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Longitudinal analysis of proinflammatory and anti-inflammatory cytokines in the cerebrospinal fluid and peripheral blood of treatment-naïve first-episode psychosis patients, and their correlation with psychosis severity and cognitive impairment in sub-Saharan Africa

Lindokuhle Thela ,[1] Saeeda Paruk,[2] Bongani B Nkambule ,[3,4] Vuyokazi Ntlantsana ,[4,5] Nathlee S Abbai,[6] Zama Msibi,[7] Usha Chhagan ,[2] Andrew Tomita,[3] Thirusha Naidu,[8] Sanele Nkosi,[1] Bonginkosi Chiliza [9]

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For numbered affiliations see end of article.

Correspondence to
Lindokuhle Thela;
thelal@ukzn.ac.za

ABSTRACT

Background Inflammation is indicated as one of the factors that play a role in the development of schizophrenia, with several studies having found considerable inconsistencies in their results. Few have investigated the role of inflammation in primary psychosis in blood and cerebrospinal fluids simultaneously, the aim of this study being to investigate the expression of blood and cerebrospinal fluid inflammatory cytokines in treatment-naive first-episode psychotic participants.

Methods and analysis This is a combined cross-sectional and prospective observational study, which is currently taking place in Durban, South Africa, will recruit 60 participants (30 cases and 30 matched controls). The primary objective is to describe baseline CSF and longitudinal expression/levels of inflammatory cytokines in the blood in persons diagnosed with first-episode psychosis (FEP) for 12 months. The secondary objective is to describe the associations between inflammatory cytokines and psychosis severity, neurocognitive performance, antipsychotic response and metabolic changes at different time points (baseline, 3, 6 and 12 months).

Interventions We will collect the sociodemographic details of all participants, and the Positive and Negative Symptoms Scale, Patient Health Questionnaire-9, Childhood Trauma Scale, Repeatable Battery for the Assessment of Neuropsychological Status Update, metabolic markers and inflammatory markers (venous blood and lumbar puncture cerebrospinal fluid) for those with FEP. Data from matched controls will only be collected at one point and no follow-ups (cross-sectional).

Ethics and dissemination The study protocol has been approved by the University of KwaZulu-Natal Biomedical Research Ethics Committee (BREC/00004714/2022). The study is nested in an ongoing study titled the burden of HIV and Psychosis in an African setting: a longitudinal study of HIV-infected and non-infected patients with First-Episode Psychosis (BREC 571/18). The results will be actively disseminated through peer-reviewed journal publications and conference presentations.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ The study design is a case-control.
⇒ Small sample size.
⇒ Combined cross-sectional and prospective study.
⇒ Some measures are cross-sectional.
⇒ Purposeful sampling.

INTRODUCTION

Schizophrenia is a severe form of mental illness that is associated with considerable disability that often starts insidiously between the second and third decades of life, a course that tends to show progression with symptom severity.[1] It is among the top 10 leading causes of disability in persons between the ages of 15 and 44 years,[2] with interventions in the first episode of psychosis being associated with better outcomes.[3] However, the longitudinal stability of a diagnosis following the first episode of psychosis is variable.[4 5] There are no validated biomarkers for the diagnosis and prognosis of schizophrenia,[6] and the heterogeneous nature of schizophrenia justifies the

need for biomarkers to be used during the diagnosis and management of the patients.

Burden of schizophrenia

Schizophrenia is a severe, debilitating mental illness that is linked with 1.1% of disability-adjusted years and 2.8% of years lived with disability.[2] It is linked with an increased likelihood of unemployment, homelessness and isolation, and thus poor overall quality of life,[7] and with high rates of non-communicable disease and premature death. The lifespan of an affected person is 10–15 years shorter than the general population.[8] Having a psychotic disorder is linked to a two-to-threefold increased risk of developing cardiometabolic disorders (eg, diabetes mellitus and dyslipidaemia) compared with the general population.[9] The interventions to address and improve outcomes in schizophrenia fall short of addressing the complexity of the disease's clinical spectrum, with the majority of patients requiring long-term support and being unable to live independently.[10] While antipsychotics, the gold standard treatment of schizophrenia, are effective in treating positive symptoms, such as delusions and hallucinations, their effect in treating negative and cognitive symptoms remains minimal, leading to overall poor outcomes.[11] Therefore, there is a need for new treatment interventions supported by schizophrenia biomarkers, such as those related to the inflammatory model of schizophrenia pathogenesis.

