An Exploratory Analysis of Associations Between Psycho-Social Factors and Systemic Inflammation among South African Youth

by
Ashley Henry

B.Sc., University of Winnipeg, 2016

Capstone Project Submitted in Partial Fulfillment of the
Requirements for the Degree of
Master of Public Health

in the
Master of Public Health Program,
Faculty of Health Sciences

CONTENTS

ABBREVIATIONS AND TERMS

Abbreviation	*Full Name*	*Other names*
AIDS	Acquired Immunodeficiency Syndrome	
AVENA Study group	Alimentación y Valoración del Estado Nutricional en Adolescentes (Food and Nutritional Status in Adolescents	
AYAZAZI	Zulu language word for “knowing themselves”; name of source cohort study	
BMI	Body Mass Index	
CCR5	C-C chemokine receptor type 5	
CD4	Cluster of determination 4	
CES-D 10	Center for Epidemiologic Studies Short Depression 10-item scale	
CRP	C Reactive Protein	
DMPA	Depot medroxyprogesterone acetate	
HIV	Human Immunodeficiency virus	HIV-1
HPP	HIV Pathogenesis Programme	
HSV-2	Herpes Simplex Virus Type 2	
IFN-α2	Interferon alpha 2	IFN-α
IFN-γ	Interferon gamma	
IL-1α	Interleukin 1 alpha	
IL-1β	Interleukin 1 beta	
IL-4	Interleukin 4	
IL-6	Interleukin 6	
IL-10	Interleukin 10	
IL-12(p40)	Interleukin 12 p40	
IL-12(p70)	Interleukin 12 p70	
IP-10	Interferon inducible protein 10	CXCL10
IQR	Interquartile range	
MatCH	Maternal Child Health Research Unit	MRU
MIP-1β	Macrophage inflammation protein 1 beta	CCL4
PHRU	Perinatal Health Research Unit	
PSS	Perceived Stress Scale	
SA	South Africa	
SSA	Sub-Saharan Africa	
TNF-α	Tumor necrosis factor alpha	
UNAIDS	Joint United Nations Programme on HIV/AIDS	
ZAR	South African Rand	

ABSTRACT

Introduction: Psycho-social factors affect biological processes, including inflammation and immune response, yet their contribution to gender and socio-economic disparity of HIV is not well understood. In South Africa, 38% of new HIV infections occur in 15–24 year olds, with 3-times higher incidence among females. In this exploratory study, we examined associations between psycho-social factors and biomarkers of inflammation that may be linked to HIV acquisition in South African youth.

Methods: Baseline plasma and linked cross-sectional survey data were obtained from the AYAZAZI study, which enrolled 425 HIV uninfected or HIV status-unknown youth (16-24 years old; 60% female) in Durban and Soweto (2014-2016). Survey data captured social and clinical determinants of health (e.g., gender, income, food insecurity, body mass index [BMI]) and psycho-social characteristics (depression, anxiety, stress, substance use). A random, gender-stratified subset of 39 HIV-negative participants was selected. Luminex® assays were used to analyze 12 plasma biomarkers. Associations between biomarkers and social, clinical, and psycho-social factors were assessed using Spearman's rank correlation.

Results: Median age was 18 (IQR: 17-20); 19/39 were female. Consistent with prior studies, high depression symptomology scores were associated with elevated pro-inflammatory (IFN-a2, IL-1a, IL-6, IL-12(p40), MIP-1ß) and anti-inflammatory (IL-4, IL-10) cytokines (all $p<0.05$). Low BMI correlated with elevated pro-inflammatory (IFN-a2, IFN-g, IL-1a, IL-1ß, IL-6, IL-12(p70), IP-10 and TNF-a) and anti-inflammatory (IL-10) biomarker levels (all $p<0.05$). Associations were also observed between some biomarkers and indicators of anxiety, food insecurity, low income, and financial responsibility for dependents, which varied between sites.

Conclusion: Results indicate that psycho-social, clinical, and socio-economic challenges are associated with inflammatory biomarker levels in South African youth. This suggests a link between social determinants of health and biological factors that modulate disease risk, possibly including inflammatory conditions associated with increased HIV transmission. Further analysis is required to confirm these results and investigate their implications for HIV prevention.

1 INTRODUCTION

1.1 STATEMENT OF PURPOSE

To contribute to understanding the interaction between biological and social determinants of HIV acquisition risk among youth, the purpose of this analysis was to measure and describe the profile of biomarkers of systemic inflammation and to explore associations between inflammation and socio-behavioural factors. This exploratory analysis was performed using blood samples and cross-sectional survey data from a cohort of adolescents and young adults (aged 16-24 years; HIV-negative or HIV status unknown) enrolled in the community-based AYAZAZI cohort study in Durban or Soweto, South Africa.

