to know these community priorities when designing my PhD studies, successfully organising a workshop such as this required me to have strong working relationships with staff at the Family Centre for Research with Ubuntu (FAMCRU) and institutional support from Stellenbosch University. I cultivated this support over time as I worked with the research team at FAMCRU, so I was best placed to deliver this workshop effectively only as I progressed through my research. In doing so, I have now produced a rich resource of community-identified research priorities available for other researchers in southern Africa to use in future.

Although I organised this workshop in the latter stages of my PhD, the lived experience and priorities of people with HIV and people with depression has nevertheless shaped my research. Towards the end of my undergraduate degree, I was awarded the highly competitive **Thomas J. Watson Fellowship**. This national Fellowship funds a few graduates “of unusual promise” from across the US each year to engage with their deepest interest on a global scale. The challenge is simple: a Watson Fellow cannot go anywhere they have gone before. As my deepest interest is in HIV, during my Fellowship I chose to work with grassroots and national HIV healthcare movements in eight countries. While working with these organisations, I learnt that mental health was rapidly becoming a key priority for people with HIV. This insight into community priorities led me to subsequently design my PhD research on the neurobiological underpinnings of depression in people with HIV.

During the course of this research, I have also worked closely with people with relevant lived experience at multiple stages:

- Through Edinburgh's Translational Neuroscience PhD programme, I partnered with a person with HIV and met with him at regular intervals. The objective of this semi-formal partnership was to facilitate knowledge exchange, enabling me to better understand my Lived Experience Partner's priorities and experiences, and for him to get a "behind the scenes" look at clinical research in HIV mental health.
- To develop the isiXhosa translation of the Patient Health Questionnaire (PHQ-9), I worked with non-profit organisations in Cape Town, South Africa to consult people with HIV and people with depression. I met with these individuals as part of the multi-step translation process to seek their input on the translation (Chapter 8).
- With help from my Lived Experience Partner, I also convened a panel of people with HIV in Brighton, UK to provide feedback on the design and participant-facing materials for INSIDE-HIV (Chapter 10). The participant-facing materials were amended in response to their feedback. Their input also factored into the ethics review process for this study: I was better able to argue for the retention of a one-page summary participant recruitment document, which the ethics review board had asked us to exclude in favour of the longer Participant Information Sheet, because the lived experience expert panel had previously provided us with feedback that this one-page document had been extremely useful for them.

Therefore, meaningful engagement with people with relevant lived experience has directly informed my PhD research. The priority setting workshop described in this Chapter was another opportunity for me to understand and highlight the priorities of the community which I hope to serve – people with HIV. The research questions identified through this workshop may inform the design of research in future. By centring the priorities of young people with HIV in future studies, we may deliver research that truly responds to the needs of this community in South Africa and beyond.

6

Aims & Methodology

HIGHLIGHTS

- The aims of this research were to find out whether inflammation contributes to the higher risk for depression in people with HIV, and whether this may be true for people in different parts of the world (and thus from different socioeconomic and cultural backgrounds). I also wanted to find out whether brain scans can be used to differentiate between people with HIV who experience severe depression and those who experience mild depression.
- For this research, I worked with three groups of participants: the COBRA cohort of older people with and without HIV in the UK and Netherlands, the GOLD cohort of young people with and without HIV in South Africa, and a small number of people with HIV and severe depression who were recruited for a study in Brighton (UK).
- In all three research studies, symptoms of depression were measured using a questionnaire called the PHQ-9, which is used worldwide and is a reliable tool to screen for depression.
- Inflammation was measured by testing blood or cerebrospinal fluid samples for certain proteins which are released by immune cells in the blood, and by using brain scans to indirectly measure concentrations or movement of certain biomolecules released by immune cells in the brain.

6.1 PROJECT AIMS

Aim 1: Evaluate the contribution of peripheral and central nervous system inflammation to the association between HIV status and depressive symptoms.

This aim was achieved through the COBRA-DI study (Chapter 7).

Aim 2: Determine whether this contribution is reproducible across different sociocultural settings.

This aim was achieved through the GOLD Depression Study (Chapter 9).

Aim 3: Explore neuroimaging signatures which may characterise people with HIV experiencing severe and persistent depressive symptoms.

This aim was achieved through the INSIDE-HIV study (Chapter 10).

6.2 COHORTS AND SETTINGS

COBRA: UK AND NETHERLANDS

The COBRA-DI study (Chapter 7) analysed data from a subset of the COmorBidity in Relation to AIDS (COBRA) cohort [37]. This cohort recruited virally-suppressed participants with HIV, and participants without HIV carefully matched to have comparable demographic characteristics, in London (UK) and Amsterdam (Netherlands) between 2013 and 2014. Participants with HIV were relatively evenly split between the two study sites (56% in Amsterdam, 44% in London), whereas a greater proportion of participants without HIV were recruited in Amsterdam (63% in Amsterdam, 37% in London). The primary aim of the cohort was to assess age-related co-morbidities in older adults (>45 years) with HIV. Of relevance to this thesis, all COBRA participants completed questionnaires measuring depressive symptoms, underwent neuroimaging, and provided blood samples in which biomarkers of inflammation, neuronal damage, and related physiological processes were quantified.

GOLD: SOUTH AFRICA

The GOLD Depression Study (Chapter 9) draws on a subset of the Adolescent Cognitive Brain Imaging (GOLD) cohort in South Africa. The GOLD cohort is an active longitudinal study investigating markers of brain health and neurocognitive outcomes in adolescents with

perinatally-acquired HIV who started ART at an early age, and demographically comparable adolescents without HIV. All participants in the GOLD cohort therefore underwent neuroimaging and neurocognitive assessments. This cohort was, in turn, a follow-up study from the landmark Children with HIV Early antiRetroviral (CHER) clinical trial, which showed for the first time that early initiation of antiretroviral therapy was safe and effective in infants with perinatally-acquired HIV [38, 39]. Participants in the GOLD cohort were recruited partly from children who had originally been in the CHER trial and partly from the local community in Cape Town, South Africa.

The GOLD Depression Study was carried out primarily at the Family Centre for Research with Ubuntu (FAMCRU). FAMCRU is a specialist infectious disease research clinic which was established early in the HIV pandemic to help children access antiretroviral medications. The clinic is embedded in a large public hospital (Tygerberg Hospital, Cape Town) and regularly carries out large-scale observational research, clinical trials, and community engagement initiatives. A major strength of the GOLD cohort was that many participants had received their HIV-related clinical care at FAMCRU throughout their lives, and therefore have strong and positive relationships with staff at the research clinic. Neuroimaging for the GOLD cohort was carried out at the Cape Universities Body Imaging Centre (CUBIC), a shared medical imaging research facility embedded within Groote Schuur Hospital (Cape Town).

INSIDE-HIV: UK

A small sample of individuals with HIV were recruited specifically for the INSIDE-HIV study (that is, they were not drawn from an existing larger cohort). These participants were recruited by clinical research staff from individuals who receive their regular clinical care at the Lawson Unit, a specialist centre offering treatment, support and ongoing care for people with HIV in Brighton (UK). Potential participants underwent screening and completed depression questionnaires at the Lawson Unit. Participants who successfully met all inclusion criteria at screening then underwent neuroimaging at the Clinical Imaging Sciences Centre (Brighton, UK) at the University of Sussex.

6.3 MEASURING DEPRESSIVE SYMPTOMS

PATIENT HEALTH QUESTIONNAIRE (PHQ-9)

The PHQ-9 is a well-validated tool for measuring depressive symptoms [40]. Total scores on the PHQ-9 range from 0 to 27 (each question being scored between 0 and 3), with a cumulative score of ≥ 4 representing the presence of at least some depressive symptoms, and a threshold of 10 commonly considered as indicating major depressive disorder [41]. The PHQ-9 has been translated into numerous languages and is used globally as a screening tool for depression [42]. It is brief (with only nine items) and may be self-administered or administered by trained non-clinical or clinical professionals, and performs equally well when delivered digitally or telephonically [43–45]. For these reasons, the PHQ-9 is an excellent triage tool to quickly screen individuals for depressive symptoms, including in resource-constrained settings. In this thesis, the PHQ-9 was used as the primary outcome measure of depressive symptoms in all three studies (COBRA-DI, GOLD Depression Study, and INSIDE-HIV).

SOUTH AFRICAN DEPRESSION SCALE (SADS)

The SADS was recently developed and validated by a research group at the University of Cape Town [46, 47]. Total scores on the SADS range from 0 to 48, with 16 questions each scored between 0 and 3, similar to the PHQ-9. The SADS offers the advantage of asking respondents about certain idiomatic expressions of depressive symptoms - such as “I was carrying a lot of weight on my shoulders”, “I had pain in my heart”, “I had deep sorrow” - in addition to the classical features of depression. The questionnaire was validated as a dual-language tool (English and isiXhosa) in samples of people with HIV in South Africa. However, the English-language version of the SADS may have broad applicability beyond this population and region, but this has not yet been confirmed. Participants in the INSIDE-HIV study completed both the PHQ-9 and SADS questionnaires, thus providing preliminary evidence of the applicability of the SADS for people outside South Africa and its convergent validity with the PHQ-9.

6.4 MEASURING INFLAMMATION: FLUID BIOMARKERS

Immune cells secrete a plethora of signalling molecules in response to inflammatory challenges. Protein concentrations of these signals may be readily measured in biofluids such as

blood or CSF. Some of these proteins, such as CRP and IL-6, are regularly used in clinical practice as biomarkers of inflammation, and have also recently been associated with symptoms of depression. However, given the sheer complexity of the human inflammatory proteome, there are numerous candidate biomarkers which may potentially be relevant to inflammation and depression, as highlighted in the scoping review in Chapter 3. Here, I provide a summary of key biomarkers of interest which were measured in the studies included in this thesis, and their roles in inflammation, immune responses to HIV, and depression. Note that quinolinic acid was not measured in any of the studies in this thesis, but has been included in this table nevertheless as it is a major component of the kynurenine pathway of tryptophan metabolism. Full forms of biomarker names are available in the **List of Abbreviations**.

Table 6.1: Key biomarkers of inflammation and related processes which may be measured in biofluids.

Biomarker	Role in Inflammation	Role in HIV	Role in Depression
BDNF	Not inflammatory, but known to influence the activity of microglia [48, 49] and astrocytes [50] via the receptor TrkB [51]; major roles are neurogenesis or synaptic plasticity, but also apoptosis, T cell survival [52]; CSF BDNF elevated in CNS infections [53]	HIV may reduce expression (via TNF- α), retrograde transport (via IL-1 β), and anterograde transport (via TNF- α) of BDNF [54, 55]; this leads to synaptic injury and neuronal degeneration [52]; however, BDNF has dose-dependent effects, so upregulation in HIV causes chronic pain [56]	Low serum BDNF in depressed patients, normalised with antidepressant therapy [57]; possibly gender-specific as observed in at least one study only in women (not men) [58]; reduced BDNF in hippocampus and pre-frontal cortex and increased BDNF in nucleus accumbens can induce depression [51]; inflammation-induced depression-like behaviours are mediated by BDNF-TrkB binding [59]
CCL2 MCP-1	/ Induced by a variety of mediators including IL-1 and IL-4, TNF- α , and IFN- γ ; produced by monocytes and macrophages; chemoattractant (recruits monocytes, memory T-cells, and dendritic cells to location of immune insult) [60]	May facilitate the recruitment of uninfected microglia and astrocytes to CNS regions that have HIV-infected monocytes [61]; may protect glia from HIV infection [62]; astrocytic release of CCL2 in response to HIV mediated by PAR-1 [63]	Increased serum concentrations linked to depression [64]; meta-analyses show higher MCP-1 in MDD [65, 66]; some conflicting evidence [49, 67]

Biomarker		Role in Inflammation	Role in HIV	Role in Depression
CCL ₃ MIP-1 α	/	Chemoattractant; produced in CNS mainly by astrocytes [68]; inducible by IL-1 β ; CCR ₅ is key receptor expressed on glial cells [69]	Produced by glial progenitor cells in response to HIV [70], possible role in HIV suppression (along with RANTES/CCL ₅) as both HIV and MIP-1 α bind to CCR ₅ receptor [71, 72]	Evidence is sparse, but some studies suggest that increased serum CCL ₃ may be associated with depression [66]; CCL ₃ is higher in more depressed pregnant women [73]; meta-analysis found no overall significant association with depression [74]
CCL ₅ RANTES	/	Chemoattractant; produced by microglia and astrocytes [66]; inducible by IL-1 β and TNF- α [75] or quinolinic acid [66]	Produced by glial progenitor cells in response to HIV [70]; along with MIP-1 α , considered to be a major HIV suppressive factor [71] but also promotes replication of HIV [76]; along with IP-10, strong predictor of cognitive impairment in people with HIV [77]	Some evidence of higher concentration in MDD [78]; some evidence of lower concentration [49]; meta-analysis found no overall significant association with depression [74]
sCD14		Primarily a receptor for lipopolysaccharide, pro-inflammatory [79]; regulates levels of IL-1 β , IL-6 [80]; involved in NLRP ₃ inflammasome assembly and secretion of IL-1 and IL-18 [81]	Possibly a marker of HIV-induced neuronal damage, as it correlates with CSF neopterin and NFL [82]; decreases with ART [83]	Possibly decreased in microglia as suggested by a post-mortem study [84]

Biomarker	Role in Inflammation	Role in HIV	Role in Depression
sCD16	Also known as Fc γ -RIIIb, role in inflammatory response from monocytes; regulated by and induces TNF- α [85]; CD16+ monocytes may be involved in immediate microbicidal activity [86] whereas CD14+ monocytes have capacity to function as Antigen Presenting Cells [87]	Early study suggested sCD16 in plasma may inhibit HIV from infecting monocytes via CCR3 [88]; CD16 monocyte expansion is linked to HIV disease progression [89]; CD16 monocytes have higher migratory capacity, which may support HIV infiltration [90]	No difference in MDD [84, 91]; symptom severity negatively correlated with CD16:CD56 ratio [92]
sCD163	Receptor for haptoglobin (e.g. IgG) [90]; primarily a marker for monocytes and macrophages with anti-inflammatory potential, induced by anti-inflammatory mediators such as glucocorticoids and IL-10 [93, 94]	Post-mortem study shows higher plasma CD163 in HIV, correlating with markers of synaptodendritic damage [MAP-2] and microglial activation [HLA-DR] [95]; differential expression on subsets of monocytes [96]; elevated plasma CD163 correlates with cognitive impairment in people with HIV [97]	Higher CD163 associated with depression in people with HIV [26], including after controlling for antidepressant use; ketamine antidepressant therapy may induce CD163 expression in monocytes [98]; post-mortem study shows downregulation of <i>CD163</i> mRNA in microglia [84]

Biomarker	Role in Inflammation	Role in HIV	Role in Depression
CRP	Marker of acute-phase response; promotes phagocytosis and monocyte proliferation [99]; can be pro- or anti-inflammatory [100]; produced by hepatocytes, secreted into plasma; induced by cytokines such as IL-6, IL-1 β , TNF- α [100]; correlates strongly with TNF- α and IL-6 [101]	HIV may lead to CRP production as a “terminal product of the inflammation cascade”; increased risk of cardiovascular disease in people with HIV is associated with increased CRP [102, 103]	Increased CRP linked to depressive symptoms [104] and to symptom density, which predicts treatment-resistant depression [105]; correlates with other biomarkers such as IL-6 and TNF- α [101, 106]; direction of association is unresolved: twin study suggests depression predicts CRP [107], but meta-analysis suggests CRP predicts depression [108]
CXCL ₁₀ / IP-10	Chemoattractant; receptor is CXCR ₃ (thus related to MIG), primarily induced by IFN- γ , produced by astrocytes and microglia in CNS [109]; also inducible by IL-1 β and TNF- α [110]; correlates with serum CRP [111]	CSF concentrations associated with HIV viral load [112]; along with MIG and CXCL ₁₁ , significantly upregulated in HIV infection and predicts disease progression [113]	Trend towards higher IP-10 in depression, specifically in people with HIV [114]; increased concentration in MDD, decreases with treatment [115]; however, meta-analysis found no overall significant association with depression [74]
CXCL ₉ / MIG	Chemoattractant; receptor is CXCR ₃ (thus related to IP-10), primarily induced by IFN- γ [109] but also inducible by quinolinic acid, TNF- α , IL-1 β ; correlates with serum CRP [111]	Along with IP-10 and CXCL ₁₁ , significantly upregulated in HIV infection and predicts disease progression [113]	Trend towards higher MIG in depression, specifically in people with HIV [114]; however, meta-analysis found no overall significant association with depression [74]

Biomarker	Role in Inflammation	Role in HIV	Role in Depression
IL-18	Produced by microglia and astrocytes [116] in response to immune insults via IFN- γ ; stimulates natural killer cells and lymphocytes; may be mediator of sickness behaviours along with IL-1 β [117]; induces TNF- α , IL-6, IL-1 β , MCP-1 [118]	HIV infection enhances production of IL-18 which activates the inflammasome and increases the number of target cells [119]; imbalance between IL-18 and its antagonist (IL-18BP) leading to increased free IL-18 [120];	Some studies suggest it is increased [121, 122] and correlated with symptom severity (BDI) [123] and reversed by antidepressant treatment [123, 124]; another suggests decreased and not correlated with symptom severity (HAM-D) [125]
IL-1 β	Produced by microglia [48] and activated by caspase-1 (which in turn is activated by the NLRP3 inflammasome); enhances T cell activation and antigen recognition [126]; induces pro-inflammatory cytokines and chemokines [127]	Increased levels of IL-1 β in early HIV infection [120]	Increased in MDD [78, 128] and correlated with severity [123, 129], decreased with antidepressant treatment [123, 124]
IL-6	Produced by microglia [48]; induces acute phase inflammation (CRP); induced by TNF- α , IL-1 β [130]	Produced by astrocytes in response to HIV infection [131], HIV infection enhances production of IL-6 in monocytes [132]; likely via HIV-1 Tat [131]	Association observed but with varied strengths [78, 104, 106, 122, 124, 125, 133, 134]; twin study and meta-analysis shows IL-6 may be risk factor rather than consequence [107, 108]; some studies specifically show link with IL-6 but not CRP [67, 134]

Biomarker	Role in Inflammation	Role in HIV	Role in Depression
Kyn:Trp	Trp can be catabolised to either serotonin (by tryptophan hydroxylase) or, during an inflammatory response, to Kyn (by indoleamine 2,3-dioxygenase [IDO]) [135, 136]; Kyn:Trp ratio is essentially an index of IDO activity and a marker of neuroinflammation [137]; increase in Kyn:Trp ratio is inducible by IL-6, TNF- α , IL-1 β , IFN- α [138, 139]	HIV leads to increased IDO activity; IDO activity results in immune suppression, especially of CD4 ⁺ T cells [140, 141] – possibly a feedback loop	Increased Kyn:Trp linked to greater depression severity (possibly because greater Kyn production results in lower Trp availability for production of serotonin) [142], although evidence is inconsistent [143]; increased CSF Kyn correlated with depression severity [144] but evidence is conflicting [145]
Neopterin	Byproduct of the tetrahydrobiopterin synthesis pathway, stimulated by IFN- γ [146]; serves as a sensitive marker of neuroinflammation in conditions such as viral and bacterial CNS infections [137, 147]; CSF neopterin associated with increased Kyn:Trp [148]	CSF concentrations increased with HIV and decreased (but not normalised) with ART [149, 150]; increased neopterin correlated with cognitive impairment for women with HIV [83]	Meta-analysis indicates higher blood neopterin in people with depression [151]

Biomarker	Role in Inflammation	Role in HIV	Role in Depression
NFL	Not inflammatory, but marker of neuronal injury [152] and correlates with neuroinflammation [153]	Biomarker of CNS injury in HIV [154]; negatively associated with neurocognition in people with HIV and declines with ART [155] (but some contradictory evidence exists [156]);	People with MDD show significantly higher concentrations [157]; predictor of risk for depression in people recovering from strokes [158, 159]
Quinolinic acid	Synthesised from Trp via Kyn pathway, inducible by IL-6, TNF- α , IL-1 β [138, 139]; potentially a marker of neuroinflammation [137]	HIV viral proteins cause microglia to produce Quin [160]; CSF Quin levels associated with HIV RNA and driven by HIV infection, reduced by ART [161]; also linked to HIV-associated neurocognitive disorder [162]; Quin production causes MCP-1 production and increased CCR5 production, thus driving HIV proliferation [162]	Absolute concentrations increased in untreated depression and correlate with depression severity [144, 145]

Biomarker	Role in Inflammation	Role in HIV	Role in Depression
S100A8/A9 / Calprotectin	Expressed by neutrophils and macrophages as a calcium ion sensor [163]; leading to production of IL-6, MCP-1, TNF- α [164–166]; induces neutrophil chemotaxis [167] but also inhibits neutrophil oxidative metabolism [168]	Peripheral neutrophils of people with HIV display impaired chemotaxis and have an exaggerated response to immune insults and reduced inhibition by S100A8/A9 [169]; release of S100A8 may mediate reactivation of HIV replication in macrophage reservoirs [170]	Some recent evidence suggesting higher concentrations in people with depression [164, 171], but not studied widely
TNF- α	Produced by macrophages including microglia [48, 172]; inducible by bacterial lipopolysaccharide and immune mediators, including IFN- α [172]; induces chemokines including IL-8, MCP-1 and IP-10 [173]; receptors are TNFR ₁ and TNFR ₂ [172]; TNFR ₁ stimulation can lead to inflammatory or apoptotic responses, especially activation and nuclear localisation of NF κ B, while TNFR ₂ supports lymphoid cell activation, migration, and proliferation [173, 174]	Produced by microglia in response to HIV infection [175, 176]; in early infection, TNF- α is protective (e.g. promoting RANTES, which has anti-HIV functions); in chronic infection, TNF- α leads to increased transcription/activation of HIV, and HIV proteins (Nef, Tat, Vpr) exhibit functional mimicry of TNF- α [177, 178]	Increased in MDD [122] and correlates with depressive symptoms [25, 125, 129, 134]; anti-TNF- α therapy reduces depression severity [179]

Biomarker	Role in Inflammation	Role in HIV	Role in Depression
YKL-40 C3L1	/ Role not well-studied, possibly antigen sensitisation and macrophage activation [180]; correlates with CSF neopterin and NFL [181]; produced mainly by astrocytes and inducible by IL-1 β and TNF- α [182, 183]	Higher CSF YKL-40 in HIV-associated dementia and particularly in untreated HIV [181, 184]; may not correlate with HIV clinical parameters but correlates with CSF neopterin, IP-10, and NFL in chronic untreated infection [184]	Higher CSF levels in women with suicide ideation [185]; higher serum YKL-40 at baseline in MDD + alcohol use, normalises during recovery [106]

6.5 MEASURING INFLAMMATION: NEUROIMAGING BIOMARKERS

PROTON MAGNETIC RESONANCE SPECTROSCOPY (^1H -MRS)

MRS is a safe (non-ionising) and non-invasive neuroimaging technique that enables the quantification of metabolites in localised brain regions *in vivo*. In proton MRS, a magnetic field and radiofrequency pulse sequence is applied to the brain, which causes hydrogen nuclei (protons) in water and other molecules to emit radiofrequency signals. These signals yield MR spectra, in which amplitudes of peaks from voxels placed at certain locations represent the concentrations of corresponding neurometabolites [186]. Concentration of a neurometabolite can be determined relative to another endogenous metabolite (generally total creatine, which is abundant in the brain and yields a strong signal in MRS spectra) by normalising the spectral peak to the peak for the reference metabolite. Alternatively, water-referenced concentration of a neurometabolite can be determined by normalising the spectral peak to the water signal in the same voxel, although this requires further adjustment for tissue fraction volumes within the voxel of interest [187].

Of particular interest in the context of neuroinflammation are the metabolites choline (Cho, generally quantified as the sum of glycerophosphocholine and phosphocholine [GPC + PCh]) and myo-inositol (mI). Cho is elevated with cell membrane turnover, while mI is upregulated in activated glial cells [188, 189]. As a result, these neurometabolites are considered indirect measures of neuroinflammation. Recent MRS studies have shown that mI and Cho concentrations in the brain are altered in people with HIV compared to people without HIV [190, 191].

In this thesis, concentrations of Cho and mI (relative to creatine and water) were used as indirect measures of neuroinflammation in COBRA-DI, the GOLD Depression Study, and INSIDE-HIV. In addition, several other neurometabolites (not necessarily associated with neuroinflammation) were also quantified for INSIDE-HIV, as the same MRS spectrum can yield concentrations of multiple neurometabolites via distinct peaks.

DIFFUSION-WEIGHTED MAGNETIC RESONANCE SPECTROSCOPY (DW-MRS)

DW-MRS is an emerging technique which combines the principles of MRS, which provides information on the concentrations of neurometabolites (which are often cell type-specific), with those of diffusion-weighted magnetic resonance imaging, which provides information

on the diffusion of molecules intra- and extra-cellularly. DW-MRS therefore enables the quantification of intracellular diffusion of neurometabolites as Apparent Diffusion Coefficients (ADCs) calculated from DW-MRS spectra [192]. ADC of Cho was recently shown to be sensitive to experimentally-induced neuroinflammation in humans, increasing after administration of lipopolysaccharide in healthy volunteers [193]. Increase in creatine ADC has been reported in multiple sclerosis and neuropsychiatric lupus, both conditions characterised by neuroinflammation [194, 195]. Taken together, these recent findings suggest that the intracellular diffusion of certain neurometabolites, as quantified by DW-MRS, may serve as a marker of neuroinflammation. In this thesis, DW-MRS was used as an indirect measure of neuroinflammation in participants with HIV in the INSIDE-HIV study.

DYNAMIC CONTRAST-ENHANCED MAGNETIC RESONANCE IMAGING (DCE-MRI)

The blood-brain barrier (BBB) is the interface which regulates the transport of biomolecules between the CNS and the periphery. Systemic inflammation can result in degradation of the BBB, leading to greater permeability and a loss of homeostasis [196, 197]. To measure BBB permeability in humans, a common approach is to image the brain after administering a tracer intravenously, thereby tracking the movement of the tracer across the BBB. In DCE-MRI, participants undergo neuroimaging before, during, and after intravenous administration of a (typically Gadolinium-based) contrast agent [198]. Using this data, a volume transfer rate constant (K_{trans}) can be calculated, which represents the rate of transfer of the contrast agent from the blood plasma into the extravascular-extracellular space and thus is a measure of the permeability of the BBB [199]. K_{trans} was recently observed to be significantly higher in people with major depressive disorder and positively correlated with severity of depressive symptoms [200]. Similarly, K_{trans} is higher in people with HIV and neurocognitive impairment compared to people without HIV [201]. In this thesis, DCE-MRI was used to measure BBB permeability in participants with HIV in the INSIDE-HIV study.

7

COBRA-DI: Older Adults in UK and Netherlands

HIGHLIGHTS

- This study analysed data from 125 people with HIV and 79 people without HIV based in London and Amsterdam. The data available from these participants included the severity of their depressive symptoms, as well as concentrations of 15 proteins in their blood plasma, 12 proteins in their cerebrospinal fluid, and two biomolecules in two regions of their brains measured using brain scans.
- People with HIV in this study were at greater risk for experiencing symptoms of depression than people without HIV, even when statistically removing the possible effects of sociodemographic factors measured in this study (age, sex, ethnicity, and years of education received).
- When calculating this risk for depression in participants with HIV, if the effects of certain biomarkers of inflammation were also removed, depression risk was decreased by at least 10%. This suggests that these biomarkers may be contributing to this risk for depression. These biomarkers were MIP-1 α and IL-6 in cerebrospinal fluid, and MIG and TNF- α in blood. All of these proteins are involved in inflammation.
- This study thus suggests that inflammation may at least partly contribute to the higher risk for depression in people with HIV.

7.1 INTRODUCTION

PREVIOUS CHAPTERS HAVE SUMMARISED the evidence supporting the hypothesis that inflammatory biomarkers may contribute to depressive symptoms in people with HIV. This hypothesis was tested using data available from the COmorBidity in Relation to AIDS (COBRA) cohort. In collaboration with the COBRA cohort steering committee, I developed a statistical analysis plan to assess the possible mediating roles of inflammatory biomarkers (neuroimaging, blood plasma, and CSF) on the relationship between HIV status and Any Depressive Symptoms (defined as PHQ-9 score > 4) using step-wise logistic regression. First, the odds for Any Depressive Symptoms in participants with HIV (compared to participants without HIV) were calculated, then these odds were calculated when statistically controlling for the effects of individual biomarkers of inflammation to obtain a % change in odds for depressive symptoms. This indirect approach was used to identify biomarkers which contributed at least 10% of the odds for depressive symptoms in this sample.

7.2 RESEARCH ARTICLE: *TRANSLATIONAL PSYCHIATRY*

ARTICLE OPEN



Biomarkers of central and peripheral inflammation mediate the association between HIV and depressive symptoms

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People living with HIV are at increased risk for depression, though the underlying mechanisms for this are unclear. In the general population, depression is associated with peripheral and central inflammation. Given this, and since HIV infection elicits inflammation, we hypothesised that peripheral and central inflammatory biomarkers would at least partly mediate the association between HIV and depressive symptoms. People living with HIV ($n = 125$) and without HIV ($n = 79$) from the COmorBidity in Relation to AIDS (COBRA) cohort were included in this study. Participants living with and without HIV had similar baseline characteristics. All participants living with HIV were on antiretroviral therapy and were virally suppressed. Plasma, CSF, and brain MR spectroscopy (MRS) biomarkers were measured. Using logistic regression models adjusted for sociodemographic factors, we found that participants with HIV were more likely to have Any Depressive Symptoms (Patient Health Questionnaire [PHQ-9] score >4) (odds ratio [95% confidence interval] 3.27 [1.46, 8.09]). We then sequentially adjusted the models for each biomarker separately to determine the mediating role of each biomarker, with a $>10\%$ reduction in OR considered as evidence of potential mediation. Of the biomarkers analysed, MIG (-15.0%) and TNF- α (-11.4%) in plasma and MIP1- α (-21.0%) and IL-6 (-18.0%) in CSF mediated the association between HIV and depressive symptoms in this sample. None of the other soluble or neuroimaging biomarkers substantially mediated this association. Our findings suggest that certain biomarkers of central and peripheral inflammation may at least partly mediate the relationship between HIV and depressive symptoms.

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INTRODUCTION

Mental health is a key priority in improving the overall quality of life for people living with HIV. Depression, in particular, is one of the most common psychiatric disorders in people living with HIV. Early studies estimated that the prevalence of depression amongst people living with HIV is twice as high as that in the general population [1], though heterogeneity in screening tools used in studies makes a rigorous meta-analysis elusive [2]. More recent evidence has shown that HIV infection is associated with an increased likelihood of depression [3]. This risk may be exacerbated by “premorbid” depression, i.e., high prevalence of depression even before seroconversion in disenfranchised populations which are disproportionately affected by HIV. Amongst people living with HIV, smoking, drug use, excess alcohol use, or a

recent sexually transmitted infection are further associated with greater prevalence of depressive symptoms [4]. Prevalence rates of depression also tend to differ by factors such as gender, socioeconomic status, or geography. Notably, the risk of depression is higher amongst women living with HIV and amongst people in the Global South [5, 6]. These high rates of depression impact the quality of life and ability to access care for people living with HIV. However, we do not currently fully understand why people living with HIV face this elevated risk for depression. Therefore, it is critical to examine (neuro)biological and psychosocial mechanisms that may drive this increased risk for depression. Understanding these underlying mechanisms may enable the discovery of predictive biomarkers as well as potential therapeutic targets for scalable interventions [7].

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In the general population, depression is increasingly being recognised as comprising multiple distinct subtypes—constellations of behavioural disturbances, genetic risk factors, and neurobiological deficits—rather than a monolithic condition [8]. One such subtype of depression is associated with inflammation in the periphery and the central nervous system (CNS) [9]. Several sickness behaviours, such as fatigue, reduced appetite, and loss of interest or pleasure (motivational anhedonia), are shared across depression and inflammatory conditions [10]. People who experience depression exhibit increased concentrations of circulating pro-inflammatory cytokines and chemokines [11, 12]. Administration of anti-inflammatory medication as monotherapy or in conjunction with other antidepressant treatments can alleviate depressive symptoms [13]. Taken together, this evidence indicates that inflammation may play a role in the pathogenesis of depression, or at least a subtype of depression characterised by sickness behaviours. Although the precise relationship between neuroinflammation and depressive symptoms remains unclear, it is possible that neuroinflammatory responses contribute to neurotransmitter dysfunction, hypothalamus-pituitary-adrenal (HPA) axis dysregulation, and impaired hippocampal neurogenesis to generate features that are common to depression and sickness [14].

An inflammation-mediated subtype of depression may have important implications for our understanding of HIV-associated depression. The brain is a key reservoir for HIV infection, including for people living with HIV who are virally suppressed on antiretroviral therapy. HIV viral proteins and viral DNA elicit a neuroinflammatory response in the brain, characterised by sustained microglial activation [15]. Furthermore, systemic inflammation persists in spite of suppressive antiretroviral therapy [16, 17]. Given this, it is possible that HIV-induced peripheral and central inflammation may contribute to the increased risk for depression amongst people living with HIV and represent a promising screening and therapeutic target.

This study leveraged data from a well-characterised cohort of people living with and without HIV with similar demographic characteristics to evaluate the contributions of biomarkers of both central and peripheral inflammation to the association between HIV and depressive symptoms. We hypothesised that peripheral and central inflammatory biomarkers would at least partly mediate the association between HIV status and depressive symptoms.

METHODS

Cohort

The COMorBidity in Relation to AIDS (COBRA) study was a prospective cohort of adults living with HIV in London (UK) and Amsterdam (Netherlands) recruited between 2013 and 2014 [18]. At enrolment, participants living with HIV in the cohort were aged 45 or over, virally suppressed, and on ART for at least 12 months. The cohort also included demographically-similar participants without HIV. All participants underwent a comprehensive battery of cognitive testing, blood and cerebrospinal fluid (CSF) sample collection for biomarker measurement, and neuroimaging at a baseline visit and a follow-up visit separated by 2 years [19]. This study was approved by the institutional review board of the Academic Medical Center (AMC) of the University of Amsterdam (reference number NL 30802.018.09) and a UK Research Ethics Committee (REC) (reference number 13/LO/0584 Stanmore, London). The current analysis included baseline data collected as part of the COBRA study.

Patient Health Questionnaire

Depressive symptoms were measured using the nine-item Patient Health Questionnaire (PHQ-9). The PHQ-9 is a well-validated questionnaire that assesses self-reported frequency of depressive symptoms over the preceding two weeks [20]. The questionnaire is scored between 0 and 27, with a score of 10 commonly used as a cut-off to screen for major depressive disorder (MDD) [21]. All potential participants for the COBRA study previously completed a self-administered PHQ-9 questionnaire as part of the study

screening. Individual items and total scores were recorded for each participant. Participants whose total score on the PHQ-9 at a screening visit was higher than 15 were ineligible for the COBRA cohort. For the current analysis, PHQ-9 score was summarised as a dichotomous variable (PHQ-9 score >4 classified as “Any Depressive Symptoms” as suggested by Kroenke et al. [20]) and as a continuous variable.

Plasma biomarkers

Plasma concentrations of various biomarkers were previously determined for participants in the COBRA cohort, as described in detail elsewhere (Supplementary File S1 in [19]). Immunoturbidimetry was used to determine high-sensitivity CRP concentrations. Enzyme-linked immunosorbent assays (ELISAs) were used to determine the plasma concentrations of I-FABP, sCD14, sCD163, neopterin, and sCD16. A single molecular array (SIMOA) assay was used to measure plasma concentration of NFL. High-performance liquid chromatography was used to determine plasma concentrations of tryptophan and kynurenine, from which a kynurenine-to-tryptophan (Kyn:Trp) ratio was calculated.

An expanded set of pro-inflammatory cytokines and chemokines was previously measured only in a subset of 78 participants [19]. These 78 participants were randomly selected with equal numbers across COBRA age groups (45–50 years, 51–55 years, 56–60 years, 61–65 years, 66–70 years), except for the oldest age group (>70 years) where few individuals were available. For this subset, human magnetic Luminex assay was used to determine plasma concentrations of IL-6 and TNF- α (pro-inflammatory cytokines), and MIG/CXCL9, IP-10/CXCL10, MCP-1/CCL2, MIP1 α /CCL3, and RANTES/CCL5 (chemokines).

CSF biomarkers

Concentrations of biomarkers were likewise previously measured in cerebrospinal fluid (CSF), as described in detail elsewhere (Supplementary File S1 in [19]). ELISAs were used to determine the CSF concentrations of sCD14, sCD163, neopterin, and NFL. Liquid chromatography was used to determine the CSF Kyn:Trp ratio. As with plasma biomarkers, in a subset of 78 participants, Luminex assay was used to determine CSF concentrations of IL-6, TNF- α , MIG/CXCL9, IP-10/CXCL10, MCP-1/CCL2, MIP1 α /CCL3, and RANTES/CCL5.

Magnetic resonance spectroscopy

Proton magnetic resonance spectroscopy (^1H -MRS, or MRS) data was acquired to assess concentrations of neurometabolites. Parameters of neuroimaging acquisition have been reported in detail elsewhere (Supplementary File S2 in [19]). For image acquisition during baseline COBRA study visits, a Siemens 3T Verio scanner was used at the London site, while Philips 3T Intera and 3T Ingenia scanners were used at the Amsterdam site. Single-voxel MRS data were acquired for two regions: frontal white matter (FWM) and putamen. Concentrations of choline [Cho] and myo-inositol [mI] were determined for these regions [22].

A summary of biomarkers included in this study, along with the number of participants for whom each biomarker was assessed, is provided in Table 1.

Statistical analysis

We summarised participant characteristics using counts (proportions) for categorical variables and medians (interquartile ranges [IQRs]) for continuous variables. Univariate comparisons by HIV status were conducted using Wilcoxon-rank sum, Chi-squared (with Yates correction), Cochran-Armitage trend, and Fisher's exact tests, as appropriate.

Following the assumptions of previous COBRA analyses, participants with biomarker values out of range were assumed to have a biomarker value of half the detection limit (e.g., if the detection limit was 0.30, then a value of 0.15 was assumed). The number of participants for whom this assumption was made for each biomarker is shown in Supplementary File 1. For biomarkers with a lower limit of 0 (namely high-sensitivity CRP), zero values were set to 0.01 so that the value could be log₂-transformed (note that this only affected one participant in the dataset).

To assess correlations between concentrations of biomarkers measured in this sample, we determined Spearman's rank correlation coefficients (ρ) using pairwise complete observations. We calculated correlations between all possible biomarker pairs, and additionally for subsets of plasma-plasma, CSF-CSF, plasma-CSF, and MRS biomarkers. Plasma-CSF correlations were calculated for the full sample, and for subgroups of participants by HIV status and depressive symptom status.

Table 1. Summary of neuroimaging, plasma, and cerebrospinal fluid (CSF) biomarkers of (neuro)inflammation included in the study.

		Marker of	FWM	N	Putamen	N
Neurometabolites	Myo-inositol	Glial cell activation	x	161	x	63
	Choline	Glial cell activation	x	184	x	128
		Marker of	Plasma	N	CSF	N
Soluble biomarkers measured in all participants, where possible	CRP	Acute phase response	x	204		
	I-FABP	Intestinal barrier integrity	x	202		
	Kyn:Trp Index	IDO-1 activity	x	203	x	202
	Neopterin	Inflammation	x	203	x	202
	NFL	Neuronal injury	x	202	x	203
	sCD14	Monocyte activation	x	201	x	202
	sCD16	Monocyte activation	x	202		
	sCD163	Monocyte activation	x	204	x	203
		Marker of	Plasma	N	CSF	N
Soluble biomarkers measured in a subset of participants	IL-6	Inflammation	x	78	x	78
	IP-10/CXCL10	Chemotaxis	x	78	x	78
	MCP-1/CCL2	Chemotaxis	x	78	x	78
	MIG/CXCL9	Chemotaxis	x	78	x	78
	MIP1 α /CCL3	Chemotaxis	x	78	x	78
	RANTES/CCL5	Chemotaxis	x	78	x	78
	TNF- α	Inflammation	x	78	x	78

FWM frontal white matter.

Some soluble biomarkers were measured in all participants, where possible. An expanded set of pro-inflammatory cytokines and chemokines was measured only in a subset of $n = 78$ participants. The number of participants for whom data were available for each biomarker is indicated. 'x' indicates that a biomarker was measured in the respective brain region or biofluid, whereas '-' indicates that it was not.

To assess the main effect of HIV status on the prevalence of "Any Depressive Symptoms", we used logistic regression models to estimate the odds ratio (OR) (95% confidence interval [CI]) for the association between HIV status and Any Depressive Symptoms for our full sample with outcome data available ($N = 204$), with the reference OR = 1.00 for participants without HIV. To assess the main effect of HIV status on each biomarker, we used linear regression models for the outcome $\log_2$ -transformed biomarker concentration, fitted separately for each biomarker and adjusted for sociodemographic factors (age, sex, ethnicity, and years of education).

For our primary analyses, we used logistic regression to explore the potential mediating role of biomarkers on the association between HIV status and "Any Depressive Symptoms". We first adjusted the model for sociodemographic factors: age (years, continuous), sex, ethnicity, and years of education (continuous). Models involving any neurometabolite measures were further corrected for MRI scanner. The sample size considered for each model is reported, as this varied based on the availability of data for each biomarker of interest. To determine whether any biomarkers mediated the relationship between HIV status and Any Depressive Symptoms, we then sequentially adjusted the model for each ($\log_2$ -transformed) biomarker separately. We report the OR for the association between HIV status and Any Depressive Symptoms for all models, along with profile-likelihood CIs. Individual biomarkers for which adjustment resulted in a >10% reduction in the OR were considered potential mediators of the association between HIV status and Any Depressive Symptoms. A reduction (rather than an increase, which may more likely reflect confounding) in the effect estimate after adjustment is commonly used to identify potential mediator variables [23].

For our sensitivity analyses, we used linear regression to explore the main effect of HIV status on depressive symptom severity, with PHQ-9 score as a continuous outcome. The same sequential adjustments for sociodemographic factors and individual biomarkers (with each biomarker tested in a separate model) were made for the sensitivity analyses as described for the primary analyses.

All analyses were conducted using listwise deletion, only including participants with data available on all variables used in an analysis. Analyses were performed using R version 4.1.0. A detailed description of assumptions made as part of these analyses is available in Supplementary File 2. We did not adjust for multiple testing, as we focus on the strengths

of observed associations and consistency of results with sensitivity analyses, rather than on statistical significance.

RESULTS

Participant characteristics

We included $N = 204$ participants for whom data were available on PHQ-9 score and at least one biomarker. Of these, $n = 125$ were participants living with HIV and $n = 79$ were demographically comparable controls. Sociodemographic, lifestyle, and HIV-specific characteristics, as well as the PHQ-9 responses, are summarised in Table 2. The median age [IQR] of participants was 57 [51–62] years, 92.6% of participants were male, 91.7% were White, and 82.8% were men who have sex with men. The median [IQR] years of education was 15 [13–16] years. All participants living with HIV were on combination antiretroviral therapy (cART) and had plasma HIV-RNA < 200 copies/mL.

Participants living with and without HIV were similar on most baseline sociodemographic and lifestyle characteristics, with notable exceptions: participants living with HIV were less likely to be White (88.1% vs 97.5%, $p = 0.03$) and current alcohol users (77.6% vs 92.4%, $p = 0.03$) compared to participants without HIV.

Correlations between CSF and plasma inflammatory biomarkers

The correlation matrix for soluble biomarkers which were measured in both plasma and CSF is shown in Fig. 1. Correlations are also reported for subgroups: people with HIV and Any Depressive Symptoms (maximum sample $n = 33$), people with HIV and No Depressive Symptoms (maximum sample $n = 92$), people without HIV and with Any Depressive Symptoms (maximum sample $n = 9$), and people without HIV and with No Depressive Symptoms (maximum sample $n = 70$). Note that, given smaller sample sizes in subgroups, certain biomarkers had identical values for all participants, resulting in an incalculable correlation

Table 2. Summary of selected baseline characteristics and outcome data (PHQ-9 score/categories) by HIV status for participants from the COBRA cohort included in the current study.