Inflammation in the neurobiology of schizophrenia

Psychosis neurobiology is complex, with various theories proposed for its pathophysiology, including immune dysregulation and neuroinflammation.[12 13] Compelling evidence has shown that the presence of inflammatory cytokines can be used as a surrogate biomarker of schizophrenia clinical phenotype and course.[14]

Patients with schizophrenia have been shown to have higher expression of cytokines, such as interleukin (IL)-6, 8, 10, C-reactive protein (CRP) and tumour necrosis factor-alpha (TNF-α), compared with health-matched controls.[15] In some cohorts, high levels of IL-6 at the onset of the illness are a negative prognostic marker,[15] while a high level of IL-8 has been linked with poor antipsychotic response in affected persons.[14] However, there is a paucity of data reporting on immune cell activation profiles and surface markers in FEP cases. Findings from a postmortem study of schizophrenia patients showed a correlation between neuroimmune activation, where several cell markers of microglia and age-related T-lymphocyte activation were observed, supporting the hypothesis of the modified neuroimmune environment.[16]

A meta-analysis reported high rates of neuroinflammatory markers in the brains of people with schizophrenia versus controls during postmortem, those with the condition showing histological and molecular features of inflammation.[13] The pooled estimate of microglia density was significantly higher in the brains of patients with schizophrenia compared with healthy controls (p=0.0028), with an overall increase in the expression of proinflammatory genes in the former compared with the latter (p=0.0052).[13] Another meta-analysis of in vivo microglial activity imaging studies reported that patients living with schizophrenia had higher rates of tracer binding of the translocator protein than healthy controls, which increased during microglial activation.[17]

Schizophrenia-related inflammation risk factors

There is evidence that inflammation plays a role in the pathogenesis from an early age, probably during the stages of neurodevelopment, and shows that genetic and environmental factors contribute to schizophrenia's immune dysregulation.[18 19] Evidence of the genetics role in immune dysregulation and schizophrenia has been researched extensively and shown that polymorphism in the major histocompatibility locus on chromosome 6 is associated with an increased risk of developing the condition. In a meta-analysis of European ancestry subjects (8008 patients vs 19 077 controls), the polymorphism on chromosome 6p22.1 was associated with schizophrenia development,[20] while rates of HLA polymorphism were reported to be higher in Japanese and Tunisian patients with schizophrenia.[21 22]

Inflammation arising because of environmental risk factors was discovered many decades ago. An association between prenatal maternal infections, such as viruses, and the development of schizophrenia in the offspring of these mothers paved the way for understanding the role of inflammation in schizophrenia. There are multiple lines of evidence showing that high inflammatory circulating cytokines in the mother during pregnancy are a predictor of schizophrenia in their offspring.[23] Allswede *et al* (2016) also found evidence that people who developed schizophrenia were most likely to be born to mothers with elevated inflammatory cytokine IL-8 in the second trimester compared with healthy controls.[24]

One of the most plausible environmental risk factors of schizophrenia is exposure to childhood trauma.[25–27] One of the models that explains its development in individuals who report childhood traumatic experiences is its role in promoting immune dysregulation. There is evidence from a longitudinal study that childhood exposure to traumatic experiences is associated with elevations in inflammation levels almost 20 years later.[28] The association between childhood traumatic experiences and chronic inflammation is independent of clinical comorbidities.[29] In adults with schizophrenia who reported childhood trauma, there is a significant elevation in the peripheral CRP and TNF-α.[30] Childhood trauma has been linked with an increase in the levels of IL-6, which negatively correlated with brain-derived neurotrophic factors and the hippocampal volume in patients who developed psychosis.[31]

Aims

This study aims to investigate the expression of inflammatory cytokines in the blood and cerebrospinal fluid (CSF) of treatment-naive first-episode psychotic patients.