1.2 THE SOUTH AFRICAN HIV EPIDEMIC

HIV/AIDS has disproportionately burdened the South African population since the beginning of the global epidemic. Statistics South Africa (2017) estimated that 4.64% of South African youth aged 15-24 were living with HIV in 2016. Adolescents and young adults aged 16-24 years are estimated to account for 50% of new HIV infections in South Africa (UNAIDS, 2010). Adolescents and young adults have been identified as a key population in the South African epidemic based on both socio-behavioural and biological factors making them more susceptible to HIV infection (UNAIDS, 2010).

HIV acquisition risk is affected by the social environment, behavioural factors, and biological processes. This dynamic, and complex environment is one in which over 590,000 youths, aged 15 to 24, were infected with HIV in 2017 worldwide (UNICEF, 2017). Identifying risk factors, both socio-behavioural and biological, is essential for HIV prevention efforts targeting this population.

The most recent South African National HIV Prevalence Survey (2017) found a higher prevalence of HIV infection (16.6%) among Black South Africans compared to other racial

groups. The survey also found a three times greater prevalence in females compared to males aged 20 to 24 (15.6% and 4.8%, respectively). Household income, education, and other indicators of socioeconomic status have varied in their association with HIV over time during the evolution of the epidemic in South Africa. However, currently, lower income has been broadly associated with higher HIV prevalence (Bunyasi & Coetzee, 2017). Socioeconomic factors have been linked to behavioural risk factors for HIV. Lower income has been linked to an increased likelihood of engaging in transactional sex, or age-disparate relationships (Steinert et al., 2017). Lower levels of education have been linked to higher numbers of sexual partners, inconsistent condom use, early sexual debut, and age-disparate relationships. These socio-behavioural risk factors for HIV also affect adolescents and young adults. Cluver, and colleagues (2016) recently surveyed adolescents aged 10 to 17 in two South African provinces and found that issues of food insecurity, income, education, and mental health struggles made adolescents more likely to engage in behaviours associated with high HIV risk like transactional sex and inconsistent condom use. However, the 2012 South African National HIV Prevalence Survey found that more youth (15-24) reported condom use (58.4%) than older age groups, and 45.7% of youth reported consistent condom use. Youth and adults experience variations in the social and behavioural environments contributing to HIV acquisition risk as they age.

Biological sex and gender are important determinants of HIV risk. Females are more susceptible to contracting HIV per-exposure than men for several social and biological reasons. Women have been shown to be particularly vulnerable in youth. Women of all ages have a larger susceptible surface area for genital infection and some studies have found a greater number of receptors for HIV to bind, facilitating infection (Yi, Shannon, Prodger, McKinnon, & Kaul, 2012; Lajoie, Mwangi, & Fowke, 2017; Prodger et al., 2012). Young women are more prone to small tears in the vaginal wall during intercourse, and vulnerable columnar epithelial cells are more prominent on the endocervix (Yi, Shannon, Prodger, McKinnon, & Kaul, 2012). Social determinants specific to the South African context such as income inequity make young

women more likely to engage in transactional sex and relationships with older men contributing to a reduced ability to negotiate condom use and safe sex ultimately increasing their risk of acquiring HIV (Harrison, Colvin, Kuo, Swartz & Lurie, 2015).

1.3 INFLAMMATORY BIOMARKERS

Inflammation is a healthy and natural process in the regulation of the human body's defense against infection. This process is implemented and regulated by a dynamic milieu of many different biomarkers, known as cytokines and chemokines, some of which promote inflammation and some of which inhibit inflammation. Generally, cytokines are the messengers between cells that affect how a cell will function, and chemokines attract cells to a particular location, affecting where a cell will function. These functions are broadly outlined in **Table 1** for those cytokines or chemokines included in this analysis.