Variable n (%) or median (interquartile range)	Overall N = 204	Participants without HIV N = 79	Participants with HIV N = 125	p value
<i>General baseline characteristics</i>				
Study Site				0.66
Amsterdam	124 (60.8%)	50 (63.3%)	74 (59.2%)	
London	80 (39.2%)	29 (36.7%)	51 (40.8%)	
Age (years)	57 (51, 62)	57 (52, 64)	55 (51, 62)	0.25
Sex at birth				>0.99
Female	15 (7.4%)	6 (7.6%)	9 (7.2%)	
Male	189 (92.6%)	73 (92.4%)	116 (92.8%)	
Men who have sex with men	169 (82.8%)	62 (78.5%)	107 (85.6%)	0.26
Ethnicity				0.03
Black-African	17 (8.3%)	2 (2.5%)	15 (12.0%)	
White	187 (91.7%)	77 (97.5%)	110 (88.0%)	
Years of Education	15 (13, 16)	16 (14, 16)	14 (13, 16)	0.19
Current smoker	58 (28.4%)	20 (25.3%)	38 (30.4%)	0.53
Current alcohol use	173 (84.8%)	73 (92.4%)	100 (80.0%)	0.03
Use of recreational drugs in the past 6 months	59 (28.9%)	18 (22.8%)	41 (32.8%)	0.17
Ever injected drugs	5 (2.5%)	0 (0.0%)	5 (4.0%)	0.16
<i>HIV-specific characteristics</i>				
Years since HIV diagnosis			14.8 (9.0, 18.7)	
Currently on any form of combination antiretroviral therapy (cART)			125 (100.0%)	
Duration of cART (years)			11.9 (7.3, 15.5)	
HIV-RNA viral load <200 copies/mL			125 (100.0%)	
Prior AIDS event			40 (32.0%)	
Current CD4 + T-cell count (cells/ μ L)			615 (472, 798)	
Nadir CD4 + T-cell count (cells/ μ L)			180 (100, 250)	
<i>PHQ-9 score</i>				
Total Score	2 (0, 4)	1 (0, 3)	2 (1, 5)	0.005
PHQ-9 > 4 ("Any Depressive Symptoms")	42 (20.6%)	9 (11.4%)	33 (26.4%)	0.02

Univariate comparisons by HIV status were conducted using Wilcoxon-rank sum, Chi-squared (with Yates correction), Cochran-Armitage and Fisher's exact tests, as appropriate.

coefficient due to lack of variability (i.e., the standard deviation of 0). Further matrices for correlations between imaging biomarkers only, plasma-plasma biomarkers only, CSF-CSF biomarkers only, and all soluble biomarkers are available in Supplementary File 3.

Overall, concentrations of soluble biomarkers were only weakly or moderately correlated, if at all (mean $[M] = 0.07$, standard deviation $[SD] = 0.17$, range -0.46 to 0.62). Moderate-to-strong positive correlations were observed between plasma Kyn:Trp ratio and plasma neopterin ($\hat{\rho} = 0.62$), CSF IP-10 and CSF neopterin ($\hat{\rho} = 0.58$), and CSF sCD14 and CSF sCD163 ($\hat{\rho} = 0.58$). Notably, a cluster of moderate positive correlations was observed for plasma IL-6 with MIP1- α (CSF, $\hat{\rho} = 0.46$), MCP-1 (plasma, $\hat{\rho} = 0.40$), and MIG (plasma, $\hat{\rho} = 0.46$; CSF, $\hat{\rho} = 0.40$), and for CSF IL-6 with MIP1- α (CSF, $\hat{\rho} = 0.53$), MCP-1 (CSF, $\hat{\rho} = 0.50$), and MIG (CSF, $\hat{\rho} = 0.47$).

Correlations between plasma and CSF concentrations of a single biomarker, represented by the diagonal in Fig. 1, tended to be slightly higher than the overall correlation of soluble biomarkers ($M = 0.24$, $SD = 0.27$, range $= 0.46$ to 0.51). The strongest correlations observed, which were still only moderate, were positive correlations between plasma and CSF IP-10 ($\hat{\rho} = 0.51$), NFL ($\hat{\rho} = 0.50$), and IL-6 ($\hat{\rho} = 0.47$) and a negative correlation between

plasma and CSF RANTES ($\hat{\rho} = -0.46$). There were no strong correlations between plasma and CSF concentrations of any other soluble biomarkers, or between the putamen and frontal white matter (FWM) measures of neurometabolites (Supplementary File 3).

Association of HIV separately with depressive symptoms and inflammation

There was a relatively low severity of depressive symptoms across all participants in this sample, with a median [IQR] PHQ-9 score of 2 [0, 4]. Despite this, depressive symptom scores were higher amongst participants living with HIV compared to participants without HIV ($p = 0.005$), with the prevalence of "Any Depressive Symptoms" also being higher in this group (26.4% vs 11.4%, $p = 0.02$, Table 2). The full range of PHQ-9 scores was 0 to 22, with four participants living with HIV reporting a PHQ-9 score >15 at the study visit (but not at the screening visit).

In the full sample, the crude (unadjusted) OR (95% CI) for the association between HIV status and Any Depressive Symptoms was 2.79 (1.30, 6.54). After adjusting for age, sex, ethnicity, and years of education, the adjusted OR increased to 3.27 (1.46, 8.09).

We found associations between HIV status and plasma concentrations of CRP, I-FABP, Kyn:Trp ratio, neopterin, NFL,

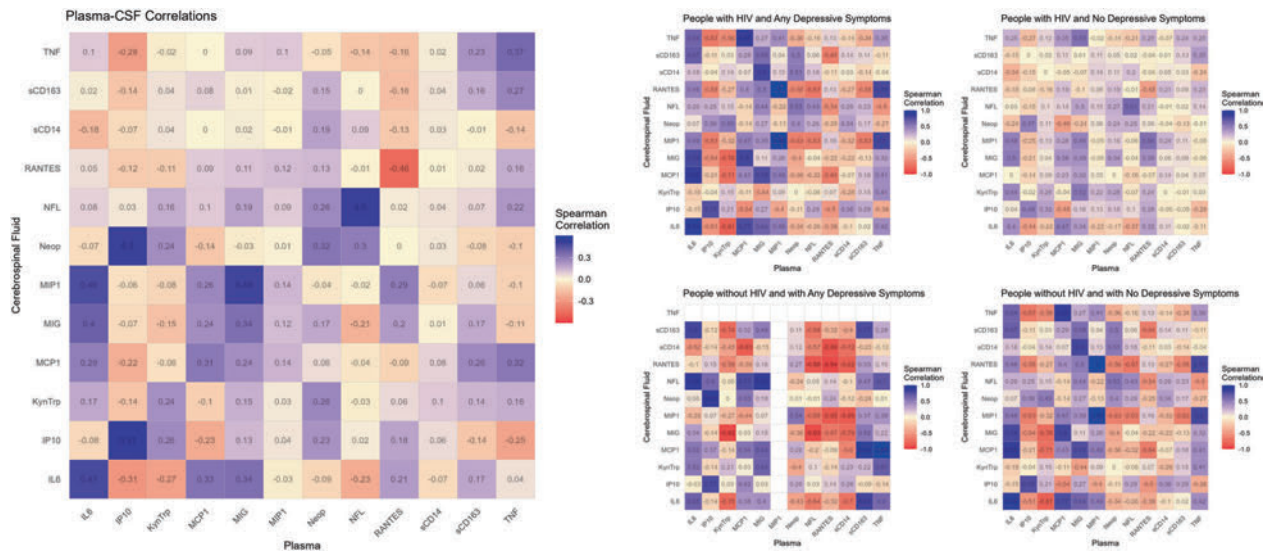


Fig. 1 Correlations between cerebrospinal fluid (CSF) and plasma concentrations of soluble biomarkers measured in the current study. Correlations are shown for the full sample, and for subgroups of participants: people with HIV and Any Depressive Symptoms (maximum sample $n = 33$), people with HIV and No Depressive Symptoms (maximum sample $n = 92$), people without HIV and with Any Depressive Symptoms (maximum sample $n = 9$), and people without HIV and with No Depressive Symptoms (maximum sample $n = 70$). For correlations in the full sample, the strength of correlations are graded across a 3-point scale selected to range from the 5th and 95th percentiles of the correlation coefficients to optimise visual comparisons: -0.6 (in deep red), 0.0 (in light yellow), and $+0.6$ (in deep blue). For correlations in subgroups, the grading scale was: -1.0 (in deep red), 0.0 (in light yellow), and $+1.0$ (in deep blue).

sCD14, sCD16, sCD163, and TNF- α , and between HIV status and CSF concentrations of IP-10, neopterin, and Kyn:Trp ratio, (all $p < 0.05$, Supplementary File 4). Mean concentrations of these biomarkers were all higher amongst participants living with HIV compared to those without HIV.

Mediation by biomarkers of peripheral and central inflammation

The odds ratios for the association between HIV and Any Depressive Symptoms, before and after adjusting for each biomarker separately, are shown in Table 3. Of the biomarkers analysed, our criterion for potential mediation was met for plasma MIG (change in OR_{HIV} : -15.0%), plasma TNF- α (-11.4%), CSF MIP1- α (-21.0%), and CSF IL-6 (-18.0%) (Fig. 2). None of the other plasma, CSF, or neuroimaging biomarkers met our criterion of greater than 10% reduction in OR.

Conclusions from sensitivity analyses in which the PHQ-9 score was modelled as a continuous outcome were consistent with those of our main analyses (Supplementary File 5).

DISCUSSION

In this study, we aimed to determine whether biomarkers of peripheral and central inflammation may mediate the risk for depressive symptoms in HIV. We observed that the prevalence and severity of depressive symptoms were significantly greater amongst participants living with HIV than those without HIV, as were the concentrations of several plasma and CSF inflammatory biomarkers. These findings indicate that HIV status was associated both with increased risk for depressive symptoms and increased systemic and central nervous system inflammation. Crucially, we show that four biomarkers of inflammation—MIG and TNF- α in plasma, and MIP1- α and IL-6 in CSF—are potential mediators of the association between HIV status and depressive symptoms, as their inclusion in the regression models attenuated the odds ratio for this association. Taken together, our findings offer support for the hypothesis that the association between HIV and depressive symptoms may at least in part be mediated by biomarkers of central and peripheral inflammation.

Despite the biologically plausible conceptual links between neuroinflammation, depression, and HIV (described in detail elsewhere [24]), few studies have previously attempted to quantitatively test this hypothesis. Amongst participants living with HIV (but not those without HIV), Saloner et al. [25] observed a trend between depression severity and composite neuroinflammation scores while Woods et al. [26] similarly saw a significant association between specific dimensions of depression and plasma BDNF. In line with our findings in the current study, Musinguzi et al. [27] previously demonstrated that participants living with HIV recruited in Uganda who exhibited increased TNF- α were at significantly higher risk for depression. Our study offered the additional advantage of replicating this mediation effect in a sample of participants living with and without HIV. Given that TNF- α appears to mediate the relationship between HIV and depressive symptoms in two independent and demographically distinct samples, it is plausible that the effect of plasma TNF- α on this association is robust and reliable.

We found CSF IL-6 to have a considerable mediating role in depressive symptoms, which is compatible with previous evidence consistently implicating IL-6 in the risk for depression, although our study is one of the first to demonstrate this specifically in the context of HIV. We also saw sub-threshold but notable mediation of the association between HIV and depressive symptoms by plasma IL-6. The association between IL-6 and depression has been observed repeatedly with varying strengths [28]. Longitudinal twin studies and meta-analyses indicate that elevation in IL-6 may be a risk factor leading to depression, rather than a consequence of depression [29, 30]. Our findings therefore suggest that IL-6 may play a role in mediating the risk for depression in people living with HIV.

We found a moderate correlation between IL-6 concentrations in blood and CSF. We also observed moderate correlations for both plasma and CSF IL-6 with a cluster of chemokines, which suggests that the pro-inflammatory cytokine IL-6 may play an important role in chemotactic cascades in the periphery and the CNS. These results are compatible with findings from a previous study which similarly observed moderate correlations between blood and CSF IL-6 and between IL-6 and certain chemokines

Table 3. Odds ratios (OR, with 95% confidence interval [CI]) for the association between HIV status and Any Depressive Symptoms, before and after adjustment for each biomarker fitted separately.

Biomarker	N	N Any Depressive Symptoms	OR (95% CI) for Any Depressive Symptoms, adjusted for HIV status and sociodemographic factors	
			Before adjusting for biomarker	Adjusted for biomarker
Neurometabolites ^a				
Myo-inositol				
Frontal White Matter	161	29	2.96 (1.12, 9.13)	3.23 (1.19, 10.15)
Putamen	63	20	5.19 (1.32, 27.63)	5.55 (1.35, 30.35)
Choline				
Frontal White Matter	184	36	2.75 (1.17, 7.20)	2.91 (1.21, 7.74)
Putamen	128	29	7.46 (2.26, 35.53)	8.54 (2.50, 41.47)
Soluble biomarkers measured in all participants, where possible				
Plasma				
CRP	204	42	3.27 (1.46, 8.09)	3.05 (1.34, 7.61)
I-FABP	202	41	3.81 (1.65, 9.91)	3.98 (1.62, 10.86)
Kyn:Trp	203	41	3.76 (1.63, 9.78)	3.63 (1.54, 9.59)
Neopterin	203	41	3.76 (1.63, 9.78)	3.50 (1.44, 9.45)
NFL	202	41	3.16 (1.41, 7.80)	2.94 (1.29, 7.31)
sCD14	201	40	3.01 (1.34, 7.49)	3.22 (1.39, 8.20)
sCD16	202	42	3.36 (1.50, 8.30)	3.27 (1.44, 8.18)
sCD163	204	42	3.27 (1.46, 8.09)	3.62 (1.59, 9.11)
CSF				
Kyn:Trp	202	41	3.07 (1.37, 7.57)	3.04 (1.34, 7.62)
Neopterin	202	41	3.07 (1.37, 7.57)	2.84 (1.25, 7.11)
NFL	203	41	3.13 (1.40, 7.80)	3.15 (1.40, 7.82)
sCD14	202	41	3.08 (1.38, 7.62)	3.14 (1.39, 7.78)
sCD163	203	41	3.13 (1.40, 7.72)	3.28 (1.46, 8.13)
Soluble biomarkers measured in a subset of 78 participants				
Plasma				
IL-6	78	13	1.67 (0.46, 6.58)	1.51 (0.40, 6.01)
IP-10/CXCL10	78	13	1.67 (0.46, 6.58)	1.68 (0.45, 6.69)
MCP-1/CCL2	78	13	1.67 (0.46, 6.58)	1.67 (0.46, 6.59)
MIG/CXCL9	78	13	1.67 (0.46, 6.58)	1.42 (0.38, 5.63)
MIP1- α /CCL3	78	13	1.67 (0.46, 6.58)	1.74 (0.47, 7.00)
RANTES/CCL5	78	13	1.67 (0.46, 6.58)	1.55 (0.41, 6.20)
TNF- α	78	13	1.67 (0.46, 6.58)	1.48 (0.39, 5.98)
CSF				
IL-6	78	13	1.67 (0.46, 6.58)	1.37 (0.35, 5.59)
IP-10/CXCL10	78	13	1.67 (0.46, 6.58)	1.75 (0.46, 7.16)
MCP-1/CCL2	78	13	1.67 (0.46, 6.58)	1.67 (0.45, 6.58)
MIG/CXCL9	78	13	1.67 (0.46, 6.58)	1.66 (0.45, 6.58)
MIP1- α /CCL3	78	13	1.67 (0.46, 6.58)	1.32 (0.35, 5.26)
RANTES/CCL5	78	13	1.67 (0.46, 6.58)	1.64 (0.45, 6.47)
TNF- α	78	13	1.67 (0.46, 6.58)	1.83 (0.49, 7.31)

All models were adjusted for age, sex, ethnicity and years of education.

^aModels which included neurometabolite measures were further corrected for scanner.

amongst people living with HIV [31]. Most other soluble biomarkers were only weakly or moderately correlated with each other in our sample. Correlations between plasma and CSF concentrations of individual biomarkers were slightly stronger on average, though still only moderate, which may be in part because

participants living with HIV in this study were virally suppressed as measured by an undetectable plasma HIV-RNA load [32]. These findings may suggest that multiple inflammatory biomarkers may carry unique information about facets of the inflammatory cascade.

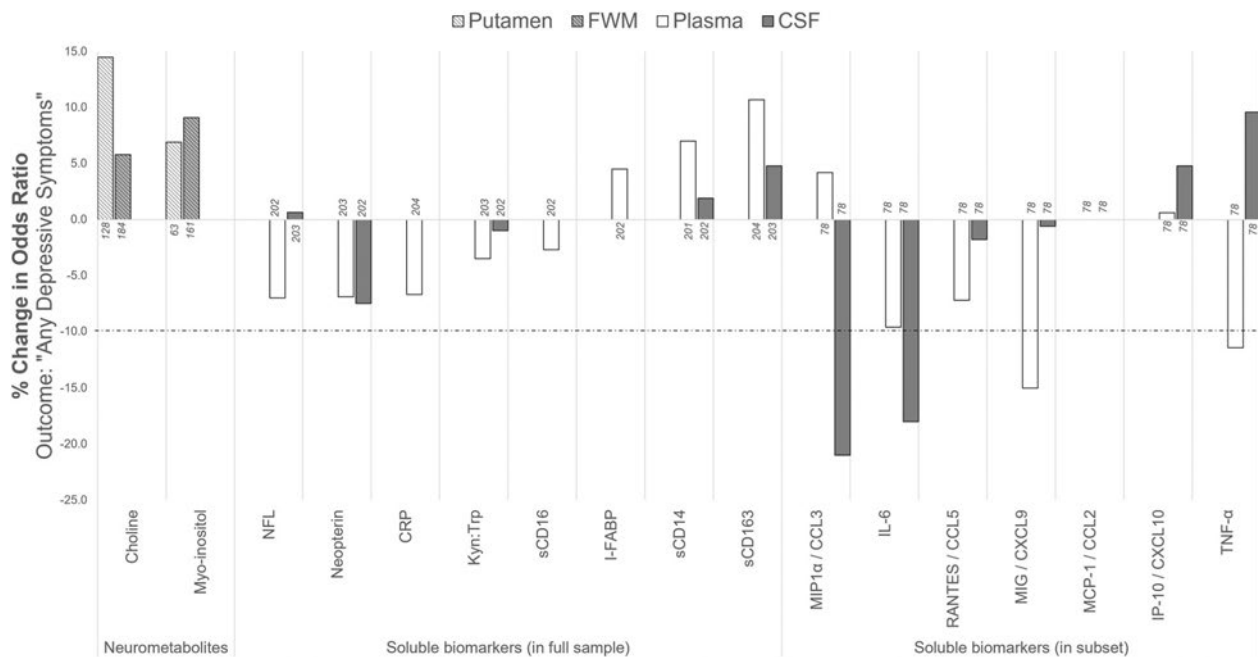


Fig. 2 Mediation of the association between HIV status and Any Depressive Symptoms by adjusting for each biomarker separately. Sample sizes for which data were available for each biomarker are indicated at the x axis next to each bar. The dotted line marks a 10% reduction in the odds ratio, which represents our criterion for potential mediation.

A novel and exciting finding in our exploratory study is the substantial mediation of the association between HIV and depressive symptoms by CSF MIP1- α . Although the functions of MIP1- α in chemotaxis and HIV suppression have been closely studied [33–35], few studies have attempted to understand what role, if any, this chemokine may play in depression. In fact, a recent meta-analysis investigating the role of chemokines in depression found only six eligible studies that tested associations between MIP1- α and depression [12]. This meta-analysis reported that blood MIP1- α concentrations were increased in depressed vs non-depressed individuals, but only when including participants who were physically healthy (i.e., without co-morbid physical illness). We observed a fairly large attenuation of the risk for depressive symptoms by CSF MIP1- α in a sample of participants living with and without HIV. Our findings thus suggest that MIP1- α may be a useful and underexplored biomarker for depression, especially in people living with HIV.

Notably, we did not find that the neuroimaging biomarkers measured in our study (ml and Cho in putamen and FWM) attenuated the odds ratio for the association between HIV status and depressive symptoms. These results indicate that alterations in concentrations of ml and Cho, which are often considered markers of glial cell activation and cellular metabolism [36], did not mediate the relationship between HIV and depressive symptoms in this sample. However, others have previously noted that changes in ml and Cho concentrations, as measured by MRS, may be caused by competing mechanisms and not specifically reflective of neuroinflammation [37, 38]. More sensitive techniques such as diffusion-weighted MRS, which has recently been shown to be sensitive to an experimental model of neuroinflammation in humans [39], may thus be used in future to clarify the relationship between imaging biomarkers of neuroinflammation and depressive symptoms in people living with HIV.

A few potential limitations must be considered in the interpretation of our findings. Our threshold for >10% reduction in OR for the association between HIV and depressive symptoms, which we used to define potential mediators in this study, may be considered low. Since this study was cross-sectional, we cannot make inferences about causality and cannot entirely rule out the possibility of other

confounding variables which may lead to an artificial association of some of these biomarkers with the outcome. Additionally, the biomarkers included in this study were fixed by parent study design and chosen for their relevance to immune dysfunction, metabolism, and neuronal damage in age-associated non-communicable co-morbidities amongst people living with HIV. Some key biomarkers of inflammation and neurogenesis such as IL-1 β and BDNF, which have been associated with depressive symptoms in people living with HIV, were not measured in this study but warrant investigation in future research [26, 40]. Concentrations of certain biomarkers such as MIP1- α in plasma and RANTES and TNF- α in CSF were near the detection limit for the majority of participants, which further limits these analyses.

Further limitations relate to the size and characteristics of the sample included in this study. The COBRA study protocol excluded participants with a total PHQ-9 score >15 at screening, thus these analyses were restricted to people without severe depressive symptoms, and most participants meeting criteria for “Any Depressive Symptoms” reported mild or moderate depressive symptom severity. Particularly for some biomarkers in this study, the sample of people with Any Depressive Symptoms was quite small, so some of these analyses may be underpowered. Given the small sample sizes in subgroups for correlation analyses (especially for participants without HIV and with Any Depressive Symptoms), limited meaningful comparisons between biomarker correlations in the full sample and in subgroups can be made. All participants with HIV were on antiretroviral medication, with potential side-effects such as fatigue which overlap with depressive symptoms, thus potentially confounding our observations. Participants needed to be willing to attend additional study visits and were encouraged to undergo lumbar puncture, which may further have introduced some selection bias. Finally, the demographic characteristics of this cohort were largely fixed by study design and were chosen to reflect the epidemiology of older adults living with HIV in the UK and the Netherlands at the time of recruitment (2013–14). As a result, this cohort was comprised primarily of White men who have sex with men, which is not representative of the global population of people living with HIV. This lack of gender

and ethnic diversity in our sample limits generalisation to other populations, including women living with HIV and people living with HIV in the Global South.

Despite these limitations, our study offers novel and significant implications for our understanding of HIV-associated depression. Through this exploratory study, we offer evidence for the mediating role of pro-inflammatory cytokines (TNF- α , IL-6) and chemokines (MIP1- α , MIG) in the association between HIV and depressive symptoms. This is one of the first studies to specifically investigate the interrelationship between systemic and neuroinflammation, depressive symptoms, and HIV in a relatively large sample of participants living with HIV and carefully selected demographically-similar controls. We also report a rich dataset of correlations between plasma and CSF concentrations of a wide range of inflammatory biomarkers, which is often missing from similar studies, and which may inform future research design and biomarker discovery. This study particularly adds value to the ongoing efforts for the discovery of clinically useful biomarkers for depression, especially in the context of HIV, by revealing MIP1- α , an underexplored biomarker, as a notable mediator of the risk for HIV-associated depression. It will be important to replicate these findings in larger and more diverse cohorts and with participants who exhibit greater depressive symptom severity. We did not include interaction terms or stratify our analyses to explore any variable-specific trends due to the limited sample size and small number of participants with Any Depressive Symptoms, but future studies with larger samples may investigate age- and sex-specific trends. In addition, we did not explore possible synergistic or antagonistic effects of biomarkers in this study, but future studies may analyse the effects of multiple biomarkers in combination on the relationship between HIV and depressive symptoms. Future studies may also investigate the precise mechanisms by which the inflammatory biomarkers highlighted in our study influence the risk for depression, perhaps by exploring possible links between these biomarkers and microglial activation, synaptodendritic injury and neurogenesis, or serotonin depletion.

DATA AVAILABILITY

The datasets presented in this article are not readily available. Data sharing has been restricted by the Medisch Ethische Toetsingscommissie and the UK National Research Ethics Service (NRES) because the data underlying this study contains sensitive and potentially identifying information. Requests for data sharing, however, can be made on a case-by-case basis following the submission of a concept sheet as per instructions on the project website (<http://fp7-cobra.eu/>). Once submitted, the proposed research/analysis will undergo review by the COBRA Steering Committee for evaluation of the scientific value, relevance to the study, design and feasibility, statistical power and overlap with existing projects. If the proposed analysis is for verification/replication, data will then be made available. If the proposed research is for novel science, upon completion of the review, feedback will be provided to the proposer(s). In some circumstances, a revision of the concept may be requested. If the concept is approved for implementation, a writing group will be established consisting of the proposers (up to 3 persons that were centrally involved in the development of the concept) and members of the COBRA group (or other appointed cohort representatives). All persons involved in the process of reviewing these research concepts are bound by confidentiality. Medisch Ethische Toetsingscommissie, Academisch Medisch Centrum, Universiteit van Amsterdam Academisch Medisch Centrum, XT4-140 Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands. UK National Research Ethics Service (NRES), Charing Cross Hospital, Fulham W6 8RF, London, UK.

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AUTHOR CONTRIBUTIONS

AMRL conceptualised this sub-study and wrote the manuscript with supervision from JHV. NB carried out the statistical analyses, with supervision from CAS. AMRL, JHV, NB, and CAS designed and revised the statistical analysis plan. All other authors were involved in COBRA study design, data collection, and analysis as described in the acknowledgements. All authors reviewed and approved the manuscript.

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COMPETING INTERESTS

AMRL, NB, DS, FW, NAK and JHV have no competing interests to declare. JA has received financial support to attend scientific conferences from MSD, Janssen UK and Gilead Sciences. HZ has served at scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, Alzinova, ALZPath, Annexon, Apellis, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Passage Bio, Pinteon Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Cellectric, Fujirebio, Alzecure, Biogen, 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). MG has received research grants from Gilead Sciences and Janssen-Cilag 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. PR through his institution has received independent scientific grant support from Gilead Sciences, Janssen Pharmaceuticals Inc., Merck & Co and Viiv Healthcare, and has served on scientific advisory boards for Gilead Sciences, Viiv Healthcare, and Merck & Co., for which honoraria were all paid to his institution. AW has received honoraria or research grants on behalf of Imperial College London or been a consultant or investigator in clinical trials sponsored by Bristol-Myers Squibb, Gilead Sciences, GlaxoSmithKline, Janssen-Cilag, Roche and Viiv Healthcare. CAS has received funding for the membership of Data Safety and Monitoring Boards, Advisory Boards and for the preparation of educational materials from Gilead Sciences and Viiv Healthcare.

ADDITIONAL INFORMATION

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7.3 CONCLUSIONS

In this study, participants with HIV showed an increased risk for depressive symptoms and increased inflammation compared to participants without HIV. The increased risk for depressive symptoms in participants with HIV was driven (at least in part) by four biomarkers of inflammation: MIG and TNF- α in plasma, and MIP-1 α and IL-6 in CSF. The odds for depressive symptoms were not notably attenuated when controlling for the effects of choline and myo-inositol, suggesting that neurometabolites may not be involved in this mediation. Together, these results support the central hypothesis that inflammation may partly contribute to the higher risk for depressive symptoms in people with HIV.

Some potential limitations of this study are noted here. First, the sample for this study was overwhelmingly White (92%), male (93%), and older (median age 57 years). This narrow demographic make-up reflects the issues highlighted in Chapter 4 and may substantially limit the generalisability of these findings to other populations, such as women with HIV. Second, the statistical approach for this study, which was designed in collaboration with the COBRA statistical team, allows only an indirect quantification of potential mediation. In this study, the % change in odds ratio for depressive symptoms after controlling for the effects of a biomarker was considered an indirect measure of mediation. A relatively arbitrary threshold for this % change in odds ratio ($>10\%$) was defined to indicate potential mediation. The rationale for this low threshold, as described in Chapter 2, is that a small degree of mediation (if any) is anticipated, since the biomarkers used in this study are non-specific measures of inflammation and inflammation may be only one of several processes contributing to the risk for depression. It may be inferred that a % change in odds ratio for depressive symptoms as large as 20.9% (observed for CSF MIP-1 α) may strongly suggest a significant underlying mediation effect. Nevertheless, a major limitation of the COBRA-DI study was that the statistical significance of the measure of potential mediation could not be directly quantified. These limitations were partly addressed through the GOLD Depression Study (Chapter 9).

8

isiXhosa translation of the PHQ-9

HIGHLIGHTS

- isiXhosa is the first language for over 8 million people in South Africa. Despite this, there was no publicly available version of the PHQ-9 (a commonly used screening test for depression) in isiXhosa.
- An isiXhosa translation of the PHQ-9 was developed in four steps: (1) initial translation by two independent professional translators, (2) changes made by mental health researchers and clinicians, (3) review by people with lived experience of depression and/or HIV, and (4) back-translation by another two translators. At all stages, the people involved in this process were fluent in both English and isiXhosa.
- A total of 47 young people completed this translated questionnaire. If someone scored high on one question, they were also likely to score high on other questions.
- All participants had also completed another questionnaire called the ASEBA Youth Self Report form, which also asks about symptoms of depression. People responded similarly across both these questionnaires.
- Together, these results tell us that this translation of the PHQ-9 works correctly to identify symptoms of depression. This translation is now freely available online for anyone to use.

8.1 INTRODUCTION

THE NINE-ITEM PATIENT HEALTH QUESTIONNAIRE (PHQ-9) is a useful measure of depressive symptoms because it is brief, easy to administer, and cost-effective. Translations of the PHQ-9 have been validated in many languages globally, and it is recommended as a Common Measure in Mental Health Science to allow for global comparisons in depression research. At the Family Centre for Research with Ubuntu (FAMCRU), we work with many young people whose first language is isiXhosa. Although isiXhosa is spoken by over 10 million speakers in southern Africa, when designing the GOLD Depression Study (Chapter 9), I discovered that there was no isiXhosa version of the PHQ-9. Research groups and clinics tended to develop ad hoc translations when needed, or bilingual researchers would translate the questionnaire on the spot when administering the PHQ-9. This variability could substantially impact research findings and limit comparisons across studies.

To address this gap, I led the development of an open-access isiXhosa translation of the PHQ-9 in a pre-registered pilot study (Stage 1 Registered Report available at DOI: [10.3389/fpsyg.2022.840912](https://doi.org/10.3389/fpsyg.2022.840912)). A four-step transcultural translation framework was used to develop this translation. This isiXhosa PHQ-9 was then administered to young people in the GOLD cohort who spoke isiXhosa at home, and who had previously completed the ASEBA Youth Self Report (YSR) form in their preferred language, which was used as a reference standard. Using this data, the reliability, criterion validity, convergent validity, and construct validity of this isiXhosa PHQ-9 were assessed. This translation was used as a measure of depressive symptoms in the GOLD Depression Study (Chapter 9).

8.2 REGISTERED REPORT: *BRAIN AND NEUROSCIENCE ADVANCES*

isiXhosa translation of the Patient Health Questionnaire (PHQ-9) shows satisfactory psychometric properties for the measurement of depressive symptoms [Stage 2]

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Abstract

Depression is a debilitating illness, and stigma associated with it often prevents people from seeking support. Easy-to-administer and culturally-inclusive tools can allow for early screening for depressive symptoms in primary care clinics, especially in resource-limited settings. In this pre-registered pilot study (Stage 1 Report available at DOI: 10.3389/fpsy.2022.840912), we produced an open-access isiXhosa-language version of the nine-item Patient Health Questionnaire (PHQ-9), a well-validated measure of depression incidence and severity, using a transcultural translation framework. We validated this isiXhosa PHQ-9 in a sample of $N=47$ adolescents living with and without HIV in Cape Town, South Africa who speak isiXhosa at home. Reliability, convergent validity, and criterion validity were assessed, with T scores on the Achenbach System of Empirically Based Assessment Youth Self Report (YSR) form completed previously as reference standard. Our isiXhosa PHQ-9 exhibited satisfactory reliability, with Cronbach's $\alpha = 0.866$, inter-item correlations ranging from 0.229 to 0.730, and mean item-total correlation of 0.69. PHQ-9 score and Withdrawn/Depressed component T scores on the Youth Self Report were moderately associated (Spearman's $\rho = 0.40, p = 0.011$), indicating acceptable convergent validity. The isiXhosa PHQ-9 showed satisfactory criterion validity (area under the receiver operating characteristic curve, $AUC=0.706$), but these analyses were under-powered. Principal component analysis revealed a one-factor solution, with 45.8% of variance explained by the first principal component and all factor loadings above conventional thresholds. Our isiXhosa translation of the PHQ-9 thus exhibited satisfactory psychometric properties in this pilot validation study and performed comparably to other PHQ-9 versions validated in different languages in African and global contexts. This questionnaire may serve as an invaluable culturally-inclusive screening tool for measuring depressive symptoms among isiXhosa speakers. Caution must be exercised as screening tools including the PHQ-9 may over- or underestimate prevalence of depression. Further validation in larger, independent cohorts may enable wider use of our isiXhosa PHQ-9 as a screening tool in clinics, research studies, and mental health non-profits who serve amaXhosa.

Keywords

Depressive symptoms, major depressive disorder (MDD), psychometric testing, translation, multicultural tools

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Introduction

'If I show you where I'm struggling, I feel you have been exposed to my weakness', said Siyanda, a young Xhosa man, in relation to the cultural expectation for Xhosa men to manage mental health issues without seeking support (Williams, 2019: 56). The constellations of behavioural and somatic issues that comprise major depressive disorder (MDD, or depression) are estimated to affect between 4.4% and 5.0% of people globally (Ferrari et al., 2013; World Health Organization (WHO), 2017). Biological and psychosocial factors can predispose individuals to developing MDD (Dobson and Dozois, 2011). Living with a chronic human immunodeficiency virus (HIV) infection, especially, significantly increases the risk of depression (Rezaei et al., 2019), with prevalence of depression among people living with HIV

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estimated to be as high as 50% (Bhatia and Munjal, 2014; Wang et al., 2018). Given the stigma associated with depression across cultures, people with depression may struggle to access mental healthcare resources, as Siyanda suggests. However, early referral and interventions have substantial benefits for the treatment and prevention of depression (Callander et al., 2017; Rapee, 2013). It is, therefore, imperative to continue to refine tools for early screening of depression and increase their accessibility in primary care clinics.

The Xhosa people ('amaXhosa') comprise a diverse cultural group who speak variations of isiXhosa. The vast majority of amaXhosa live in the Eastern Cape and Western Cape provinces of South Africa (Government Communications, 2020). Over the last three centuries, European colonial expansion into the South African heartland occurred at the expense of indigenous populations, including amaXhosa, culminating in systematic disenfranchisement during the apartheid regime (Clark, 2014; Stapleton, 1993). Under the apartheid government, especially, amaXhosa suffered severe marginalisation as Afrikaans was the favoured language in government and education in place of indigenous languages such as isiXhosa (Kaschula, 2008). As a result of these institutional barriers, it is difficult for people to access quality healthcare in isiXhosa today (Levin, 2006; Vergunst et al., 2015). In order to reduce this healthcare inequity faced by amaXhosa, it is crucial that clinical resources are made available to them in their home language.

There is no word for 'depression' in isiXhosa, but that does not mean that this debilitating illness is not found among the amaXhosa. In fact, the prevalence of depression in this group, estimated at 6.9%, is significantly higher than global estimates for depression in the general population (Baron et al., 2017). Substantial intra-group variability exists, with, for instance, a significantly higher prevalence rate of 31.9% among women in Khayelitsha during pregnancy and 12 weeks postpartum (Hung et al., 2014). Despite this, few isiXhosa-language tools exist for diagnosis of depression. An early study translated the Edinburgh Postnatal Depression Scale (EPDS) into isiXhosa and found satisfactory internal consistency (De Bruin et al., 2004), but this scale is limited in its target population. Another study validated an isiXhosa version of the Centre for Epidemiological Studies Depression Scale in a more general population group (Baron et al., 2017). The ongoing development and validation of the 16-item South African Depression Scale (SADS) in isiXhosa is particularly promising, especially for amaXhosa living with HIV (Andersen et al., 2020, 2021). However, the trade-off in developing such a tool is that limited comparisons can be made between scores from the SADS and other established scales until it is validated in other countries and cultures, thereby restricting synthesis of results across global studies.

The nine-item Patient Health Questionnaire (PHQ-9) is a useful measure for depression incidence and severity (Kroenke et al., 2001; Kroenke and Spitzer, 2002). It has been validated as a diagnostic tool in clinical samples (Beard et al., 2016) as well as the general population (Martin et al., 2006), where it shows a high degree of convergent validity with other depression scales such as the Beck Depression Inventory (BDI). Furthermore, the PHQ-9 is easy to administer, especially in resource-limited settings (Hancock and Lerner, 2009), and exhibits similar results whether it is self-administered or carried out by an interviewer (Pence et al., 2012; Pinto-Meza et al., 2005). It is shown to be

useful to screen for depression in African populations (Adewuya et al., 2006) and in people living with HIV (Cholera et al., 2014; Monahan et al., 2009). Numerous validation studies for translations of the PHQ-9 have demonstrated its value as a depression scale in samples across the world (Lotrakul et al., 2008; Mwangi et al., 2020; Woldetensay et al., 2018). Given this global body of evidence, the PHQ-9 represents a measure of depression that could facilitate cross-cultural comparisons of depression pathology better than population- or geography-specific scales such as the EPDS or SADS. Despite these many advantages, to the best of our knowledge, an isiXhosa-language version of the PHQ-9 has not yet been validated and made publicly available. Baron et al. (2017) report findings from an isiXhosa version of the PHQ-9, but this version is not available alongside the study except by request to the authors. Given the utility of the PHQ-9 in rapid screening of depressive symptomatology, a freely available isiXhosa-language PHQ-9 may be an invaluable mental health triage tool for clinicians serving amaXhosa.

In this pilot study, we aimed to produce and validate one of the first open-access isiXhosa-language versions of the PHQ-9 depression scale. The protocol for this study was pre-registered in the Stage 1 Registered Report found at DOI: 10.3389/fpsy.2022.840912. This isiXhosa PHQ-9 was produced using a transcultural translation framework and administered to a cohort of adolescents living with and without HIV in Cape Town, South Africa. Responses on the PHQ-9 were compared against those on the Achenbach System of Empirically Based Assessment (ASEBA) Youth Self Report (YSR) forms as a reference standard. Our primary hypothesis was that the isiXhosa-language PHQ-9 will exhibit satisfactory reliability, measured as internal consistency using Cronbach's α . We also hypothesised that this translation of the PHQ-9 will show acceptable convergent validity (correlation coefficient for PHQ-9 and YSR scores) and diagnostic accuracy (area under Receiver Operating Characteristic (ROC) curve (AUC) for PHQ-9 vs YSR). This pilot study aimed to pave the way for larger-scale, independent validation of this isiXhosa-language PHQ-9 and add an easy-to-administer, culturally inclusive questionnaire to the local clinician's toolbox.

Methods

Participants

Participants for this study were recruited through the Adolescent Cognitive and Brain Imaging (GOLD) cohort at Stellenbosch University and Tygerberg Hospital in Cape Town, South Africa. The GOLD study, which draws on the cohort of participants in the landmark Children with HIV Early antiretroviral therapy (CHER) trial, includes adolescents living with HIV who were initiated on antiretroviral therapy (ART) early in life (Cotton et al., 2013). The CHER cohort is active and regularly willing to contribute to sub-studies, with as many as 80 children living with HIV and 80 age-matched HIV-controls participating in recent sub-studies (Van Biljon et al., 2021).

Inclusion criteria. Participants were included in this study if they were younger than 18 years old and spoke isiXhosa at home.

Sample size estimation. *A priori* estimation of sample size in validation studies for psychometric tools is remarkably low

(Anthoine et al., 2014). For our a priori sample size estimation (described in the Stage 1 Registered Report), we utilised a web-based sample size calculation tool for reliability studies (Arifin, 2018), available at https://wnarifin.github.io/ssc_web.html, with the primary outcome of interest as the Cronbach's α reliability coefficient for the translated PHQ-9. The *a priori* sample size estimation showed that a sample size of $N=19$ would be necessary to detect a Cronbach's $\alpha=0.65$ (indicative of a moderate reliability) at 80% power for the nine-item questionnaire.

Ethical considerations. We received written informed assent from participants and written informed consent from participants' parents or legal guardians in their language of choice for inclusion in the study before participation. The study was conducted in accordance with the Helsinki Declaration and Good Clinical Practice (GCP) standards. The protocol for this study was approved by the Stellenbosch University Human Research Ethics Committee (N21/10/116_Substudy N19/10/135). Any participants who reported experiencing feelings of self-harm or suicide ideation or scored greater than 15 on the PHQ-9 were referred to a trained psychologist or social worker on staff for psychological support.

Materials

Patient Health Questionnaire. The English-language version of the PHQ-9 was designed with slight adaptations from the original version (Kroenke et al., 2001). The adaptations, intended primarily to improve comprehensibility of the scale to adolescents in 2022, were as follows: in item 7, we replaced 'reading the newspaper' with 'reading', and in the final question, we replaced 'if you checked off *any* problems' with 'if you chose a number higher than 0'. The isiXhosa-language version of the PHQ-9 was created from this English version using a transcultural translation framework (see Procedure). Both the English-language and isiXhosa-language versions of the PHQ-9 are freely available in Supplemental materials.

YSR form. The ASEBA YSR form (Rescorla, 2005) measures behavioural issues representing syndromes such as 'Withdrawn/Depressed', 'thought problems', and 'rule-breaking behaviour'. The YSR has been validated as a measure of behavioural issues among adolescents (Ebesutani et al., 2011), including in several studies in southern and eastern Africa (Zieff et al., 2022). In this study, we used data obtained from participants in the GOLD study during previous clinic visits when a validated bilingual (English and isiXhosa) YSR form was administered via an interview by a trained Research Assistant. During these visits, participants were asked (in the language in which they are most comfortable, English or isiXhosa) whether they think they have exhibited any of the behaviours in question over the past 6 months. These responses were used as a reference standard to compare with the translated version of the PHQ-9. T scores for the Withdrawn/Depressed component within Syndrome Scales of the YSR were used to determine participants' depressive symptoms and classify participants as 'clinically depressed', 'borderline', or 'non-depressed' using ASEBA standards.

Procedure

Setting. The study site for participant recruitment and data collection was the Family Centre for Research with Ubuntu (FAMCRU), Ward J8, Tygerberg Hospital, Department of Paediatrics and Child Health, Faculty of Medicine and Health Sciences, Stellenbosch University.

Transcultural isiXhosa translation of the PHQ-9. The process of transcultural translation of psychometric tools involves steps to ensure that translated questionnaires remain accurate, relevant, and culturally acceptable (Kaiser et al., 2013; Kohrt et al., 2016). We adopted this systematic methodology to translate the PHQ-9 into isiXhosa using four steps:

1. Translation from English into isiXhosa by two independent bilingual (English and isiXhosa) speakers;
2. Review of isiXhosa translation by a panel of mental health experts, including research staff and clinical professionals, who speak both languages;
3. Review of translation by a co-production panel involving individuals living with HIV and/or with a lifetime history of depression and who speak both languages; and
4. Blinded back-translation from isiXhosa into English by two additional independent bilingual translators.