Objectives and hypothesis

We hypothesise that

Chronic low-grade inflammation is associated with the severity of psychosis and short-term clinical outcomes in primary psychosis.

The objectives are as

1. To describe baseline levels of inflammatory cytokines in CSF.
2. And its associations with the longitudinal expression/levels of inflammatory cytokines in the blood and clinical symptoms in persons diagnosed with first-episode psychosis (FEP) over 12 months.

The primary outcome

The identification of baseline inflammatory cytokine profiles in CSF that are associated with longitudinal changes in blood cytokine levels and clinical symptoms in individuals with FEP, providing insights into the role of immune system activity in the progression of psychosis.

METHODS

Design

This is a combined cross-sectional and prospective study being conducted in four hospitals in Durban, as part of a larger study called *the burden of HIV and Psychosis in an African setting: A longitudinal study of HIV-infected and non-infected patients with First Episode Psychosis.* [32] This study is underway and started enrolling participants in September 2024. The study anticipates completing recruitment in September 2025. We are recruiting participants with a diagnosis of a primary psychotic disorder as defined in the Diagnostic Statistical Manual-5 (DSM-5).[33] Participants who fulfil the requirements are assessed for eligibility to participate in the study by a psychiatrist for capacity to consent before they are enrolled.

Setting of the study

The study is recruiting participants from four general hospitals in Durban, South Africa, that provide outpatients and inpatient psychiatric services to the surrounding communities, with a population of approximately 4.2 million.

Sampling

Case participants

Patients diagnosed with a first episode of psychosis are approached and invited to take part in the study through consecutive sampling.

Control participants

Patients undergoing spinal anaesthesia (epidural block) for elective surgery who are matching the sociodemographic profile of the case participants are invited to participate in the study through a consecutive sampling method.

Table 1 Inclusion and exclusion criteria for case participants

Inclusion criteria	Exclusion criteria
Females and males	Secondary psychotic disorder
18–48 years old	Presence of infectious disease (eg, HIV, TB and syphilis)
DSM-5 primary psychotic disorders	LP contraindication (eg, raised intracranial pressure, platelets $<150 \times 10^{9}$/L, INR>1.5 s)
<6 weeks of antipsychotic treatment	Anti-inflammatory drug use
Capacity to consent to participate	Pregnant
	Presence of an autoimmune disorder
	Presence of a neurological disorder or illness

DSM-5, Diagnostic Statistical Manual-5; LP, lumbar puncture.

Inclusion and exclusion criteria

Case participants

All patients presenting with a first episode of psychosis are considered eligible to participate in the study provided they meet the following inclusion criteria: all genders, 18–48 years old, confirmed non-organic psychotic disorder, less than 6 weeks exposure to antipsychotic medications, proven capacity to consent (and consenting). Potential participants are excluded from the study if they are pregnant, have lumbar puncture (LP) contraindications, have infections (eg, HIV, TB and syphilis), are currently using anti-inflammatory medications, autoimmune disorders or have neurological illnesses (table 1).

Control participants

All patients undergoing elective surgical procedures under epidural anaesthesia are considered eligible to participate in the study if they meet the following criteria: all genders, 18–48 years old, no previous diagnosis of mental illness and able to consent to participate in the study. The participant will be *excluded* from the study if they are pregnant, (2) have infections (eg, HIV, syphilis, TB), pre-existing neurological disorders (eg, traumatic brain injury and epilepsy) and current or chronic use of anti-inflammatory medications (table 2).