Chronic elevation of inflammation-promoting biomarkers, known as pro-inflammatory cytokines, has been linked to elevated risk of acquiring HIV, and poorer outcomes in HIV infection, in addition to several other poor health outcomes (Aiello & Kaplan, 2009; Albert, Glynn, Buring, & Ridker, 2006; Gimeno, Brunner, Lowe, Rumley, Marmot, & Ferrie, 2007; Elliot & Chapman, 2016; Loucks et al, 2006; Welsh, Woodward, Rumley, & Lowe, 2008). Pro-inflammatory cytokines included in this analysis are IL-1α, IL-1β, TNF-α, IL-6, IL-12, IFN-α2, and IFN-γ. Higher levels of these cytokines in tissues exposed to HIV make it easier for HIV to penetrate the specific cells necessary for infection (Monastero & Pentyala, 2017). Anti-inflammatory cytokines include IL-10, and IL-4. These cytokines moderate the effects of pro-inflammatory cytokines and so must increase in response to increases in their pro-inflammatory counterparts. However, cytokines are part of a cascade of responses that may ultimately influence either pro- or anti-inflammatory effects. Even IL-10 has been shown to have pro-inflammatory effects at high concentrations (Monastero & Pentyala, 2017). In addition to the cytokines already described, two chemokines are included in this analysis, MIP-1β and IP-10.

Chemokines, though similar in many ways to cytokines, function to attract cells to a specific location. Chemokines are generally found most concentrated near their source and dissipate more quickly than cytokines (Liebenberg et al., 2017). MIP-1β and IP-10 have been implicated in attracting cells that are vulnerable to HIV infection to genital tissue (Liebenberg et al., 2017).

Defining a normal range for a given cytokine is difficult because it varies with age, from person-to-person, over time throughout the day, in response to subtle and often unobservable physiological processes, hormonal cycles, and other factors (Monastero & Pentala, 2017, Huijbregts et al., 2013). Elevated levels of pro-inflammatory cytokines will trigger the production of anti-inflammatory cytokines to reach a balance in the system. The levels of cytokines observed in plasma are only comparable to other levels observed in plasma. A common threshold used for clinically defining a normal range of concentrations for a given cytokine is two standard deviations away from the mean or median of the specific population in consideration.

While it is known that the immune system matures over time, less is known about how that process of maturation affects the HIV acquisition risk of youth. Studies have investigated differences in mucosal immunity and inflammation between adults and youth but none were identified that characterized specific differences in circulating cytokines (Kyongo et al., 2015). Foo, Nakagawa, Rhodes, and Simmons (2016) conducted a meta-analysis of studies on the effects of sex hormones on immune function and found that immune suppression was consistently associated with testosterone levels in males, and that oestrogen levels in females had a more complex relationship with immune function. Levels of these hormones are elevated in adolescence, but the role of this difference in the pathogenesis of HIV in adolescents has not been investigated.

Hormonal contraception and hormone variation associated with the menstrual cycle and pregnancy also affects cytokine levels in females. Depot medroxyprogesterone acetate (DMPA), a progesterone analog sold as Depo-Provera, has been shown to inhibit secretion of both pro- and anti-inflammatory cytokines (Huijbregts et al., 2013). The luteal phase of the menstrual

cycle, associated with increasing progesterone and decreasing estradiol, and DMPA are both associated with multiple alterations in the immune response contributing to increased susceptibility to HIV (Wessels, Felker, Dupont, & Kaushic, 2018).

Much of the work linking external factors to elevated indicators of inflammation is based in cardiovascular research with older adults in high-income countries (Aiello & Kaplan, 2009; Albert, Glynn, Buring, & Ridker, 2006; Gimeno, Brunner, Lowe, Rumley, Marmot, & Ferrie, 2007; Elliot & Chapman, 2016; Loucks et al, 2006; Welsh, Woodward, Rumley, & Lowe, 2008). Associations of elevated inflammation with depression and obesity have also been established by this research (Elliot, Turiano, & Chapman, 2016; Gabbay et al., 2009; Dahl et al., 2014; Herder et al., 2007; Mitchell, & Goldstein, 2014; Stepanikova, Oates, & Bateman, 2017). However, the social and biological environments within which South African youth navigate their sexual lives is very different, limiting the generalizability of previous research to the South African context. Moreover, limited previous research has explored broader linkages between socio-behavioural determinants, including the social determinants of health.