The translated version was refined after each step to preserve the core meaning and purpose of each item and incorporate culturally specific idioms describing the affective components measured by the questionnaire where possible. Notably, these refinements were made to accommodate variations in language used by people in different geographical locations and to ensure that the translations were accessible to people who have not received a formal education. Modifications were reconciled in consultation with the translators and review panel involved in this process. The final isiXhosa-language version of the PHQ-9 is available in Supplemental materials.

We also translated a demographic questionnaire, which included inclusive and diverse options for various gender identities and sexual orientations in both English and isiXhosa. This demographic questionnaire is available in Supplemental materials.

Co-production. Knowledge co-production, or participatory research methods, involves incorporating insights from individuals with lived experience of the conditions being studied. For this study, we recruited four isiXhosa-speaking individuals as a focus group to review and provide feedback on the isiXhosa translation of the PHQ-9 during the initial stages of this study. Two adults and two adolescents with lived experience of HIV and/or depressive symptoms were recruited through the FAMCRU clinic and the non-profit Inala Mental Health Foundation. These individuals were invited to review the isiXhosa PHQ-9 during Step 3 of the transcultural translation process and suggest edits to improve the accessibility, comprehensibility, and cultural sensitivity of the translation. Co-producers were reimbursed for their time.

Participant recruitment and informed consent. Participants were recruited through the GOLD study at the Family Centre

for Research with Ubuntu (FAMCRU), Tygerberg Hospital. Adolescents living with or without HIV who met this study's inclusion criteria were contacted by research staff to ascertain interest in participation in this study. A trained member of the research staff discussed the study procedures in person with the potential participant's parent or legal guardian in their preferred language. Informed consent and assent forms were available in two versions (English and isiXhosa). Parents or legal guardians of the participants were asked to read and review the consent form. If participants agreed to take part in this study, written informed consent was received from parents or legal guardians in advance of the study procedures and verified by a doctor on staff. All participants were also required to assent to the study procedures. Participants were reimbursed for their travel costs to visit the clinic for the study in line with Stellenbosch University policies.

Psychometric testing. Participants were provided a private space for psychometric testing. A member of the research staff briefed each participant on the procedures involved in the study and receive informed assent. The research staff confirmed that the participant would prefer to respond to questionnaires in isiXhosa and offered them a choice between filling out the questionnaire themselves in writing or having the research staff ask them the questions verbally (for participants who may not be able to write or have a preference for spoken isiXhosa over written isiXhosa). If participants chose to complete the psychometric testing in writing, they were asked to fill out a short demographic questionnaire and the translated version of the PHQ-9 in writing (paper and pencil). If participants chose to complete the questionnaire verbally, the member of the research staff read each item on the questionnaire exactly as written in a neutral manner and wrote down the participant's response, without judgement or comment. In either case, the research staff member was available to the participant for any clarifications to ensure participants understood what each question was asking. Once participants completed the demographic questionnaire and the PHQ-9, the research team member verified that all nine items of the PHQ-9 were completed. The research team member calculated the total score on the PHQ-9 as the sum of the scores from individual items and input responses into a secure electronic database (Project RedCap), which included built-in quality control checks. Data were handled in accordance with the Protection of Personal Information Act (South Africa) and the UK General Data Protection Regulation (GDPR) Act.

Statistical approach

All statistical analyses were performed using R version 4.2.1. Participant demographic characteristics were summarised using proportions (*n* and %) for categorical variables, and medians and interquartile ranges (IQRs) for continuous variables.

To determine the reliability of the isiXhosa PHQ-9, we calculated Cronbach's α as a measure of internal consistency for the translated version. Inter-item (excluding correlation of an item with itself) and item-total score correlations were also calculated. To determine convergent validity for the PHQ-9, Spearman's correlation coefficients were calculated for the total PHQ-9 scores and T scores for the Anxious/Depressed and Withdrawn/

Depressed syndrome scales on the YSR. To determine the criterion validity of this PHQ-9 version, we compared participant responses on the isiXhosa PHQ-9 with the Withdrawn/Depressed scale on YSR forms as a reference standard, to calculate diagnostic sensitivity and specificity, predictive values (PPV/NPV), likelihood ratios (PLR/NLR), and Youden's Index. Participants with a Withdrawn/Depressed T score in borderline ($65 \leq T \leq 69$) or clinical ($T \geq 70$) range were assigned values of 1 (representing cases), and all other participants ($T < 65$) were assigned values of 0 (representing controls). An ROC curve was produced and AUC for the PHQ-9 was calculated to assess diagnostic performance in comparison to the YSR. Finally, individual item analyses were conducted to determine whether mean scores on each item of the PHQ-9 differed between participants who met clinical or borderline depression thresholds and those who did not, as categorised by withdrawn/depressed T scores on the YSR. For item-level analyses, that is, comparing mean scores between the isiXhosa PHQ-9 and a global pooled sample and comparing mean scores between participants classified using the YSR as having Borderline or Clinical Depression and those having No Depression, *p*-values were corrected using the false discovery rate (FDR) method to adjust for multiple comparisons.

Deviations from Stage 1 Registered Report

Certain deviations from the protocol described in the Stage 1 Registered Report are noted. First, the Stage 1 Registered Report indicated that only adolescents living with HIV would be recruited. However, given that the validity of the PHQ-9 is not specific to this population and to ensure the maximum possible power for our analyses, we included both adolescents living with and without HIV in this study. Second, the Stage 1 Registered Report indicated that the PHQ-9 and YSR scores would be separated by no more than 3 months, but this was not possible due to practical constraints in participant scheduling. We, therefore, report the median absolute time difference between the YSR and PHQ-9 administration. In all cases, the most recent YSR scores available for each participant were included for analysis, and the YSR and PHQ-9 were administered as closely as possible, as was indicated in the registered protocol. To ensure that these deviations from the registered protocol did not substantially impact our findings, we conducted sensitivity analyses, which are described below.

Exploratory analyses

In addition to pre-registered analyses described above, we carried out descriptive analyses of group differences, as our sample included both participants with and without HIV. Group differences in demographic characteristics were assessed using Pearson's chi-square test or Fisher's exact test for categorical variables, and Wilcoxon rank sum test for continuous variables. Group differences were assessed by HIV status, comparing participants with HIV and participants without HIV, and depressive symptom status, comparing participants with total PHQ-9 score > 4 ('Some Depressive Symptoms') and those with score ≤ 4 ('Minimal Depressive Symptoms'; Kroenke et al., 2001).

We also investigated the construct validity and factor structure of our isiXhosa-language PHQ-9. We conducted a principal

component analysis (R function principal with varimax rotation) to calculate eigenvalues and factor loadings for each item on the questionnaire. A scree plot was produced using variance explained for each component to confirm number of factors. Tucker's coefficient of factor congruence was calculated to assess the congruence between factor loadings in our sample with other reported PHQ-9 validation studies. To ascertain whether the variability in factor loadings for each item in our translation may be attributed to the small sample size in our pilot study, a bootstrap analysis was run (R function 'boot') with 1000 sets of bootstrapped factor loadings based on our observed factor loadings. Using the bootstrap output, bias corrected accelerated 95% confidence intervals (CIs; BCa, R function boot.ci, type='bca') were calculated for all nine items.

To demonstrate the comparability of our isiXhosa-language PHQ-9 with other versions of the PHQ-9, in addition to describing the inherent psychometric properties of our translation, we also present comparisons with published psychometric properties of other PHQ-9 versions in African and global contexts. These include a seTswana version in South Africa (Bhana et al., 2015), an English version in Kenya (Monahan et al., 2009), a Portuguese version in Mozambique (Cumbe et al., 2020), a Swahili version in Tanzania (Fawzi et al., 2019), and a recent multi-country, multi-language validation study (Bianchi et al., 2022).

Sensitivity analyses for deviations from Stage 1 Registered Report. To ensure that the inclusion of participants without HIV in our study did not unduly influence our findings, we conducted sensitivity analyses for reliability and construct validity, following the same procedures as above, for the subset of participants with HIV only. To ensure that greater time difference between PHQ-9 and YSR administration did not unduly influence our findings, we conducted sensitivity analyses for convergent validity using partial Spearman's correlation coefficients, which controlled for time between PHQ-9 and YSR administration. We also conducted sensitivity analyses for criterion validity with the same procedures as above, for only the subset of participants for whom absolute time difference between PHQ-9 and YSR administration was less than 100 days.

Results

Participant characteristics

We included $N=47$ participants in this pilot validation study (see Table 1). Median (IQR) age of participants was 16 (15, 16) years. Participants were relatively evenly split by gender (47% girls), $\chi^2(1) = 0.20, p = 0.662$. All participants were currently enrolled in school, ranging in grade from Grade 7 to Grade 11. All participants identified as Black/African ($n=47, 100\%$) and most identified as heterosexual ($n=44, 94\%, \chi^2(2) = 76.9, p < 0.001$). Participants living with HIV ($n=35, 74.5\%$) were all receiving ART, and 91.4% were virally-suppressed (viral load < 200 copies/ μ L; see Table 2).

Demographic characteristics by subgroups of HIV status and depressive symptoms are reported in Table 1. The majority of participants were adolescents living with HIV ($n=35, 74.5\%$), although as the utility of the PHQ-9 as a screening tool for depressive symptoms is not limited to people living with HIV, we

did include participants without HIV ($n=12, 25.5\%$) in this study. Participants did not differ on demographic characteristics when stratified by HIV status, except participants with HIV were slightly older, and a greater proportion of participants without HIV were boys.

Median (interquartile range (IQR)) total score on the PHQ-9 was 3 (0, 7), with a range of 0–17. Of the full sample, $n=16$ (34%) participants met the criterion for 'some depressive symptoms' (total PHQ-9 score > 4). Participants did not differ on demographic characteristics when stratified by depressive symptoms, except participants with some depressive symptoms were more likely to report having used recreational drugs in the past 6 months.

Median (IQR) absolute time difference between administration of the PHQ-9 and YSR was 92 (52, 131) days.

Reliability

Cronbach's α for our isiXhosa PHQ-9 was 0.866, suggesting high internal consistency. Inter-item correlations ranged from 0.229 to 0.730 (see Figure 1). Mean (standard deviation (SD)) scores on each item, and mean inter-item correlations (excluding correlations of an item with itself) are shown in Table 3. Mean item-total correlation was 0.688.

Item-level mean scores for our isiXhosa PHQ-9 were comparable to those from a large ($N=58,472$) validation study recently carried out, which reported PHQ-9 data pooled from participants in seven countries (France, Germany, Israel, New Zealand, Spain, Switzerland, and the United States) and administered in five languages (French, German, English, Hebrew, Spanish; Bianchi et al., 2022; see Figure 2). Welch's t -tests were used to compare means between the two data sets, which revealed that mean scores on only Question 4 (loss of energy) differed significantly between our data set and the larger pooled validation data set, $t(29) = -7.11, P_{FDR} < 0.001$ (all other $P_{FDR} > 0.05$). Mean score on Question 4 in our sample ($M=0.43, SD=0.54$) was lower than that in the sample of Bianchi et al. ($M=1.13, SD=0.99$).

In all, our isiXhosa PHQ-9 exhibited satisfactory reliability, and item-level scores were comparable to previously published findings.

Convergent validity with the YSR

There was a moderate significant association between total PHQ-9 score and T score on the Withdrawn/Depressed component of the YSR (Spearman's $\rho = 0.44, p = 0.002$). The correlation between total PHQ-9 score and T score on the Anxious/Depressed component of the YSR was not significant (Spearman's $\rho = 0.121, p = 0.419$). Thus, the isiXhosa PHQ-9 demonstrated acceptable convergent validity with the Withdrawn/Depressed component of the YSR, but not with the Anxious/Depressed component.

Criterion validity

Using T score thresholds, $n=5$ participants met borderline criteria and $n=4$ met clinical criteria on the Withdrawn/Depressed YSR component. The ROC curve for the isiXhosa PHQ-9 is shown in Figure 3. Area under the ROC curve (AUC) (95% CIs)

Table 1. Demographic characteristics for all participants included in the study, and for subgroups by HIV status and depressive symptoms.

Characteristic	Overall N=47	Participants with HIV n=35	Participants without HIV n=12	p-value	Some depressive symptoms n=16	Minimal depressive symptoms n=31	p-value
Age (years), Median (IQR) ^a	16.0 (15.0, 16.0)	16.0 (15.0, 16.0)	14.5 (14.0, 15.2)	0.006	16.0 (15.0, 16.0)	16.0 (15.0, 16.0)	> 0.9
Grade in School, n (%) ^b				0.100			0.4
7	3 (6.4)	1 (2.9)	2 (17)		2 (12)	1 (3.2)	
8	8 (17)	6 (17)	2 (17)		4 (25)	4 (13)	
9	11 (23)	6 (17)	5 (42)		2 (12)	9 (29)	
10	20 (43)	17 (49)	3 (25)		6 (38)	14 (45)	
11	5 (11)	5 (14)	0 (0)		2 (12)	3 (9.7)	
Gender, n (%) ^b				0.015			0.3
Boy	25 (53)	15 (43)	10 (83)		7 (44)	18 (58)	
Girl	22 (47)	20 (57)	2 (17)		9 (56)	13 (42)	
Ethnicity, n (%) ^b							> 0.9
Black/African	47 (100)	35 (100)	12 (100)		16 (100)	31 (100)	
Sexual orientation, n (%) ^b				> 0.9			0.3
Bisexual/Pansexual	1 (2.1)	1 (2.9)	0 (0)		1 (6.2)	0 (0)	
Gay/Lesbian/Homosexual	2 (4.3)	2 (5.7)	0 (0)		1 (6.2)	1 (3.2)	
Straight/Heterosexual	44 (94)	32 (91)	12 (100)		14 (88)	31 (97)	
Alcohol use (yes), n (%) ^c	10 (21)	6 (17)	4 (31)	0.4	3 (19)	7 (23)	> 0.9
Cigarette smoking (yes), n (%) ^c	2 (4.3)	2 (5.7)	0 (0)	> 0.9	1 (6.2)	1 (3.2)	> 0.9
Recreational drug use (yes), n (%) ^c	5 (11)	5 (14)	0 (0)	0.3	4 (25)	1 (3.2)	0.037

'Some Depressive Symptoms' indicates PHQ-9 score > 4, and 'Minimal Depressive Symptoms' indicates PHQ-9 score ≤4. Bold text indicates a *p* value less than 0.05. HIV: human immunodeficiency virus; IQR: interquartile range.

^aWilcoxon rank sum test.

^bPearson's chi-square test.

^cFisher's exact test.

Table 2. HIV-related clinical characteristics for participants living with HIV included in the study.

HIV-related clinical characteristic	Participants with HIV, n=35
Viral load < 200 copies/μL, n (%)	32 (91.4)
Absolute CD4 count, median (IQR)	788.5 (622.2, 987.2)
(Missing)	3
Antiretroviral regimen, n (%)	
ABC/3TC combination tablets	1 (2.9)
Atazanavir	3 (8.6)
DTG	1 (2.9)
F/TAF	1 (2.9)
Lopinavir + Ritonavir (LPV/r, Kaletra)	3 (8.6)
TLD combination tablets	26 (74)

HIV: human immunodeficiency virus; IQR: interquartile range.

was 0.706 (0.476, 0.937), indicating acceptable discrimination. Characteristics of the ROC curve are shown in Table 4. A threshold of PHQ-9 score ≥ 10 provided satisfactory specificity of 94.74% but poor sensitivity of 44.44%, with a Youden's Index of 1.39. Item-level analyses (see Figure 4) indicated that participants with a borderline or clinical T score on the Withdrawn/Depressed component of the YSR scored significantly higher on

most items of the isiXhosa PHQ-9 ($P_{FDR} < 0.05$), except on questions about sleep changes ($P_{FDR} = 0.055$) and psychomotor changes ($P_{FDR} = 0.704$).

Due to the small number of participants meeting criteria for borderline or clinical depression as indicated by the YSR, analyses of criterion validity were under-powered. A *post hoc* power analysis estimated that the AUC of 0.706 with 9 cases and 38 controls was detected with only 50% power. Therefore, the isiXhosa PHQ-9 demonstrated acceptable criterion validity against the YSR as a reference standard, but these analyses were under-powered and warrant replication in a larger sample.

Exploratory analyses: construct validity

Given previous findings in the literature, we expected to see a one-factor solution for our isiXhosa PHQ-9. The scree plot (see Figure 5) indicated one dominant dimension with a substantial decrease in eigenvalues between the first and second principal component, and small decreases thereafter (eigenvalues: 4.51, 1.12, 1.03, 0.80, 0.55, 0.49, 0.24, 0.16, and 0.09). The proportion of variance explained by the first principal component was 49.6%. The scree plot of variance explained for our isiXhosa PHQ-9 was comparable to that for Monahan et al. (2009).

Factor loadings ranged from 0.52 to 0.92 (thus exceeding the 0.40 cut-off (Howard, 2016)) and item-total correlations ranged

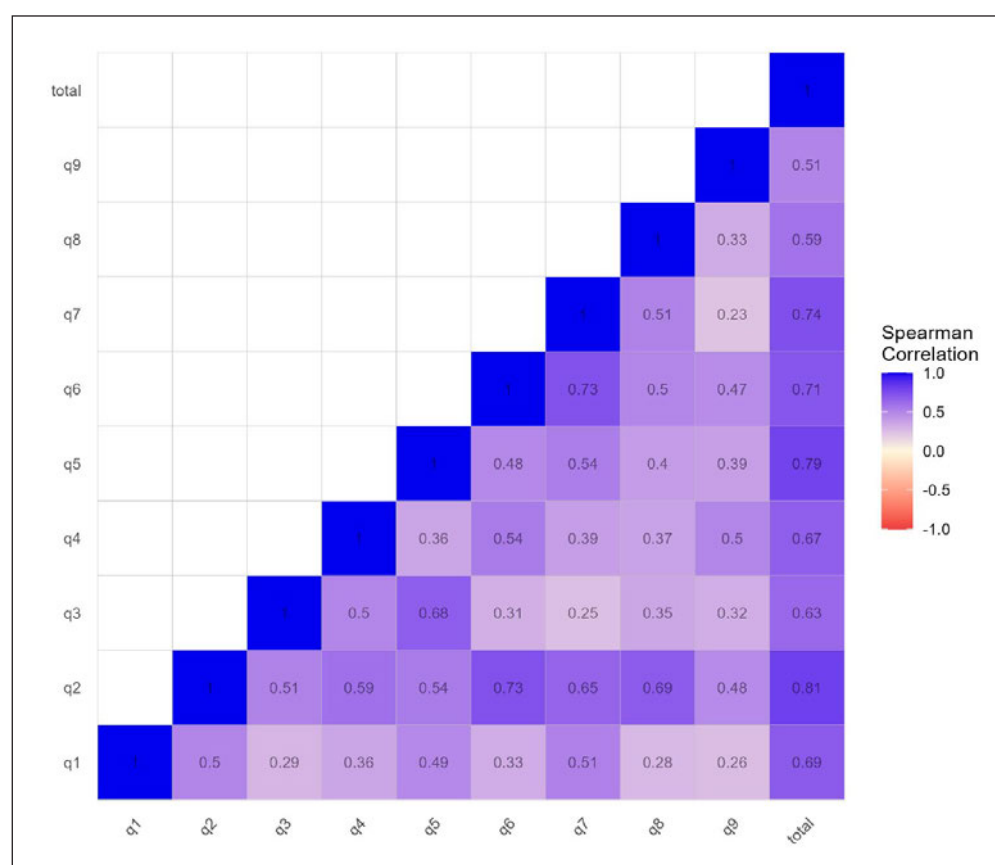


Figure 1. Inter-item correlations for the isiXhosa PHQ-9.

Spearman's rank correlation coefficients (ρ) were determined using pairwise complete observations in our sample. Strength of correlations are graded across a 3-point scale: -1.0 (in deep red), 0.0 (in light yellow), and $+1.0$ (in deep blue).

Table 3. Mean score with standard deviation (SD) and inter-item correlation for each item of the isiXhosa PHQ-9.

Question	Item	Mean	SD	Inter-item correlations
q1	Anhedonia	0.617	0.765	0.376
q2	Depressed mood	0.574	0.950	0.587
q3	Sleep changes	0.532	0.929	0.401
q4	Loss of energy	0.426	0.542	0.451
q5	Appetite changes	0.702	1.082	0.483
q6	Low self-esteem	0.404	0.712	0.511
q7	Trouble concentrating	0.468	0.687	0.476
q8	Psychomotor changes	0.298	0.587	0.428
q9	Suicide ideation	0.191	0.537	0.371

SD: standard deviation.

from 0.51 to 0.81 (thus exceeding the 0.30 cut-off; see Table 5). Factor loadings across various items of the isiXhosa PHQ-9 were more variable (i.e. had a larger range) than observed in other versions of the PHQ-9 (see Figure 6). However, Tucker's coefficients of factor congruence indicated high congruence between factor loadings in our sample and those reported by Monahan et al. (2009) ($\phi = 0.98$) and Cumbe et al. (2020) ($\phi = 0.98$). To

determine whether this variability in factor loadings may be attributed to our smaller sample size, we calculated 95% CIs with 1000 sets of bootstrapped factor loadings for all nine items. For most items, with the exception of Question 2 (depressed mood), the factor loadings reported in at least one of these two previous studies fell within the CIs of the bootstrap realisations, suggesting that the variability in factor loadings for our version may have arisen due to the small sample size of our pilot study (Supplemental materials). Overall, we observed that our isiXhosa PHQ-9 demonstrated a one-factor structure with satisfactory factor loadings and item-total correlations.

Sensitivity analyses

To assess whether the inclusion of participants without HIV in this study may have influenced our findings, we conducted sensitivity analyses for the subset of participants with HIV ($n = 35$) only. Sensitivity analyses of reliability showed that the Cronbach's α for participants with HIV only was 0.861. Mean item-total correlation was 0.640. Mean (standard deviation (SD)) scores on each item, and mean inter-item correlations (excluding correlations of an item with itself), were comparable to the full sample and are available in Supplemental materials. Sensitivity analyses of construct validity using principal component analysis indicated one dominant dimension, with 49.0% of variance

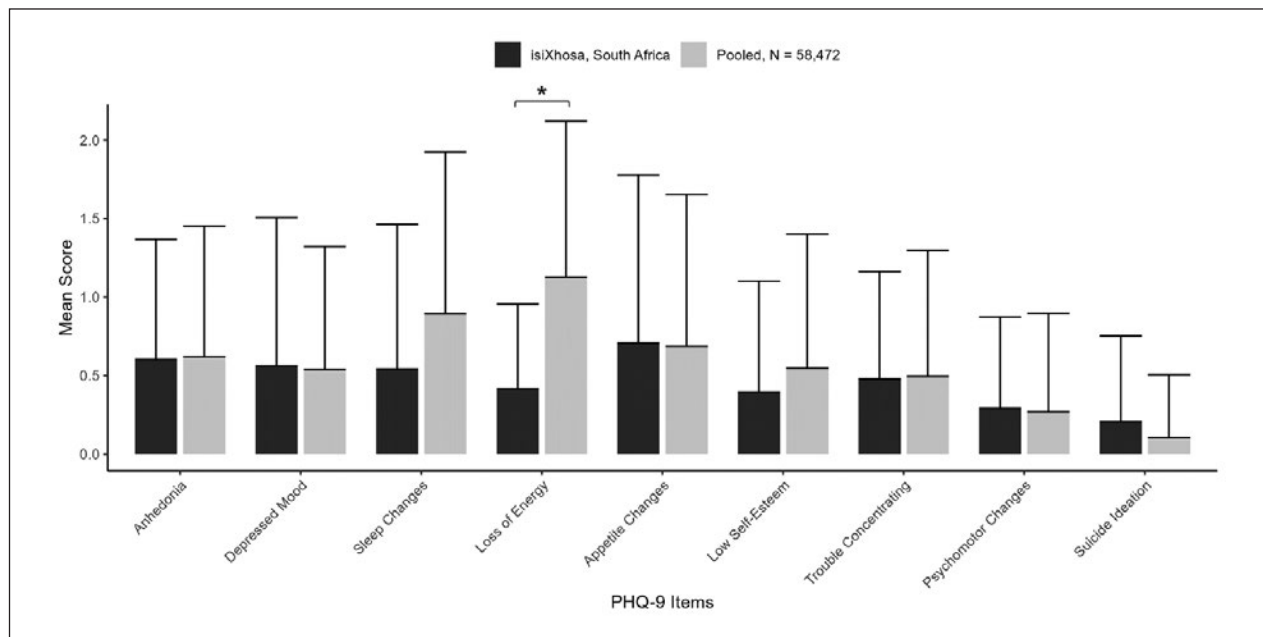


Figure 2. Mean scores on each item of the PHQ-9.

Mean scores are compared between our isiXhosa PHQ-9 and means from a large pooled sample reported by Bianchi et al. (2022). Error bars depict standard error of the mean (SEM).

*Indicates a significant difference in group means (Welch's t-test, FDR-corrected $p < 0.05$).

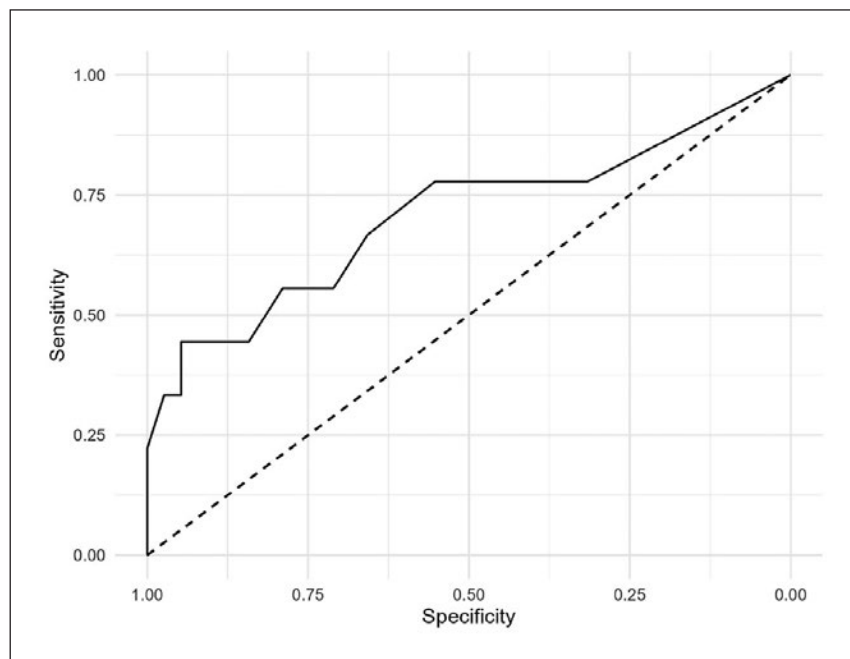


Figure 3. Receiver Operating Characteristic (ROC) curve for the isiXhosa PHQ-9.

Area under the ROC curve (AUC) with 95% confidence intervals was 0.671 (0.400, 0.942).

explained by the first principal component, thus comparable to the full sample. Eigenvalues, factor loadings, and scree plot of variance explained for this subset are available in Supplemental materials. In all, reliability and construct validity of the isiXhosa

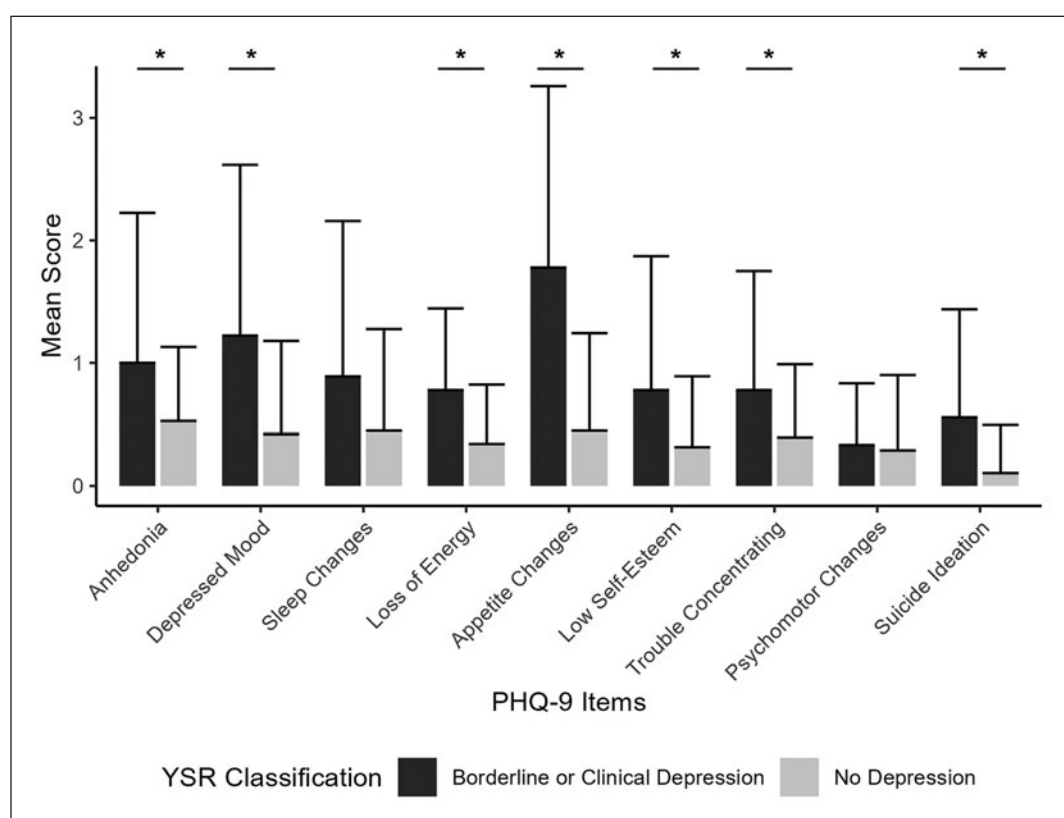
PHQ-9 was comparable among participants with HIV only and the full sample.

To assess whether the greater time difference between PHQ-9 and YSR administration may have influenced our findings, we conducted further sensitivity analyses. For convergent validity,

Table 4. Diagnostic performance of the isiXhosa PHQ-9 in comparison with the Youth Self Report form as a reference standard.

Threshold	Sensitivity %	Specificity %	PPV	NPV	PLR	NLR	1-Sensitivity	1-Specificity	Youden's Index
≥ 1	77.78	31.58	0.21	0.86	1.14	0.70	0.22	0.68	1.09
≥ 2	77.78	44.74	0.25	0.89	1.41	0.50	0.22	0.55	1.23
≥ 3	77.78	55.26	0.29	0.91	1.74	0.40	0.22	0.45	1.33
≥ 4	66.67	65.79	0.32	0.89	1.95	0.51	0.33	0.34	1.32
≥ 5	55.56	71.05	0.31	0.87	1.92	0.63	0.44	0.29	1.27
≥ 6	55.56	73.68	0.33	0.88	2.11	0.60	0.44	0.26	1.29
≥ 7	55.56	78.95	0.38	0.88	2.64	0.56	0.44	0.21	1.35
≥ 8	44.44	84.21	0.40	0.86	2.81	0.66	0.56	0.16	1.29
≥ 9	44.44	89.47	0.50	0.87	4.22	0.62	0.56	0.11	1.34
≥ 10	44.44	94.74	0.67	0.88	8.44	0.59	0.56	0.05	1.39
≥ 11	33.33	94.74	0.60	0.86	6.33	0.70	0.67	0.05	1.28
≥ 14	33.33	97.37	0.75	0.86	12.67	0.68	0.67	0.03	1.31
≥ 16	22.22	100.00	1.00	0.84	Inf	0.78	0.78	0.00	1.22

PPV: positive predictive value, NPV: negative predictive value, PLR: positive likelihood ratio, NLR: negative likelihood ratio.

**Figure 4.** Mean scores on each item of the isiXhosa PHQ-9 by withdrawn/depressed classification on the YSR.

Participants with borderline or clinical T scores on the YSR were pooled. Error bars depict standard error of the mean (SEM).

*Indicates FDR-corrected p -value < 0.05 .

we calculated partial Spearman's correlation coefficients adjusted for absolute time difference between the two assessments. Findings remained consistent, with a moderate significant association between total PHQ-9 score and withdrawn/depressed T score (Spearman's $\rho = 0.45$, $p = 0.002$), but no significant correlation between total PHQ-9 score and anxious/depressed T score

(Spearman's $\rho = 0.12$, $p = 0.417$). Finally, we assessed criterion validity in only the subset of participants for whom the PHQ-9 and YSR were administered less than 100 days apart ($n = 25$). Of this subset, $n = 6$ participants met criteria for clinical or borderline depression on the YSR. The ROC curve and diagnostic performance characteristics for this subset are available in

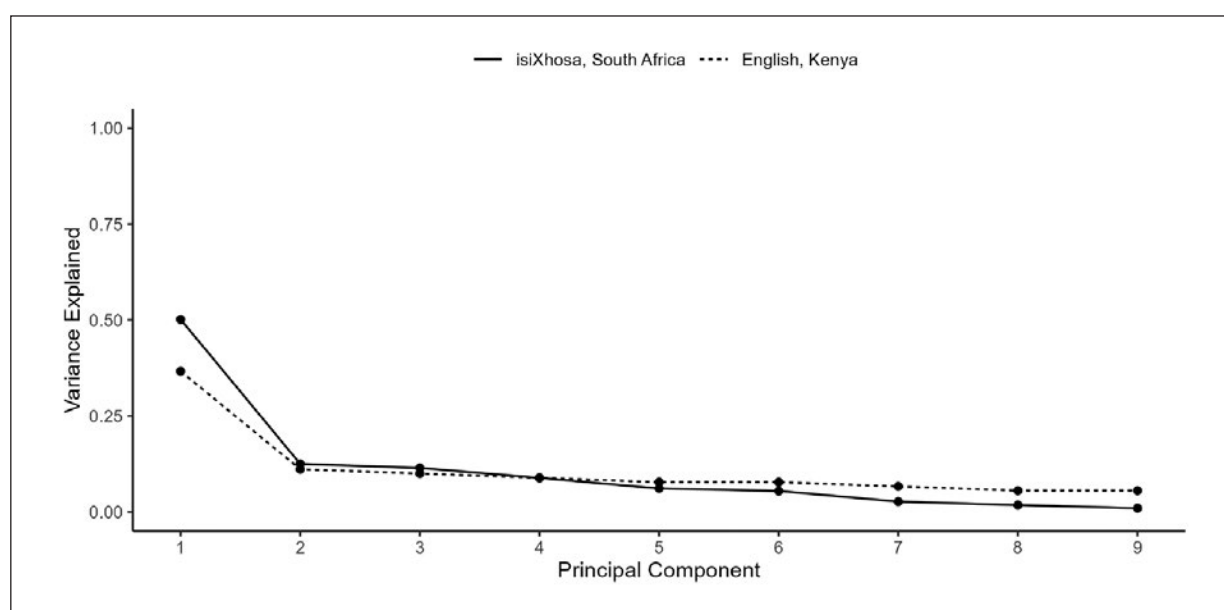


Figure 5. Scree plot of variance explained by each principal component of the PHQ-9.

Variances explained are plotted for our isiXhosa PHQ-9 and for an English version validated in Kenya Monahan et al. (2009) for comparison.

Table 5. Factor loadings and item-total correlations for each item of the isiXhosa PHQ-9.

Question	Item	Factor loading	Item-total correlations
q1	Anhedonia	0.54	0.69
q2	Depressed mood	0.92	0.81
q3	Sleep changes	0.67	0.63
q4	Loss of energy	0.71	0.67
q5	Appetite changes	0.77	0.79
q6	Low self-esteem	0.83	0.71
q7	Trouble concentrating	0.68	0.74
q8	Psychomotor changes	0.52	0.59
q9	Suicide ideation	0.64	0.51

Supplemental materials. AUC (95% CIs) was 0.79 (0.54, 1.00), indicating satisfactory discrimination. A threshold of PHQ-9 score ≥ 7 yielded 84.2% sensitivity and 66.7% specificity, with Youden's Index of 1.51. Therefore, diagnostic performance of the isiXhosa PHQ-9 was slightly better among participants for whom the YSR and PHQ-9 were administered closer together.

Discussion

In this Stage 2 Registered Report, we produced an isiXhosa translation of the PHQ-9 and assessed its psychometric properties. Crucially, we have demonstrated that our isiXhosa PHQ-9 exhibits psychometric properties comparable to those of other PHQ-9 versions in Africa and beyond. Internal consistency ($\alpha = 0.864$) observed for our isiXhosa PHQ-9 is compatible with previous reports of Cronbach's α of 0.76 (South Africa (Bhana et al., 2015)), 0.78 (Kenya (Monahan et al., 2009)), and 0.83

(Tanzania (Fawzi et al., 2019)) for the PHQ-9 in other African contexts. Similarly, the factor structure of our isiXhosa PHQ-9 was comparable with other versions in African nations (Kenya (Monahan et al., 2009) and Mozambique (Cumbe et al., 2020)). Overall, we have shown that our isiXhosa PHQ-9 performs comparably to other versions, which supports the adoption of this translation into research and clinics.

Item-level mean scores in our study were comparable with the large, global sample reported by Bianchi et al. (2022). We only observed a significant difference in mean score on Question 4, concerning fatigue and loss of energy, which participants in our study reported less frequently than participants in the pooled sample. One possible explanation for this difference may be an effect of age. While our study included only adolescents (median age 16 years), the pooled sample included primarily older adults (mean age 43 years), with many non-adolescent participants even in the youngest subgroup (private correspondence with Dr Bianchi). Older participants may be more likely to report fatigue and loss of energy, which may explain the difference in mean score between the two samples.

A key limitation of this study is the small sample size and low prevalence of depressive symptoms. Despite the increased overall power afforded to the study by our larger-than-planned sample size, the proportion of participants who were classified as 'clinically depressed' or 'borderline depressed' on the YSR was extremely small. As a result, assessments of criterion validity were underpowered. Larger validation studies in future are necessary to assess criterion validity of this isiXhosa PHQ-9 with sufficient power. Another potential limitation is that the diagnostic performance of our isiXhosa PHQ-9 was compared against the YSR as a reference standard, rather than a clinical diagnostic interview, and that the PHQ-9 and YSR were not administered on the same day. The YSR has been shown to predict a 'true' diagnosis of depression (i.e. determined using an interview by a

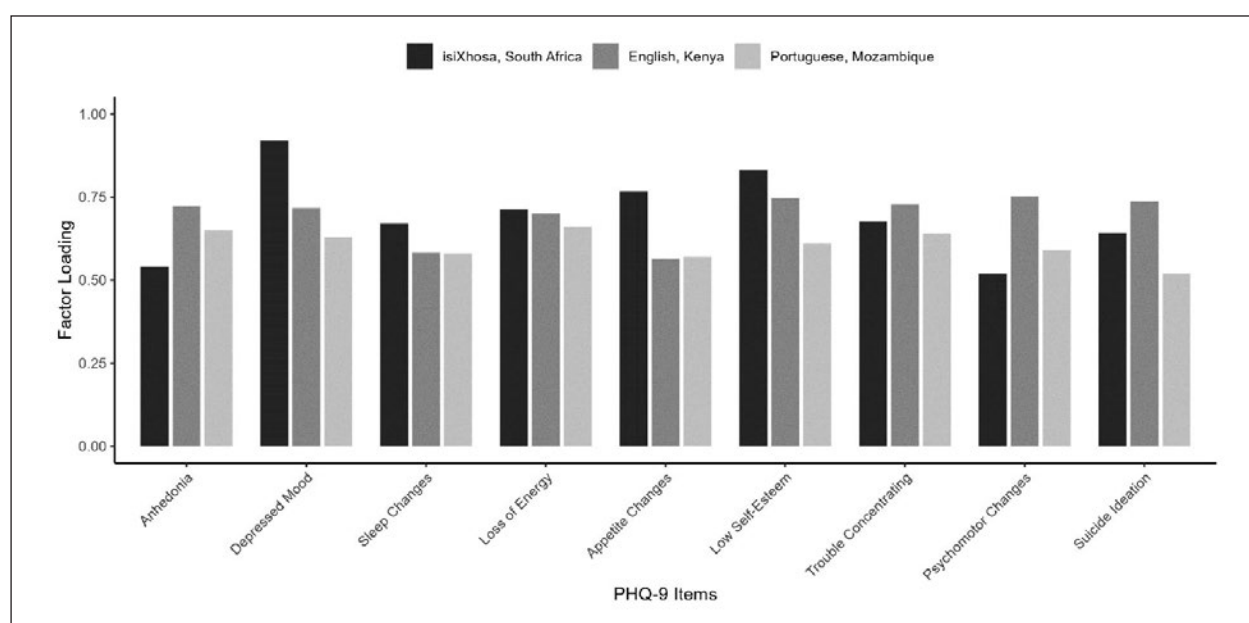


Figure 6. Factor loadings for each item of the PHQ-9.

Factor loadings are plotted for our isiXhosa PHQ-9, an English version validated in Kenya Monahan et al. (2009), and a Portuguese version validated in Mozambique Cumbe et al. (2020).

psychiatrist) with acceptable sensitivity and specificity (Geibel et al., 2016). Nevertheless, future studies may assess the criterion validity of the isiXhosa PHQ-9 against a clinical interview rather than a screening tool such as the YSR.

However, it must be noted that the PHQ-9 itself is a screening tool, which may support triage for people experiencing depressive symptoms, before a comprehensive diagnostic evaluation by a clinician is carried out. Although defining a cut-off on the PHQ-9 can enable putative diagnosis of MDD, this questionnaire is not in fact sufficient for diagnosis. More broadly, measurement of depressive symptoms – including using screening tools such as the PHQ-9 – suffers from substantial methodological and theoretical challenges, which impair our ability to accurately detect depression. (Fried et al., 2022) Depending on the cut-off chosen (and there is no universal consensus on the ‘correct’ cut-off), the PHQ-9 may severely under- or over-estimate the prevalence of depression. (A simulation of the extent of this error may be found at <http://depression-screening100.com/phq/>.) Moreover, measurement of depressive symptoms on a continuous scale, rather than categorical incidence of MDD, can be useful in identifying symptom clusters, tracking subtle changes in symptom severity, or assessing efficacy of anti-depressant interventions beyond a strict diagnostic binary. For these reasons, there is intrinsic value in the assessment of the utility of the isiXhosa PHQ-9 as a screening tool for a continuous spectrum of depressive symptoms, and in comparison with other screening tools, rather than an over-emphasis on comparisons with clinical diagnostic tools.

At the time of registration of our Stage 1 Registered Report, there was no openly available isiXhosa translation of the PHQ-9. Since then, another research group has independently produced a similar isiXhosa-language PHQ-9, which merits discussion. Marlow et al. (2023) also used a transcultural translation framework to produce their version of the PHQ-9. They

then assessed the criterion validity of their PHQ-9 in comparison with the Kiddie Schedule for Affective Disorders and Schizophrenia (K-SADS) in a sample of 302 adolescents in South Africa. They reported high discrimination ($AUC=0.88$) for their overall sample when comparing their PHQ-9 with the K-SADS. Given that translation of the PHQ-9 by Marlow et al. was produced with a similar methodology and with comparable adjustments to the original English as our isiXhosa PHQ-9, these independent results lend further support to the utility of the isiXhosa PHQ-9. The study by Marlow et al benefits from a much larger sample size than ours, suggesting that their analyses of criterion validity may be more sufficiently powered. Conversely, our study offers the advantage of assessing additional psychometric properties (beyond criterion validity) for the isiXhosa PHQ-9, including reliability, convergent validity with the YSR, and factor structure. In addition, our study and statistical analysis plans were pre-registered to minimise any risk of bias towards positive results. Together, our study and that by Marlow et al. independently support the utility of the isiXhosa PHQ-9 as a tool for measuring the incidence and severity of depressive symptoms.

isiXhosa is a primarily oral language, and many amaXhosa (particularly young people) are more comfortable with spoken isiXhosa than written isiXhosa. In keeping with this, many of our adolescent participants requested that the isiXhosa PHQ-9 be read aloud to them. Assessments of emotional or behavioural states may be most accurate when conducted in participants’ home language and in a format in which they are most comfortable. We have, therefore, identified the need to standardise and optimise this process. One solution is to produce an audio-recorded version of the isiXhosa PHQ-9, which may be administered digitally (in an app format) with recordings playing alongside the text of the questions. In future, we hope to produce

this app-based version of the isiXhosa PHQ-9, which may allow access to this screening tool for participants who can speak (but not read) isiXhosa, as well as reducing staff burden and minimising inter-interviewer variability.