STUDY PROCEDURE

Recruitment

The study is recruiting participants in two arms (namely case participants and matched controls). All participants

Table 2 Inclusion and exclusion criteria for matched control

Inclusion criteria	Exclusion criteria
Females and males	History of mental illness.
18–48 years old	Use of anti-inflammatory medication.
Undergoing spinal anaesthesia	Presence of a neurological disorder.
Capacity to consent to participate	Presence of an infectious disease (HIV, TB, syphilis)
	Pregnant
	Presence of an autoimmune disorder

are recruited from the wards (patients ho are admitted in the hospital).

The recruitment of participants is conducted purposively. Treating clinicians refer potential participants to the principal investigator (PI, Psychiatrist). The PI evaluates the potential participants using the established inclusion and exclusion criteria (tables 1 and 2) to determine eligibility for the study. Eligible participants are invited to participate in the study. Written informed consent is obtained from each participant prior to their involvement in the research.

Case participants are inpatients and are being recruited in the ward during admission. This is to ensure that all necessary tests are done adequately, and all participants can be observed for any potential adverse event arising from the LP.

Matched controls are being recruited from an orthopaedics ward, specifically patients admitted for elective lower limb surgery. This is a convenience sampling method since these patients will receive spinal anaesthesia, during which a sample of CSF will be collected by the anaesthesiologist. The participants are healthy patients scheduled for elective surgical operations involving the lower limb.

Data collection

Data collection is underway, and data collection takes place at four time points (cross-sectional and longitudinally) (table 3): baseline, 3, 6 and 12 months. Data

Table 3 Study intervention and timepoints

Intervention		Intervention time points			
		Case and control participants	Case participants		
			Months		
Tool	Description	Baseline	3	6	12
Sociodemographic	Information about participants, for example, age, gender, education	✓	–	–	–
MINI-International Neuropsychiatric Interview (MINI) V.7	Structured diagnostic interview to confirm a psychotic disorder according to the DSM-5 classification	✓	×	×	×
Positive and Negative Syndrome Scale (PANSS) (Kay *et al* 1987)	Instrument used to measure the severity of negative and positive symptoms commonly associated with psychosis	✓	✓	✓	✓
Childhood Trauma Questionnaire Short (Bernstein *et al* 2003)	Retrospective measure used to obtain information about childhood traumas	✓	×	×	×
Neuropsychometric battery for cognition (RBANS)	Assess the cognitive performance	X	✓	×	✓
Extrapyramidal Symptom Rating Scale (ESRS)	Scale used to measure drug-induced movement disorders	✓	✓	✓	✓
WHO encounter	Pathways to mental healthcare and duration of untreated psychosis	✓	×	×	×
Venous blood	Analysis of inflammation +HIV ELISA and full blood count	✓	✓	✓	✓
Lumbar puncture cerebrospinal fluid	Analysis of inflammation	✓	×	×	×

Table 3. The procedure and tools used in collecting data at different timepoint of the study.
DSM-5, Diagnostic and Statistical Manual-5.

collection is done by means of clinical interviews, physical examination results and clinical rating scales. Blood is collected at all time points, including a baseline CSF sample.

To minimise distress and fatigue, the interviews are limited to approximately 2 hours per data collection session with the participant, where possible. Data collection during the 3 and 12 month visits will be conducted over 2 days (in outpatients) due to the length of formal neurocognitive assessments, which take approximately 2 hours to complete, the study procedure being summarised in table 3. Each participant will be compensated for all expenses incurred during participation, such as transport and food, and fees associated with the involvement in the research (for inconvenience and discomfort during participation).

Demographic details

Sociodemographic data are collected at the time of enrolment using a sociodemographic tool that has been developed for this study.

Clinical assessment

A physical and neurological examination is done for all participants, with specific attention being placed on excluding contraindications for LP. Blood pressure, pulse, body mass index and waist circumference measurement are also done.

RESEARCH TOOLS

Five validated tools are used to obtain data about the participants' psychological state at the four time intervals of the study, starting with baseline, these being the Mini-International Neuro-psychiatric Interview (MINI) V.7.02, Positive and Negative Syndrome Scale (PANSS), Childhood Trauma Questionnaire (CTQ), Extrapyramidal Symptom Rating Scale, Repeatable Battery for the Assessment of Neuropsychological Status, WHO Encounter form and blood and LP CSF collection.

Mini-International Neuro-psychiatric Interview (MINI) V.7.02

The MINI was developed by clinicians to evaluate 17 disorders based on the DSM criteria (APA 2013). The MINI is designed as a short and accurate structured psychiatric interview for multicentre clinical trials and epidemiological studies. It can also be used as the first step in outcome tracking in non-research clinical settings. Apart from anxiety disorders and bulimia nervosa, this instrument has shown good reliability and validity in other psychiatric disorders.[34] According to Petterson and colleagues, MINI has a global median satisfaction rating of 80 for patients and 86 for interviewers. In addition, patients, general practitioners and therapists found MINI effective in treating patients appropriately. The average MINI duration in this study was 26 min.[35] The MINI has been found to be an acceptable research tool in the South African (SA) population.[36]

Positive and Negative Syndrome Scale (PANSS)

The PANSS was developed as a practical method for assessing symptom clusters in schizophrenia and has been used in the SA population before.[37] The PANSS has been found to have a high level of inter-rater reliability.[38] The PANSS consists of 30 items subdivided into three subscales: positive, negative and general psychopathology.[39] Each of the 30 items is scored between 0 and 7, with 7 denoting the most severe symptoms. Most items forming the positive and negative subscales of the PANSS have been shown to perform very well in identifying the symptoms and severity.[40]

Childhood Trauma Questionnaire (CTQ-SF)

The CTQ is a self-report tool used as a screening tool for traumatic experiences during childhood. Twenty-eight items in the CTQ screen for five types of traumas, namely, emotional abuse, physical abuse, sexual abuse, emotional neglect and physical neglect. The CTQ had been shown to have good test–retest reliability and has been used in the SA population before.[41]

Extrapyramidal Symptom Rating Scale (ESRS)

The ESRS is used to evaluate drug-induced dyskinesia's presence and severity. The tool consists of items that assess parkinsonism, akathisia, dystonia and tardive dyskinesia.[42] The scale rates the medication-induced movement disorders based on severity and frequency, ranging from 0 to 6.[42] Both subjective and objective measures of the four types of movement abnormalities are rated. The scale also places the Clinical Global Impressions from 0 to 8 (absent–extremely severe).[43] The ESRS has been used in the SA population.[44]

Repeatable Battery for the Assessment of Neuropsychological Status (RBANS)

The Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) was developed to identify and characterise abnormal cognitive decline in older adults and as a neuropsychological screening battery for younger patients.[45] Ten subtests allow screening of the five cognitive domains: immediate memory, visuospatial/constructive, language, attention, and delayed memory.[46]

The duration of the battery administration takes about 30 min. The battery consists of five cognitive domains. The RBANS is also a helpful screening tool for neurocognitive impairment in schizophrenia.[47] The overall score of the RBANS has good reliability in schizophrenia. In approximately 25 min, this test provides reliable and clinically useful information about neurocognitive function, making it ideal for screening persons diagnosed with schizophrenia. RBANS can also be used in non-clinical settings.[47 48]

WHO encounter form

The WHO encounter form determines the pathway to care for mental health. The three main sections in the self-report tool include basic information about the current healthcare visit or admission, the decision to seek help