In this pilot study, we have demonstrated that our isiXhosa translation of the PHQ-9 exhibits satisfactory psychometric properties. Larger, independent validation studies are necessary to further characterise the diagnostic performance of this isiXhosa PHQ-9 as our analysis of criterion validity was under-powered, although the study by Marlow et al. (2023) using a similar translation suggests that the isiXhosa PHQ-9 may show acceptable diagnostic performance. Given the brevity and ease of administration of the PHQ-9, and the transcultural translation framework, which we used to produce this translation, our isiXhosa PHQ-9 may be an invaluable tool for measuring depressive symptoms in clinics, research groups, and mental health non-profits serving isiXhosa speakers.

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Author contributions

A.M.R.-L. and B.L. conceptualised and designed the study. T.H., B.F., K.v.W., F.L., and S.K. carried out informed consent procedures and data collection. A.M.R.-L. designed the statistical approach and carried out statistical analyses, and wrote the initial draft of the article. B.L. supervised the design and conduct of the study. All authors critically reviewed and approved this article.

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Data availability

Data generated in this study (anonymised to protect participant identity) and R code used for data analysis are available publicly at github.com/arishmr/isixhosa-phq9.

Declaration of conflicting interests

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Supplemental material

Supplemental material for this article is available online.

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8.3 CONCLUSIONS

In this pilot study, the isiXhosa translation of the PHQ-9 was shown to exhibit satisfactory psychometric properties. Internal consistency, item-level mean scores, and factor structure of the isiXhosa PHQ-9 were comparable with other language versions validated in African nations and other global contexts. A particular strength of this pilot study was the adoption of the Registered Report format, which meant that the study protocol and statistical analysis plan were publicly available before data collection was begun. This enabled other researchers to find this translation project even as it was ongoing; several researchers had requested to use this translation before it was published freely alongside the Stage 2 Registered Report. Reporting of the findings from this pilot study was also commended by a peer reviewer for the Stage 2 article as a “very good example of transparent reporting and thorough evaluation of an important psychometric tool”. This adoption of an open research practice, together with a comprehensive set of psychometric analyses, has therefore lent substantial credibility and support to this translation.

Notable limitations of this study are that we did not compare the performance of the isiXhosa PHQ-9 with a clinically validated diagnostic tool as reference standard, the time difference between administration of the isiXhosa PHQ-9 and the YSR (used as reference standard), and the small sample size of this pilot study. Due to these limitations, the results from this study serve as a preliminary exploration of the psychometric properties of the isiXhosa PHQ-9, rather than a formal validation of this translation. With validation in larger, independent cohorts, this isiXhosa PHQ-9 may be used for measurement or triage for depressive symptoms by research groups, clinics, and non-profit organisations serving isiXhosa speakers. By producing this isiXhosa PHQ-9, I have helped address a critical gap in mental healthcare resources for a community which continues to be disenfranchised in healthcare and research due to the legacy of apartheid.

9

GOLD Depression Study: Adolescents in South Africa

HIGHLIGHTS

- This study involved 42 young people with HIV who started ART early in life and 18 young people without HIV based in Cape Town. Participants completed the PHQ-9 in their preferred language as a measure of the severity of their depressive symptoms. Concentrations of 15 proteins in their blood serum and two molecules in three regions of their brains (measured using brain scans) were analysed as biomarkers of inflammation. Cerebrospinal fluid proteins were not analysed in this study, because collecting a cerebrospinal fluid sample is an invasive procedure, whereas blood samples are often collected as part of regular clinic visits.
- Young people with HIV were more likely to report feeling symptoms of depression. Young people with HIV also had different concentrations of some markers in their blood and brain on average than young people without HIV. However, concentrations of these biomarkers were not linked to the severity of people's depressive symptoms.
- In this study, biomarkers of inflammation did not significantly contribute to the increased risk for depressive symptoms in young people with HIV. This may possibly be because of the much smaller number of participants in this study (making it more difficult to detect subtle biological relationships), or because not many participants in this study had severe depression (which may be more strongly linked to inflammation).

9.1 INTRODUCTION

IN CHAPTER 7, FOUR BIOMARKERS OF INFLAMMATION were shown to be potential mediators of the increased risk for depressive symptoms amongst older adults with HIV in the UK and the Netherlands. To effectively translate these findings globally, these mediation effects must be reproducible in other, demographically distinct samples. Therefore, I next carried out a similar study in a sample of young people with and without HIV in Cape Town, South Africa recruited from the Adolescent Cognitive Brain Imaging (“GOLD”) cohort. Participants in this *GOLD Depression Study* completed the PHQ-9 as a measure of depressive symptoms in either English or isiXhosa (as produced and validated in Chapter 8). Choline and myo-inositol were measured in three brain regions using MRS, and 16 inflammatory proteins were quantified in blood serum using immunoassays. Using this data, I aimed to determine the mediation effects of neuroimaging and blood biomarkers of inflammation on the association between HIV status and depressive symptom severity in this sample.

9.2 RESEARCH ARTICLE: *PLOS ONE*

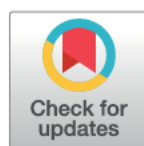
RESEARCH ARTICLE

Multi-modal analysis of inflammation as a potential mediator of depressive symptoms in young people with HIV: The GOLD depression study

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Data Availability Statement: Data generated in this study is available in processed and anonymised form via the Edinburgh DataShare service at <https://doi.org/10.7488/ds/7507>. R code used for statistical analysis is available at <https://github.com/arishmr/gold-depression-study>.

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Abstract

People living with HIV are at three times greater risk for depressive symptoms. Inflammation is a notable predictor of depression, and people with HIV exhibit chronic inflammation despite antiretroviral therapy. We hypothesised that inflammatory biomarkers may mediate the association between HIV status and depressive symptoms. Participants ($N = 60$, 53% girls, median [interquartile range (IQR)] age 15.5 [15.0, 16.0] years, 70% living with HIV, of whom 90.5% were virally-suppressed) completed the nine-item Patient Health Questionnaire (PHQ-9). We measured choline and myo-inositol in basal ganglia, midfrontal gray matter, and peritrigonal white matter using magnetic resonance spectroscopy, and 16 inflammatory proteins in blood serum using ELISA and Luminex™ multiplex immunoassays. Using structural equation mediation modelling, we calculated standardised indirect effect estimates with 95% confidence intervals. Median [IQR] total PHQ-9 score was 3 [0, 7]. HIV status was significantly associated with total PHQ-9 score ($B = 3.32$, $p = 0.022$). Participants with HIV showed a higher choline-to-creatine ratio in the basal ganglia than those without HIV ($\beta = 0.86$, $p_{FDR} = 0.035$). In blood serum, participants with HIV showed higher monocyte chemoattractant protein-1 (MCP-1, $\beta = 0.59$, $p_{FDR} = 0.040$), higher chitinase-3 like-1 (YKL-40, $\beta = 0.73$, $p_{FDR} = 0.032$), and lower interleukin-1beta (IL-1 β , $\beta = -0.67$, $p_{FDR} = 0.047$) than those without HIV. There were no significant associations of any biomarkers with total PHQ-9 score. None of the indirect effects were significant, mediating <13.1% of the association.

(Medical Research Travel Grant) awarded to AMRL. Neuroimaging and infrastructure for this study was supported by NIH Grant R01HD099846 for the GOLD cohort (co-principal investigators: AvdK, EM, and BL). Blood biomarker analysis was supported by seed funding from the SAMRC/SU Extramural Unit on the Genomics of Brain Disorders awarded to AMRL. SRC was supported by a Sir Henry Dale Fellowship, jointly funded by the Wellcome Trust and the Royal Society (221890/Z/20/Z). There was no additional external funding received for this study.

Competing interests: The authors have declared that no competing financial interests exist.

Findings remained consistent when accounting for age, gender, and time between neuroimaging and PHQ-9 administration. Using a robust analytical approach in a community-based sample, we have shown that participants living with HIV reported greater depressive symptoms than those without HIV, but we did not find that neuroimaging and blood biomarkers of inflammation significantly mediated this association. Further studies with participants experiencing severe depression may help to elucidate the links between HIV, inflammation, and depression.

Introduction

People living with HIV are three times more likely to experience depressive symptoms compared to people without HIV [1]. In African countries, where the global majority of people living with HIV are located [2], 3.63 million people living with HIV were estimated to have major depressive disorder (MDD), resulting in loss of 1.57 million disability-adjusted life years [3]. The prevalence of suicide deaths amongst people living with HIV (10.2/1000 persons) is almost 100 times higher than the global incidence of suicide deaths in the general population (0.11/1000 persons) [4]. Young people (i.e. children and adolescents) living with HIV are similarly at elevated risk for depressive symptoms compared to young people without HIV [5, 6]. This risk is higher amongst women and girls, as well as those who experience stigma, food or housing insecurity, or other forms of social deprivation [7, 8]. Depression thus represents a substantial challenge for people living with HIV, especially for those who are socioeconomically marginalised.

Identifying mechanisms underlying this increased risk for depressive symptoms in people living with HIV may enable the development of effective interventions to treat depression in this community. In a subset of the general population (including young people), depression is associated with inflammation [9, 10]. People with depression exhibit increased concentrations of biomarkers of inflammation, including cytokines such as interleukin-6 (IL-6) and tumour necrosis factor- α (TNF- α) [11]. Early-life increases in the inflammatory biomarker IL-6 can predict the total number of depressive episodes later in life, even when accounting for gender, body mass index, and socioeconomic status [12]. People living with HIV exhibit chronic inflammation, evidenced by increased cytokine release and monocyte activation and alterations in neurometabolites such as choline (Cho) and myo-inositol (mI), which persist despite antiretroviral therapy (ART) [13–15]. Together, this evidence suggests that the increased risk for depression amongst people living with HIV may be driven by inflammation.

Inflammatory biomarkers which mediate a significant proportion of the association between HIV status and depressive symptoms may be useful targets for antidepressant interventions. The cytokines IL-6 and TNF- α have frequently been reported as being associated with depressive symptoms in people living with HIV [16]. In a recent study, we showed that controlling for the effects of certain cytokines and chemokines in blood and cerebrospinal fluid (CSF) attenuated the odds for depressive symptoms in people living with HIV by at least 10% [17]. These findings offered early evidence that these inflammatory biomarkers may at least partly mediate the association between HIV status and depressive symptoms. In the current study, we aimed to directly test whether inflammatory biomarkers mediate a significant proportion of this association in a sample of young people living with perinatally-acquired HIV and demographically comparable young people without HIV. We hypothesised that HIV

status would be significantly associated with depressive symptoms, and that inflammatory biomarkers would significantly mediate this association.

Materials and methods

Participant recruitment

For this cross-sectional, observational study, participant recruitment was carried out between May and December 2022 at the Family Centre for Research with Ubuntu (FAMCRU) at Tygerberg Hospital in Cape Town, South Africa. We recruited a subset of participants from the Adolescent Cognitive Brain Imaging (GOLD) cohort. The GOLD cohort is an active longitudinal study investigating brain health and neurocognitive outcomes in adolescents living with perinatally-acquired HIV and demographically comparable adolescents without HIV, which followed on from the landmark Children with HIV Early antiretroviral (CHER) trial [18].

Participants were eligible for this study if they were younger than 18 years old and able to provide informed assent, with a parent or legal guardian able to provide informed consent. Participants living with HIV were eligible if they were currently receiving ART. To minimise selection bias, we did not target potential participants using previous history of depressive symptoms. Participants with a recent history of co-occurring infections (e.g. tuberculosis, HIV encephalopathy) were excluded from the study.

All participants completed a demographic questionnaire in their preferred language (English or isiXhosa). For participants living with HIV, we also received access to their clinical records, including the latest HIV viral load, CD4 cell counts (which are no longer routinely measured and thus not available for all participants), and ART. All participants were compensated for their travel expenses to attend the study visit.

This study was conducted in accordance with internationally recognised standards for ethical research and the International Conference on Harmonisation (ICH E6), and was approved by the Health Research Ethics Committee (HREC) of Stellenbosch University (N21/10/116_Sub Study N19/10/135) and the School of Psychology, Philosophy, and Language Sciences Research Ethics Committee (201-2122/3) of the University of Edinburgh. All participants provided written informed assent, and their parents or primary caregivers provided written informed consent, in the language of their choosing (English or isiXhosa).

Patient Health Questionnaire (PHQ-9)

To quantify depressive symptoms, participants completed the nine-item Patient Health Questionnaire (PHQ-9) in their preferred language (English or isiXhosa). We developed the isiXhosa translation of the PHQ-9 using a transcultural translation framework for this study. This isiXhosa PHQ-9, the English-language version, and results from our validation study are available elsewhere [19]. Participants were provided a private space to complete the questionnaire, where they could complete the questionnaire themselves or ask for a trained member of the research staff to read out the questions and record their responses. Responses from the PHQ-9 were digitally captured following quality assurance checks. Any participants who scored >15 total or indicated suicide ideation on item 9 of the PHQ-9 were referred for further support to a qualified social worker or psychologist on staff.

Neuroimaging biomarkers

To quantify neuroinflammation, participants underwent proton magnetic resonance spectroscopy (MRS). Our neuroimaging biomarkers of interest were choline-containing compounds (glycerophosphocholine + phosphocholine), which are associated with cell membrane

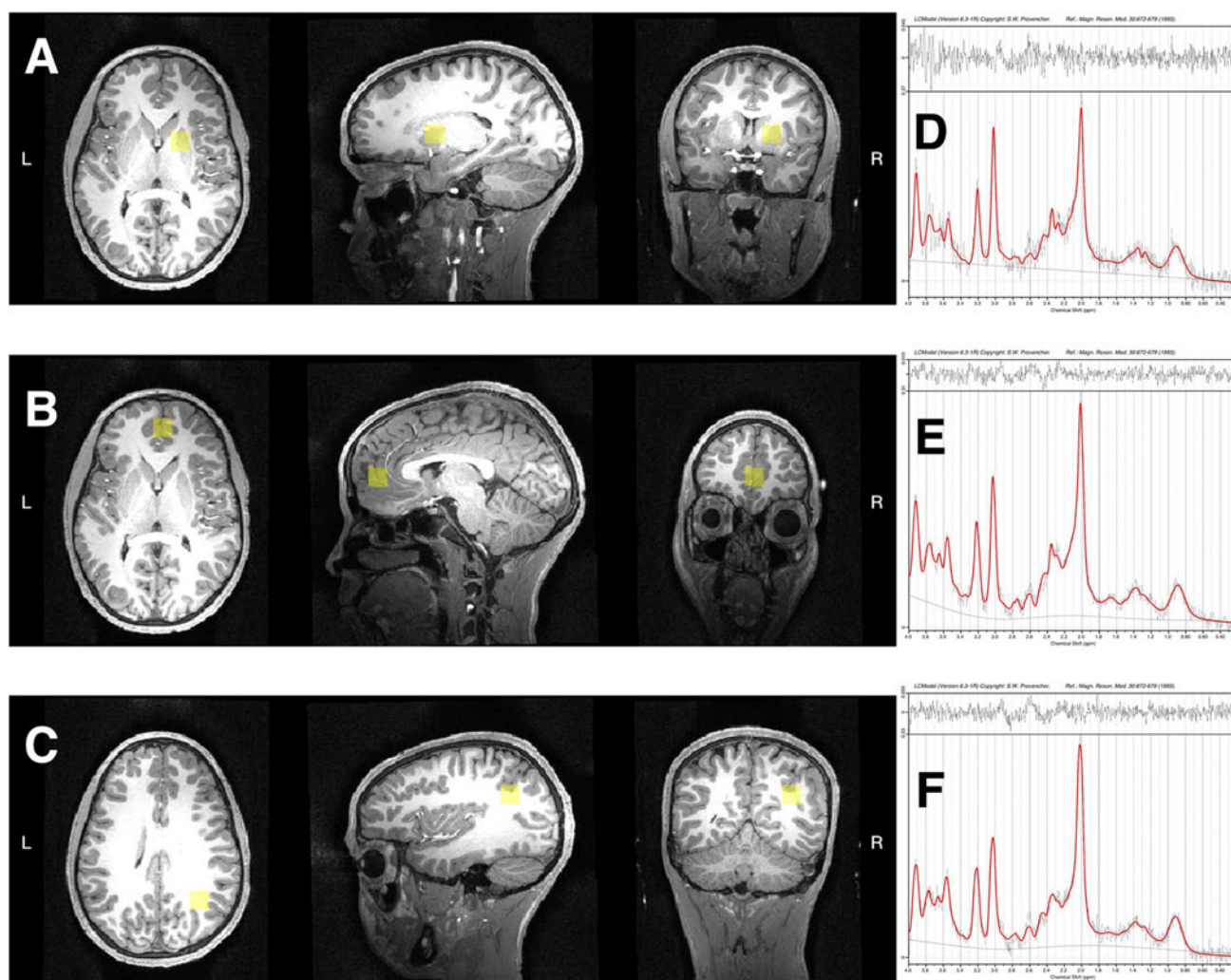


Fig 1. Voxels of interest and representative MRS spectra from one participant. (A–C) Positions of the voxels of interest (VOI, in yellow) in the (A) basal ganglia (BG), (B) midfrontal gray matter (MFGM), and (C) peritrigonal white matter (PWM), and (D–F) corresponding MRS spectra from the VOIs in (D) BG, (E) MFGM, and (F) PWM. Voxel placement images were produced using Gannet software: <https://www.fil.ion.ucl.ac.uk/spm/software/spm12/>.

<https://doi.org/10.1371/journal.pone.0298787.g001>

turnover, and myo-inositol, which is associated with glial cell activation. Both choline and myo-inositol are used as biomarkers of neuroinflammation [20].

Detailed methods for MRS image acquisition and processing for the GOLD cohort at age 11 have been reported elsewhere, and the same protocol was used in the current study [21]. Participants underwent scanning in a 3T Skyra scanner at the Cape Universities Body Imaging Centre (CUBIC) at the University of Cape Town. Single voxel MRS was acquired in three brain regions: basal ganglia (BG), midfrontal gray matter (MFGM), and peritrigonal white matter (PWM) (Fig 1). These brain regions were fixed by parent study design, and not necessarily selected for their association with depressive symptoms. Concentrations of choline and myo-inositol were determined in these regions as ratios to total water and to total creatine using LCModel version 6.3 via the method described previously [22]. Cases were excluded from analysis if any of the following quality control criteria were met: metabolite standard deviation (%SD) > 20%; full width at half maximum (FWHM) > 0.08; or signal-to-noise ratio

(SNR) < 8. Apart from implementing these quality control criteria, we did not exclude any outliers from the MRS data.

Blood biomarkers

To quantify peripheral inflammation, we collected blood samples from all participants at the same study visit during which the PHQ-9 was administered. Blood samples were allowed to clot, then centrifuged at 1500x g for 10 minutes to extract serum. Serum samples were frozen and stored at -80°C within 2 hours of collection.

Neurofilament light (NFL) concentrations in serum were determined using a human NFL enzyme-linked immunosorbent assay (ELISA) (BioTechne, Minneapolis, USA, catalogue number NDP2-81184). The assay was run according to manufacturer instructions in two replicates by two independent researchers with no sample dilution. One replicate failed to yield a satisfactory standard curve and was thus discarded. Absorbances from the NFL ELISA were converted to concentrations using a four parameter logistic (4-PL) curve.

Concentrations of remaining biomarkers of interest were determined using premixed human magnetic Luminex assays (BioTechne, Minneapolis, USA, catalogue number LXSAHM) analysed using a Luminex Bio-Plex 200 system (Bio-Rad, Hercules, USA). C-reactive protein (CRP) and soluble CD14 were detected using one 2-plex kit with a 1:200 sample dilution. Brain-derived neurotrophic factor (BDNF) and regulated upon activation normal T-cell expressed and secreted (RANTES/CCL5) were detected using one 2-plex kit with a 1:2 sample dilution. Soluble CD163, interleukin-1beta (IL-1β), IL-18, IL-6, interferon gamma-induced protein 10 (IP-10/CXCL10), monocyte chemoattractant protein 1 (MCP-1/CCL2), monokine induced by interferon gamma (MIG/CXCL9), macrophage inflammatory protein-1alpha (MIP-1α/CCL3), S100A8, tumour necrosis factor-alpha (TNF-α), and chitinase-3-like protein 1 (YKL-40/CHI3L1/Hc gp-39) were detected using one 11-plex kit with a 1:2 sample dilution. All assays were run according to manufacturer instructions and in two replicates by two independent researchers. One replicate of the BDNF/RANTES assay and one replicate of the CRP/CD14 assay failed to yield a satisfactory standard curve and were thus discarded. Both replicates of the 11-plex assay yielded satisfactory standard curves.

Biomarker concentrations from Luminex assays were quantified by the instrument software (BioPlex Manager) using a five-parameter logistic (5-PL) curve. Where both replicates were quantified reliably, we averaged the two values. Where one replicate was quantified reliably and one was out-of-range (OOR) but a predicted value was computed, we retained only the reliably quantified replicate. Where both replicates were OOR but predicted values were available, we retained the replicate with the best-fitting standard curve, or if standard curves for both replicates were equally satisfactory, we averaged both predicted values. Where both replicates were below the detection range of the assay and no predicted values were computed, we assumed a value that was half the detection limit for that biomarker. Imputed values were assumed in this way for 36 cases for S100A8 and 5 cases for MIP-1α, but for no other biomarkers.

Statistical approach

All analyses were conducted in R v.4.2.1. Demographic and HIV-related clinical characteristics were summarised using proportions (n and %) for categorical variables and medians and interquartile ranges (IQRs) for continuous variables. Normality of continuous variables was assessed using the Shapiro-Wilk test. Group differences between participants with and without HIV were assessed using Pearson's chi-squared test or Fisher's exact test (as appropriate) for categorical variables and Wilcoxon rank sum test for continuous variables.

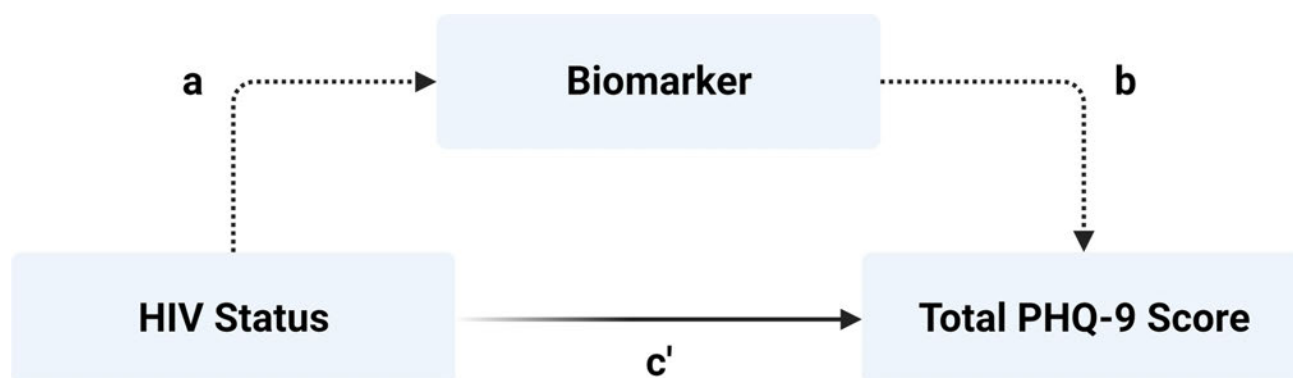


Fig 2. Path diagram for structural equation model (SEM). Our primary outcome of interest was the indirect effect (representing mediation—dotted lines) of each biomarker on the association between HIV status and total PHQ-9 score, indicated by path *ab*. The direct effect is represented by path *c'*.

<https://doi.org/10.1371/journal.pone.0298787.g002>

There was no missingness in PHQ-9 data. After implementing exclusion criteria for the neuroimaging data, one case in the BG had a repeat measurement (carried out on the same day), from which the second measurement was retained and the first was excluded from analysis. In the PWM, seven cases had missing values, which were excluded from analysis.

In the Luminex immunoassays, data was missing for one participant for CRP and CD14, and one participant for BDNF and RANTES. These values were excluded from analysis. To detect outliers in the blood biomarker dataset, we identified values separately for each biomarker which were below the 1st percentile or above the 99th percentile for that biomarker. We then used the Rosner's test for multiple outliers to test whether these values were true outliers; if so, these values were excluded. Outliers were excluded in this way for: BDNF (1 outlier), CD163 (1), CRP (1), IL-18 (2), IL-6 (1), IP-10 (2), MCP-1 (1), MIG (1), MIP-1 α (1), and TNF- α (2).

Linear regression models were used to assess the main effect of HIV status on total PHQ-9 score for all participants, before and after adjusting for age (years, continuous) and self-identified gender (boy/girl).

Structural equation modelling (function `sem` in package `lavaan` v.0.6–16) was used to investigate the mediating effect of our biomarkers of interest on the main association between HIV status and total PHQ-9 score. The path diagram for the model is shown in Fig 2. Continuous variables (total PHQ-9 score, age, time difference between PHQ-9 and neuroimaging, and biomarker concentrations) were scaled before being input into models. For each biomarker separately, we determined standardised effect estimates with 95% confidence intervals (CIs) and *p* values using 1,000 bootstraps. Estimates were calculated for the direct effect of HIV status on total PHQ-9 score (*c'* path), effect of HIV status on biomarker (*a* path), effect of biomarker on total PHQ-9 score (*b* path), total effect of HIV status on total PHQ-9 score, indirect effect of HIV status on total PHQ-9 score through the biomarker (*ab* path), and proportion of the association between HIV status and total PHQ-9 score mediated by the biomarker (expressed as % difference between the total effect and *c'*). Our primary outcome of interest was the indirect effect (*ab* path), which represents the mediation effect of the biomarker on the association between HIV status and depressive symptoms. Given that PHQ-9 scores were not normally distributed, we also ran SEM (adjusted for age and gender) with robust maximum likelihood estimation ("MLM") for neuroimaging and blood biomarkers.

We also aimed to test the extent to which each biomarker might contribute unique, additive mediation effects beyond all other biomarkers, resulting in an aggregate increase in the

proportion of the association between HIV status and depressive symptoms that could be explained by neuroimaging or blood biomarkers. We therefore ran multiple mediation SEM separately for neuroimaging and blood biomarkers, in which all biomarkers of the respective category were entered as simultaneous correlated mediators.

Statistical models were adjusted for age (years, continuous, scaled) and gender (boy/girl). Models involving neuroimaging biomarkers were also adjusted for the absolute time difference (days, continuous, scaled) between PHQ-9 administration and neuroimaging. All *p* values in SEM were corrected for multiple comparisons using the False Discovery Rate (FDR) method, and we report these FDR-corrected *q* values. We conducted a *post hoc* power analysis for our mediation effects using the Monte Carlo test in the pwrss shiny app v.0.3.1 (<https://pwrss.shinyapps.io/index/>) [23].

Finally, given the importance of assessing sex or gender differences to close the gender data gap in health research [24, 25], we also tested for differences in neuroimaging and blood biomarkers based on participants' self-identified gender. Using linear regression models adjusted for age, we then explored whether there may be any gender differences in the mediating effects of inflammatory biomarkers on the association between HIV status and depressive symptoms.

Results

Participant characteristics

We recruited *N* = 60 participants, of whom *n* = 42 (70%) were participants living with HIV and *n* = 18 (30%) were participants without HIV. Summary demographic characteristics for participants are shown in Table 1. Participants were young people with a median (IQR) age of

Table 1. Summary of demographic characteristics self-reported by participants, stratified by HIV status.

Characteristic	Overall <i>N</i> = 60	Participants	Participants	p-value
		with HIV <i>n</i> = 42	without HIV <i>n</i> = 18	
Age (years), Median (IQR) ¹	15.5 (15.0, 16.0)	16.0 (15.0, 16.0)	15.0 (14.0, 15.0)	<0.001*
Gender, <i>n</i> (%) ²				0.4
Boy	28 (47%)	18 (43%)	10 (56%)	
Girl	32 (53%)	24 (57%)	8 (44%)	
Ethnicity, <i>n</i> (%) ²				0.3
Black/African	55 (92%)	37 (88%)	18 (100%)	
Coloured	5 (8.3%)	5 (12%)	0 (0%)	
Sexual orientation, <i>n</i> (%) ²				0.8
Bisexual/Pansexual	2 (3.3%)	1 (2.4%)	1 (5.6%)	
Gay/Lesbian/Homosexual	3 (5.0%)	2 (4.8%)	1 (5.6%)	
Straight/Heterosexual	55 (92%)	39 (93%)	16 (89%)	
Alcohol use, <i>n</i> (%) ³	15 (25%)	10 (24%)	5 (28%)	0.8
Cigarette smoking, <i>n</i> (%) ³	4 (6.7%)	4 (9.5%)	0 (0%)	0.3
Recreational drug use, <i>n</i> (%) ³	7 (12%)	6 (14%)	1 (5.6%)	0.7
Language of Administration, <i>n</i> (%) ²				0.2
English	13 (22%)	7 (17%)	6 (33%)	
isiXhosa	47 (78%)	35 (83%)	12 (67%)	

¹ Wilcoxon rank sum test

² Pearson's Chi-squared test

³ Fisher's exact test

* *p* < 0.05

<https://doi.org/10.1371/journal.pone.0298787.t001>

Table 2. Summary of clinical characteristics for participants living with HIV ($n = 42$).

Characteristic	Value
Viral Load < 200 copies/mL, n (%)	38 (90.5%)
CD4+ T-cell count (cells/ μ L), Median (IQR)	778 (625.75, 1011.75)
(Missing)	4
Antiretroviral Therapy Regimen, n (%)	
Tenofovir disoproxil fumarate + Lamivudine + Dolutegravir (TLD)	29 (69%)
Lopinavir + Ritonavir (LPV/r)	5 (12%)
Atazanavir	4 (9.5%)
Abacavir + Lamivudine (ABC/3TC)	1 (2.4%)
Dolutegravir	1 (2.4%)
Efavirenz (EFV)	1 (2.4%)
Emtricitabine + Tenofovir alafenamide (F/TAF)	1 (2.4%)

<https://doi.org/10.1371/journal.pone.0298787.t002>

15.5 (15.0, 16.0) years, relatively evenly split by gender (53% girls), and most participants self-identified as Black/African (92%) and heterosexual (92%). A modest proportion of participants reported alcohol use (25%) and a small proportion reported cigarette (6.7%) or recreational drug (12%) use in the preceding six months.

Clinical characteristics of participants living with HIV are shown in [Table 2](#). All participants living with HIV were on ART, with 90.5% being virally suppressed (i.e. with an HIV viral load <200 copies/mL at the most recent clinical evaluation). Participants with and without HIV were comparable on all demographic characteristics, except participants with HIV were older than those without HIV ($W = 178$, $p < 0.001$).

Median [IQR] total PHQ-9 score in the overall sample was 3 [0, 7]. Amongst participants living with HIV, median [IQR] total PHQ-9 score was 3.5 [0.25, 8], whereas amongst participants without HIV, median [IQR] total PHQ-9 score was 1.5 [0, 3]. Median [IQR] absolute time difference between PHQ-9 administration and neuroimaging was 92 [52, 130] days.

Number of participants for whom data was included for each biomarker is shown in [Table 3](#).

Association between HIV status and depressive symptoms

We first assessed the direct effect of HIV status on depressive symptoms, quantified as total PHQ-9 score. There was a significant association between HIV status and depressive symptoms ($B = 3.14$, $\beta = 0.66$, $p = 0.017$), such that participants living with HIV reported higher severity of depressive symptoms than those without HIV ([Fig 3](#)). Findings remained consistent after adjusting for age and gender ($B = 3.32$, $\beta = 0.70$, $p = 0.022$).

Correlations between inflammatory biomarkers

Concentrations of neuroimaging biomarkers were moderately correlated, with mean (standard deviation) absolute Spearman correlation of $|\rho| = 0.30$ (0.21) and a range of -0.21 to 0.82 ([Fig 4A](#)). Modest correlations were observed for Cho:Cr between the BG and MFGM ($\rho = 0.61$), Cho and mI referenced to water in the MFGM ($\rho = 0.67$), and Cho and mI referenced to creatine in the PWM ($\rho = 0.67$).

Concentrations of blood biomarkers were only weakly or moderately correlated, if at all, with mean (standard deviation) absolute Spearman correlation of $|\rho| = 0.14$ (0.10) and a range of -0.27 to 0.45 ([Fig 4B](#)). The strongest observed correlations, which were still only moderate, were between IL-6 and soluble CD163 ($\rho = 0.45$) and IL-6 and CRP ($\rho = 0.41$).

Table 3. Biomarkers assessed in this study and number of participants for whom data was included for each biomarker.

Biomarker			Participants with HIV (n)	Participants without HIV (n)
Neuroimaging biomarkers				
Choline	Creatine Referenced	Region		
		BG	34	17
		MFGM	37	18
	Water Referenced	PWM	27	15
		BG	34	17
		MFGM	37	18
		PWM	27	15
Myo-inositol	Creatine Referenced	BG	34	17
		MFGM	37	18
		PWM	27	15
	Water Referenced	BG	34	17
		MFGM	37	18
		PWM	27	15
Blood biomarkers				
BDNF			40	18
CD14			41	18
CD163			41	18
CRP			40	18
IL-1β			42	18
IL-18			40	18
IL-6			41	18
IP-10			40	18
MCP-1			41	18
MIG			40	18
MIP-1α			41	18
RANTES			41	18
S100A8			40	18
TNF-α			42	18
YKL-40			40	18

BDNF: brain-derived neurotrophic factor; BG: basal ganglia; CD: soluble cluster of differentiation; CRP: C-reactive protein; IL: interleukin; IP-10: interferon gamma-induced protein 10; MCP-1: monocyte chemoattractant protein 1; MFGM: midfrontal gray matter; MIG: monokine induced by interferon gamma; MIP-1 α : macrophage inflammatory protein-1 alpha; PWM: peritrigonal white matter; RANTES: regulated upon activation normal T cell expressed and secreted; TNF- α : tumour necrosis factor alpha; YKL-40: chitinase-3 like-1 protein.

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Biomarker correlations separately for participants with and without HIV, as well as correlations between all neuroimaging and blood biomarkers, are available in [S1 Fig](#).

Neuroimaging biomarkers as mediators of the HIV-depression association

Concentrations of choline and myo-inositol (referenced to creatine and water) in each brain region stratified by HIV status and as a function of total PHQ-9 score are shown in [Fig 5A](#) and [5B](#). Mean concentrations (ratio) and standard deviation (SD) for each biomarker, referencing protocol, and brain region is available in [S1 Table](#).

In SEM adjusted for age, gender, and absolute time difference in days between PHQ-9 administration and neuroimaging ([Fig 5C](#)), there was a significant association between HIV status and choline referenced to creatine in the basal ganglia, β (95% CI) = 0.86 (0.36, 1.52), $q = 0.035$. Participants with HIV thus showed a higher choline-to-creatine ratio in the BG than

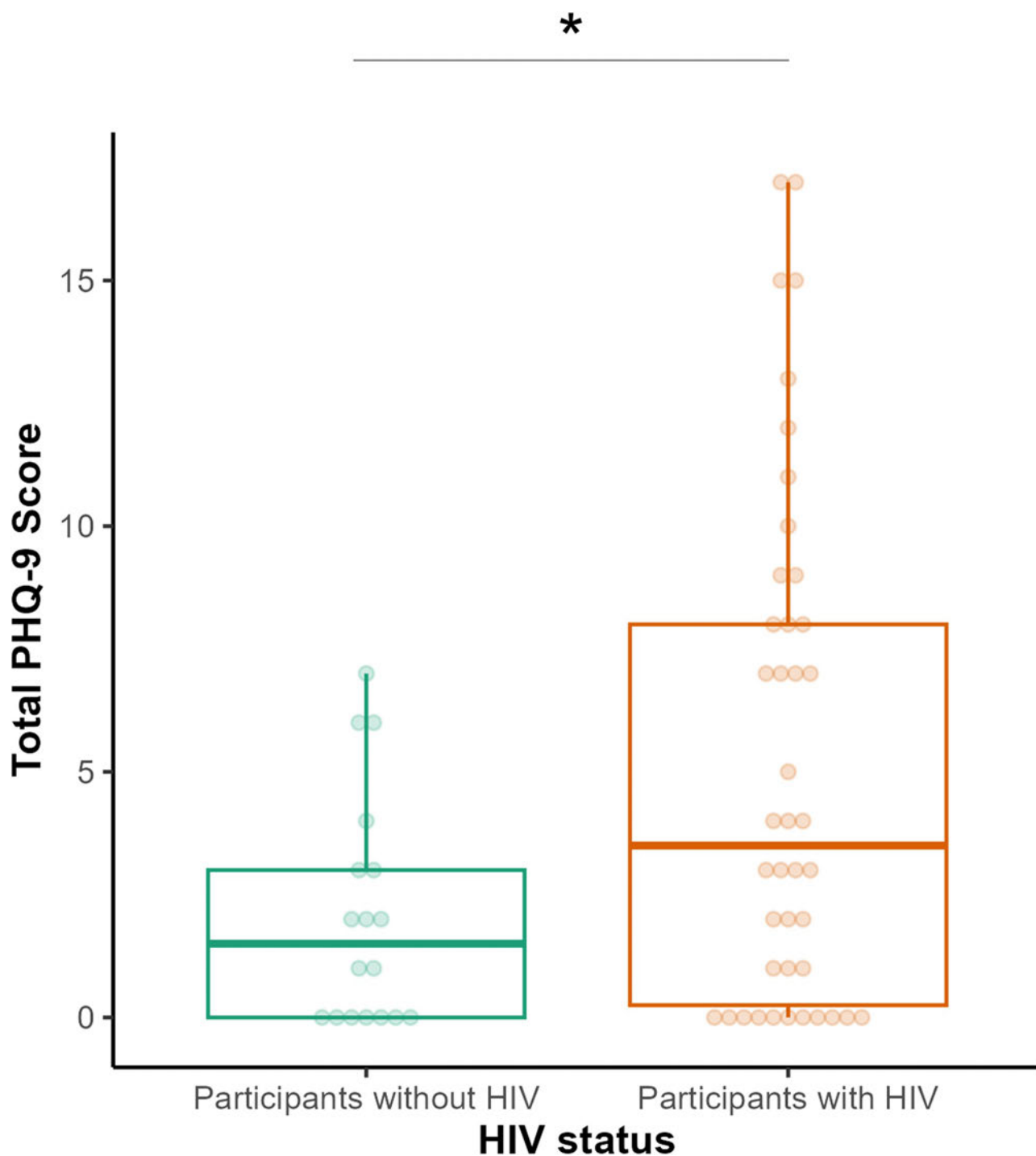


Fig 3. Self-reported depressive symptoms (quantified as total PHQ-9 score) for participants stratified by HIV status. The middle line in each box plot represents the median, with the upper and lower hinges representing the third and first quartile, respectively. * indicates $p < 0.05$.

<https://doi.org/10.1371/journal.pone.0298787.g003>

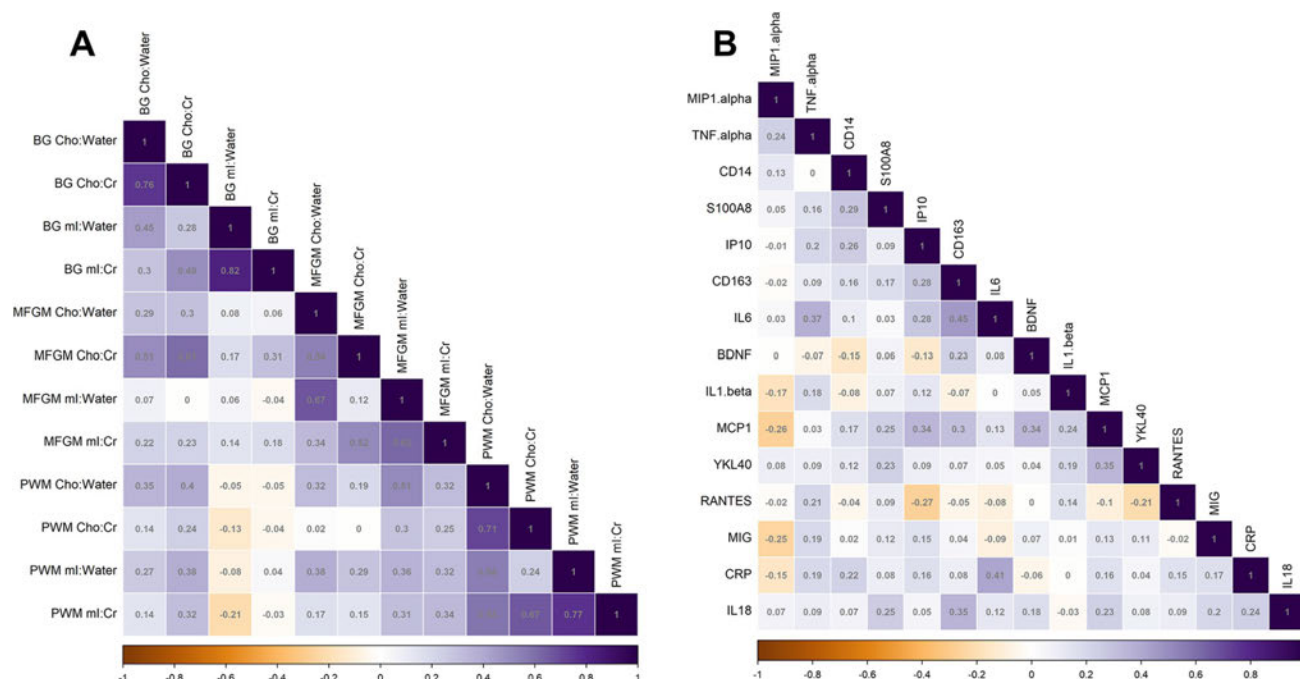


Fig 4. Spearman's correlations (ρ) between (A) neuroimaging biomarkers and (B) blood biomarkers measured in this study. The strength of correlations is graded across a 3-point scale: -1.0 (in orange), 0.0 (in white), and $+1.0$ (in purple). For blood biomarkers, the correlation matrix is ordered by hierarchical clustering to optimise visual comparisons.

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those without HIV. There were no other significant associations between HIV status and the neuroimaging biomarkers, and no significant associations between these biomarkers and total PHQ-9 score (all other $\beta < |0.65|$, $q > 0.05$). Indirect effect estimates for these biomarkers were not significant, mediating $<13.1\%$ of the association between HIV status and total PHQ-9 score (all $\beta < |0.09|$, $q > 0.05$).

We ran multiple mediation SEM in which all six MRS biomarkers (choline and myo-inositol in the BG, MFGM, and PWM each) were entered as simultaneous correlated mediators, separately for biomarkers referenced to creatine and to water. Compared to the largest mediation signals of individual neuroimaging biomarkers (13.1% for creatine-referenced choline in the BG and 12.7% for creatine-referenced myo-inositol in the MFGM), all creatine-referenced neuroimaging biomarkers together mediated 46.32% of the association between HIV status and depressive symptoms, though this mediation was also non-significant (indirect effect β (95% CI) = 0.31 (-0.29, 1.28), $q = 0.77$). Similarly, all water-referenced neuroimaging biomarkers together mediated 9.22% of this association, and this mediation was also non-significant (indirect effect β (95% CI) = 0.06 (-0.38, 0.90), $q = 0.99$).

Blood biomarkers as mediators of the HIV-depression association

NFL was undetectable in our human NFL ELISA for all except two participants. We therefore did not carry out any further statistical analyses with NFL data.

Concentrations of blood biomarkers by HIV status and total PHQ-9 score are shown in Fig 6A and 6B. Mean (SD) absolute concentrations (pg/mL) for each biomarker are available in S2 Table.

In SEM adjusted for age and gender (Fig 6C), there was a significant association between HIV status and IL-1 β , β (95% CI) = -0.67 (-1.33, -0.17), $q = 0.047$, such that participants with

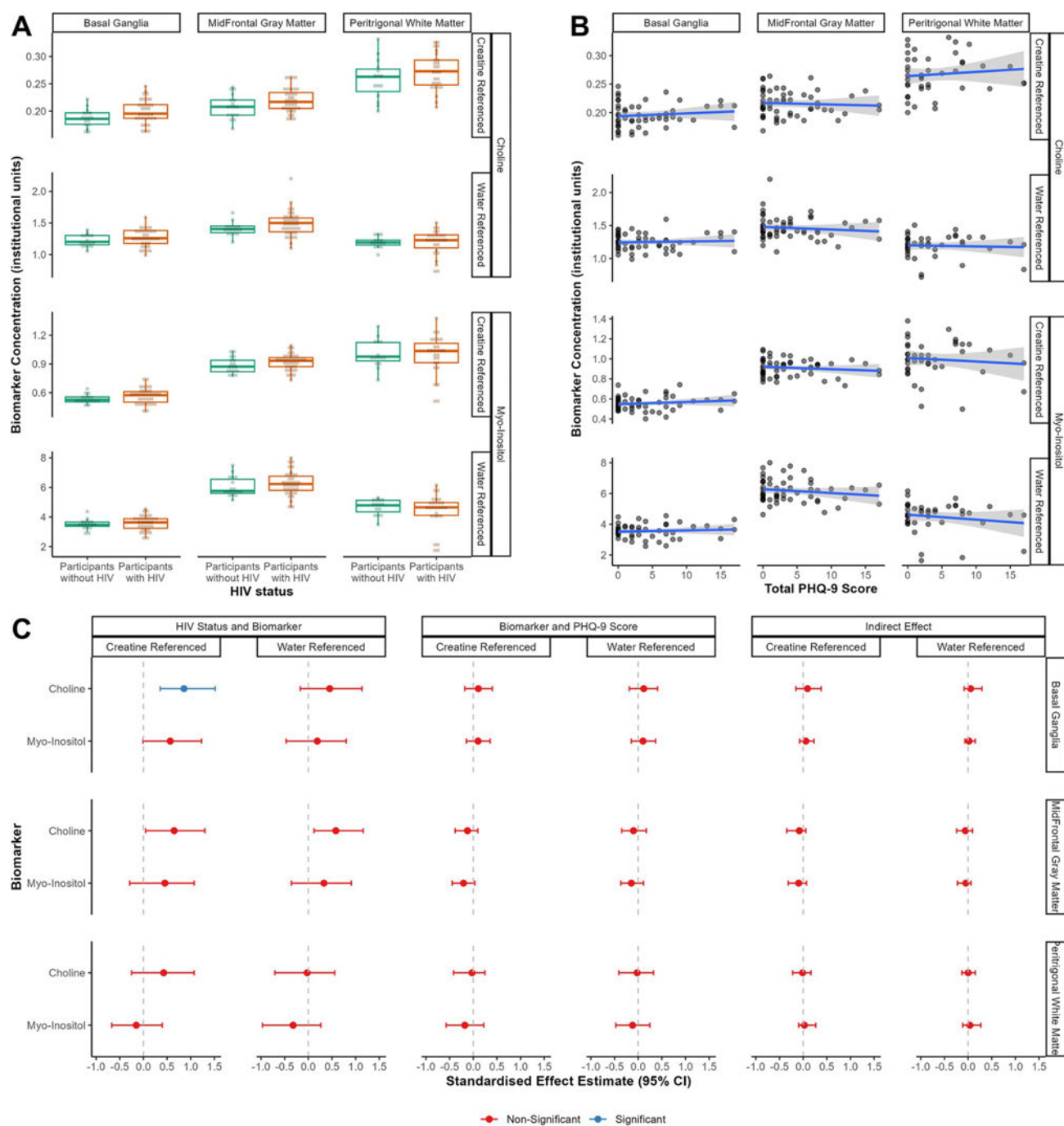


Fig 5. Concentration (institutional units) of choline and myo-inositol, across two referencing protocols and three brain regions, by (A) HIV status, and (B) total PHQ-9 score. Values shown in (A) and (B) are unadjusted for covariates. (C) Standardised effect estimates with 95% confidence intervals (CI) for structural equation modelling (SEM) involving the neurometabolites choline and myo-inositol across two referencing protocols and three brain regions, adjusted for age, gender, and absolute time difference (in days) between PHQ-9 administration and neuroimaging. Path estimates which are significantly different from zero (after correcting p values for multiple comparisons) are shown in blue.

<https://doi.org/10.1371/journal.pone.0298787.g005>

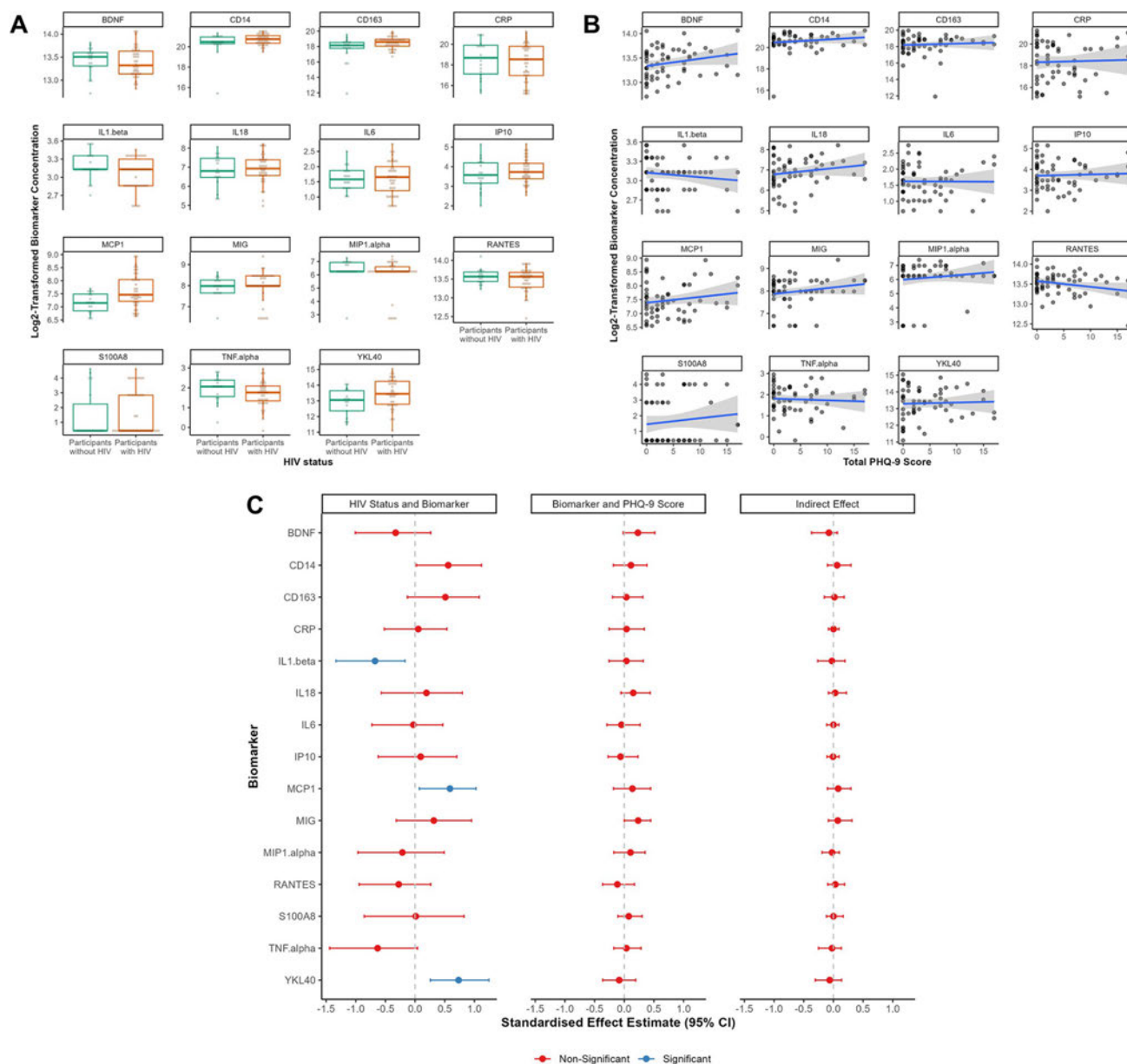


Fig 6. Concentration (log2-transformed) of blood biomarkers by (A) HIV status, and (B) total PHQ-9 score. Values shown in (A) and (B) are unadjusted for covariates. (C) Standardised effect estimates with 95% confidence intervals (CI) for structural equation modelling (SEM) involving biomarkers quantified in blood serum, adjusted for age and gender. Path estimates which are significantly different from zero (after correcting p values for multiple comparisons) are shown in blue.

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HIV showed lower concentrations of IL-1 β than those without HIV. There were also significant associations between HIV status and MCP-1, β (95% CI) = 0.59 (0.07, 1.03), q = 0.040 and YKL-40, β (95% CI) = 0.73 (0.26, 1.24), q = 0.003, such that participants with HIV showed higher concentrations of these biomarkers than those without HIV. There were no other significant associations between HIV status and blood biomarkers measured in this study, and no significant associations of these biomarkers with total PHQ-9 score (all other β < |0.63|).

$q > 0.05$). Indirect effect estimates for these biomarkers were not significant, mediating $<11.0\%$ of the association between HIV status and total PHQ-9 score (all $\beta < |0.08|$, $q > 0.05$).

Given the modest correlations across blood biomarkers, we ran a multiple mediation SEM in which all 15 blood biomarkers were entered as simultaneous correlated mediators. Compared to the largest mediation signal of individual blood biomarkers (11% for MCP-1 and 10.5% for BDNF), all blood biomarkers together mediated 16.25% of the association between HIV status and depressive symptoms, though this mediation was also non-significant (indirect effect β (95% CI) = -0.11 (-0.98, 0.66), $q = 0.99$).

Unadjusted SEM analyses for neuroimaging and blood biomarkers are available in [S2–S3 Figs](#). Findings were consistent across unadjusted and adjusted analyses for both neuroimaging and blood biomarkers, except that the association between IL-1 β and HIV status was not significant before adjusting for age and gender, β (95% CI) = -0.54 (-1.12, -0.03), $q = 0.130$. All path estimates, 95% CIs, and p values for our SEM analyses (unadjusted, adjusted, and only including participants with HIV who were virally suppressed) are available in [S3 Table](#). Findings remained consistent when excluding the $n = 4$ participants with HIV who had an HIV viral load >200 copies/mL. We also ran SEM adjusted for age and gender with the MLM estimator to account for non-normality in the outcome (PHQ-9 scores). Effect sizes and confidence intervals for all path estimates were largely consistent between the two sets of analyses, although path estimates for blood biomarkers did not survive FDR correction in models with the MLM estimator ([S4 Table](#)).

A *post hoc* power analysis for the largest absolute mediation effect in our findings ($\beta = -0.09$ for creatine-referenced myo-inositol in the MFGM) revealed that this effect was detected with 31% power.

There were several nominally significant differences in neuroimaging and blood biomarkers differed based on participants' gender, including water-referenced choline in the basal ganglia and IL-1 β and YKL-40 in serum (though these associations did not survive FDR correction; [S4 Fig](#)). We then tested whether there may be any gender differences in the mediating effects of inflammatory biomarkers on the association between HIV status and depressive symptoms. We found no significant interactions between gender and HIV status on biomarker concentrations or between gender and biomarker concentrations on total PHQ-9 score ([S5 Table](#)). Given this lack of significant gender interactions in path a and path b of our mediation model, we did not further assess the interaction of gender with our indirect effect of interest.

Discussion

In a sample of young people living with and without HIV, we found that HIV status is associated with depressive symptoms. When accounting for age and gender, participants with HIV on average scored 3.32 points higher on the PHQ-9 compared to participants without HIV. Participants with HIV also exhibited significantly lower IL-1 β but higher MCP-1 and YKL-40 concentrations in blood serum. However, associations between biomarkers (in blood or brain) and PHQ-9 were entirely null. Thus, we did not find support for our hypothesis that inflammatory biomarkers mediate the association between HIV status and depressive symptoms: none of the neuroimaging or blood serum biomarkers measured in our study mediated a significant proportion of this association.

After adjusting for age and gender, one neuroimaging biomarker (choline referenced to creatine in the basal ganglia) and three inflammatory biomarkers in blood serum (IL-1 β , MCP-1, and YKL-40) were significantly associated with HIV status. We have previously observed higher choline levels in basal ganglia of children with HIV compared to those without HIV in the CHER cohort, and a similar association was observed in the current study [26].

However, as this effect was not observed for choline referenced to water, it is possible that this association was confounded by alterations in creatine concentrations. Moreover, alterations in choline and myo-inositol concentrations may be driven by mechanisms other than neuroinflammation, such as alcohol use [27]. Concentrations of choline may also be influenced by other physiological processes which impact cell membrane turnover, including myelination, which occurs rapidly during adolescence [28, 29]. Therefore, the lack of associations between HIV status and neurometabolites measured in this study may be partly attributed to possible confounding mechanisms and the non-specific nature of these biomarkers.

In alignment with existing evidence, we saw that participants with HIV exhibited higher MCP-1 than those without HIV [30]. We also found that participants with HIV showed higher YKL-40 compared to those without HIV. YKL-40 is a relatively novel marker associated with astrocytic activation and neurodegeneration [31]. Few studies to date have compared YKL-40 concentrations between people with and without HIV. Hermansson et al. [32] found that YKL-40 concentrations in CSF did not differ between participants with and without HIV. Guha and colleagues [33] recently also observed no significant difference in plasma or CSF concentrations of YKL-40 by HIV status, but significantly higher YKL-40 in CSF (compared to people without HIV) specifically in people with HIV with a CSF HIV viral load higher than 50 copies/mL [34]. Notably, these studies were carried out in older adults. We report the first evidence that YKL-40 in blood serum is significantly higher ($\beta = 0.73$) in young people living with perinatally-acquired HIV compared to demographically comparable participants without HIV. This difference in serum YKL-40 may be indicative of ongoing astrocytic activation and neuronal injury in participants with HIV despite suppressive ART.

We observed that IL-1 β was significantly lower in participants with HIV compared to participants without HIV. Absolute differences in median IL-1 β concentration between participants with and without HIV were small (8.43 pg/mL vs 9.25 pg/mL respectively). However, the strength of association between HIV status and IL-1 β was fairly large, both before ($\beta = -0.54$) and after ($\beta = -0.67$) adjusting for age and gender. This finding is unexpected, as previous studies have generally observed higher IL-1 β in serum of participants with HIV, including those who are virally suppressed [35, 36]. Notably, at least one study has reported lower IL-1 β in participants with HIV compared to those without HIV, but this measurement was in cervicovaginal lavage and not in blood [37]. IL-1 β and IL-18 are both secreted as a result of inflammasome activation, and thus are expected to be correlated [38]. However, in our sample, there was almost no correlation between IL-1 β and IL-18 (Spearman's $\rho = -0.03$), which suggests possible 'masking' of IL-1 β concentrations. One potential explanation for lower IL-1 β observed in participants with HIV may be higher levels of receptor expression on certain cells in these participants, leading to receptor binding of the cytokine and thus removal of IL-1 β from the serum. However, this is speculative given that many previous studies have observed higher IL-1 β in participants with HIV, and future replication of these findings is necessary to clarify the direction of the association between HIV status and IL-1 β .

Although we observed HIV-related differences in concentrations of certain neuroimaging and blood biomarkers, none of the biomarkers measured in this study were significantly associated with total PHQ-9 score. For neuroimaging biomarkers, this may be in part because the brain regions in which these metabolites were measured in this study were not necessarily selected for their importance in the pathogenesis of depression. Previous findings have suggested that depressive symptoms may be associated with alterations in choline concentrations in the prefrontal cortex and putamen, or myo-inositol concentrations in the anterior cingulate cortex and prefrontal cortex [39]. It will therefore be important to examine these brain regions in future studies of depressive symptoms in young people with HIV. Furthermore, although several previous studies have observed significant associations between inflammatory

biomarkers and depressive symptoms in people with HIV, this research has largely been carried out in adults with HIV acquired later in life [16], whereas our study focused on young people living with perinatally-acquired HIV. Since perinatal HIV infection in the brain results in distinct and persistent alterations in white matter microstructure [40–42], it is possible that depressive symptoms in our participant sample were driven by these neuroanatomical changes rather than neurometabolite concentrations.

Soluble inflammatory biomarkers such as IL-6 and TNF- α have been frequently observed to be associated with depressive symptoms in other studies, so the lack of statistically significant associations in our sample was surprising [16]. We recruited a community-based sample and thus observed a ‘natural’ spread of depressive symptoms in our sample, with a few participants reporting severe depressive symptoms, while the majority reported no or low depressive symptom severity (median PHQ-9 score of 3). This prevalence of depressive symptoms is comparable with previous studies; for instance, in a larger sample of adults with HIV in which we recently showed a potential mediating role for inflammatory biomarkers, we observed a median [IQR] PHQ-9 score of 2 [0, 4] [17]. Nevertheless, the relatively low prevalence of depressive symptoms in our sample may explain why we did not detect any significant associations with inflammatory biomarker concentrations, and, by extension, why we did not find any significant mediation by inflammatory biomarkers of the association between HIV status and depressive symptoms.

Certain limitations of the current study are noted. We had a small sample size ($N = 60$), which may have limited our ability to reliably detect true underlying associations, as demonstrated by the post hoc power analysis which indicated that we could detect the largest mediation effect in our study with only 31% power [43]. We only measured inflammation and depression at a single time-point, thus no inferences can be made about the temporality of the associations between these variables. Additionally, as the PHQ-9 is a screening (not diagnostic) tool, measurement of depressive symptoms using this tool instead of a more comprehensive interview may also have influenced our findings. Although we endeavoured to schedule participant study visits close to the neuroimaging visits, in practice, MRS acquisition and PHQ-9 administration were separated by about 3 months for most participants. This time difference limits our interpretation of the association between PHQ-9 score and neuroimaging biomarkers of inflammation, since depressive symptoms can change transiently even over the course of a few weeks [44]. However, we aimed to partly mitigate this limitation by including this time difference as a statistical covariate in our analysis. Concentrations of inflammatory biomarkers in blood, and NFL in particular, may be measured by more sensitive immunoassays such as single molecule array (SIMOA). Finally, while we controlled for the effects of age and gender in our analyses, our findings may have been influenced by other potential confounders such as socioeconomic status or antiretroviral regimens, which can also impact inflammation and risk for depression [45, 46].

Despite these limitations, our study offers some important advantages. Research on the mental health of people living with HIV has relied on primarily White, primarily male samples, with many studies in the field involving up to 90% White and male participants [47]. Our study adds much-needed diversity to this field, with our participant sample being primarily Black/African and gender-diverse (53% girls). Our participant sample is comparable to the population of young people living with HIV in South Africa and globally, as the majority of these young people are Black/African and girls [48, 49]. Previous research has largely focused on describing associations between inflammatory biomarkers and depressive symptoms. We take this work one crucial step further by explicitly assessing whether inflammatory biomarkers mediate depressive symptoms in people with HIV. Identifying biomarkers which mediate this association will support the development of diagnostic or therapeutic targets for depression in this community. We used structural equation modelling (a robust approach to

mediation analysis) to investigate this question, with a rigorous analytical pipeline which accounted for multiple comparisons and key sociodemographic factors. Finally, we assessed the mediating role of inflammation using multiple modalities: neurometabolites (with two separate referencing protocols) and blood serum proteins (measured using latest-generation high-sensitivity immunoassays), lending further robustness to our null findings.

We found no evidence that inflammatory biomarkers significantly mediated the association between HIV status and depressive symptoms in a sample of young people. Future studies may seek to explore the associations we observed between inflammatory biomarkers and HIV status, particularly those for IL-1 β and YKL-40, and investigate these mediation effects in larger samples. Studies focusing on participants with severe depressive symptoms may help to clarify whether inflammatory biomarkers mediate a significant proportion of the association between HIV and clinical depression. Longitudinal studies tracking the trajectories of depressive symptoms and inflammatory biomarkers in young people with HIV may enable us to determine whether inflammation predicts depression or vice versa in this population. Our sample comprised young people living with perinatally-acquired HIV who were initiated on ART early. Future work may thus seek to determine whether early (versus delayed) ART initiation plays a role in dampening any associations between inflammation and depressive symptoms in young people with HIV. Identifying significant neurobiological mediators of the association between HIV status and depression may reveal useful therapeutic targets. Nevertheless, the contributions of antiretroviral medication and psychosocial factors such as stigma, discrimination, or socioeconomic adversity must be given equal consideration when exploring possible mechanisms driving the risk for depression in people with HIV.

Supporting information

S1 Fig. Spearman's correlations between neuroimaging biomarkers, blood biomarkers, and all biomarkers separately for participants with and without HIV.

(TIF)

S2 Fig. Unadjusted standardised effect estimates for SEM involving neuroimaging biomarkers.

(TIF)

S3 Fig. Unadjusted standardised effect estimates for SEM involving blood biomarkers.

(TIF)

S4 Fig. Unadjusted standardised effect estimates for associations of all biomarkers with participant age, gender, and HIV status.

(TIF)

S1 Table. Summary statistics for neuroimaging biomarkers.

(XLSX)

S2 Table. Summary statistics for blood biomarkers.

(XLSX)

S3 Table. All path estimates for SEM (adjusted and unadjusted) for neuroimaging and blood biomarkers.

(XLSX)

S4 Table. All path estimates for SEM (adjusted) for neuroimaging and blood biomarkers using robust maximum likelihood estimation (MLM).

(XLSX)

S5 Table. Standardised effect estimates for interactions between gender and HIV status on biomarker concentrations or between gender and biomarker concentrations on total PHQ-9 score.

(XLSX)

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9.3 CONCLUSIONS

Through the GOLD Depression Study, I attempted to address certain limitations of the COBRA-DI study (Chapter 7). Participants in the GOLD Depression Study were primarily Black/African and relatively evenly split by gender, thus offering a sample which is more comparable to the global population of people with HIV. I used structural equation modelling in the GOLD Depression Study to quantify the indirect (mediation) effects and assess their statistical significance, which was not possible in the COBRA-DI study due to data ownership and sharing limitations. Conversely, the COBRA-DI study had a much larger sample size (and therefore higher statistical power) than the GOLD Depression Study. Therefore, these studies offer complementary advantages and together enabled me to test the central hypothesis of this thesis.

The potential mediation effects of inflammatory biomarkers in COBRA-DI and the GOLD Depression Study may be compared by employing similar statistical approaches. In COBRA-DI, step-wise logistic regression was used to determine the odds ratio for the association between HIV status and Any Depressive Symptoms ($\text{PHQ-9} \geq 4$) before and after adjusting for each biomarker separately. In the subset of 78 participants for whom an expanded biomarker panel was run, adjusting for four biomarkers resulted in a $>10\%$ reduction in this odds ratio, which was considered to be an indication of potential mediation (Figure 9.1A). To compare these findings with the GOLD Depression Study, I ran step-wise linear regression models to determine the standardised effect estimate for the association between HIV status and total PHQ-9 score before and after adjusting for each biomarker separately for these 60 participants. In these models, a $>10\%$ reduction in the standardised effect estimate was observed when adjusting for creatine-referenced choline in the basal ganglia (-13.1%) and MCP-1 in blood serum (-11.0%) (Figure 9.1B). Thus, if using only the step-wise regression approach to mediation analysis with a similar threshold as COBRA-DI, one may conclude that Cho/Cr in the BG and MCP-1 in serum were potential mediators of depressive symptoms in the GOLD Depression Study sample.

An advantage of the structural equation modelling (SEM) approach is that, in addition to estimating the magnitude of the mediation effect, SEM allows for the direct quantification of the significance of this effect. Using SEM with bootstrapped confidence intervals in the GOLD Depression Study, I showed that although creatine-referenced choline (13.1%) in the basal ganglia and MCP-1 (11.0%) in blood serum mediated $>10\%$ of the association between

HIV status and PHQ-9 score, the estimates of these mediating effects had very wide 95% confidence intervals (Cho/Cr in BG: -28.4%, 65.0%; MCP-1: -16.1%, 57.6%) and thus non-significant p values ($p_{FDR} = 0.98$ for both). Therefore, the statistical approach in the GOLD Depression Study allowed me to demonstrate that the mediation effects of biomarkers measured in this study were not significant.

This comparison must be interpreted with caution and does not necessarily negate the findings from the COBRA-DI study. Several differences in the design and methodology – most notably the demographically distinct samples and the choice of biofluids in which inflammatory biomarkers were quantified – limit a direct comparison between the two studies. Despite these differences, the chemokine MIG mediated a comparable proportion of the relationship between HIV status and depressive symptoms in COBRA-DI (15.0%, measured in blood plasma) and the GOLD Depression Study (9.8%, measured in blood serum), which lends support to the hypothesis that certain underlying inflammatory processes may be mediating the risk for depressive symptoms across these distinct populations of people with HIV.

Crucially, the largest mediation effect estimated amongst soluble inflammatory biomarkers in the GOLD Depression Study (11.0% for BDNF in serum) was much smaller than the largest mediation effect estimated in COBRA-DI (20.9% for MIP-1 α in CSF). It is therefore plausible that these larger mediation effects observed (particularly for CSF biomarkers) in COBRA-DI may indicate statistically significant mediation. At the same time, small mediation effects such as those observed in the GOLD Depression Study would be reasonable in the context of inflammatory depression, as no single inflammatory biomarker is expected to fully account for the risk for depression in people with HIV. Larger samples can enable much greater precision when computing point estimates and assessing the statistical significance of these mediation effects. For this reason, it is important to continue to explore this relationship in larger and more diverse participant cohorts.

In summary, in the GOLD Depression Study, I assessed the potential mediation effects of neuroimaging and blood biomarkers of inflammation on the association between HIV status and depressive symptoms in a sample of adolescents in Cape Town, South Africa. Participants with HIV reported greater depressive symptoms and alterations in certain inflammatory biomarkers compared to participants without HIV. However, none of these biomarkers were significantly associated with the severity of depressive symptoms in this sample, and therefore none of the biomarkers significantly mediated the relationship between HIV status and depressive symptoms. These null findings could partly be attributed to the relatively low

prevalence of depressive symptoms in this sample or the low statistical power in this study. Equally, it is possible that in this sample there was no true underlying mediation of depressive symptoms by inflammatory biomarkers in the blood or brain. Investigating these mediation effects in larger samples, particularly those including a greater proportion of young people experiencing severe depressive symptoms, may help clarify the potential mediation role of inflammation in depression amongst young people with HIV.

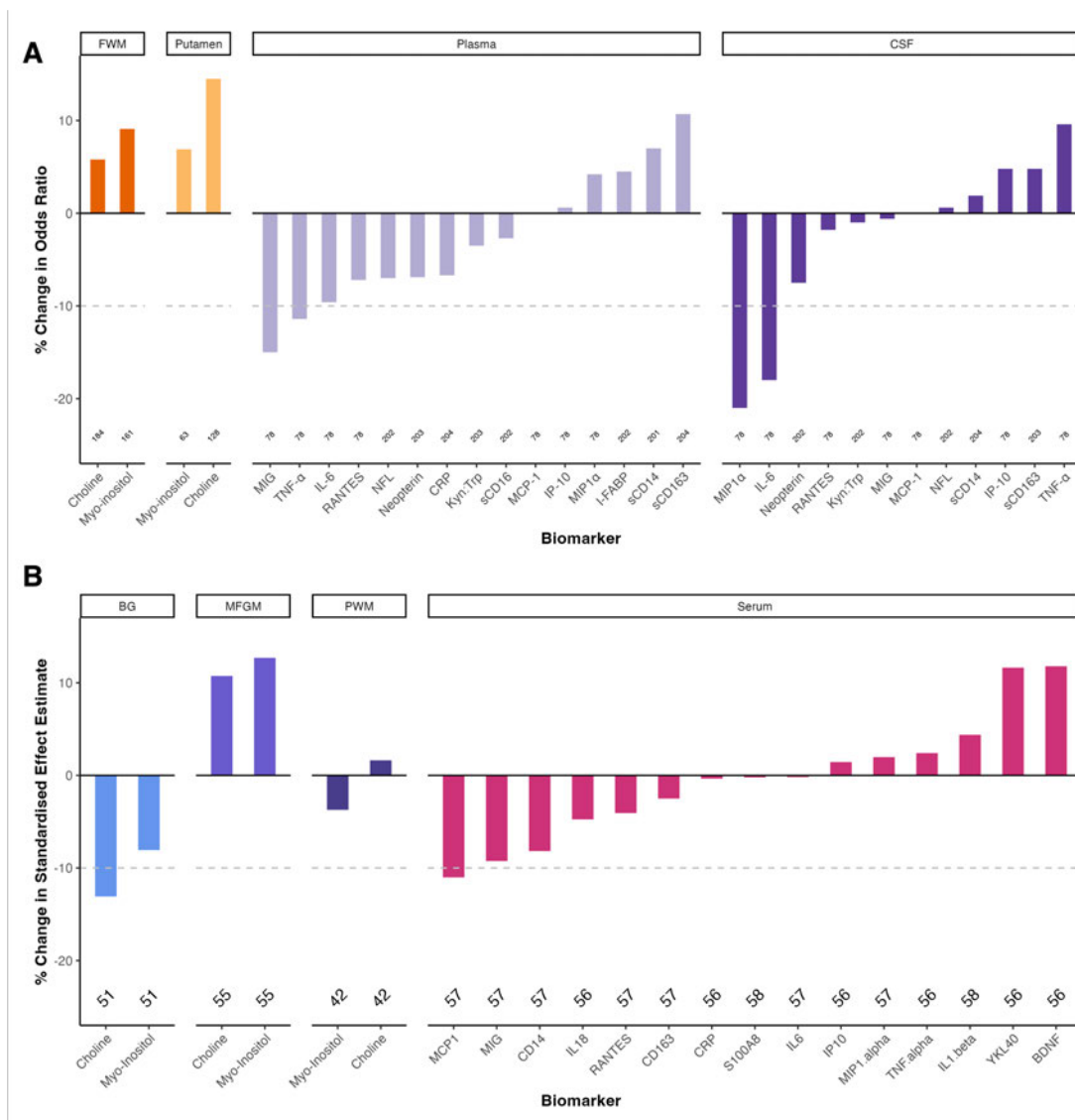


Figure 9.1: Comparison of potential mediation by inflammatory biomarkers in COBRA-DI (older adults in UK, Netherlands) and the GOLD Depression Study (young people in South Africa). (A) Change in odds ratio for the association between HIV status and Any Depressive Symptoms (PHQ-9 ≥ 4) when adjusting (separately) for each biomarker. Neurometabolites were quantified relative to creatine in frontal white matter (FWM) and putamen, and soluble biomarker concentrations were determined in blood plasma and cerebrospinal fluid (CSF). (B) Change in standardised effect estimate for the association between HIV status and total PHQ-9 score when adjusting (separately) for each biomarker. Neurometabolites were quantified relative to creatine in basal ganglia (BG), midfrontal gray matter (MFGM), and peritrigonal white matter (PWM), while soluble biomarker concentrations were determined in blood serum. Number of participants for whom data was available for each biomarker is indicated above the x axis.

10

INSIDE-HIV: Severe Depression and HIV

HIGHLIGHTS

- I explored whether the concentrations or rates of movement of certain molecules in the brain may be linked to the severity of a person's symptoms of depression.
- A total of 20 adults with HIV, 11 of whom were living with severe and persistent symptoms of depression, took part in this study. Participants went through several types of brain scans, including diffusion-weighted MRS, which estimates the rate of movement of molecules in the brain non-invasively. This is the first study to use diffusion-weighted MRS in people with HIV.
- I found that participants with more severe symptoms of depression showed higher rates of movement of creatine, a molecule linked to energy consumption and neuroinflammation. Energy consumption can increase in immune cells in the brain when they are activated, and higher movement of creatine has been found in people with conditions such as neuropsychiatric lupus which involve neuroinflammation.
- This study provides some early evidence that severe symptoms of depression in people with HIV may involve increases in energy consumption (which in turn may possibly indicate increased inflammation) in the brain, but as it was a small exploratory study, more research is needed to confirm and better understand these findings.

10.1 INTRODUCTION

DEPRESSION RESULTS IN THE GREATEST GLOBAL DISEASE BURDEN amongst neuropsychiatric conditions [202]. People with depression present with varied symptom profiles and, in up to 55% of these individuals, symptoms persist despite receiving first-line antidepressants [203]. Given this heterogeneity, we now understand depression to comprise a constellation of distinct subtypes, rather than a monolithic disease. Identifying and targeting the relevant neurobiological mechanisms may enable effective interventions for each subtype of depression.

Inflammation is increasingly being recognised as a mechanism underlying a subtype of depression. Early-life increases in biomarkers of inflammation can predict the total number of depressive episodes later in life [10], and people who exhibit inflammation are more likely to be resistant to standard antidepressant treatments [11]. Conversely, anti-inflammatory medications can alleviate depressive symptoms [13, 14]. Together, this evidence suggests that inflammation may drive a subtype of depression, termed “inflammatory depression”.

People with HIV are at three times greater risk for depression than people without HIV [2]. People with HIV also exhibit peripheral and central nervous system inflammation, evidenced by increased cytokine release in blood and microglial activation in the brain, which persists despite antiretroviral therapy (ART) [16, 17]. In addition to neuroinflammation, people with HIV also show increased blood-brain barrier (BBB) permeability [204], which is in turn implicated in the pathophysiology of depression in the general population [205]. Disruption of the BBB (via enlargement of the choroid plexus) has been observed to overlap with increases in neuroinflammation (indicated by greater TSPO binding) in people with depression [206]. It is thus possible that the increased risk for depression amongst people with HIV is linked, at least in part, to increased neuroinflammation and BBB permeability. However, people with HIV living with severe depression are often excluded from studies of HIV-related co-morbidities, resulting in limited understanding of whether neuroinflammation or BBB dysfunction may contribute to the severity of depression in this community.

Measuring neuroinflammation directly in humans remains challenging. One indirect in vivo measure of neuroinflammation is proton magnetic resonance spectroscopy (1H-MRS or simply MRS), which is used to measure concentrations of neurometabolites in localised brain regions non-invasively. Of relevance to neuroinflammation, the metabolites choline

(Cho) and myo-inositol (mI) – whose increased concentrations are widely considered to be indicative of cell membrane turnover and glial cell activation, respectively – can be quantified using MRS [207].

However, there are several challenges in using Cho or mI concentrations as biomarkers of neuroinflammation: elevations in Cho and mI may be confounded by variables such as participant age or alcohol consumption [188, 208], and changes in Cho concentrations may reflect processes such as (de)myelination in addition to neuroinflammation [209]. Therefore, more sensitive and specific non-invasive measures of neuroinflammation are needed to precisely characterise the relationship between neuroinflammation and depression.

Diffusion-weighted magnetic resonance spectroscopy (DW-MRS) is an emerging technique that can overcome some of the limitations of standard MRS. This non-invasive neuroimaging modality allows for the measurement of the intracellular diffusion rates (quantified as Apparent Diffusion Coefficients, ADCs) of neurometabolites in localised brain regions [210]. By combining the microstructural sensitivity of diffusion-weighted MR imaging (DW-MRI) with the compartmentalisation of neurometabolites, DW-MRS can offer higher specificity than DW-MRI to processes that selectively affect specific cell populations, such as neuroinflammation (microglia and astrocytes) and neurodegeneration (neurons) [211]. DW-MRS was recently shown to be sensitive to experimentally-induced neuroinflammation in humans, and may thus represent a useful non-invasive tool to explore the relationship between neuroinflammation and depression [193]. Measuring neuroinflammation and determining its influences on long-term clinical outcomes is of critical importance in people with HIV. DW-MRS may therefore be a promising technique for research involving people with HIV, but has never before been applied in this context.

In this pilot study, I aimed to explore the neuroimaging signatures of severe and persistent depressive symptoms amongst people with HIV. The primary objective was to determine the relationship between neurometabolite diffusion and the severity of depressive symptoms in people with HIV using DW-MRS. Secondary objectives were to explore the relationships between (1) neurometabolite concentrations and depressive symptom severity, (2) BBB permeability and depressive symptom severity, and (3) neuroimaging parameters and blood proteins indicative of inflammation and neurological dysfunction. A tertiary objective was to assess the validity of the South African Depression Scale (SADS), a recently developed measure of depressive symptoms, in a UK-based participant sample.

10.2 METHODS

PARTICIPANTS

For this cross-sectional pilot study, adults with HIV who were receiving their regular clinical care at the Lawson Unit (Royal Sussex County Hospital) were recruited in Brighton, UK between May 2023 and March 2024. All participants provided written informed consent prior to enrolment in the study. This study received ethical approval from the London–Surrey Borders Research Ethics Committee (reference 22/LO/0546).

Principal inclusion criteria for this study were: participants aged 18 years or older with a documented HIV-1 infection, currently receiving a stable antiretroviral regimen, and virally suppressed (plasma HIV RNA <50 copies/mL documented at screening). Participants were recruited into one of two study groups based on their Patient Health Questionnaire (PHQ-9) score at screening: high depressive symptom severity (“HD”) or low depressive symptom severity (“LD”). Participants were eligible for the HD group if they had a PHQ-9 score ≥ 15 and a current or previous history of receiving antidepressant medication, or eligible for the LD group if they had a PHQ-9 score ≤ 7 .

Principal exclusion criteria for this study included lack of capacity to provide informed consent, active hepatitis C or B infection on most recent documented laboratory tests, or a PHQ-9 score at screening outside the criteria of the LD or HD groups (i.e. $7 < \text{PHQ-9} < 15$). Participants were also excluded if they had active neurological or neurodegenerative diseases, or non-affective psychiatric disorders unrelated to depression (schizophrenia, obsessive compulsive disorder, trauma- or stress-related disorders). Finally, participants with any contraindications to MRI or the Gadolinium-based contrast agent (e.g. implanted medical devices, claustrophobia, or renal impairment documented at screening) were also excluded from this study.

QUESTIONNAIRES

At a screening visit, all participants completed the nine-item Patient Health Questionnaire (PHQ-9) as a measure of current depressive symptom severity. The PHQ-9 is a brief and well-validated screening tool which tests for key features of depression, including anhedonia, fatigue, and suicidality [40]. Although the PHQ-9 is not a diagnostic tool, a PHQ-9 score ≥ 10 offers high sensitivity and specificity for the detection of major depressive disorder [212].

All participants also completed a sociodemographic and lifestyle questionnaire, designed to provide inclusive options for self-identification.

In addition to the PHQ-9, participants also completed the South African Depression Scale (SADS). The SADS has been recently developed and validated as a dual-language (English and isiXhosa) screening tool for depressive symptoms. [46, 47] An advantage of the SADS is that it has been designed to include core features of depression which are also assessed by the PHQ-9, such as sleep disturbances and anhedonia, as well as more idiomatic expressions of depressive symptoms, such as “thinking too much” or “carrying a lot of weight on my shoulders”, which the PHQ-9 does not necessarily evaluate. An English-language version of the SADS was administered to all participants for a preliminary assessment of its acceptability in populations outside South Africa. All participants were residing and receiving their regular HIV-related clinical care in Brighton, and none identified as Xhosa.

Participants had access to a dedicated HIV mental health service including psychologists and psychiatrists, and were referred to this service if they exhibited suicide ideation or other symptoms which required referral on any of the depressive symptom screening tools.

NEUROIMAGING

Neuroimaging acquisition and processing was carried out by collaborators (Prof Itamar Ronen and Dr Nicholas G. Dowell) at the Clinical Imaging Sciences Centre of the Brighton & Sussex Medical School; the methods for acquisition and processing described in this section were thus provided by these collaborators. All participants underwent neuroimaging on a 3T Siemens Prisma MR scanner (Erlangen, DE) and 64 channel array receiver coil. Following a scout image and a T_1 weighted anatomical image, a 2cm (RL) x 1.5cm (FH) x 3cm (AP) volume of interest (VOI) was positioned on the bilateral dorsal anterior cingulate cortex (ACC) (Figure 10.1).

MRS and DW-MRS were used to assess neurometabolite concentrations and diffusivity. This study focused on the anterior cingulate cortex (ACC), as inflammation in the ACC has previously been associated with depression in the general population [139, 213]. MRS data was acquired using a semi-LASER sequence (TE/TR = 30/3000 ms, 64 averages, GOIA-WURST pulses). DW-MRS data was acquired with the same sequence, equipped with a bipolar diffusion weighting scheme [210] with three mutually orthogonal DW directions and three b values (0, 1863 and 3856 s/mm²), 32 averages per DW condition and TE/TR =

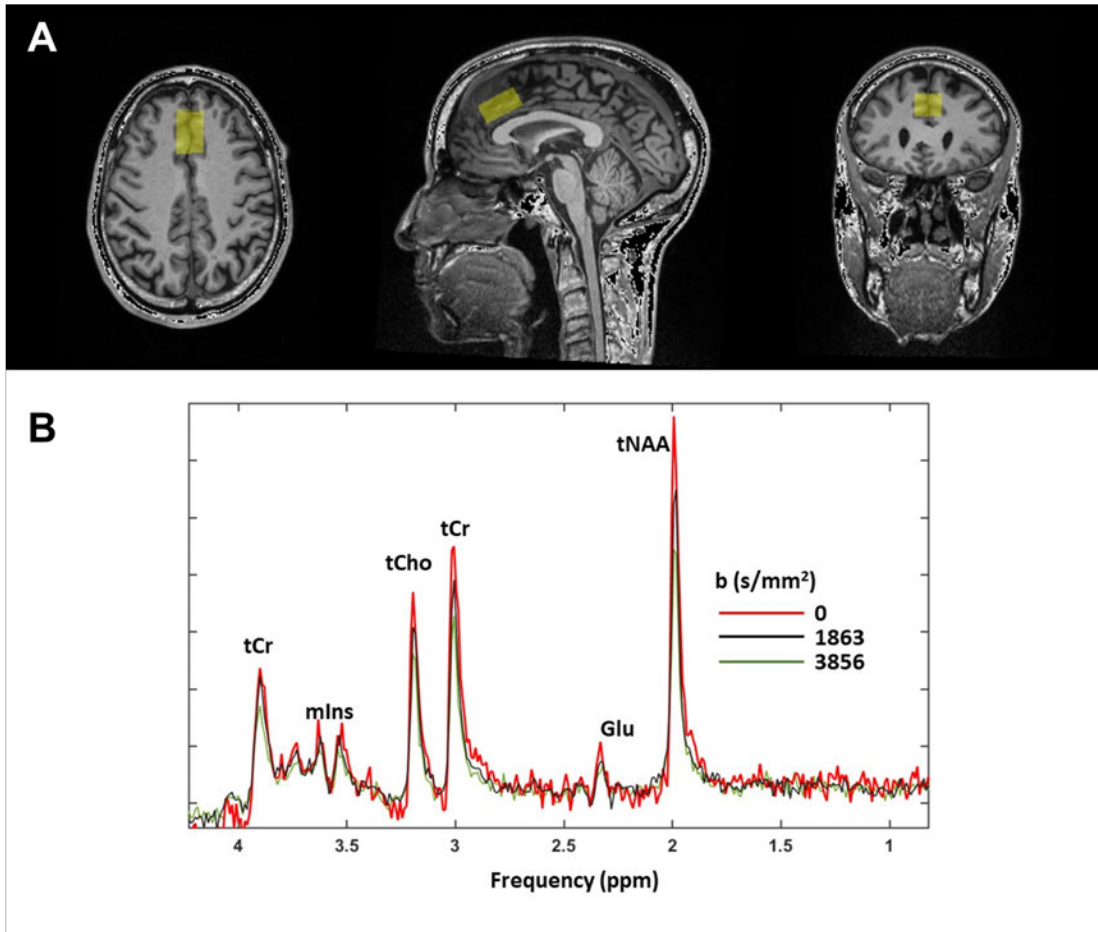


Figure 10.1: (A) Position of the volume of interest (VOI, in yellow) in the anterior cingulate cortex, and (B) representative DW-MRS spectra from this VOI at three b-values. (tNAA = total NAA, Glu = Glutamate, tCr = total creatine, tCho = total choline, mIns = myo-inositol.)