Table 4 Venous and CSF processing and storage

Sample type	Tube	Amount	Processing	Sample of interest	Storage at –81°C
Venous blood	SST	5 mL	Centrifuge: 2000 rpm, room temperature, 20 min, 0 break.	Serum	Serum: aliquot samples of 500 µL into cryovials Store at –81°C
	Citrate	2.7 mL	Centrifuge: 700 rpm, room temperature, 20 min, 0 break.	Plasma	Plasma: aliquot samples of 500 µL into cryovials Store at –81°C
	Citrate	2.7 mL	Conical falcon tube, add 3 mL density gradient medium, carefully layer 1.5 mL of blood. Centrifuge: 700 Relative Centrifugal Force (RCF), room temperature, 20 min, 0 break.	Peripheral blood mononuclear cells	Carefully extract the PBMC. Mix PBMC with Phosphate-buffered saline (PBS) at a ratio of 1:2 (PBMC: PBS). Aliquots of 500 µL in cryovials
CSF	Citrate	2.7 mL	Gently rotate the tube to ensure that the CSF is mixed with citrate in the tube.	–	Aliquot samples of 500 µL into cryovials Store at –81°C

Table 4. The procedure for collecting, processing and storage of blood and cerebrospinal fluid samples.

CSF, cerebrospinal fluid; PBMC, peripheral blood mononuclear cells; PBS, Phosphate-buffered saline; RCF, Relative Centrifugal Force; SST, serum separator tubes.

and the path from the first mental healthcare provider contact to the recent visit. The tool can be helpful when trying to quantify the duration of untreated psychosis in clinical research. For example, the tool has been used in past studies researching psychosis in South Africa to determine the duration of untreated psychosis.[49]

Blood and lumbar puncture cerebrospinal fluid collection

A qualified medical doctor collects blood and LP CSF samples in the afternoon between 12:00 PM and 2:00 PM. The venous blood will be drawn first, followed by CSF sampling within 30 min. The matched control samples are collected during spinal anaesthesia (before administering the anaesthetic agent). Plasma and peripheral blood mononuclear cells (PBMCs) will be isolated from whole blood using density gradient centrifugation methods and serum will be prepared using serum separator tubes. Table 4 provides a summary of blood and CSF collection and storage.

PRIMARY OUTCOMES OF INFLAMMATION

Analysis of the inflammatory markers in blood and cerebrospinal fluid

A range of T-helper 1 and T-helper 2 cytokines (eg, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12/IL-23, IL-13, IL-15, IL-16) will be analysed from plasma and CSF samples at baseline, 3, 6 and 12 months. The cytokines will be measured using cytometric bead arrays.

Immunophenotyping of peripheral blood T cells

PBMCs will be isolated using Ficoll-Plaque within 2 hours of blood collection. T-cells will be isolated from PBMCs and CSF samples using multicolour flow cytometry. The flow cytometry panel will consist of cell surface markers including CD3, T-cell receptor (TCR) αβ chains, CD4, CD8, CD25, CD45RA and CD45RO.[50] All flow cytometry files will be analysed using the Kaluza Analysis Software (Beckman Coulter).

Statistics analysis

Statistical analysis will be conducted using SPSS V.24. All participants will be allocated a study code when enrolling in the study. The data will be transferred and analysed using SPSS V.24. We will apply visual (eg, quantile–quantile (Q–Q) plot), descriptive (eg, skewness and kurtosis) and statistical tests (Shapiro-Wilk Test given the proposed small sample size) to check for the normality of data before applying any parametric tests (such as t-test or analysis of variance).

Correction for multiple comparisons will be integrated into the proposed study. More specifically, within the context of our proposed study that involves pairwise comparisons of clinical outcomes on dependent samples across more than two assessment periods, we will consider the Wilcoxon Signed-Rank Test,[51] with various methods being explored, including Bonferroni correction.

Managing the missing data

There are three patterns of missing data, namely (a) missing completely at random (MCAR), (b) missing at random (MAR) and (c) missing not at random (MNAR). We will apply Little's test[52] to determine MCAR and covariate-dependent missingness. We will test the plausibility of MAR by t-test (or alternative non-parametric methods) between the group with complete data and that with missing data.[53] Depending on the outcomes, we will manage the missing data by means of either listwise deletion, pairwise deletion, mean substitution or regression-based multiple imputation. Although the percentage of missing data can significantly influence the choice/effectiveness of imputation methods, we plan on applying multiple imputation[54] for MCAR/MAR data. Finally, under the MNAR assumption, we will conduct sensitivity analysis to examine how different assumptions about the missing data mechanism can impact the results.[55–57]

Intergroup analysis

First, the analysis will describe participants' sociodemographic profiles in the case and matched control cohorts. The sociodemographic and clinical characteristics that are continuous variables will be summarised using means (SD) and medians, depending on the distribution, while for the categorical variables, proportion (%) will be reported.

Second, the baseline analysis will compare the expression of blood and CSF in each cohort by using a t-test. The intergroup differences between the cases and controls with respect to cytokine expressions in blood and CSF will be assessed, with a p-value of <0.05 being regarded as statistically significant.

Case participants analysis

At baseline, a Pearson's correlation analysis between immunological markers, negative symptoms and cognitive impairment will be conducted, after which a longitudinal analysis of immunological changes will be described to determine changes over time using a t-test. Third, a multiple-factor analysis to compare the changes in blood cytokines, negative symptoms, cognitive symptoms, and metabolic variables (BMI, cholesterol, and glucose) at 12 months will be conducted.

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Sample size and power

We designed the study using a post hoc power estimate and opted for a total of 60 participants at 1:1 ratio for case and matched controls (30 per group), which gives a medium to large effect size of 65%, with 80% power at a p value set at <0.05 (CI of 95%). The rationale for using a post hoc estimate includes the following: multiple time point follow-up for a year, low incidence of first episode psychosis, stringent inclusion criteria, timelines and the moderate-to-high-risk intervention (LP). A reasonable sample should be >25 to conduct mixed-effect linear regression analysis, with an estimated attrition rate of 20% (often reported to be approximately 15% in long follow-up studies of psychosis). An additional nine case participants will be enrolled to accommodate the 20% attrition rate reported in mental health research.[58]

Data monitoring and management

Participants are assigned unique codes to ensure anonymity, which are maintained throughout the study. Data are entered and stored in Research Electronic Data Capture (RedCap), and analyses will be conducted using SPSS V.24, with all physical files being locked in a protected cabinet in the Department of Psychiatry at the University of KwaZulu Natal. Only the research team has access to the collected data, which is password-protected, the data being retained for up to 5 years before being deleted or shredded.

Ethics and dissemination

This study received full ethical approval from the University of KwaZulu-Natal Biomedical Research Ethics Committee (BREC/00004714/2022) as part of an ongoing longitudinal prospective study: *The burden of HIV and Psychosis in an African setting: A longitudinal study of HIV-infected and non-infected patients with First Episode Psychosis (BREC 571/18)*.[32] The study also received full approval from the KZN Provincial Department of Health, and permission was obtained to conduct the studies from the four hospital research ethics committees. Participation in the study is voluntary and requires that the participants fulfil the criteria for capacity to consent and provide written informed consent before participation. The study findings will be shared through peer-reviewed publications and conference presentations. Data will be stored in

RedCap, adhering to FAIR data principles for accessibility and long-term curation. Data and materials will be shared with other parties in accordance with ethical and legal standards.

Patient advocacy and community participation

There was no patient or public involvement in designing this study.

DISCUSSION

Risk management

The study involves conducting an LP at baseline, this being an invasive procedure, with measures to mitigate the risks of complications being that all will be done by a qualified medical specialist with relevant training in LP procedure. All participants are assessed to exclude the contraindications for LPs, these being increased intracranial pressure (by clinical history, neurological examination and funduscopy), ensuring that the clotting parameters are appropriate for LP by checking the clotting parameters (INR <1.5 s, platelets count>150×10^9/L, the absence of use of medication that leads to bleeding), as well as the absence of spinal deformities and infections at the site of LP. The LP is done under sterile conditions using a 21-gauge pencil-point atraumatic needle, this having the lowest incidence of complications compared with other spinal needles. To reduce pain, a local anaesthetic agent will be administered at the LP site, with the amount of CSF being limited to not more than 3 mL, the threshold for side effects associated with CSF depletion being 30 mL.[59] Post LP, participants rest in bed for at least 30 min. They are monitored in the hospital, and the common side effects, such as post-LP headaches, are managed by administering caffeine and paracetamol. Each participant receives an information sheet about LP and what to do if complications occur, and the investigator's contact information is provided in case of an emergency.