80ms/3 heart beats (cardiac synchronization with pulse oximeter unit). From the DW-MRS spectra, ADCs for three metabolites were quantified. From the MRS data, concentrations of nine metabolites were calculated relative to total creatine and concentrations of ten metabolites were calculated relative to total water, correcting for T_2 and water content differences between gray matter, white matter and CSF. Only metabolites for which Cramer Rao Lower Bounds (CLRB) was <20% for all participants and all DW conditions were considered.

To assess BBB permeability, whole-brain dynamic contrast enhanced MRI (DCE-MRI) was acquired with a T_1 -weighted gradient echo pulse sequence: 100 volumes, time resolution 9.34s, FA=7deg, voxel size=2.5mm³, total acquisition time=15.5mins. Dotarem (gadoterate meglumine; 0.1mMol/kg) was injected after 90s of baseline scanning. Volume transfer rate constant (K_{trans} , a proxy for BBB permeability) was extracted by fitting the Patlak model to the data. Segmentation of the mean baseline DCE images was performed with SPM to yield a whole-brain ROI, and FMRIB Software Tools (FSL) was used to extract mean whole-brain K_{trans} values from the maps. Thalamus ROI was taken from the Harvard-Oxford Subcortical Atlas and transformed into participant (native) space using Advanced Normalization Tools (ANTs) and a diffeomorphic transformation.

BLOOD PROTEINS

To quantify peripheral inflammatory and neurological biomarkers, blood samples were collected from all participants at the same study visit during which the PHQ-9 was administered. Blood samples were allowed to clot for 30 minutes at room temperature, then centrifuged at 2000xg for 10 minutes to extract serum. Serum samples were aliquoted and stored at -80°C within 2 hours of collection at the Royal Sussex County Hospital Clinical Research Laboratory (Brighton, UK). Samples were subsequently shipped in a single batch on dry ice to the University of Edinburgh Biomolecular & Assay Core (Edinburgh, UK). Protein quantification was carried out in a single batch using Invitrogen™ ProcartaPlex Human Simplex and Combinable Panels (Thermo Fisher Scientific, UK) according to manufacturer's instructions. Samples were diluted 1:500 for the CRP Simplex Panel and 1:100 for the YKL-40 Simplex Panel, with no sample dilutions for other analytes. Panels were analysed using a Luminex™ xMAP™ INTELLIFLEX DR-SE System (Thermo Fisher Scientific, UK) and analyte concentrations in samples were obtained from standard curves using five-parameter logistic (5-PL) curves in the instrument software, BioPlex Manager.

I assessed all samples in triplicate, with values averaged across replicates for each analyte and sample. Only cases with coefficient of variation (%CV) <20% across the three replicates were included. Where all replicates for a sample were below the detection range of the assay, a value that was half the minimum detection limit for that analyte was assumed, and where all replicates were above the detection range, a value twice the maximum detection limit for that analyte was assumed. Imputed values were assumed in this way for IL-17A (1 case), IFN- α (7), IL-1 α (6), IL-1 β (6), IL-8 (6), and MIP-1 α (6), all below detection range, and for CRP (one case, above detection range), but for no other analytes.

STATISTICAL ANALYSIS

Statistical analyses were performed in R v4.3.2. Group comparisons for participant characteristics were performed using Wilcoxon's rank-sum tests (for continuous variables) or Pearson's χ^2 tests (for categorical variables). Spearman's correlations (ρ) were calculated for values of DW-MRS and DCE-MRI parameters with PHQ-9 score and for blood proteins with PHQ-9 score and all neuroimaging parameters. Partial Spearman's correlations adjusted for fractional white matter volumes were calculated for MRS parameters with PHQ-9 score. In sensitivity analyses, neuroimaging parameters and blood proteins were compared between HD and LD groups using Wilcoxon's rank-sum tests. All p -values (except for comparisons of participant characteristics) were corrected for multiple comparisons using the False Discovery Rate method to yield corrected q values. Finally, the internal consistency of the SADS was assessed using Cronbach's α , convergent reliability with the PHQ-9 using Pearson's correlation coefficient (r), and item-level performance using descriptive statistics and item-total correlations. Given that this was a pilot study with a small sample, when interpreting these findings I explored the magnitude of the correlations as well as their statistical significance to avoid an overemphasis on p values [214]. I used Funder & Ozer's [215] framework for interpreting the effect sizes, with correlations greater than 0.30 considered large effects, while noting that very large effects may likely be overestimated due to limited statistical power.

10.3 RESULTS

PARTICIPANTS CHARACTERISTICS

Participants were $N = 20$ people with HIV, with median [interquartile range (IQR)] age of 50 [45, 54] years, of whom 90% self-identified as men and 80% as White (**Table 1**). Median [IQR] PHQ-9 score overall was 17.0 [2, 21]. Participants in the HD group ($n = 11$) reported significantly higher depressive symptom severity than those in the LD group ($n = 9$), with median [IQR] PHQ-9 score of 21 [20, 22] compared to 2 [1, 4] ($p < 0.001$) (**Figure 10.2**). Both groups were comparable on most sociodemographic characteristics, except participants in the HD group reported fewer years of formal education (14 vs 19 years, $p = 0.013$). As anticipated by the study design, experience of treatment for depression differed between groups: a significantly greater proportion of participants in the HD group reported ever taking antidepressants (100% vs 44%, $p = 0.008$) and currently receiving psychotherapy (45% vs 0%, $p = 0.038$). Participants in the HD group also showed lower absolute CD4 count than those in the LD group (606 vs 855, $p = 0.023$). Participants were receiving a wide range of ART and antidepressant regimens, which did not differ significantly by group (**Supplementary Table D.1**).

PSYCHOMETRIC PROPERTIES OF THE SADS

The tertiary objective for this study was to assess the psychometric performance of the SADS in a UK-based sample. Results from these analyses were published separately in *BMJ Mental Health*. The SADS exhibited high internal consistency, Cronbach's α (95% confidence interval [CI]) = 0.975 (0.955, 0.988). Total score on the SADS was strongly and significantly correlated with total score on the PHQ-9 ($r = 0.97$, $p < 0.001$) (**Figure 10.3**). Item-level analysis indicated that most items of the SADS were strongly correlated with the total score (**Table 10.2**). However, scores on SADS10 ("I had pain in my heart") and SADS11 ("I cried") were skewed towards zero and showed poor corrected item-total correlation ($r = 0.67$ for SADS10 and $r = 0.48$ for SADS11). Findings remained consistent when only including participants who self-identified as male.

Characteristic	Overall, N = 20	High Depression Severity, N = 11	Low Depression Severity, N = 9	p-value
Age (years), Median (IQR)	50.5 (45.0, 54.0)	51.0 (47.0, 54.0)	49.0 (45.0, 54.0)	0.8
Sex, n (%)				0.9
Female	1 (5.0%)	1 (9.1%)	0 (0%)	
Male	19 (95%)	10 (91%)	9 (100%)	
Gender, n (%)				0.9
Man	18 (90%)	9 (82%)	9 (100%)	
Man & Non-binary	1 (5.0%)	1 (9.1%)	0 (0%)	
Woman	1 (5.0%)	1 (9.1%)	0 (0%)	
Sexual Orientation, n (%)				0.6
Gay / lesbian / homosexual	17 (85%)	10 (91%)	7 (78%)	
Straight / heterosexual	3 (15%)	1 (9.1%)	2 (22%)	
Ethnicity, n (%)				0.5
Black - African	1 (5.0%)	0 (0%)	1 (11%)	
Latino / Latina / Latinx / Hispanic	1 (5.0%)	1 (9.1%)	0 (0%)	
Mixed race	1 (5.0%)	0 (0%)	1 (11%)	
White - Non-UK	3 (15%)	1 (9.1%)	2 (22%)	
White - UK	13 (65%)	8 (73%)	5 (56%)	
White - UK & Black - Caribbean	1 (5.0%)	1 (9.1%)	0 (0%)	
Years of Education, Median (IQR)	16.5 (13.5, 19.3)	14.0 (11.5, 16.0)	19.0 (17.0, 20.0)	0.013
Smoking, n (%)	1 (5.0%)	1 (9.1%)	0 (0%)	0.9
Alcohol Use, n (%)	13 (65%)	7 (64%)	6 (67%)	0.9
Recreational Drug Use, n (%)	2 (10%)	2 (18%)	0 (0%)	0.5
Currently Taking Antidepressants, n (%)	13 (65%)	9 (82%)	4 (44%)	0.2
Ever Taken Antidepressants, n (%)	15 (75%)	11 (100%)	4 (44%)	0.008
Currently Receiving Psychotherapy, n (%)	5 (25%)	5 (45%)	0 (0%)	0.038
Ever Received Psychotherapy, n (%)	12 (60%)	9 (82%)	3 (33%)	0.065
HIV RNA Viral Load 40 copies/mL, n (%)	20 (100%)	11 (100%)	9 (100%)	
CD4 Count, Median (IQR)	686.5 (558.0, 804.0)	606.0 (515.0, 709.0)	855.0 (689.0, 966.0)	0.023

Table 10.1: Sociodemographic and clinical characteristics of participants overall, and stratified by depressive symptom severity group. Participant groups were determined at screening using the Patient Health Questionnaire (PHQ-9) score as High Depression Severity (HD, PHQ-9 ≥ 15 and history of using antidepressant medication) or Low Depression Severity (LD, PHQ-9 ≤ 7). Participants could select multiple genders, sexual orientations, or ethnicities. HIV RNA viral load and CD4 count were extracted from most recent available clinical record for each participant.

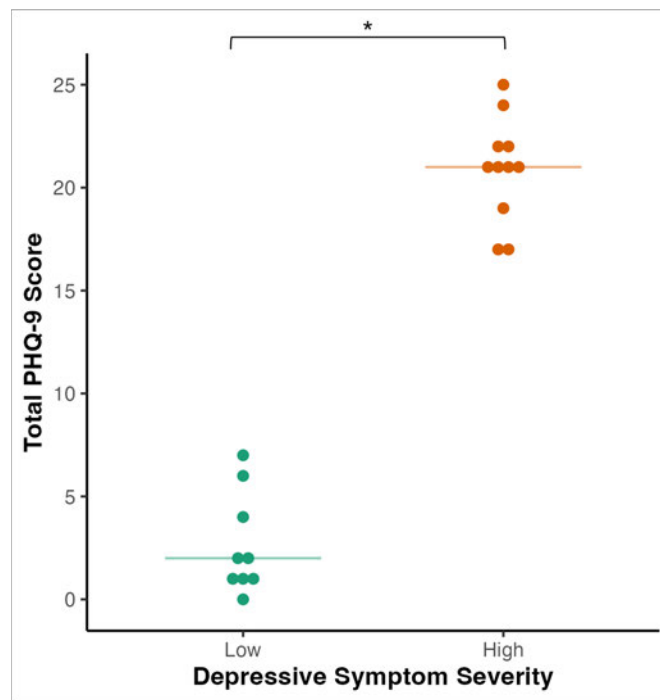


Figure 10.2: Distribution of total score on the nine-item Patient Health Questionnaire (PHQ-9) for high depressive symptom severity (HD) and low depressive symptom severity (LD) groups. Horizontal lines indicate group medians. * indicates $p < 0.05$.

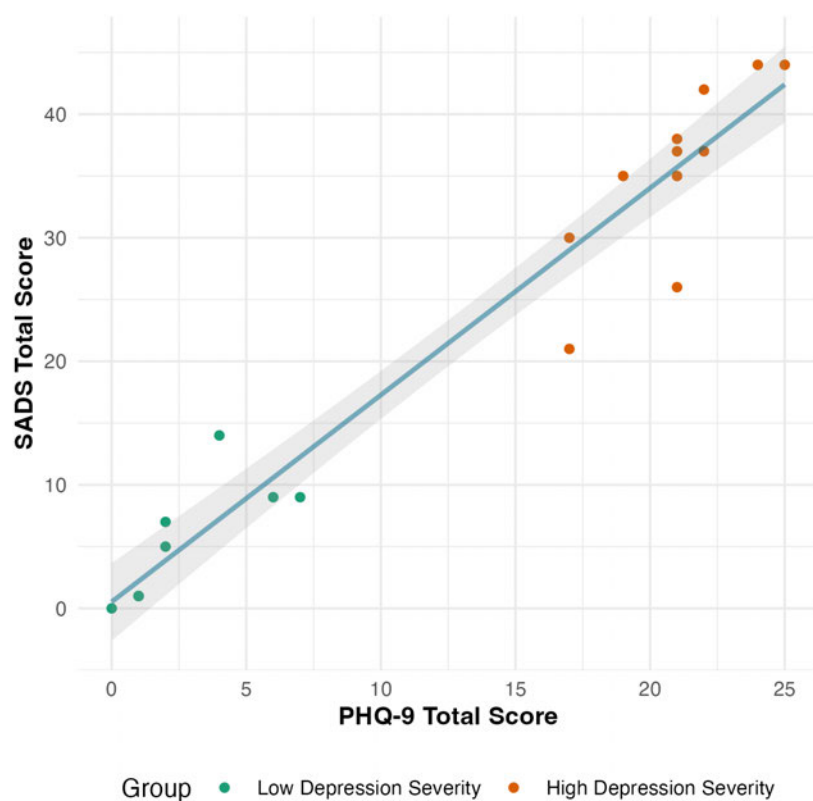


Figure 10.3: Correlation between total score on the South African Depression Scale (SADS) and total score on the nine-item Patient Health Questionnaire (PHQ-9) for 20 adults with HIV in Brighton, UK, with 95% confidence interval displayed in grey.

Item	Mean	Standard Deviation	Item-Total Correlation	Corrected Item-Total Correlation
SADSo1	1.5	1.24	0.93	0.92
SADSo2	1.5	1.19	0.74	0.71
SADSo3	1.9	1.25	0.89	0.87
SADSo4	1.5	1.28	0.86	0.84
SADSo5	1.6	1.19	0.93	0.92
SADSo6	1.4	1.35	0.79	0.76
SADSo7	1.4	1.14	0.93	0.92
SADSo8	1.45	1.32	0.88	0.86
SADSo9	1.4	1.23	0.9	0.88
SADSI0	0.7	1.03	0.7	0.67
SADSI1	0.65	0.93	0.52	0.48
SADSI2	1.35	1.09	0.9	0.88
SADSI3	1.55	1.23	0.96	0.95
SADSI4	1.3	1.38	0.85	0.82
SADSI5	1.5	1.32	0.9	0.88
SADSI6	1.1	1.21	0.9	0.88

Table 10.2: Item-level analysis of the South African Depression Scale (SADS) for 20 adults living with HIV in Brighton, UK.

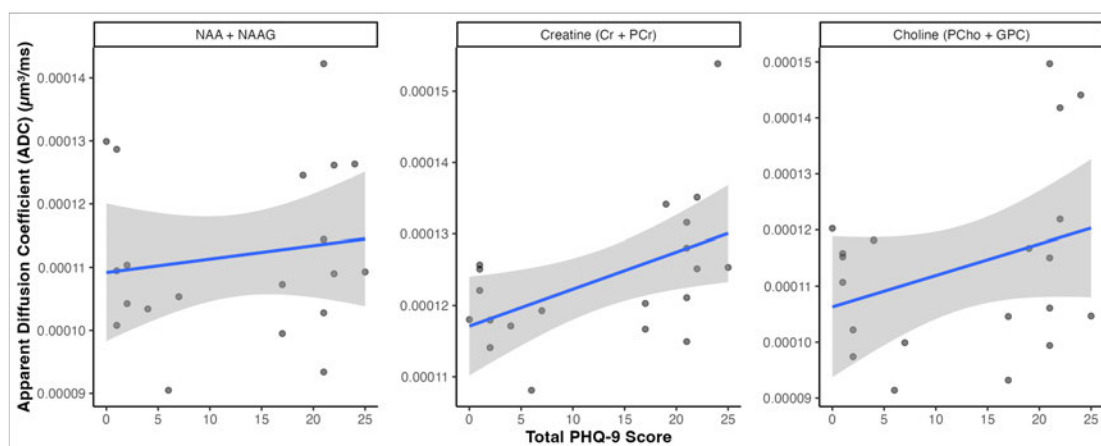


Figure 10.4: Neurometabolite diffusion and depressive symptom severity. Correlation between measures of neurometabolite diffusion (apparent diffusion coefficient, ADC) in the anterior cingulate cortex quantified using diffusion-weighted magnetic resonance spectroscopy and depressive symptom severity (total PHQ-9 score).

NEUROMETABOLITE DIFFUSION AND DEPRESSIVE SYMPTOM SEVERITY

Summary statistics for all neuroimaging parameters are available in **Supplementary Table D.2**. Diffusion of neurometabolites in the ACC was quantified as the apparent diffusion coefficients (ADCs, **Figure 10.4**) using diffusion-weighted MRS. ADC for creatine was significantly and positively correlated with PHQ-9 score ($\rho = 0.48$, $p = 0.034$). However, this correlation did not survive FDR correction ($q = 0.102$). There was also a modest correlation of choline ADC ($\rho = 0.24$) with PHQ-9 score, but this was not statistically significant ($q = 0.467$). ADC for NAA was not correlated with PHQ-9 score ($\rho = 0.07$, $q = 0.768$).

As years of education and CD4 count differed significantly between the participant groups, partial Spearman's correlations adjusted for these variables (separately) were calculated for DW-MRS parameters (**Supplementary Table D.3**). Findings remained consistent after adjusting for CD4 cell counts. After adjusting for years of education, the correlation between ADC of creatine and PHQ-9 score was no longer significant ($\rho = 0.25$, $p = 0.303$, $q = 0.909$). ADC of creatine was significantly correlated with years of education, $\rho = -0.60$, $p = 0.005$. All correlation coefficients and associated p values are available in **Supplementary Table D.4**.

NEUROMETABOLITE CONCENTRATIONS AND DEPRESSIVE SYMPTOM SEVERITY

Concentrations of neurometabolites in the ACC were quantified relative to total creatine (**Figure 10.5**) and total water (**Supplementary Figure D.2**) using standard MRS. After ad-

justing for fractional white matter volume, creatine-referenced aspartate was significantly and moderately correlated with PHQ-9 score ($\rho = 0.54$, $p = 0.016$), though this correlation did not survive FDR correction ($q = 0.115$). Water-referenced aspartate also trended towards a correlation with PHQ-9 score ($\rho = 0.43$, $p = 0.065$, $q = 0.527$). None of the other metabolite concentrations, either referenced to creatine or water, were significantly associated with PHQ-9 score (all $|\rho| < 0.30$, $q > 0.05$). Findings remained largely consistent when adjusting for years of education or CD4 count (**Supplementary Table D.3**).

BLOOD-BRAIN BARRIER PERMEABILITY AND DEPRESSIVE SYMPTOM SEVERITY

DCE-MRI data was not acquired for one participant who chose to withdraw from the scanning, and thalamus ROI was not available for one additional participant. Volume transfer rate constant (K_{trans}) for the whole-brain ROI was not significantly correlated with PHQ-9 score ($\rho = -0.15$, $q = 0.537$). Similarly, K_{trans} for the thalamus ROI was not significantly correlated with PHQ-9 score, though the magnitude of this correlation was larger than that for the whole-brain ROI ($\rho = -0.38$, $q = 0.257$) (**Figure 10.6**). Findings remained consistent when adjusting these correlations for years of education or CD4 count (**Supplementary Table D.3**).

NEUROIMAGING AND BLOOD PROTEINS

I quantified 20 inflammatory and neurological proteins in blood serum samples, summary statistics for which are available in **Supplementary Table D.2**. Of these 20 proteins, only NGF- β showed a significant correlation with PHQ-9 score ($\rho = 0.48$, $p = 0.032$), though this did not survive FDR correction ($q = 0.640$) (**Supplementary Figure D.3**).

I then explored whether the neuroimaging parameters measured in this study may be correlated with these peripheral markers of inflammation and neurological dysfunction (**Figure 10.7**). While I focused on the magnitudes of these correlations when interpreting these findings, those correlations which met the threshold for statistical significance before FDR correction are highlighted in **Supplementary Figure D.4**. ADCs of metabolites quantified using DW-MRS did not correlate strongly with any blood proteins (all $|\rho| < 0.43$, $p > 0.075$). Whole-brain K_{trans} was negatively correlated with IL-12p70 ($\rho = -0.60$, $p = 0.009$) and ICAM-1 ($\rho = -0.57$, $p = 0.016$). Creatine-referenced concentrations of several metabolites were also strongly correlated with peripheral markers, most notably lactate with BDNF ($\rho =$

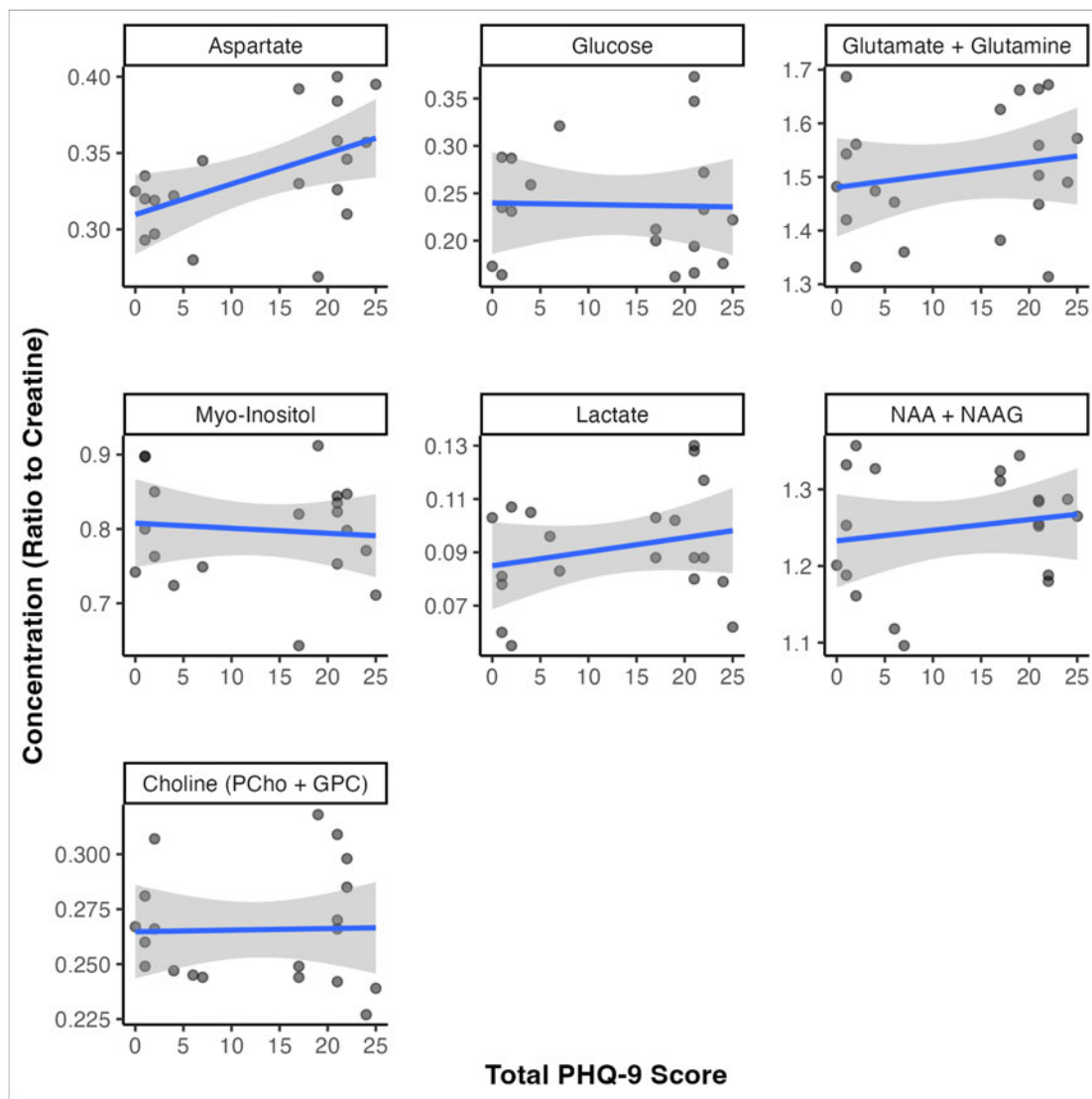


Figure 10.5: Neurometabolite concentrations and depressive symptom severity. Correlation between measures of neurometabolite concentrations (relative to total creatine) in the anterior cingulate cortex quantified using magnetic resonance spectroscopy and depressive symptom severity (total PHQ-9 score).

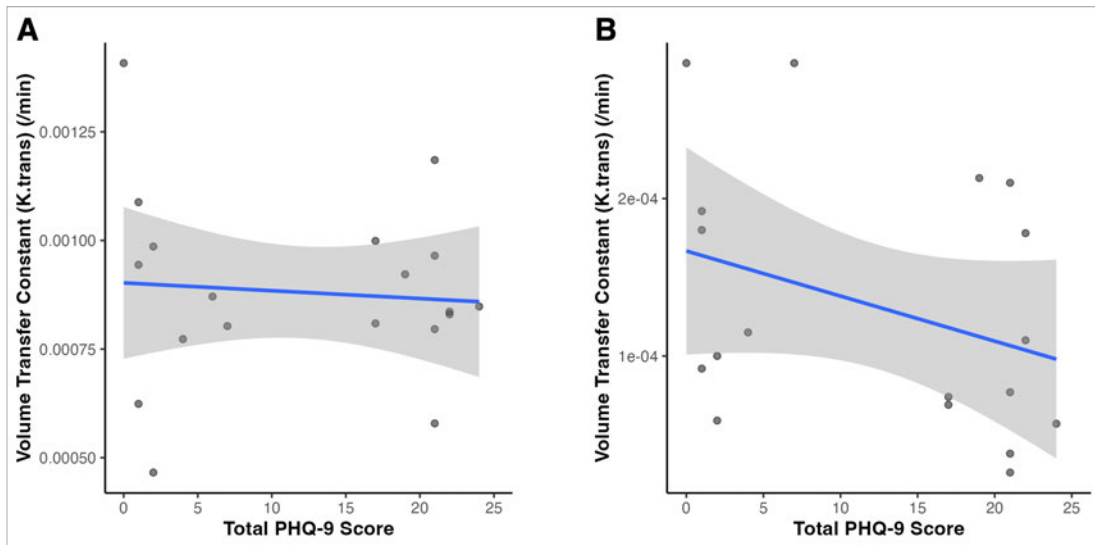


Figure 10.6: Blood-brain barrier permeability and depressive symptom severity. Correlation between measures of blood-brain barrier permeability (volume transfer rate constant, K_{trans}) quantified using dynamic contrast-enhanced magnetic resonance imaging for the (A) whole brain ($n = 19$) and (B) thalamus ($n = 18$) and depressive symptom severity (total PHQ-9 score).

-0.75 , $p < 0.001$) and CRP ($\rho = -0.64$, $p = 0.008$), and myo-inositol with MIP-1 α ($\rho = 0.63$, $p = 0.006$). After FDR correction, these correlations were no longer statistically significant (all $q \geq 0.05$), though creatine-referenced lactate trended towards a significant correlation with BDNF ($q = 0.050$).

10.4 DISCUSSION

In this exploratory study, diffusion-weighted MRS was applied for the first time in a small sample of people with HIV. I aimed to explore the relationships of neuroimaging parameters with the severity of depressive symptoms in people with HIV.

CREATINE DIFFUSION

A key finding from this study is the moderate positive correlation of creatine ADC (measured using DW-MRS) with depressive symptom severity, indicating that participants with more severe depressive symptoms exhibited higher intracellular diffusion of creatine in the ACC. In recent DW-MRS studies, increased creatine ADC has been reported in patients with multiple sclerosis [194] and neuropsychiatric lupus [195], both conditions characterised by

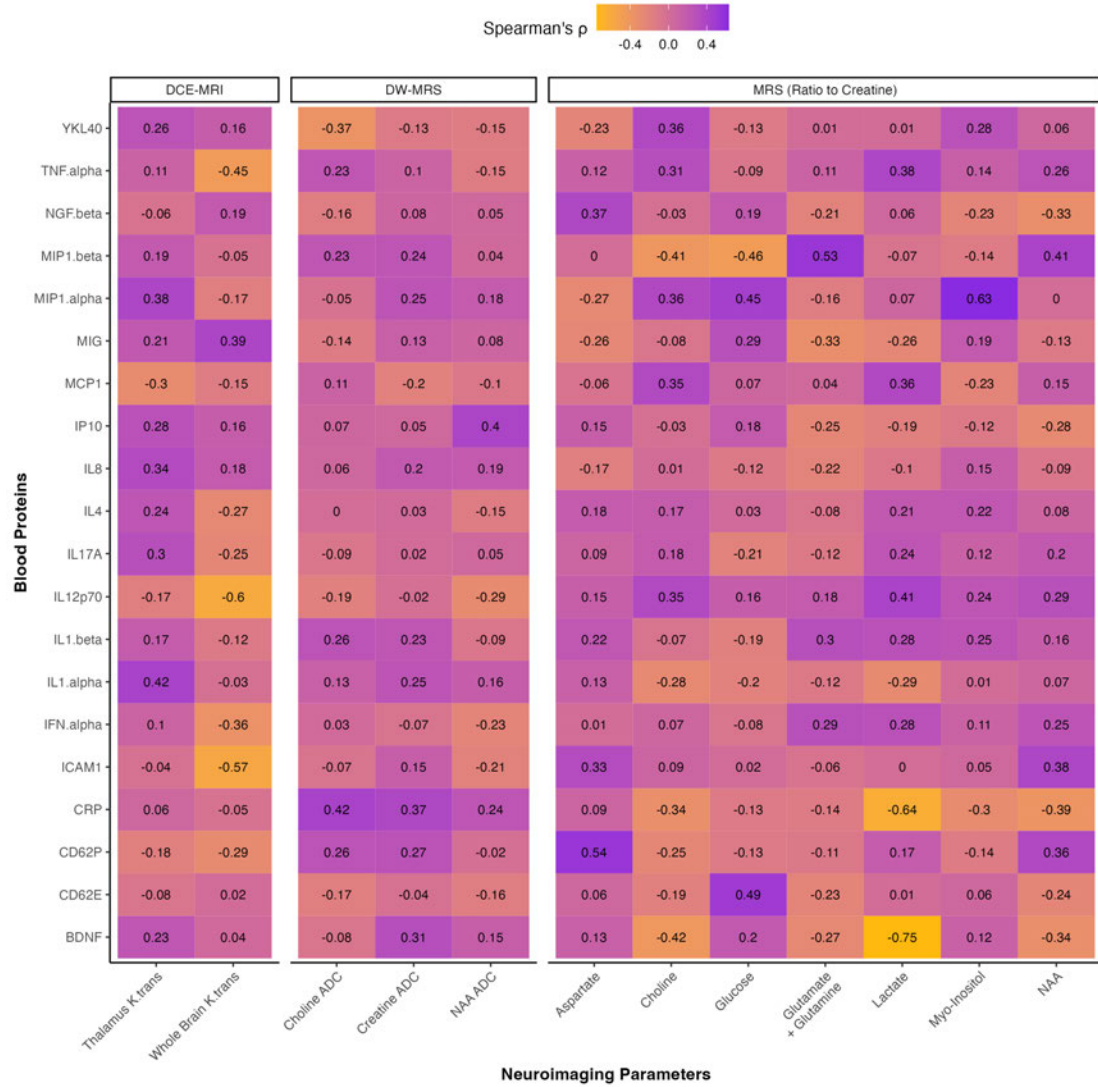


Figure 10.7: Neuroimaging parameters and blood proteins. Heatmap of Spearman's correlations for neuroimaging parameters with concentrations of inflammatory and neurological proteins quantified in blood serum. Dynamic contrast-enhanced MRI (DCE-MRI) was used to assess blood-brain barrier permeability (volume transfer rate constant, K_{trans}) in the thalamus and whole brain, diffusion-weighted MRS used to assess intracellular diffusion (apparent diffusion coefficient, ADC) of neurometabolites in the anterior cingulate cortex, and standard MRS used to assess concentrations (relative to creatine) of neurometabolites in the anterior cingulate cortex. Proteins in blood were quantified using Luminex™ immunoassays. Strength of correlations is represented by a colour gradient from 1.0 (bright yellow) to +1.0 (bright purple), with in-laid text in tiles indicating these magnitudes.

neuroinflammation. Additionally, increase in creatine ADC has been associated with increase in metabolic activity in the visual cortex in a functional DW-MRS study [216]. Creatine concentrations are shown to be higher in glial cells than in neurons, and animal models with SIV infection (the non-human primate analogue of HIV) revealed increased creatine concentrations concurrent with neuroinflammatory responses [188, 217]. Given this previous evidence, the intracellular diffusion of creatine may be considered an indirect indicator for neuroimmunometabolic alterations. In this context, these preliminary findings suggest that severe and persistent depression in people with HIV may involve underlying hypermetabolism and neuroinflammation.

Notably, the correlation between creatine ADC and depressive symptom severity was attenuated when accounting for years of education, whereas creatine ADC was strongly and negatively correlated with years of education ($\rho = -0.60$). Years of education is a well-established proxy for socioeconomic status, higher levels of which can be protective against depression [218]. Conversely, lower socioeconomic status is associated with increased inflammation [219], poorer metabolic health [220], and wider risk factors for depression, such as early life adversity or trauma [221]. Results from this study thus suggest that socioeconomic status may be correlated with creatine diffusion in the brain (possibly through cross-talk with inflammatory or metabolic pathways) and in turn influence depressive symptom severity.

BLOOD-BRAIN BARRIER PERMEABILITY

A secondary objective for this study was to explore the relationship of blood-brain barrier permeability (measured using DCE-MRI) with depressive symptom severity. I did not find significant correlations between the volume transfer rate constant (K_{trans} , a proxy BBB permeability), and depressive symptom severity. Increased BBB permeability has previously been reported in people with HIV, with strong evidence that HIV viral proteins may result in reduced integrity of the BBB [201, 222]. In people with depression, as well, increased BBB permeability has been observed, including in the thalamus [200]. However, I did not find a significant correlation between BBB permeability and depressive symptom severity. Since all participants in this study had been with HIV for several years, long-term compromise of BBB integrity due to HIV may perhaps overshadow any subtler correlations between BBB permeability and depressive symptoms. Indeed, the median K_{trans} value for this participant sample ($0.848 \times 10^{-3} \text{ min}^{-1}$) was higher than that recently reported in the group of participants with

major depressive disorder ($0.557 \times 10^{-3} \text{ min}^{-1}$) [200]. Thus, the lack of correlation between BBB permeability and depressive symptom severity observed in this study may perhaps be attributed to the stronger underlying BBB impacts of HIV compared to more temporally fluctuating impacts of depressive symptoms.

NEUROIMAGING PARAMETERS AND BLOOD PROTEINS

I also observed nominally significant correlations between certain neurometabolites in the ACC and inflammatory or neurological proteins in blood serum. In particular, lactate concentration in the ACC was strongly correlated with peripheral BDNF concentration. Increase in lactate concentrations in the ACC has been reported in depression as well as other psychiatric conditions such as bipolar disorder and schizophrenia [223–225]. Results from this study extend these findings and report a possible link between increased brain lactate concentrations, indicative of hypermetabolism, and decreased peripheral BDNF concentrations, a well-established marker for depression. This correlation was strongly negative ($\rho = -0.75$); correlations of such magnitudes are unlikely in relationships between biological and behavioural variables, and may not be reproducible in larger samples [215]. At the same time, this correlation trended towards significance even after correction for multiple comparisons in a large family (7×20) of *t*-tests, which suggests that it may warrant further investigation in larger studies. I also found that myo-inositol concentration in the ACC was strongly and positively correlated with peripheral MIP-1 α , a chemokine involved in the inflammatory response. This finding aligns with previous studies which have reported associations between brain myo-inositol concentrations (measured via standard MRS in the basal ganglia) and peripheral chemokine concentrations in people with HIV, and suggests an overlap between astrocytic activation in the brain and the peripheral inflammatory response [226].

PSYCHOMETRIC PROPERTIES OF THE SADS

The tertiary objective was to assess the psychometric performance of the South African Depression Scale (SADS) in the UK context. I observed high internal consistency for the SADS and high convergent validity between the SADS and PHQ-9 in this small UK-based sample of adults with varying depressive symptomatology. I also found that the SADS exhibited satisfactory item-level psychometric properties in this sample, except for two items (SADS₁₀ and SADS₁₁). Notably, SADS₁₀ (“I had pain in my heart”) was included in this scale to

reflect a culturally-specific idiom of distress and showed the highest factor loading amongst SADS items [46]. Thus, the poor item-total correlation that I observed for this item in this UK sample was somewhat expected, and suggests that SADS₁₀ may be specific to the South African (or more generally, African) context and not “translate” well to expressions of depressive symptomatology in UK samples.

In this sample, SADS₁₁ (“I cried”) exhibited the poorest item-total correlation. This participant sample was majority male, with all participants except one self-identifying as male. Gender roles, especially in cultures in the Global North, forbid crying for men [227], resulting in a measurable gender-based difference in reporting of crying as a symptom of depression [228]. It is thus possible that the poor item-total correlation for SADS₁₁ observed in this study was driven by the demographic (sex/gender) composition of the sample rather than being an artifact of cultural context. Interestingly, all other items on the SADS displayed satisfactory psychometric properties in this pilot study, including SADS₁₆ (“I had deep sorrow”). SADS₁₆ was added to the scale in consultation with isiXhosa-speaking mental health providers, and asks about a component of depression (“*isingqala*”, loosely translated to “deep sorrow”) which is specific to the isiXhosa language [46]. Nevertheless, this item showed a high item-total correlation in this study, suggesting that this idiomatic expression of depression may still be relevant among English-speaking participants in the UK.

Therefore, I found that the SADS performed satisfactorily as a screening tool for depressive symptoms in the UK context. This study was limited by a small sample size and consequently limited statistical power, thus being restricted to preliminary psychometric analyses. Despite these notable limitations, this pilot study offers early evidence that the SADS may be useful in the UK context. Establishing the utility of the SADS in populations outside South Africa is important for several reasons. First, this evidence can enable direct comparisons between studies of depressive symptoms carried out in South Africa (using the SADS) and outside South Africa (using other tools such as the PHQ-9). Second, it is possible that culturally-inclusive tools such as the SADS may be effective at detecting depressive symptoms in communities of African origin residing in the UK and other countries in the Global North. Finally, as suggested by the item-level analyses, some idiomatic expressions of depressive symptoms may be understood by people across diverse cultural groups. By applying a screening tool that includes such idiomatic expressions in different populations, we may better distinguish between culturally-specific and culturally-agnostic features of depression.

Future work may seek to determine whether applying the SADS in other contexts may

require excluding the highly culturally-specific item SADS₁₀, and whether this tool may be particularly useful for exploring depressive symptoms amongst African immigrant communities in the UK. In this instance, participants were not asked directly about their perceptions of the idiomatic expressions of depression included in the SADS, but future studies should explore these perceptions, including through qualitative research methods. Validating the use of this screening tool in larger samples outside South Africa may enable cross-cultural comparisons of depressive symptoms.

LIMITATIONS OF THIS STUDY

As this was an exploratory study, it had a small sample size ($N = 20$), which is a major caveat to these results. Small sample sizes result in issues such as reduced statistical power to detect true underlying effects, possible underestimation or inflation of true effect sizes, and risk of overfitting [229, 230]. Therefore, these findings must be interpreted with caution. I have attempted to mitigate some of these issues by using simple correlation analyses rather than more complex models, limiting the number of covariates to one (or two, in case of standard MRS data) to minimise the risk of overfitting, and reporting FDR-corrected q values to account for multiple comparisons. As DW-MRS is an emerging technique, the sample size of this study is also largely comparable to previous studies involving this imaging modality [193–195, 210]. Importantly, the correlations reported in this study did not survive FDR correction, but the magnitudes of these correlations suggest that at least some of these effects may be indicative of true underlying relationships. Very large effect sizes – especially in exploratory research linking biological and behavioural variables – are unlikely to be fully replicable in larger samples [215]. Nevertheless, as DW-MRS has never been applied in the context of HIV before, these findings (when interpreted with appropriate caveats) may offer useful directions for future research, despite the small sample size of the current study.

Additional limitations for this study include its cross-sectional design, which limits any inferences about the causality or temporality of relationships between neurometabolite diffusion and depressive symptom severity. This participant sample was also primarily White (80%), male (90%), and older (median age 50 years). This sample is broadly representative of the population of people with HIV in Brighton, UK. Nevertheless, these findings may not be immediately generalisable to other population groups, including women with HIV and people with HIV in the Global South.

10.5 CONCLUSIONS

Despite these limitations, this study makes a novel contribution to the literature by exploring neuroimaging signatures of severe and persistent depressive symptoms in people with HIV, including by applying DW-MRS for the first time in this population. This study offers early evidence that worse depressive symptom severity in people with HIV may be correlated with neuroimmunometabolic dysfunction, evidenced by increased diffusion of creatine, likely reflecting hypermetabolism and neuroinflammation. Future research may attempt to overcome some of the limitations of this exploratory study, by replicating these findings in larger and more diverse participant samples and assessing changes in DW-MRS parameters alongside changes in depressive symptom severity longitudinally. Regional differences exist in alterations in metabolite concentrations in the context of depression [231] or HIV [232], and although investigations of metabolite diffusion in these contexts are sparse, it is plausible that this diffusion may also vary by brain region. While this exploratory study focused on the ACC, future studies should thus investigate these imaging parameters in other brain regions implicated in depression, such as the prefrontal cortex or putamen, and consider whole-brain spectroscopic imaging approaches. It will also be useful to compare neurometabolite diffusion between participants with and without HIV who are experiencing clinical depression, to determine whether the DW-MRS signatures of severe depressive symptoms observed in this study may be specific to people with HIV. In summary, intracellular diffusion of neurometabolites as quantified using DW-MRS may be useful, non-invasive, and novel parameters to explore the neurobiological mechanisms underlying depression, including in people with HIV.

11

Discussion

HIGHLIGHTS

- In this research, I found some evidence that inflammation may contribute to the higher risk for depression in people with HIV.
- During this project, I summarised evidence from existing research in the field for other scientists and led the collection of new data in three studies (Chapters 7-10). I also secured funding for this work, carried out testing on blood samples, and analysed data using statistical techniques.
- Results from this research should be interpreted in light of some important limitations, including the small sample sizes (which reduce the confidence with which we can draw conclusions) and the indirect nature of the biomarkers of inflammation measured in these studies.
- Despite these limitations, this research makes some important contributions to our understanding of the role of inflammation in the high risk for depression in people with HIV. Some advantages of this research include involving people with lived experience at different stages, testing the research question in two very different populations, and sharing data and analysis code transparently.
- With further research, the biomarkers highlighted in these studies might allow us to identify a person's risk for depression based on their level of inflammation, or match them to the most appropriate antidepressant medication as early as possible.

11.1 MOTIVATION FOR THIS RESEARCH

MY OVERARCHING GOAL IS TO HELP IMPROVE the health and well-being of people with HIV, many of whom face the compounding challenges of multi-morbidity and socioeconomic deprivation. Mental health issues in this community are associated with considerable excess mortality, independent of HIV treatment success [233]. Depression, in particular, results in significant and disproportionate disease burden amongst people with HIV, who are at three times greater risk for depression and nearly 100 times greater risk for suicide death compared to people without HIV [2, 4].

The research described in this thesis focused on understanding the contribution of inflammation to the high prevalence of depression in people with HIV. In the general population, inflammation is a notable predictor of depression [10, 234]. Given that people with HIV exhibit chronic inflammation even when receiving successful antiretroviral therapy, I hypothesised that inflammation may be a mediating mechanism underlying the co-morbidity of HIV and depression. I tested this hypothesis using clinical research studies involving people with and without HIV. Participants in these studies completed self-report questionnaires which assessed the severity of their depressive symptoms. Inflammation was quantified by measuring inflammatory proteins in the blood or CSF (using immunoassays) and neurometabolites in the brain (using MRS).

11.2 KEY CONCLUSIONS

To underpin the original research described in this thesis, I first synthesised existing evidence on the relationships between inflammation, depression, and HIV using narrative (Chapter 2) and scoping (Chapter 3) reviews. I found that several inflammatory biomarkers have been reported as being associated with depressive symptoms in people with HIV, though findings in this field are heterogeneous. Through my research, I wanted to extend these findings to identify biomarkers which may *mediate* (i.e. contribute mechanistically to) the risk for depressive symptoms, rather than merely being associated with these symptoms. In addition to this evidence synthesis, I also produced scholarship demonstrating approaches to integrating principles of equity and inclusion and meaningful engagement with people with relevant lived experience in this field.

In **COBRA-DI** (Chapter 7), I analysed data from a sample of $N = 204$ older adults based in London and Amsterdam. I found that participants with HIV had a greater risk for depression, but this risk was attenuated by at least 10% when statistically controlling for the effects of four markers of inflammation: IL-6, TNF- α , MIP-1 α , or MIG, though I did not formally test for significance of these mediation effects. These results suggested that these soluble inflammatory biomarkers may be potential mediators of the increased risk for depression in people with HIV. The neurometabolites choline and myo-inositol, measured via MRS in this study as proxies for neuroinflammation, did not mediate this relationship.

I then recruited $N = 60$ young people in Cape Town, 70% of whom were living with perinatally-acquired HIV, to measure depressive symptoms and inflammation in the **GOLD Depression Study** (Chapter 9). In this sample, I also found that participants with HIV reported greater severity of depressive symptoms and exhibited significantly altered concentrations of certain inflammatory markers in blood and in the basal ganglia of the brain. However, using structural equation modelling, I showed that these inflammatory biomarkers did not mediate a significant proportion of the relationship between HIV status and depressive symptom severity in this sample.

To deliver this study in Cape Town, I also developed and validated an **isiXhosa translation of the Patient Health Questionnaire (PHQ-9)** in a pre-registered study (Chapter 8). This translation demonstrated satisfactory reliability, criterion validity, convergent validity with the Youth Self Report form, and construct validity. This isiXhosa PHQ-9 is now freely available for use in research and clinics serving isiXhosa-speaking communities.

Finally, I recruited $N = 20$ participants with HIV for an exploratory study called **INSIDE-HIV** (Chapter 10). This study focused on neuroimaging signatures of severe and persistent depression in people with HIV. Working closely with neuroimaging experts and using an emerging technique called diffusion-weighted magnetic resonance spectroscopy (DW-MRS), I found that the intracellular diffusion of creatine and myo-inositol - which may indicate hypermetabolism and neuroinflammation - were correlated with depressive symptom severity.

Therefore, in my doctoral research, I combined depression screening tools, inflammatory biomarkers, and leading-edge statistical approaches to provide some evidence for a mediating role for inflammation in the risk for depression in people with HIV. In doing so, my research applies the principles of immunopsychiatry - an emerging discipline exploring the contribution of immunological dysfunction to psychiatric conditions - to HIV mental health, building on the intuitive conceptual links between HIV, inflammation, and depression.

11.3 CONTRIBUTION TO KNOWLEDGE GENERATION

In Chapters 2 and 3, I outlined the evidence linking HIV, inflammation, and depression which motivated this research. Following is an updated conceptual framework that integrates the findings described in this thesis into the existing literature.

DEPRESSION AND PERIPHERAL INFLAMMATION IN PEOPLE WITH HIV

In line with previous studies, I found evidence that several soluble inflammatory biomarkers in blood were significantly associated with HIV status in both COBRA-DI (Chapter 7) and the GOLD Depression Study (Chapter 9). In COBRA-DI, the concentrations of CRP, I-FABP, neopterin, NFL, soluble CD14, soluble CD16, soluble CD163, and TNF- α differed significantly between groups, whereas in the GOLD Depression Study, this was the case for IL-1 β , MCP-1, and YKL-40. Interestingly, the inflammatory biomarkers significantly associated with HIV status did not overlap between the two studies. This may perhaps be attributed to the major differences in age, gender, and mode of HIV acquisition across the two cohorts, resulting in different inflammatory profiles. Regardless, the altered concentrations of inflammatory biomarkers in people with HIV observed in these studies is compatible with previous research [235].

I aimed to extend previous work by investigating whether inflammatory biomarkers may mediate (rather than being associated with) depressive symptoms in people with HIV. In COBRA-DI, two inflammatory markers in blood (MIG and TNF- α) were shown to be potential mediators of the risk for depressive symptoms. In the GOLD Depression Study, none of the soluble biomarkers mediated a significant proportion of the relationship between HIV status and depressive symptoms - in fact, none of these biomarkers were significantly associated with depressive symptoms at all. This result is surprising, as associations between depressive symptoms and inflammatory biomarkers have been reported widely, including in adolescents [236, 237]. This relationship had not previously been explored in adolescents with HIV; the findings from the GOLD Depression Study thus suggest that there may not be a strong association between inflammation and concurrent depressive symptoms in this population, but this must be interpreted with caution due to sample size limitations. In total, findings from this research indicate that certain peripheral inflammatory biomarkers may partly mediate the risk for depressive symptoms in people with HIV, though this could not be replicated amongst adolescents.

DEPRESSION AND NEUROINFLAMMATION IN PEOPLE WITH HIV

Concentrations of inflammatory biomarkers in CSF were used as proxy measures of neuroinflammation in the COBRA-DI study (Chapter 7). Here, I found that CSF biomarkers were potential mediators of the risk for depressive symptoms in people with HIV. In fact, the largest magnitudes of the mediation effects observed in this study were for CSF biomarkers (MIP-1 α and IL-6), suggesting a strong mediation effect by these putatively neuroinflammatory biomarkers. No previous studies had explored associations between neuroimaging markers of inflammation and depressive symptoms in people with HIV (Chapter 3). In COBRA-DI and the GOLD Depression Study (Chapter 9), standard MRS was used to quantify concentrations of neurometabolites commonly considered biomarkers of neuroinflammation. These studies revealed that choline and myo-inositol concentrations in a limited set of brain regions largely did not differ based on participants' HIV status, and did not mediate a significant proportion of the association between HIV status and depressive symptoms. The INSIDE-HIV study (Chapter 10), in which diffusion-weighted MRS was used to quantify diffusion of these neurometabolites, showed that depressive symptom severity was positively correlated with creatine diffusion, a possible marker of hypermetabolism and neuroinflammation. Overall, these results suggest that there may be a link between depression and neuroinflammation in people with HIV, particularly as indicated by soluble biomarkers in CSF, though concentrations of neurometabolites in localised brain regions do not seem to contribute significantly to this relationship.

CROSS-CULTURAL VALIDATION OF DEPRESSION SCREENING TOOLS

Robust and reproducible mental health research is contingent upon the quality of self-report measures for mental health outcomes. A major contribution of this research is the psychometric assessment of screening tools for depression. I led the development of the first isiXhosa-language PHQ-9 and validated this translation in a sample of young people in Cape Town, South Africa (Chapter 8). Prior to the development of this translation, research groups and clinicians typically translated the questionnaire *ad hoc* as necessary. The validated, freely available translation of the PHQ-9 produced in this research will ensure that future research arising from the use of the PHQ-9 in amaXhosa communities is standardised and reproducible. Results from the pilot validation study demonstrated that the psychometric performance of this isiXhosa PHQ-9 was comparable to other versions of the PHQ-9. Thus, this

translation can enable reliable comparisons between depression research involving amaXhosa in South Africa and communities in other countries or cultures.

I also conducted a preliminary assessment of the SADS in a UK-based sample (Chapter 10). I found that this screening tool, despite being developed primarily for a Xhosa population, could be useful and relevant in communities outside of South Africa as well. Through this study, I have shown that certain idiomatic expressions of depressive symptoms from one culture can still perform satisfactorily in a self-report measure in a different culture. This work lays the foundation for further development of the SADS as a depression screening tool with potentially global applications to assess culturally-specific and culturally-agnostic features of depression.

11.4 LIMITATIONS

Limitations of each study included in this thesis have been described in detail within each chapter. Here, I discuss some overarching limitations of this programme of research.

SAMPLE SIZE

A major limitation of this research - and one which has been highlighted consistently through all previous chapters - is the relatively small sample size in each study. Small research samples may lead to an under- or over-estimation of true effects, and thus partly contribute to the replication crisis in psychological and bio-behavioural research [238]. COBRA-DI (Chapter 7) included data from 204 individuals, but the expanded panel of soluble inflammatory markers was available for only 78 of these participants. The GOLD Depression Study (Chapter 9) recruited a total of 60 young people, whereas INSIDE-HIV (Chapter 10) involved 20 participants. Due to these small sample sizes, these studies lacked adequate statistical power to reliably detect associations of small magnitudes. As discussed in Chapter 9, this lack of statistical power resulted in low precision in point estimates for mediation effects in my research, which may have led to an inability to reject the null hypothesis even when the magnitudes of these effects were reasonably large.

This lack of statistical power is a common issue in both HIV mental health and immunopsychiatry research. HIV mental health studies often involve small sample sizes as the target population may be relatively small and hard to reach, whereas immunopsychiatry research can be limited in its scale by the high costs of inflammatory biomarker measurements [239]. Being

at the intersection of these fields, my research was similarly underpowered, with one of the primary reasons for the small sample sizes in the GOLD Depression Study and INSIDE-HIV being financial resources. Although I secured independent funding from the Wellcome Trust for my PhD research and supplemented this further with minority funding from other organisations, the available budget only allowed for smaller-scale investigations of inflammation and depression in participants with HIV. Future studies with larger participant cohorts may enable the estimation of these mediation effects with greater precision, resulting in increased reproducibility. Thus, well-powered investigations are needed to accurately characterise the association between inflammation and depression and help address the replication crisis in psychological research.

STUDY DESIGN

A key limitation of this research is that each of the studies described in this thesis were cross-sectional and observational by design, resulting in an inability to infer causality or temporality. The research question at the centre of this thesis is inherently causal: I hypothesised that HIV leads to inflammation, and this inflammation leads to depression, suggesting a causal chain of events [240]. However, truly establishing such causality would require experimental manipulation, for instance in a randomised controlled trial where inflammation is manipulated using immunomodulators to establish whether reducing inflammation in people with HIV results in a reduced risk for depression over time. While these experimental approaches can offer more direct causal evidence, they require extensive funding and time to follow participants long-term. An alternative approach may be to use observational studies to longitudinally follow large participant cohorts, including people at risk of (but not living with) HIV. These studies may provide some evidence on whether inflammation precedes the development of depressive symptoms in people with HIV. Following participants at risk of acquiring HIV may also enable us to determine the time lag between a person acquiring HIV, inflammation arising due to HIV infection, and any subsequent depressive symptoms. These studies would again require long follow-ups and repeated biomarker measurements, which require high financial investment. For these reasons, my research was limited to a cross-sectional, observation design with the aim of establishing preliminary evidence to support future longitudinal or experimental studies.

DEPRESSION AND INFLAMMATORY MEASURES

Throughout this thesis, the PHQ-9 was used as the primary measure of depressive symptom severity. The PHQ-9 has several advantages: it is widely used in research (thus enabling direct comparisons between studies), brief and easy to administer, and well-validated to detect symptoms of depression in a range of populations. However, the PHQ-9 is not a diagnostic tool, and thus cannot be used to directly determine whether an individual has clinical depression. Therefore, the use of screening tools such as the PHQ-9 in this research, as opposed to clinical diagnostic tools such as the Structured Clinical Interview for DSM-V (SCID-5), limit the conclusions that can be drawn from these studies about the role of inflammation in clinical depression amongst people with HIV.

Another limitation of this research is the measurement of inflammation. Inflammation is an umbrella term for a broad set of physiological responses to challenges such as infection or injury, which involves a wide range of immune cells (e.g. macrophages and lymphocytes) and signalling molecules (e.g. cytokines and chemokines) [241]. Ways of quantifying inflammation are correspondingly wide-ranging. In this thesis, the concentrations of cytokines, chemokines, and other proteins in blood were used as inflammatory biomarkers. Although these proteins can be quantified with ultra-high sensitivity (at the pg/mL scale), their concentrations are known to exhibit high intra-individual variability [242, 243]. Cytokines such as IL-6 and IL-1 β have been shown to vary considerably even within the span of a single day, and are thus more suited to assessments of acute inflammation than chronic inflammation [244]. Additionally, while these proteins can enable an assessment of peripheral inflammation, there is restricted cross-talk between the immune system of the CNS and the periphery, and thus blood proteins can provide very limited information about neuroinflammation [245]. This limitation can be partly mitigated by measuring these proteins in CSF, which more directly interacts with the CNS, as was done in COBRA-DI (Chapter 7). However, CSF biomarkers present further challenges, as lumbar punctures required to obtain CSF samples are highly invasive and difficult to ethically justify as research procedures in otherwise healthy participants. Therefore, measurements of soluble inflammatory proteins in blood or CSF can provide only limited insight into long-term inflammation or neuroinflammation.

Neuroimaging may allow for more direct assessment of neuroinflammation, but these techniques rely on non-specific markers of neurobiological processes. When using standard MRS, concentrations of choline and myo-inositol are considered to represent glial cell ac-

tivation, since these metabolites are expressed in significantly higher concentrations in glial cells than in neurons [188]. Nevertheless, connecting concentrations of these metabolites to neuroinflammation relies on an assumption that these concentrations change (only) when glial cells are activated, which has been challenged in the literature [208]. Additionally, the choice of brain region in which these markers are quantified may critically influence the findings, as concentrations of neurometabolites are not consistent across brain regions and their associations with depression, for instance, also vary by the region of interest [231]. The diffusion of these and other neurometabolites may also be quantified using apparent diffusion coefficients (ADCs) calculated from DW-MRS spectra, but as this is a relatively new technique, what physiological or pathological processes these ADCs represent precisely remains as yet unclear. Therefore, a key limitation of using neuroimaging biomarkers in this research is that the biological responses captured by changes in neurometabolite concentrations or diffusion are not fully established.

11.5 STRENGTHS

Despite these limitations, this research makes some important contributions to the literature by virtue of several key strengths. First, the statistical approach used in this thesis was robust and conservative. In these studies, I focused on magnitudes of effects (rather than only *p* values), used bootstrapped confidence intervals to compensate for small sample sizes, corrected for multiple comparisons, and conducted appropriate sensitivity analyses to account for potential confounding. These statistical techniques were intended to ensure that effect sizes were estimated with the greatest possible confidence and the likelihood of Type I (false positive) errors was minimised [246]. The reproducibility of this research is further supported by the adoption of best practices in open science, such as the Registered Report format for the validation of the isiXhosa PHQ-9. For all studies except COBRA-DI (where data ownership was governed by the cohort leadership), data and analysis code associated with this research were also shared openly with peer reviewers and alongside publications, allowing for independent review and replication of the findings. Therefore, my approach has sought to ensure that results from this research are robust and reproducible.

Throughout this thesis, I have highlighted issues of inequity in participant samples in HIV mental health, including in studies of inflammation and depression in people with HIV. A key strength of my research is that I delivered the first study investigating inflammation and

depression in young people with HIV, with a sample that was more representative of the global population of people with HIV (i.e. primarily Black/African and girls). I also used similar methods across two sociodemographically distinct samples (COBRA-DI and GOLD Depression Study) to assess whether any associations observed in one sample may be reproducible in another. Crucially, I observed mediation effect sizes close to or over the threshold of 10% for the chemokine MIG in blood amongst older adults with HIV in Europe (15.0%, Chapter 7) and young people with perinatally-acquired HIV in South Africa (9.8%, Chapter 9). This is an important and promising discovery, since finding comparable mediation effects for a biomarker across these disparate samples lends considerable support to the hypothesis that there may be shared neurobiological mechanisms partly driving the risk for depression in people with HIV across diverse sociocultural contexts.

11.6 DIRECTIONS FOR FUTURE RESEARCH

Findings from this research may be used to inform a diverse range of future investigations. First, given the diversity of inflammatory biomarkers under investigation in this field, it is critical to improve our understanding of the fundamental biological responses to HIV infection. Using cellular models, future research may aim to characterise the inflammatory proteins released by different cell types in the CNS in response to acute or chronic HIV infection. This evidence will be useful to inform the choice of inflammatory biomarkers measured in future studies, as researchers may prioritise those biomarkers which are confirmed to be released by microglia or astrocytes when infected by HIV *and* which are known to be elevated in people with depression. A recent study has approached this question creatively by collecting blood serum from people with HIV and varying degrees of depressive symptom severity, and exposing cultured human astrocytes to this blood serum [247]. This study found that cells exposed to serum from people with more severe depression exhibited decreased mitochondrial activity, suggesting dysregulated homeostasis in these astrocytes. Future research may leverage advances in *in vitro* models, including primary glial cells derived from patients, neuronal and glial co-cultures, and three-dimensional human brain organoids incorporating HIV-infected microglia to fully understand which peripherally-detectable markers may represent an HIV-related inflammatory response in the CNS [248–250].

Since *in vitro* models offer limited utility for investigating psychiatric outcomes, animal models may be used to extend these findings and better characterise the relationship between

inflammation and depression in the context of HIV. Key advantages of rodent models of HIV infection include the ability to directly measure and manipulate neuroinflammation in brain tissue, which is not often possible in humans. Using HIV transgenic rodent models, future research may thus test the effects of experimental inhibition of neuroinflammation on depression-like behavioural phenotypes. For instance, one group explored this in a mouse model by blocking MCP-1 in the hippocampus, which did not rescue depression-like behaviours in the animals [251]. More *in vivo* studies such as this targeting the expression of key cytokines such as IL-6 and TNF- α - shown in this thesis to be potential mediators of depression risk in people with HIV - could provide strong experimental evidence for a mediating role for these biomarkers. Additionally, as DW-MRS has been successfully performed in rodents previously, future research may seek to reveal the neurobiological correlates of changes in DW-MRS signals such as creatine or myo-inositol ADC. The biological significance of alterations in these ADCs (observed in Chapter 10 of this thesis) are currently unclear, but may be better understood by direct observation of microglial or astrocytic reactivity in brain tissue alongside DW-MRS signals [252].

The research in this thesis also paves the way for a host of clinical research studies to clarify the mediating role of inflammatory biomarkers in co-morbid HIV and depression. Future research should involve larger and more diverse participant cohorts and resolve the directionality of the association between inflammation and depression through longitudinal observational studies [253]. The precise role of HIV-related inflammation in subsequent depression risk may also be clarified using studies comparing young people with perinatally-acquired HIV (who may have thus experienced inflammation through critical neurodevelopmental periods), young people perinatally exposed to (but not living with) HIV, and young people with recently-acquired HIV [5, 254, 255].

In Chapter 3, I highlighted the lack of age, sex/gender, geographic, and clinical diversity in participant samples in this field. Participants in the majority of studies included in the scoping review were male, older, located in the US, and virally-suppressed. However, demographic factors such as age or sex/gender can significantly influence inflammation. For instance, older individuals exhibit immune dysregulation and increased inflammation [256], whereas resolution of the immune response after an inflammatory challenge may be more accelerated in women [257]. Additionally, significant sex differences in the relationship between inflammation and depression have previously been reported [258]. Geographic location, via differences in diet, nutrition, or adverse exposures such as air pollution, may also

influence level of inflammation or depression [259–262]. Finally, HIV-related clinical factors such as degree of viral suppression and choice of ART regimen can result in different inflammatory profiles [263, 264]. These demographic and clinical factors may thus moderate the associations between HIV, inflammation, and depression. However, these complex relationships cannot be appropriately characterised without research involving diverse participant samples that enable comparisons of moderation or mediation effects across these risk factors. Future studies exploring these relationships in people with HIV should thus test for interactions with relevant demographic and clinical factors.

To address some of the design limitations of the studies described in this thesis, I have designed a new study in collaboration with the African Cohort Study (AFRICOS). AFRICOS is an active longitudinal (2013-present) cohort of people with HIV in Nigeria, Kenya, Uganda, and Tanzania [265]. Data available in this cohort include inflammatory biomarkers in blood at baseline (enrolment) and the Center for Epidemiologic Studies Scale for Depression (CES-D) administered at 11 consecutive annual study visits for $N = 2240$ people with HIV. I will analyse this longitudinal data to test whether baseline concentrations of inflammatory biomarkers are significant predictors of an increase in CES-D score over time. I will also explore whether sociodemographic factors such as sex or socioeconomic status may moderate any associations between baseline inflammation and future depression. This study will represent the largest longitudinal analysis of inflammatory depression in people with HIV to date, thus addressing the major challenge of low statistical power in this field. Additionally, this study will also add much-needed geographic, gender, and ethnic diversity to this field, as participants in the AFRICOS Cohort are primarily Black/African and women.

To address some of the limitations of neuroimaging markers of inflammation used in this thesis, Prof. Jaime Vera and I are now extending the INSIDE-HIV study (Chapter 10). In this extension, the same participants will be invited to return for another scanning visit, which will include depression screening via the PHQ-9, demographic questionnaires, and acquisition of standard and diffusion-weighted MRS in the ACC. For most participants, this return visit will be separated from their original scanning visit by at least a year, so we expect that their depressive symptom severity may have changed in the interim. This extension will thus allow us to (1) determine whether DW-MRS parameters, which are novel and largely unexplored markers, exhibit substantial intra-individual variability over time, and (2) track possible changes in DW-MRS parameters alongside changes in depressive symptoms. Therefore, this study may allow us to better characterise the DW-MRS parameters measured in

INSIDE-HIV and their relationship with depressive symptoms.

Finally, a major output of my doctoral research - and one which contributed to my 2023 Diversity Prize - was the isiXhosa-language PHQ-9. This questionnaire is already being used by research groups and private clinics for rapid depression screening in Cape Town. Together with Prof. Heather Whalley, I have now secured funding to develop an app-based version of this isiXhosa PHQ-9, since we observed that many participants preferred for this questionnaire to be read aloud to them rather than reading it themselves. This app-based version will include audio recordings of all PHQ-9 items in isiXhosa, and is planned to be made available publicly and for free starting in August 2025. By developing this app-based version, I hope to expand access to this isiXhosa PHQ-9 to individuals with low literacy levels, reduce staff burden, and standardise the administration of this questionnaire across research groups and clinics. Future research may focus on comparing the performance of the isiXhosa PHQ-9 between the traditional pen-and-paper format and the app-based format, and exploring the economic feasibility of integrating this questionnaire into regular mental health screening in public healthcare institutions serving isiXhosa speakers.

11.7 SUMMARY AND POTENTIAL IMPACT

In this thesis, I have investigated whether inflammation may be a mediator for the increased risk for depression in people with HIV. This research was field-leading, as many of the questions I asked in these studies had not yet been tested. For instance, while several studies had explored associations between inflammation and depression in people with HIV, none had previously attempted to test whether inflammation may be a *mediating* mechanism, and similarly none had previously investigated these effects in young people with perinatally-acquired HIV. Additionally, I have produced evidence synthesis that will inform future research in this field and published theoretical and empirical articles to advance best practices in clinical research in HIV mental health. Given the novelty of this programme of research, and the limitations arising from its scale and study design, the findings from this research are preliminary. As is so often the case in clinical observational research, there is no neat set of experiments and no clear answer, only an incremental accumulation of evidence. In this spirit, this thesis provides some evidence that inflammation may indeed contribute significantly to depression in people with HIV, upon which future research must build to approach a thorough understanding of the complex relationships between these conditions.

With further validation, the biomarkers identified as potential mediators in this research may inform prediction and intervention for depression in people with HIV. Blood samples are collected routinely for HIV care and assessment of many inflammatory biomarkers in blood is already commercially feasible. If future longitudinal studies can extend the current research to establish the directionality of the relationships between blood biomarkers and depressive symptoms in people with HIV, these findings may support the use of inflammatory proteins such as IL-6, TNF- α , MIG, and MIP-1 α to predict a person's risk for depression in the near future. Characterising the potential role for inflammation in depression may also help match people to the right antidepressant treatment as quickly as possible. Elevated concentrations of inflammatory biomarkers in people with HIV and co-morbid depression may suggest an inflammatory subtype of depression, and thus help identify individuals who would benefit from anti-inflammatory treatments for depression, rather than other forms of antidepressants. Personalising treatment in this way may reduce the number of medications that an individual needs to try before they find one that works for them, and reduce the time it takes to identify this right treatment.

Finally, this “case study” in HIV may also inform future research into inflammatory depression more broadly. As people with HIV experience both chronic inflammation and high rates of depression, determining the role of inflammatory biomarkers in depression in this community may help advance a clinical model for detection and treatment of inflammatory depression that may extend more widely to people with other sources of chronic neuroinflammation, such as multiple sclerosis or Alzheimer's disease. To build on HIV as a potential model for inflammatory depression, future research may seek to identify people with HIV who may exhibit a possible inflammatory sub-type of depression. This may be achieved using screening and diagnostic tools in tandem with statistical or machine learning approaches to identify symptom clusters indicative of inflammatory depression, as has been attempted in some recent studies [266]. Studies investigating clinical outcomes such as non-response to standard antidepressants and symptom persistence or relapse as indicators of inflammatory depression are also warranted. By implementing these clinical markers together with inflammatory biomarkers as confirmatory measures, we may develop effective approaches for detecting inflammatory depression in individuals (including people with HIV) presenting with depressive symptoms in the clinic. In summary, my research provides early evidence which may in future support the implementation of immunopsychiatry approaches in people with HIV, and ultimately help reduce the burden of depression in this community.



isiXhosa PHQ-9 Stage 1 Registered Report

The following Stage 1 Registered Report was published as a public registration of our methods and statistical analysis plan for the development and validation of the isiXhosa-language PHQ-9. Results from this pilot validation study were reported in Chapter 8, alongside any deviations in data collection or analysis from the Stage 1 report.

REGISTERED REPORT: *FRONTIERS IN PSYCHIATRY*



isiXhosa Translation of the Patient Health Questionnaire (PHQ-9): A Pilot Study of Psychometric Properties [Stage 1]

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Depression is a debilitating illness, and stigma associated with it often prevents people from seeking support. Easy-to-administer and culturally-specific diagnostic tools can allow for early screening for depression in primary care clinics, especially in resource-limited settings. In this pilot study, we will produce the first open-access isiXhosa-language version of the nine-item Patient Health Questionnaire (PHQ-9), a well-validated measure of depression incidence and severity, using a transcultural translation framework. We will validate this isiXhosa PHQ-9 in a small sample of adolescents living with HIV in Cape Town, South Africa who speak isiXhosa at home. Participants have previously completed the ASEBA Youth Self Report (YSR) form, and responses from the YSR will be used as a gold standard to validate the isiXhosa PHQ-9. If validated through this Registered Report, this isiXhosa PHQ-9 may be an invaluable culturally-specific tool for clinicians serving Xhosa people in identifying clinical or sub-clinical depression.

Keywords: HIV-associated depression, major depressive disorder (MDD), psychometric testing, translation, multicultural tools

1. INTRODUCTION

“If I show you where I’m struggling, I feel you have been exposed to my weakness,” said Siyanda, a young Xhosa man, in relation to the cultural expectation for Xhosa men to manage mental health issues without seeking support (1, p.56). The constellations of behavioral and somatic issues that comprise major depressive disorder (MDD, or depression) are estimated to affect between 4.4 and 5.0% of people globally (2, 3). Biological and psychosocial factors can predispose individuals to developing MDD (4). Living with a chronic HIV infection, especially, significantly increases the risk of depression (5), with prevalence of depression amongst people living with HIV estimated to be as high as 50% (6, 7). Given the stigma associated with depression across cultures, people with depression may struggle to access mental healthcare resources, as Siyanda suggests. However, early referral and interventions have substantial benefits for the treatment and prevention of depression (8, 9). It is therefore imperative to continue to refine tools for early screening of depression and increase their accessibility in primary care clinics.

The Xhosa people comprise a diverse cultural group who speak variations of isiXhosa. The vast majority of Xhosa people live in the Eastern Cape and Western Cape provinces of South Africa (10). Over the last three centuries, European colonial expansion into the South African heartland occurred at the expense of indigenous populations, including Xhosa people, culminating in systematic disenfranchisement during the apartheid regime (11, 12). Under the apartheid

government, especially, Xhosa people suffered severe marginalization as Afrikaans was the favored language in government and education in place of indigenous languages such as isiXhosa (13). As a result of these institutional barriers, it is difficult for people to access quality healthcare in isiXhosa today (14, 15). In order to reduce this healthcare inequity faced by Xhosa people, it is crucial that clinical resources are made available to them in their home language.

There is no word for “depression” in isiXhosa, but that does not mean that this debilitating illness is not found amongst the Xhosa people. In fact, the prevalence of depression in this group, estimated at 6.9%, is significantly higher than global estimates for depression in the general population (16). Substantial intra-group variability exists, with, for instance, a significantly higher prevalence rate of 31.9% amongst women in Khayelitsha during pregnancy and 12 weeks postpartum (17). Despite this, few isiXhosa-language tools exist for diagnosis of depression. An early study translated the Edinburgh Postnatal Depression Scale (EPDS) into isiXhosa and found satisfactory internal consistency (18), but this scale is limited in its target population. Another study validated an isiXhosa version of the Centre for Epidemiological Studies Depression Scale in a more general population group (16). The ongoing development and validation of the 16-item South African Depression Scale (SADS) in isiXhosa is particularly promising, especially for Xhosa people living with HIV (19, 20). However, the trade-off in developing such a tool is that limited comparisons can be made between scores from the SADS and other established scales until it is validated in other countries and cultures, thereby restricting synthesis of results across global studies.

The nine-item Patient Health Questionnaire (PHQ-9) is a useful measure for depression incidence and severity (21, 22). It has been validated as a diagnostic tool in clinical samples (23) as well as the general population (24), where it shows a high degree of convergent validity with other depression scales such as the Beck Depression Inventory (BDI). Furthermore, the PHQ-9 is easy to administer, especially in resource-limited settings (25), and exhibits similar results whether it is self-administered or carried out by an interviewer (26, 27). It is shown to be useful to screen for depression in both African populations (28) and people living with HIV (29, 30). Numerous validation studies for translations of the PHQ-9 have demonstrated its value as a depression scale in samples across the world (31–33). Given this global body of evidence, the PHQ-9 represents a measure of depression that could facilitate cross-cultural comparisons of depression pathology better than population- or geography-specific scales such as the EPDS or SADS. Despite these many advantages, to the best of our knowledge, an isiXhosa-language version of the PHQ-9 has not yet been validated and made publicly available. [Baron et al. (16) report findings from an isiXhosa version of the PHQ-9, but this version is not available alongside the study except by request to the authors.] Given the utility of the PHQ-9 in rapid screening of depressive symptomatology, a freely-available isiXhosa-language PHQ-9 may be an invaluable mental health triage tool for clinicians serving Xhosa people.

In this pilot study, we aim to produce and validate the first open-access isiXhosa-language version of the PHQ-9 depression scale. This version will be produced using a transcultural translation framework and administered to a cohort of adolescents living with HIV in Cape Town, South Africa. Responses on the PHQ-9 will be compared against those on the ASEBA Youth Self Report (YSR) forms as a gold standard. Our primary hypothesis is that the isiXhosa-language PHQ-9 will exhibit satisfactory reliability, measured as internal consistency using Cronbach's α . We also hypothesize that this translation of the PHQ-9 will show acceptable convergent validity (Pearson's correlation coefficient for PHQ-9 and YSR scores) and diagnostic accuracy (area under Receiver Operating Characteristic curve for PHQ-9 vs. YSR). This pilot study may pave the way for larger-scale validation studies of this isiXhosa-language PHQ-9 and add an easy-to-administer, culturally-specific questionnaire to the local clinician's toolbox.

2. METHODS

2.1. Participants

Participants for this study will be recruited through the Adolescent Cognitive and Brain Imaging Study (“the GOLD study”) at Stellenbosch University and Tygerberg Hospital in Cape Town, South Africa. The GOLD study, which draws on the cohort of participants in the landmark CHildren with Early antiRetroviral therapy (CHER) trial, includes adolescents living with HIV who were initiated on antiretroviral therapy (ART) early in life (34). The CHER cohort is active and regularly willing to contribute to sub-studies, with as many as 80 children living with HIV and 80 age-matched HIV- controls participating in recent sub-studies (35).

2.1.1. Inclusion Criteria

Inclusion criteria for participants will be: HIV+ status, currently receiving antiretroviral therapy, with plasma HIV RNA < 40 copies per mL (indicating viral suppression), and speaking isiXhosa at home.

2.1.2. Sample Size Estimation

A priori estimation of sample size in validation studies for psychometric tools is remarkably low (36). We utilized a web-based sample size calculation tool for reliability studies (37), available at this link, with the primary outcome of interest as the Cronbach's α reliability coefficient for the translated PHQ-9. The *a priori* sample size estimation showed that a sample size of $N = 19$ would be necessary to detect a Cronbach's $\alpha = 0.65$ (indicative of a moderate reliability) at 80% power for the nine-item questionnaire. Therefore, we plan to recruit 20 participants for this pilot study.

2.1.3. Ethical Considerations

We will receive written informed assent from participants and written informed consent from participants' parents or legal guardians in their home language for inclusion in the study before participation. The study will be conducted in accordance with the Helsinki Declaration and Good Clinical Practice (GCP)

standards. The protocol for this study will be approved by the Stellenbosch University Human Research Ethics Committee (N21/10/116_Substudy N19/10/135).

2.2. Materials

2.2.1. Patient Health Questionnaire (PHQ-9)

The English-language version of the PHQ-9 was designed with slight adaptations from the original version (21). The adaptations, intended primarily to improve comprehensibility of the scale to adolescents in 2022, were as follows: in item 7, we replaced “reading the newspaper” with “reading,” and in the final question, we replaced “if you checked off *any* problems” with “if you chose a number higher than 0.” The isiXhosa-language version of the PHQ-9 will be created from this English version using a transcultural translation framework (see Procedure). Both the English-language and isiXhosa-language versions of the PHQ-9 will be freely available in Supplementary Materials.

2.2.2. Youth Self-Report (YSR) Form

The Achenbach System of Empirically Based Assessment (ASEBA) Youth Self-Report (YSR) form (38) measures behavioral issues representing syndromes such as “withdrawn/depressed,” “thought problems,” and “rule-breaking behavior.” The YSR has been validated as a measure of behavioral issues among adolescents (39), including in several studies in sub-Saharan Africa (40). In the current study, we will use data obtained from participants in the GOLD study during previous clinic visits when a validated bilingual (English and isiXhosa) YSR form was administered via an interview by a trained Research Assistant. During these visits, participants were asked (in the language in which they are most comfortable, English or isiXhosa) whether they think they have exhibited any of the behaviors in question over the past 6 months. These responses will be used as a gold-standard to compare with the translated version of the PHQ-9. Participants’ most recent YSR scores will be used, and the YSR and PHQ-9 will be administered as closely as possible, and not more than 3 months apart. T scores for the “Withdrawn/Depressed” component within Syndrome Scales and “Affective Problems” component within DSM-Oriented Scales produced using YSR responses will be used to determine participants’ depressive symptoms and classify participants as “clinically depressed,” “borderline,” or “non-depressed” using ASEBA standards. No new YSR responses will be collected for this study; we will use data from the most recent clinic visit.

2.3. Procedure

2.3.1. Setting

The study site for participant recruitment and data collection will be: Family Centre for Research with Ubuntu (FAMCRU), Ward J8, Tygerberg Hospital, Department of Paediatrics and Child Health, Faculty of Medicine and Health Sciences, Stellenbosch University. The basic infrastructure necessary for participant recruitment and data collection is already in place at FAMCRU.

2.3.2. Transcultural isiXhosa Translation of the PHQ-9

The process of transcultural translation of psychometric tools involves steps to ensure that translated questionnaires remain

accurate, relevant, and culturally acceptable (41, 42). We will adopt this systematic methodology to translate the PHQ-9 into isiXhosa using four steps:

1. Translation from English into isiXhosa by two independent bilingual (English and isiXhosa) speakers;
2. Review of isiXhosa translation by mental health experts and clinical care professionals who speak both languages;
3. Review of translation by a co-production panel involving adults living with HIV and adults with a lifetime history of depression; and
4. Blinded back-translation from isiXhosa into English by two additional independent bilingual translators.

The translated version will be refined after each step to preserve the core meaning and purpose of each item and incorporate culturally-specific idioms describing the affective components measured by the questionnaire where possible. Modifications will be reconciled in consultation with the translators and review panel involved in this process. The final isiXhosa-language version of the PHQ-9 will be available in Supplementary Materials.

2.3.3. Co-production

Knowledge co-production, or participatory research methods, involves incorporating insights from individuals with lived experience of the conditions being studied. We will recruit 3–4 isiXhosa-speaking co-producers as a focus group to review and provide feedback on the isiXhosa translation of the PHQ-9 during the initial stages of this study. Up to two adults living with HIV and two adults who have experienced depression in their lifetime will be recruited through the FAMCRU clinic, Stellenbosch University network, and/or Twitter advertisements. These individuals will be invited to review the isiXhosa PHQ-9 after it has undergone review by a panel of experts and caregivers and suggest edits to improve the accessibility, comprehensibility, and cultural specificity of the translation. Co-producers will be reimbursed for their time.

2.3.4. Participant Recruitment and Informed Consent

Participants will be recruited through the GOLD study at Tygerberg Hospital. Adolescents living with HIV who meet the current study’s inclusion criteria will be contacted by FAMCRU research staff to ascertain interest in participation in this study. A trained counselor will discuss the study procedures in person with the potential participant’s parent or legal guardian in their preferred language. Informed consent and assent forms will be available in two versions (English and isiXhosa). Parents or legal guardians of the participants will be asked to read and review the consent form. If participants agree to take part in the current study, written informed consent will be received from parents or legal guardians in advance of the study procedures. All participants will also be required to assent to the study procedures. Participants will be reimbursed R350 for their travel costs to visit the clinic for the study in line with Stellenbosch University policies.

2.3.5. Psychometric Testing

Participants will be provided a private space for psychometric testing. A member of the research staff will brief participants on the procedures involved in the study and receive informed assent. The research staff will then confirm that the participants would prefer to respond to questionnaires in isiXhosa and offer them a choice between filling out the questionnaire themselves in writing or having the research staff ask them the questions verbally (for participants who may not be able to write or have a preference for spoken isiXhosa over written isiXhosa). If participants choose to complete the psychometric testing in writing, they will be asked to fill out a short demographic questionnaire and the translated version of the PHQ-9 in writing (paper and pencil). If participants choose to complete the questionnaire verbally, the member of the research staff will read each item on the questionnaire in a neutral manner and write down the response, without judgement or comment. In either case, the research staff member will be available to the participant for any clarifications to ensure participants understand what each question is asking. Once participants have completed the demographic questionnaire and the PHQ-9, the research team member will verify that all nine items of the PHQ-9 have been completed. If the questionnaire is incomplete, the participant's responses will be excluded. If the responses are complete, the research team member will calculate the total score on the PHQ-9 as the sum of the scores from individual items and input responses into a secure electronic data capture programme (Project RedCap), which includes built-in quality control checks. Data will be handled in accordance with the General Data Protection Regulation (GDPR) Act that informs best practice for personal data storage and transfer in Europe.

2.4. Statistical Approach

All statistical analyses will be performed using SPSS Statistics version 25 and R version 4.0.3. To determine the reliability of the isiXhosa PHQ-9, we will calculate Cronbach's α as a measure of internal consistency for the translated version. Inter-item and item-total score correlations will also be calculated. To determine convergent validity for the PHQ-9, Pearson's correlation coefficients will be calculated for the total PHQ-9 scores and T scores for the "Anxious/Depressed" and "Withdrawn/Depressed" syndrome scales on the YSR. To determine the criterion validity of this PHQ-9 version, we will

compare participant responses on the isiXhosa PHQ-9 with the YSR forms and calculate diagnostic sensitivity and specificity, predictive values (PPV/NPV), likelihood ratios (PLR/NLR), and Youden's Index. A Receiver Operating Characteristic (ROC) curve will be produced for the isiXhosa PHQ-9 and area under the curve (AUC) will be calculated for the PHQ-9 to determine diagnostic performance in comparison to the YSR. Finally, individual item analyses will be conducted to determine whether mean scores on each item of the PHQ-9 differed between depressed, borderline, and non-depressed participants (as determined by the YSR responses).

DATA AVAILABILITY STATEMENT

Data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Stellenbosch University Human Research Ethics Committee (HREC), Study Reference N21/10/116_Sub Study N19/10/135. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

AMRL and BL conceptualized the study together. AMRL designed the study and statistical approach, and wrote the initial draft. BL refined experimental design and reviewed the manuscript. Both authors contributed to the article and approved the submitted version.

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B

SADS Psychometrics in UK Sample

The following Letter reported a subset of results from Chapter 10, involving the pilot study assessing the psychometric properties of the South African Depression Scale (SADS) in a UK-based sample.

LETTER: *BMJ MENTAL HEALTH*

Using the South African Depression Scale (SADS) to measure depressive symptoms in a UK sample

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BACKGROUND

The South African Depression Scale (SADS) was recently developed by Andersen and colleagues^{1,2} as a screening tool for depressive symptoms specific to Xhosa culture. This scale was designed to assess classical symptoms of depression which are also measured by other tools such as the 9-item Patient Health Questionnaire (PHQ-9), including sleeping issues and anhedonia. Additionally, the SADS assesses more idiomatic expressions of depression, such as 'having pain in my heart' or 'carrying a lot of weight on my shoulders', which the PHQ-9 does not capture. The SADS has so far only been validated in South African cohorts of people living with HIV, but as this questionnaire was developed in both English and isiXhosa, it is possible to apply it to populations outside South Africa. Assessing the reliability of the SADS in other countries and cultures may further support the utility of this scale and enable comparisons with studies conducted in populations outside South Africa.

METHODS

In this pilot study, we recruited $n=20$ adults living with HIV (median (IQR) age 50.5 (45.0, 54.0) years, 95% male, 80% self-identifying as White) in Brighton, UK. All participants were residing and receiving their regular HIV-related clinical care in Brighton, and none identified as Xhosa. To determine whether the SADS performed satisfactorily for participants with low or high severity of depressive symptoms, participants were recruited in two groups: low depression severity (PHQ-9 < 7, $n=9$) or high depression severity (PHQ-9 > 15 and a history of receiving antidepressant medication). All participants completed the PHQ-9 and the SADS in English during a single visit. In R v.4.3.2, we assessed the internal consistency of the SADS using Cronbach's α , convergent reliability with the PHQ-9 using Pearson's correlation coefficient (r) and item-level performance using descriptive statistics and item-total correlations. All participants had access to a dedicated HIV mental health service, including psychologists and psychiatrists, and were referred to this service if they exhibited suicide ideation or other symptoms which required referral.

RESULTS

In our UK-based sample, the SADS exhibited high internal consistency, Cronbach's $\alpha=0.975$ (95% CI 0.955, 0.988). Total score on the SADS was strongly and significantly correlated with total score on the PHQ-9 ($r=0.97$, $p<0.001$) (figure 1).

Item-level analysis indicated that most items of the SADS were strongly correlated with the total score (table 1). However, scores on SADS10 ('I had pain in my heart') and SADS11 ('I cried') were skewed towards zero and showed poor corrected item-total correlation ($r=0.67$ for SADS10 and $r=0.48$ for SADS11). Findings remained consistent when only including participants who self-identified as male.

DISCUSSION

We observed high internal consistency for the SADS and high convergent validity between the SADS and PHQ-9 in a small UK-based sample of adults with varying depressive symptomatology. We also found that the SADS exhibited satisfactory item-level psychometric properties in our sample, except for two items (SADS10 and SADS11). Notably, SADS10 ('I had pain in my heart') was originally included in this scale to reflect a culturally specific idiom of distress and showed the highest factor loading among SADS items.¹ Thus, the poor item-total correlation that we observed for this item in our UK sample was somewhat expected, and suggests that SADS10 may be specific to the South African (or more generally, African) context and not 'translate' well to expressions of depressive symptomatology in UK samples.