Methodological challenges and study limitations

The study only collects a sample of one CSF at baseline, which is a limitation. The study outcomes may be improved with serial collection of CSF as this would provide comparative data on inflammatory marker variances in the CNS with treatment initiation. However, due to the safety of vulnerable participants and ethical concerns, the study was only approved to do an LP at baseline. The matched controls are also participants who are undergoing medical care, and irrespective of their mental state, it is hard to control for factors such as geographical location and possible impulsivity. Our sample likely comes from persons who have elective lower limb surgeries, such as orthopaedic fractures, who may be likely to be high-risk takers compared to the general population, which will be a limitation and a confounding variable.

Study progress and challenges

The study is underway and started enrolling case participants from September 2023. Since then, we have encountered several challenges related to participant eligibility due to the stringent inclusion criteria. Additionally, some participants have withdrawn from the study while undergoing LPs as a very dangerous procedure that can result in them losing their mobility. There have also been instances where potential candidates could not enrol due to interference from family members despite being assessed as having sufficient capacity to consent for participation.

To date, we have successfully recruited approximately 39 case participants, taking the attrition rate into account (±20%), along with around 12 case controls. The attrition rate we are experiencing aligns with those observed in other longitudinal cohort studies. Among the participants, 26 completed 3 months, 19 completed 6 months and 8 completed the 12-month visit. We have enrolled 12 matched controls to date.

Study significance

This is the first study to investigate the immune dysregulation and neuroinflammation in persons with FEP from low-income to middle-income countries (LMIC), the study intending to bridge the gap and seek clarity about the role of the immune system in psychosis. The study results will pave the way to develop appropriate, well-designed and better-powered studies that will allow for an improved understanding of the immunology of primary psychosis, in LMIC in particular. In the long term, exploration of these biomarkers may present an opportunity to develop immunological subtypes of psychosis and predictive/prognostic markers and improve on selecting the optimal treatment in primary psychosis.

Author affiliations
[1]Psychiatry, University of KwaZulu-Natal Nelson R Mandela School of Medicine, Durban, KwaZulu-Natal, South Africa
[2]University of KwaZulu-Natal, Durban, KwaZulu-Natal, South Africa
[3]University of KwaZulu-Natal College of Health Sciences, Durban, South Africa
[4]University of KwaZulu-Natal, South Africa
[5]University of KwaZulu-Natal, Congella, KwaZulu-Natal, South Africa
[6]Clinical Medicine Laboratory, University of KwaZulu-Natal Nelson R Mandela School of Medicine, Durban, South Africa
[7]School of Laboratory Medicine, University of KwaZulu-Natal, Durban, South Africa
[8]Department of Innovation in Medical Education Canada, Ottawa University, Ottawa, Kansas, USA
[9]School of Clinical Medicine, University of KwaZulu-Natal, Durban, South Africa

X Bongani B Nkambule @Nkambuleb

Contributors All the authors made substantive contributions and were involved in the conception, design, writing and review of the manuscript. LT is the guarantor of this research project. Grammarly to check for grammatic errors.

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ORCID iDs
Lindokuhle Thela http://orcid.org/0000-0001-5483-2492
Bongani B Nkambule http://orcid.org/0000-0001-8846-1992
Vuyokazi Ntlantsana http://orcid.org/0000-0002-5882-100X
Usha Chhagan http://orcid.org/0000-0002-4436-2025
Bonginkosi Chiliza http://orcid.org/0000-0001-5417-5920

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