In our sample, SADS11 ('I cried') exhibited the poorest item-total correlation. Our participant sample was majority male, with all participants except one self-identifying as male. Gender roles, especially in cultures in the Global North, forbid crying for men,³ resulting in a measurable gender-based difference in reporting of crying as a symptom of depression.⁴ It is thus possible that the poor item-total correlation for SADS11 observed in our study was driven by the demographic (sex/gender) composition of our sample rather than being an artefact of cultural context.

Interestingly, all other items on the SADS displayed satisfactory psychometric properties in this pilot study, including SADS16 ('I had deep sorrow'). SADS16 was added to the scale in consultation with isiXhosa-speaking mental health providers, and asks about a component of depression ('isingqala', loosely translated to 'deep sorrow') which is specific to the isiXhosa language.¹ Nevertheless, this item showed a high item-total correlation in our study, suggesting that this idiomatic expression of depression may still be useful for assessing depressive symptoms among English-speaking participants in the UK.



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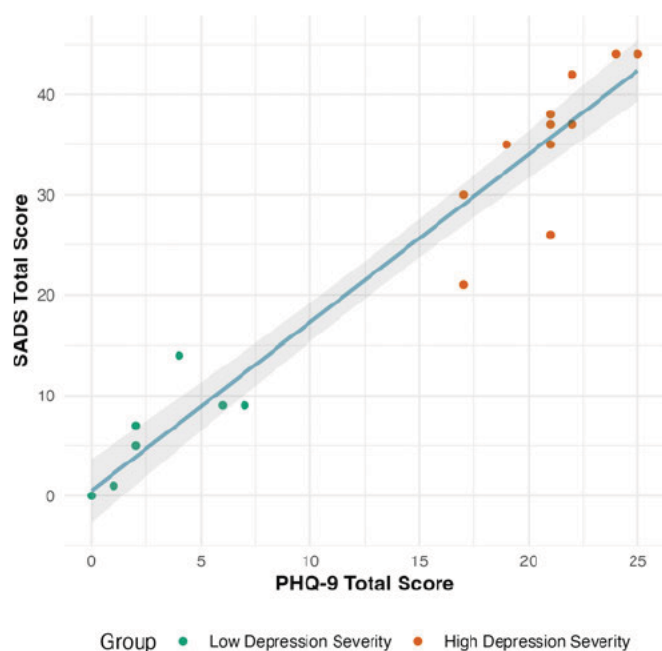


Figure 1 Correlation between total score on the South African Depression Scale (SADS) and total score on the 9-item Patient Health Questionnaire (PHQ-9) for 20 adults living with HIV in Brighton, UK, with 95% confidence interval displayed in grey.

In summary, we administered the SADS in a small sample of adults living with HIV in Brighton, UK, and found that this screening tool performed satisfactorily in the UK context. Our study was limited by a small sample size, and the majority of our participants self-identified as white. Given the small sample size and consequently limited statistical power, this study was also restricted to preliminary psychometric analyses. Despite these notable limitations, this pilot study offers early evidence that the SADS may be useful in the UK context. Establishing the utility of the SADS in populations outside South Africa is important for

Table 1 Item-level analysis of the South African Depression Scale (SADS) for 20 adults living with HIV in Brighton, UK

Item	Mean	SD	Item-total correlation	Corrected item-total correlation
SADS01	1.5	1.24	0.93	0.92
SADS02	1.5	1.19	0.74	0.71
SADS03	1.9	1.25	0.89	0.87
SADS04	1.5	1.28	0.86	0.84
SADS05	1.6	1.19	0.93	0.92
SADS06	1.4	1.35	0.79	0.76
SADS07	1.4	1.14	0.93	0.92
SADS08	1.45	1.32	0.88	0.86
SADS09	1.4	1.23	0.9	0.88
SADS10	0.7	1.03	0.7	0.67
SADS11	0.65	0.93	0.52	0.48
SADS12	1.35	1.09	0.9	0.88
SADS13	1.55	1.23	0.96	0.95
SADS14	1.3	1.38	0.85	0.82
SADS15	1.5	1.32	0.9	0.88
SADS16	1.1	1.21	0.9	0.88

several reasons. First, this evidence can enable direct comparisons between studies of depressive symptoms carried out in South Africa (using the SADS) and outside South Africa (using other tools such as the PHQ-9). Second, it is possible that culturally inclusive tools such as the SADS may be effective at detecting depressive symptoms in communities of African origin residing in the UK and other countries in the Global North. Finally, as suggested by our findings, some idiomatic expressions of depressive symptoms may be understood by people across diverse cultural groups. By applying a screening tool that includes such idiomatic expressions in different populations, we may better distinguish between culturally specific and culturally agnostic features of depression.

Future work may seek to determine whether applying the SADS in other contexts may require excluding the highly culturally specific item SADS10, and whether this tool may be particularly useful for exploring depressive symptoms among African immigrant communities in the UK. In this instance, we did not ask participants directly about their perceptions of the idiomatic expressions of depression included in the SADS, but future studies should explore these perceptions, including through qualitative research methods. Validating the use of this screening tool in larger samples outside South Africa may enable cross-cultural comparisons of depressive symptoms.

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Contributors AMRL and JHV designed the study. GD carried out participant recruitment and data collection, and AMRL carried out statistical analysis, with supervision from JHV.

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Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by the NHS London-Surrey Borders Research Ethics Committee (reference 22/LO/0546). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data generated in this study (anonymised to protect participant identity) and R code used for data analysis are available publicly at <<https://github.com/arishmr/SADS-UK-pilot>>.

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Questionnaires

Studies described in this thesis used the following self-report questionnaires to capture relevant demographic and clinical information and to measure depressive symptoms.

C.1 DEMOGRAPHIC QUESTIONNAIRES

- Demographic questionnaires in English and isiXhosa for the GOLD Depression Study, administered to adolescents living with and without HIV in Cape Town, South Africa, including culturally inclusive questions on gender identity and sexual orientation.
- Demographic questionnaire in English for INSIDE-HIV, including culturally inclusive questions on gender identity, sexual orientation, and ethnicity.

C.2 DEPRESSIVE SYMPTOMS QUESTIONNAIRES

- Nine-item Patient Health Questionnaire (PHQ-9) in English and isiXhosa. Editable versions of these questionnaires are available in the Supplementary Materials for the corresponding Stage 2 Registered Report in *Brain and Neuroscience Advances*, at DOI: [10.1177/23982128231194452](https://doi.org/10.1177/23982128231194452).
- 16-item South African Depression Scale in isiXhosa. This questionnaire was used in INSIDE-HIV (with permission from the authors) from the dual-language (English and isiXhosa) version originally validated in Cape Town and published at DOI: [10.1007/s10461-021-03305-w](https://doi.org/10.1007/s10461-021-03305-w).

GOLD DEPRESSION STUDY

Please answer these questions as honestly as possible. Your answers are confidential, and **we will never share your answers with anyone without your permission.**

Participant # _____

Date _____

1. What is your age?

--

2. What is your grade at school?

--

3. What is your gender?

Boy	Girl	Trans/Genderqueer
Other (please describe):		

4. What is your race or ethnicity?

Black/African	White/Caucasian	Coloured
Other (please describe):		

5. What is your sexual orientation?

Straight/Heterosexual	Gay/Lesbian/Homosexual	Bisexual/Pansexual
Other (please describe):		

6. In the last six months, did you drink any alcohol?

Yes	No
-----	----

If yes, how much alcohol do you usually drink in a week:

7. In the last six months, did you smoke any cigarettes?

Yes	No
If yes, how many cigarettes do you usually smoke in a day:	

8. In the last six months, did you take any recreational drugs (dagga, tik, mandrax, marijuana, ecstasy, cocaine, etc.)?

Yes	No
-----	----

UPHONONONGO LOKUDAKUMBA LWE-GOLD

Nceda phendula le mibuzo ngokunyanisekileyo kangangoko. Iimpendulo zakho ziyimfihlo, **kwaye asoze sabelane ngeempendulo zakho naye nabani na ngaphandle kwemvume yakho.**

Umthabathinxaxheba # _____

Umhla _____

1. Mingaphi iminyaka yakho?

--

2. Ufunda kweliphi ibanga esikolweni?

--

3. Sesiphi isini sakho?

Inkwenkwe	Intombazana	Umntu otshintshe isini
Okunye (nceda chaza):		

4. Uloluphi uhlanga?

Umntu oNtsundu / umAfrika	Umntu / oMhlophe	iKhaladi
Okunye (nceda chaza):		

5. Uzinxulumanisa nesiphi isini?

Ndingothandana nabasini sahlukileyo kwesam	Ndiyigeyi / Ndiyilesbhiyeni / Ndingothandana nabasini sinye nesam	Ndingothandana nabazini ezibini ezahlukeyo

6. Kwiinyanga ezintandathu (6) ezidlulileyo, uke wasela nabuphi na utywala?

Ewe	Hayi
Ukuba ngu-ewe: butywala obungakanani oqhele ubusela ngeveki:	

7. Kwiinyanga ezintandathu (6) ezidlulileyo, ingaba ukhe walitshaya na icuba?

Ewe	Hayi
Ukuba uyavuma, uqhele ukutshaya icuba elingakane ngosuku:	

8. Kwiinyanga ezintandathu ezidlulileyo, ingaba ukhe wasebenzisa naziphi na iziyobisi zokuzonwabisa (intsangu, ithikhi, imandraksi, iekhstesi, ikhokheyini, njl.njl.)?

Ewe	Hayi
-----	------

INSIDE-HIV: DEMOGRAPHICS

Participant Identification Number for this study:

1. What is your age (in years)?

--

2. What was your sex assigned at birth?

Male	Female	Intersex	Other (please specify):
------	--------	----------	-------------------------

3. What is your gender identity?

Man	Woman
Non-binary / third gender	Other (please specify):

4. Do you identify as transgender or gender non-conforming?

Yes	No
Unsure / prefer not to say	

5. What is your sexual orientation?

Gay/lesbian/homosexual	Bisexual/pansexual
Straight/heterosexual	Asexual
Other (please specify):	

6. What is your ethnicity? (You can select more than one)

White – UK	White – Non-UK
Black – African	Black – Caribbean
Arab/North African	East/South-East Asian

South Asian	Latino/Latina/Latinx/Hispanic
Other (please specify):	

7. How many years of education have you had in total?

--

8. Have you smoked cigarettes in the past 3 months?

Yes	No
-----	----

9. If yes – how many packs do you smoke in a week?

--

10. Have you drank alcohol in the past 3 months?

Yes	No
-----	----

11. If yes – how many units of alcohol in a week? A pint of beer and a glass of wine are about 2 units each, and a single serving of a spirit (whiskey, vodka, etc.) is about 1 unit each.

--

12. Have you used any recreational drugs in the past 3 months?

Yes	No
-----	----

13. Are you currently taking antidepressant medication?

Yes	No
-----	----

14. Have you ever taken antidepressant medication, whether now or in the past?

Yes	No
-----	----

15. Which antidepressant medication are you currently taking or have taken in the past?

--

16. Are you currently in therapy or counselling for depression?

Yes	No
-----	----

17. Have you ever been in therapy or counselling for depression, whether now or in the past?

Yes	No
-----	----

PATIENT HEALTH QUESTIONNAIRE (PHQ-9)

Participant # _____

Date _____

Over the *last 2 weeks*, how often have you been bothered by any of the following problems?

(use "✓" to indicate your answer)

	Not at all	Several days	More than half the days	Nearly every day
1. Little interest or pleasure in doing things	0	1	2	3
2. Feeling down, depressed, or hopeless	0	1	2	3
3. Trouble falling or staying asleep, or sleeping too much	0	1	2	3
4. Feeling tired or having little energy	0	1	2	3
5. Poor appetite or overeating	0	1	2	3
6. Feeling bad about yourself – or that you are a failure or have let yourself or your family down	0	1	2	3
7. Trouble concentrating on things, such as reading or watching television	0	1	2	3
8. Moving or speaking so slowly that other people could have noticed? Or the opposite – being so fidgety or restless that you have been moving around a lot more than usual	0	1	2	3
9. Thoughts that you would be better off dead or of hurting yourself in some way	0	1	2	3

ALL NINE ITEMS COMPLETED? Y / N

FOR OFFICE CODING _____ + _____ + _____ + _____

= TOTAL SCORE: _____

If you have chosen a number higher than 0 for any problems, how difficult have these problems made it for you to do your work, take care of things at home, or get along with other people?

Not difficult at all	Somewhat difficult	Very difficult	Extremely difficult
☐	☐	☐	☐

IPHEPHA LEMIBUZO LEMPILO YESIGULANE (PATIENT HEALTH QUESTIONNAIRE) (PHQ-9)

Umthabathinxaxheba # _____

Umhla _____

Kwiiveki ezi-2 ezidlulileyo, ingaba uhlutshwe rhoqo kangakanani yiyo nayiphi na kwezi ngxaki zilandelayo?
(sebenzisa “✓” ukubonisa impendulo yakho)

	Andikhange konke konke	lintsuku eziliqela	Ngaphezulu kweziqingatha seentsuk	Phantse yonke imihl
1. Umdla okanye ubumnandi obuncinci ekwenzeni izinto	0	1	2	3
2. Ukuziva unomoya ophantsi, udakumbile, okanye ungenathemba	0	1	2	3
3. Ukusokola ukulala, okanye ukulala ngokugqithisileyo	0	1	2	3
4. Ukuziva udiniwe okanye ungenamandla kangako	0	1	2	3
5. Ukungacaceli ukutya okanye ukutya ngokugqithisileyo	0	1	2	3
6. Ukuziva kakubi ngawe – okanye ukuba awuphumeleli okanye udanise wena okanye udanise usapho lwakho	0	1	2	3
7. Ingxaki yokuzikisa ingqondo ezintweni, ezifana nokufunda okanye ukubukela umabonakude	0	1	2	3
8. Ukuhamba okanye ukuthetha ngokucothayo kangangokuba abanye abantu babe bakuqaphele oko? Okanye okuchaseneyo noko – ukungqunga okanye ukungazinzi kangangokuba uzulazule kakhulu kunokuqhelekileyo	0	1	2	3
9. Iingcinga zokuba kungcono ubhubhe okanye ukuzenzakalisa ngandlela ithile	0	1	2	3

ZONKE IZINTO EZILITHOBA ZIGQIBEKILE?

UKUFAKWA IKHOWUDI YIOFISI: ____ + ____ + ____ + ____

EWE / HAYI

= INQAKU LILONKE: _____

Ukuba ngaba ukhethe inani elingaphezulu kwe-0 ngazo naziphi iingxaki, ezi ngxaki zenze kwanzima kangakanani kuwe ukuba wenze umsebenzi wakho, ukulungisa izinto ekhaya, okanye ukuvana nabanye abantu

Akunzimanga konke
konke

☐

Kunzinyana noko

☐

Kunzima kakhulu

☐

Kube nzima
ngokugqithisileyo

☐

SOUTH AFRICAN DEPRESSION SCALE (SADS)

Participant # _____

Date _____

Instructions: "I am going to ask you some questions about your thoughts, feelings, and behaviors in the past week. For each question, I would like you to tell me how many days in the past week you have been feeling this way."

IN THE PAST WEEK		
1. Things were not going well in my life	☐ ☐ ☐ ☐ ☐	0 days 1-2 days 3-4 days 5-7 days Decline to answer
2. I had difficulties sleeping	☐ ☐ ☐ ☐ ☐	0 days 1-2 days 3-4 days 5-7 days Decline to answer
3. I was thinking too much	☐ ☐ ☐ ☐ ☐	0 days 1-2 days 3-4 days 5-7 days Decline to answer
4. I did not want to be around people	☐ ☐ ☐ ☐ ☐	0 days 1-2 days 3-4 days 5-7 days Decline to answer
5. I was feeling tired or without energy	☐ ☐ ☐ ☐ ☐	0 days 1-2 days 3-4 days 5-7 days Decline to answer
6. I was not my usual self	☐ ☐ ☐ ☐ ☐	0 days 1-2 days 3-4 days 5-7 days Decline to answer
7. I was carrying a lot of weight on my shoulders	☐ ☐ ☐ ☐ ☐	0 days 1-2 days 3-4 days 5-7 days Decline to answer
8. I had stress	☐ ☐ ☐ ☐ ☐	0 days 1-2 days 3-4 days 5-7 days Decline to answer
9. I did not feel like talking	☐ ☐ ☐ ☐ ☐	0 days 1-2 days 3-4 days 5-7 days Decline to answer

10. I had pain in my heart	☐	0 days
	☐	1-2 days
	☐	3-4 days
	☐	5-7 days
	☐	Decline to answer
11. I cried	☐	0 days
	☐	1-2 days
	☐	3-4 days
	☐	5-7 days
	☐	Decline to answer
12. I was irritated easily	☐	0 days
	☐	1-2 days
	☐	3-4 days
	☐	5-7 days
	☐	Decline to answer
13. I was sad	☐	0 days
	☐	1-2 days
	☐	3-4 days
	☐	5-7 days
	☐	Decline to answer
14. I had no appetite or I ate more than usual	☐	0 days
	☐	1-2 days
	☐	3-4 days
	☐	5-7 days
	☐	Decline to answer
15. I was not interested in the things I used to like doing	☐	0 days
	☐	1-2 days
	☐	3-4 days
	☐	5-7 days
	☐	Decline to answer
16. I had deep sorrow	☐	0 days
	☐	1-2 days
	☐	3-4 days
	☐	5-7 days
	☐	Decline to answer

When did you start feeling this way?

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ALL 16 ITEMS COMPLETED? YES / NO

TOTAL SCORE: _____



INSIDE-HIV Supplementary Materials

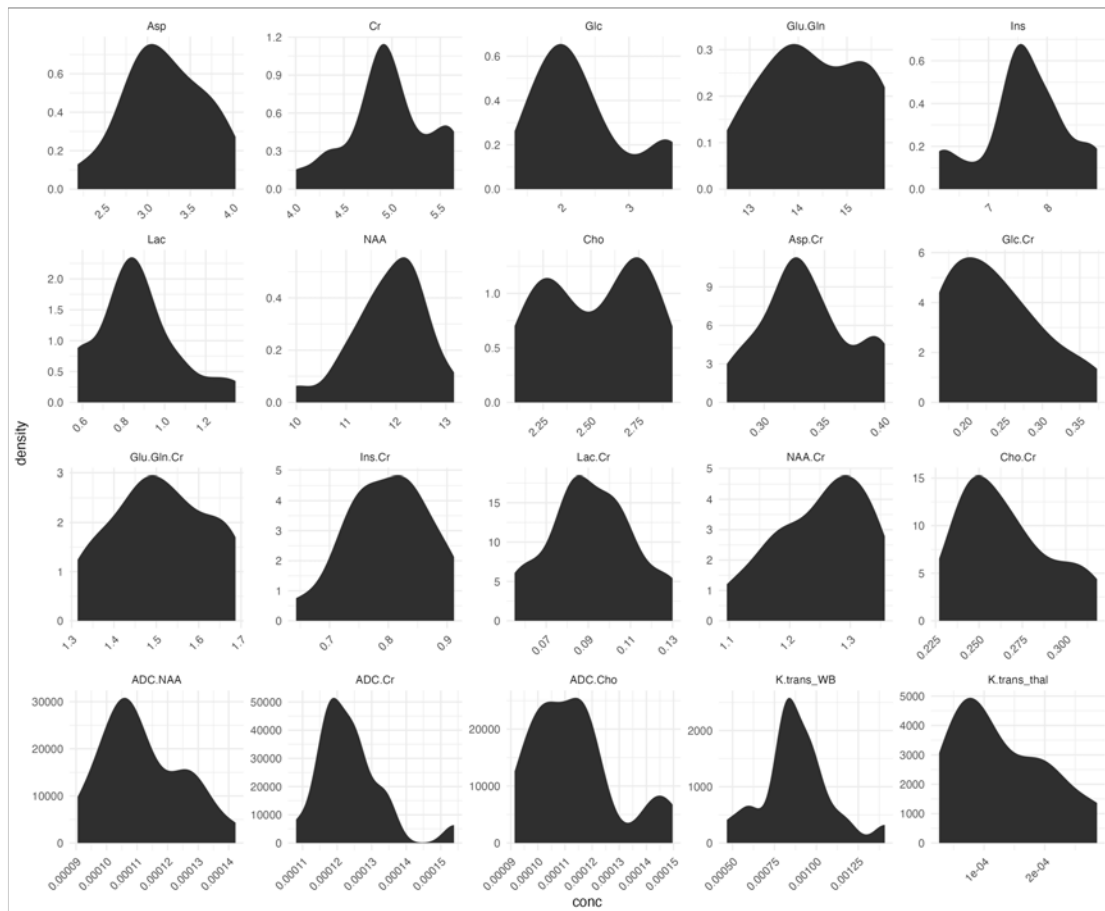


Figure D.1: Distribution of neuroimaging outcome variables.

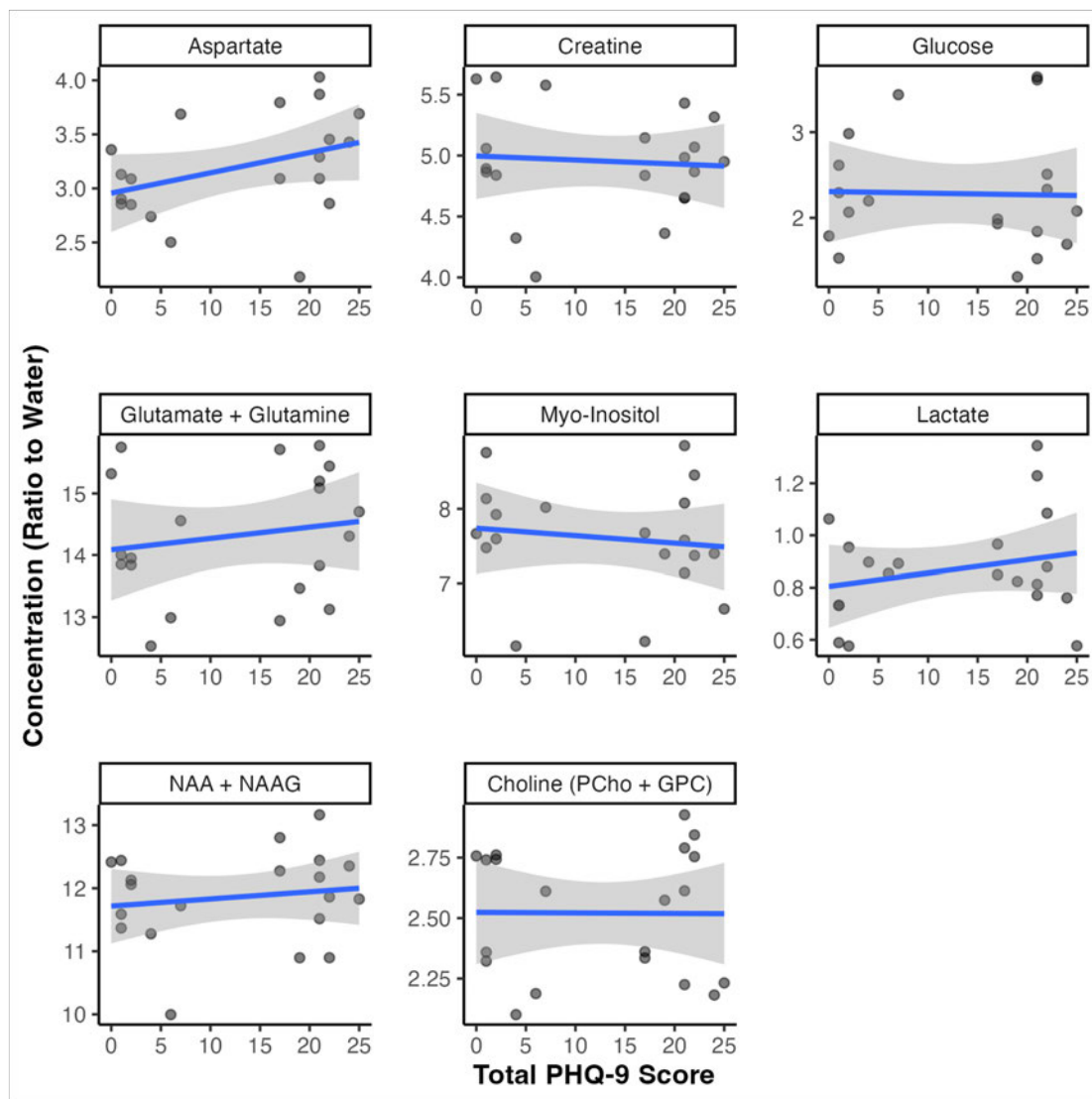


Figure D.2: Neurometabolite concentrations (relative to water) and depressive symptom severity. Correlation between measures of water-referenced neurometabolite concentrations in the anterior cingulate cortex quantified using magnetic resonance spectroscopy and depressive symptom severity (total PHQ-9 score).

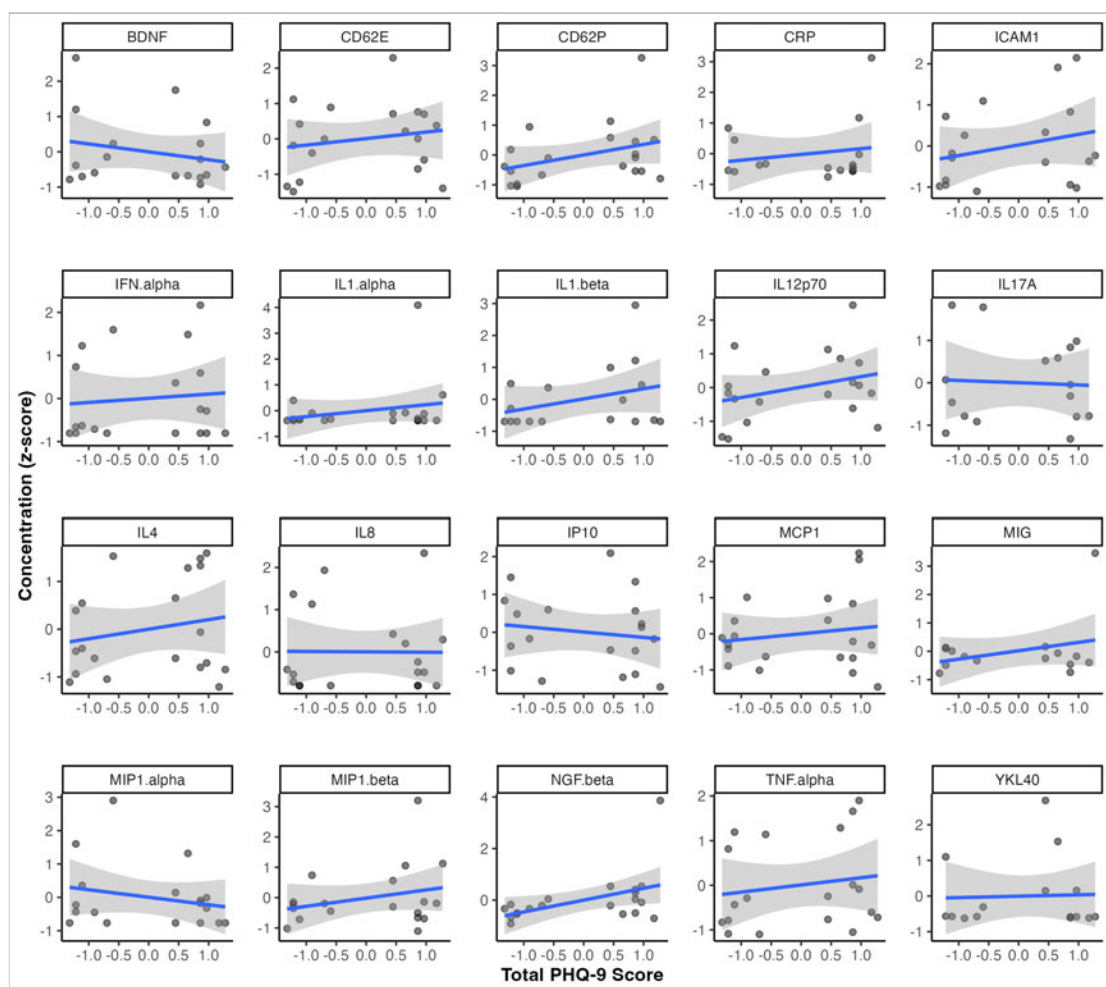


Figure D.3: Blood protein concentrations and depressive symptom severity. Correlations between concentrations of inflammatory and neurological proteins in blood serum (transformed to z-scores) quantified using Luminex™ immunoassays and depressive symptom severity (total PHQ-9 score).

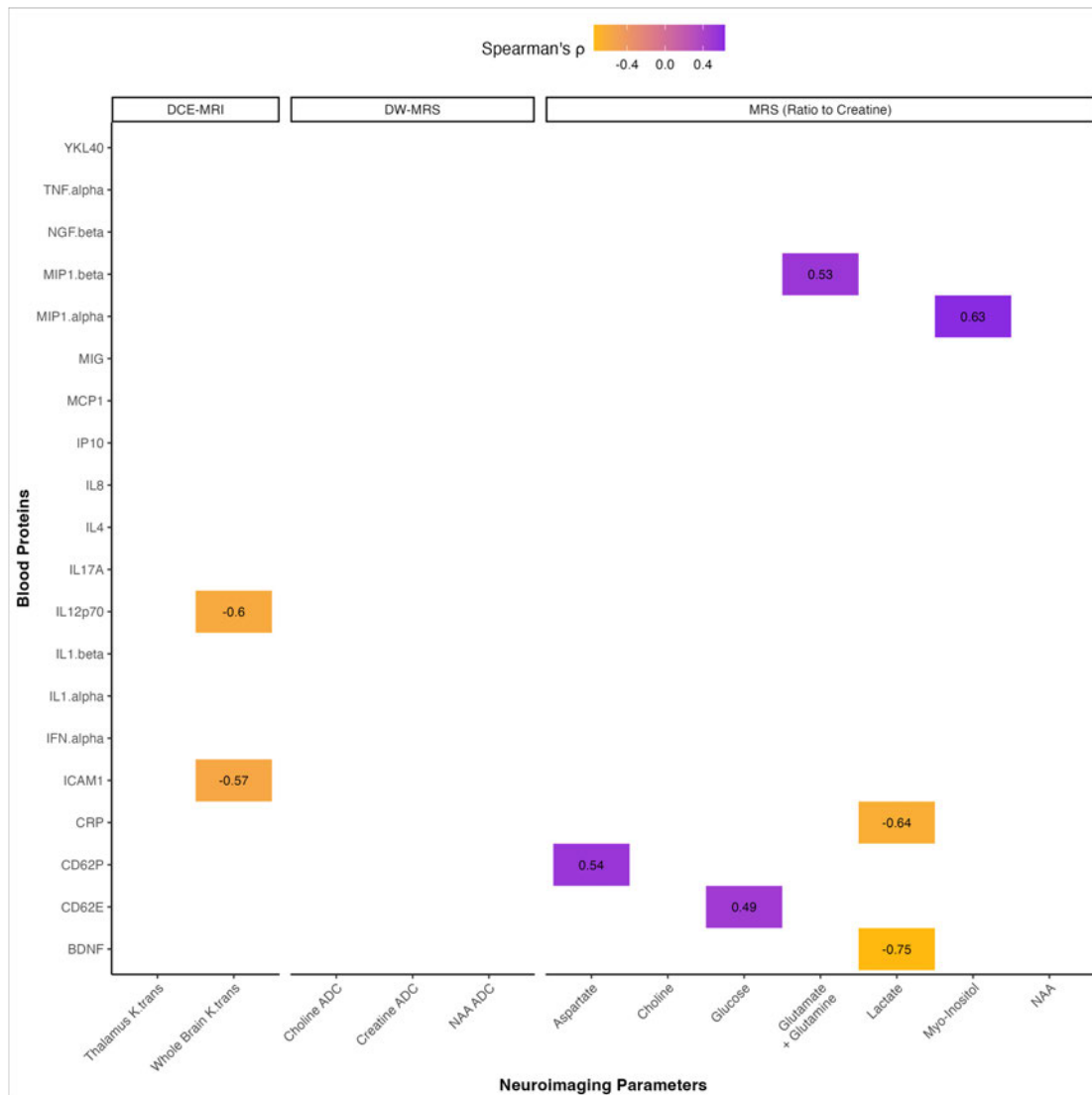


Figure D.4: Neuroimaging and blood biomarkers, significant correlations only. Spearman's correlations between concentrations of inflammatory and neurological proteins in blood serum and neuroimaging parameters. Only correlations with $p < 0.05$ are shown. In-laid text in tiles shows the magnitude of each correlation. Dynamic contrast-enhanced MRI (DCE-MRI) was used to assess blood-brain barrier permeability (volume leakage constant, K_{trans}) in the thalamus and whole brain, diffusion-weighted MRS used to assess intracellular diffusion (apparent diffusion coefficient, ADC) of neurometabolites in the anterior cingulate cortex, and standard MRS used to assess concentrations (relative to creatine) of neurometabolites in the anterior cingulate cortex. Proteins in blood were quantified using Luminex™ immunoassays.

Treatment	Overall, N = 20	High Depression Severity, N = 11	Low Depression Severity, N = 9	p-value
ART Regimen, n (%)				0.9
Delstrigo	7 (35%)	4 (36%)	3 (33%)	
Biktarvy	2 (10%)	1 (9.1%)	1 (11%)	
Cabotegravir + Rilpivirine LAI	2 (10%)	1 (9.1%)	1 (11%)	
Odefsey	2 (10%)	2 (18%)	0 (0%)	
Descovy	1 (5.0%)	1 (9.1%)	0 (0%)	
Darunavir/ Emtricitabine/ Tenofovir/ Ritonavir	1 (5.0%)	1 (9.1%)	0 (0%)	
Dovato	1 (5.0%)	0 (0%)	1 (11%)	
Genvoya	1 (5.0%)	0 (0%)	1 (11%)	
Nevirapine/ Emtricitabine/ Tenofovir	1 (5.0%)	1 (9.1%)	0 (0%)	
Rilpivirine/ Emtricitabine+Tenofovir	1 (5.0%)	0 (0%)	1 (11%)	
Triumeg	1 (5.0%)	0 (0%)	1 (11%)	
Antidepressant Regimen, n (%)				0.5
None	7 (35%)	2 (18%)	5 (55%)	
Mirtazipine	3 (15%)	1 (9.1%)	2 (22%)	
Citalopram	2 (10%)	0 (0%)	2 (22%)	
Amitriptyline	1 (5.0%)	1 (9.1%)	0 (0%)	
Citalopram/ Mirtazipine	1 (5.0%)	1 (9.1%)	0 (0%)	
Fluoxetine	1 (5.0%)	1 (9.1%)	0 (0%)	
Quetiapine	1 (5.0%)	1 (9.1%)	0 (0%)	
Sertraline	1 (5.0%)	1 (9.1%)	0 (0%)	
Venlafaxine	1 (5.0%)	1 (9.1%)	0 (0%)	
Venlafaxine / Mirtazapine	1 (5.0%)	1 (9.1%)	0 (0%)	
Vortioxetine	1 (5.0%)	1 (9.1%)	0 (0%)	

Table D.1: Antiretroviral and antidepressant treatment regimens for all participants. Participant groups were determined at screening using the Patient Health Questionnaire (PHQ-9) score as High Depression Severity (HD, PHQ-9 $\geq$ 15 and history of using antidepressant medication) or Low Depression Severity (LD, PHQ-9 $\leq$ 7). ART: antiretroviral therapy.

Parameter	MRS Referencing	<i>n</i>	Median	Q1	Q3
ADC NAA		20	0.00010914	0.00010326	0.00012495
ADC Creatine		20	0.00012161	0.00011775	0.00012628
ADC Choline		20	0.00011288	0.00010162	0.0001187
K_{trans} Whole Brain		19	0.000848	0.0007995	0.0009755
Aspartate	Creatine Referenced	20	0.33	0.32	0.36
Choline	Creatine Referenced	19	0.23	0.19	0.28
Glucose	Creatine Referenced	20	1.50	1.44	1.59
Glutamate + Glutamine	Creatine Referenced	19	0.80	0.75	0.85
Lactate	Creatine Referenced	20	0.09	0.08	0.10
Myo-Inositol	Creatine Referenced	20	1.26	1.19	1.31
NAA	Creatine Referenced	20	0.26	0.24	0.28
Aspartate	Water Referenced	20	3.11	2.86	3.51
Choline	Water Referenced	20	4.92	4.79	5.19
Creatine	Water Referenced	19	2.08	1.82	2.56
Glucose	Water Referenced	20	14.15	13.74	15.23
Glutamate + Glutamine	Water Referenced	19	7.60	7.38	8.05
Lactate	Water Referenced	20	0.85	0.75	0.96
Myo-Inositol	Water Referenced	20	11.96	11.48	12.37
NAA	Water Referenced	20	2.59	2.30	2.75
BDNF		18	181.69	115.95	342.32
CD62E		19	22408.01	15147.86	29376.33
CD62P		20	564809.63	365576.18	973399.40
CRP		16	2208.73	1402.81	5145.28
ICAM1		18	122256.52	44345.02	227382.31

IFN.alpha	19	0.27	0.10	1.56
IL1.alpha	20	0.12	0.015	0.61
IL1.beta	17	0.11	0.005	2.02
IL12p70	19	60.93	50.28	84.21
IL17A	16	10.57	5.55	17.36
IL4	20	5.02	3.70	9.34
IL8	19	0.43	0.10	1.32
IP10	19	6.82	4.77	9.47
MCP1	20	74.17	51.78	115.92
MIG	15	3.28	2.12	4.42
MIP1.alpha	18	1.12	0.44	1.64
MIP1.beta	19	50.61	39.36	64.15
NGF.beta	20	0.58	0.51	0.66
TNF.alpha	19	31.94	20.14	62.57
YKL40	15	60.75	53.46	748.74

Table D.2: Summary statistics for all neuroimaging parameters and blood proteins measured in 20 adults living with HIV in Brighton, UK.

Covariate(s)	Parameter	MRS Referencing	<i>n</i>	ρ	<i>p</i>	<i>p</i> _{FDR}
Education	ADC NAA		20	0.03	0.914	0.914
Education	ADC Creatine		20	0.25	0.303	0.909
Education	ADC Choline		20	-0.11	0.662	0.914
Education	K _{trans} Whole Brain		19	-0.08	0.752	0.752
Education	K _{trans} Thalamus		18	-0.45	0.068	0.137
Education + fWM	Aspartate	Creatine Referenced	20	0.51	0.032	0.227
Education + fWM	Choline	Creatine Referenced	20	-0.19	0.459	0.748
Education + fWM	Glucose	Creatine Referenced	19	0.04	0.893	0.893
Education + fWM	Glutamate + Glutamine	Creatine Referenced	20	0.14	0.585	0.748
Education + fWM	Lactate	Creatine Referenced	20	0.12	0.642	0.748
Education + fWM	Myo-Inositol	Creatine Referenced	19	-0.34	0.178	0.625
Education + fWM	NAA	Creatine Referenced	20	-0.13	0.614	0.748
Education + fWM	Aspartate	Water Referenced	20	0.41	0.094	0.485
Education + fWM	Choline	Water Referenced	20	0.02	0.95	0.95
Education + fWM	Creatine	Water Referenced	20	0.04	0.883	0.95
Education + fWM	Glucose	Water Referenced	19	0.04	0.888	0.95
Education + fWM	Glutamate + Glutamine	Water Referenced	20	0.15	0.548	0.95
Education + fWM	Lactate	Water Referenced	20	0.11	0.668	0.95
Education + fWM	Myo-Inositol	Water Referenced	19	-0.39	0.121	0.485
Education + fWM	NAA	Water Referenced	20	-0.07	0.796	0.95
CD4	ADC NAA		20	0.28	0.253	0.38
CD4	ADC Creatine		20	0.46	0.046	0.137
CD4	ADC Choline		20	0.03	0.893	0.893
CD4	K _{trans} Whole Brain		19	-0.09	0.735	0.735

CD ₄	K_{trans} Thalamus		18	-0.41	0.101	0.202
CD ₄ + fWM	Aspartate	Creatine Referenced	20	0.41	0.088	0.619
CD ₄ + fWM	Choline	Creatine Referenced	20	-0.15	0.559	0.973
CD ₄ + fWM	Glucose	Creatine Referenced	19	-0.08	0.746	0.973
CD ₄ + fWM	Glutamate + Glutamine	Creatine Referenced	20	0.24	0.34	0.973
CD ₄ + fWM	Lactate	Creatine Referenced	20	-0.03	0.892	0.973
CD ₄ + fWM	Myo-Inositol	Creatine Referenced	19	-0.11	0.68	0.973
CD ₄ + fWM	NAA	Creatine Referenced	20	-0.01	0.973	0.973
CD ₄ + fWM	Aspartate	Water Referenced	20	0.24	0.344	0.707
CD ₄ + fWM	Choline	Water Referenced	20	-0.19	0.442	0.707
CD ₄ + fWM	Creatine	Water Referenced	20	-0.22	0.377	0.707
CD ₄ + fWM	Glucose	Water Referenced	19	-0.08	0.751	0.858
CD ₄ + fWM	Glutamate + Glutamine	Water Referenced	20	-0.02	0.932	0.932
CD ₄ + fWM	Lactate	Water Referenced	20	-0.13	0.612	0.816
CD ₄ + fWM	Myo-Inositol	Water Referenced	19	-0.43	0.085	0.684
CD ₄ + fWM	NAA	Water Referenced	20	-0.32	0.198	0.707

Table D.3: Spearman's correlations for all neuroimaging parameters and depressive symptom severity, adjusted for years of education or CD4 count. ADC: apparent diffusion coefficient, fWM: fractional white matter volume, MRS: magnetic resonance spectroscopy.

Parameter	MRS Referencing	ρ	p	p_{FDR}
Aspartate	Creatine Referenced	0.54	0.016	0.115
Choline	Creatine Referenced	-0.13	0.599	0.973
Glucose	Creatine Referenced	0.00	0.987	0.987
Glutamate + Glutamine	Creatine Referenced	0.15	0.549	0.973
Lactate	Creatine Referenced	0.15	0.541	0.973
Myo-Inositol	Creatine Referenced	-0.10	0.695	0.973
NAA	Creatine Referenced	-0.01	0.975	0.987
Aspartate	Water Referenced	0.43	0.066	0.527
Choline	Water Referenced	-0.04	0.858	0.970
Creatine	Water Referenced	-0.12	0.638	0.970
Glucose	Water Referenced	0.01	0.970	0.970
Glutamate + Glutamine	Water Referenced	0.03	0.918	0.970
Lactate	Water Referenced	0.13	0.587	0.970
Myo-Inositol	Water Referenced	-0.29	0.243	0.970
NAA	Water Referenced	-0.08	0.741	0.970
K_{trans} Whole Brain		-0.15	0.537	0.537
K_{trans} Thalamus		-0.37	0.129	0.257
ADC Choline		0.24	0.312	0.467
ADC Creatine		0.48	0.034	0.102
ADC NAA		0.07	0.768	0.768
BDNF		-0.09	0.719	0.899
CD62E		0.10	0.674	0.899
CD62P		0.29	0.211	0.824
CRP		0.25	0.345	0.824

ICAM ₁	0.22	0.379	0.824
IFN.alpha	0.02	0.927	1.000
IL ₁ .alpha	0.14	0.567	0.899
IL ₁ .beta	0.21	0.412	0.824
IL ₁₂ p70	0.25	0.293	0.824
IL ₁₇ A	0.00	1.000	1.000
IL ₄	0.10	0.662	0.899
IL ₈	0.02	0.942	1.000
IP ₁₀	-0.24	0.314	0.824
MCP ₁	0.01	0.955	1.000
MIG	0.11	0.707	0.899
MIP ₁ .alpha	-0.17	0.501	0.899
MIP ₁ .beta	0.23	0.354	0.824
NGF.beta	0.48	0.032	0.640
TNF.alpha	0.29	0.228	0.824
YKL ₄₀	-0.28	0.306	0.824

Table D.4: Spearman's correlations for all neuroimaging parameters and blood proteins with depressive symptom severity.

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