Table 1: Overview of cytokines included in this analysis and their functions, in alphabetical order

Cytokine	Function	Interactions with other cytokines	Association with HIV pathogenesis
IFN-α2	• Pro-inflammatory effects[1] • Multiple functions coordinating response to viral infection[1]	• Inhibits production of non-specific inflammation biomarker, CRP[1]	• Restrict HIV-1 replication, reducing ability of HIV to infect host[2]
IFN-γ	• Pro-inflammatory effects[2] • Multiple functions coordinating response to viral infection[2]	• Stimulates production of IP-10[2] • Has a role in the development of macrophages which can lead to increases in TNF-α, IL-1, and IL-12[2]	• Contribute to inflammatory profile, potentially enhancing ability of HIV to replicate in early infection by attracting susceptible T cells[2,7]
IL-1α	• Pro-inflammatory cytokine[1] • Produced by activated macrophage responding to infection or cell injury[1] • Systemic reactions such as elevating body temperature in response to infection and injury[1]	• Stimulates production of non-specific inflammation biomarker, CRP[1] • Stimulates production of TNF-α[1]	• Contribute to inflammatory profile, potentially enhancing ability of HIV to replicate in early infection by attracting susceptible T cells[2,7]
IL-1β	• Pro-inflammatory cytokine[1] • Produced by activated macrophage responding to infection or cell injury[1] • Systemic reactions such as elevating body temperature in response to infection and injury[1]	• Stimulates production of non-specific inflammation biomarker, CRP[1] • Stimulates production of TNF-α[1]	• Contribute to inflammatory profile, potentially enhancing ability of HIV to replicate in early infection by attracting susceptible T cells[2,7]
IL-4	• Anti-inflammatory effects[1] • Manages the humoral immune response[1]	• Antagonist for IL-1α and IL-1β Inhibited by IL-12[1] • Inhibits production of IFN-γ[1]	• No direct HIV-related pathology • Anti-inflammatory effects would balance and regulate a higher risk inflammatory profile[7]
IL-6	• Strong pro-inflammatory effects[1]	• Stimulates production of non-specific inflammation biomarker, CRP[1]	• Contribute to inflammatory profile, potentially enhancing ability of HIV to replicate in early infection by attracting susceptible T cells [2,7]
IL-10	• Strong anti-inflammatory effects[1] • Has been shown to have pro-inflammatory effects at very high concentrations[3]	• Inhibits production of IL-12 • Inhibits IL-1α, IL-1β, IL-6, and TNF-α[1] • Inhibits production of IFN-γ[1]	• Hormonal contraceptive DMPA associated with decreased IL-10 levels in the genital tract[5] • Elevated plasma levels associated with greater risk of HIV seroconversion in serodiscordant couples[6] • Mutations in IL-10 production causing lower levels have been associated with increased susceptibility to HIV while those causing higher levels have been associated with longer periods of latency and longer time to AIDS progression[7]
IL-12 (p40)	• Pro-inflammatory effects[1] • Inactive version of IL-12[1] •	• Inhibits IL-12(p70)	• Hormonal contraceptive DMPA associated with elevated IL-12 levels in the genital tract[5]
IL-12 (p70)	• Pro-inflammatory effects[1] • Active version of IL-12[1] • Activates the cell-mediated immune response and inhibits the humoral response[1]	• Stimulates production of IFN-γ and TNF-α[1]	• Hormonal contraceptive DMPA associated with elevated IL-12 levels in the genital tract[5]
IP-10	• Chemokine[4] • Attracts T-cells when induced by IFN-γ viral response[4]	• Production is induced by IFN-γ[1]	• Elevated plasma levels associated with greater risk of HIV seroconversion in serodiscordant couples[6]

			• Elevated in genital tissue and reduced in plasma associated with greater risk in young South African women [4]
MIP-1β	• Chemokine[4] • Induces production of pro-inflammatory cytokines as part of macrophage response to bacteria[4] • Attracts multiple immune cells to origin site[4]	• Induces production of IL-1α, IL-1β, IL-6, and TNF-α[4]	• High concentrations found to be essential for establishment of SIV infection in macaques [7] • Attract vulnerable CCR5+ cells [4]
TNF-α	• Strong pro-inflammatory effects[1]	• Stimulates production of non-specific inflammation biomarker, CRP[1]	• Contribute to inflammatory profile, potentially enhancing ability of HIV to replicate in early infection by attracting susceptible T cells [2,7]

1: Zhou, Fragala, McElhaney & Kuchel, 2010; 2: Roff, Song & Yamamoto, 2014; 3: Monastero & Pentyala, 2017; 4:Liebenberg et al., 2017; 5: Louw-du Toit, Hapgood, & Africander, 2014; 6:Kahle et al., 2014; 7:Kaul et al., 2015

1.4 Socio-Behavioural Determinants of Health and Inflammation

While social and behavioural determinants alone cannot cause HIV infection, they can make exposure to the virus more likely. Recent investigations have focused on whether socio-behavioural determinants can also directly affect biological processes, making HIV infection more likely to occur per exposure event. The majority of existing literature uses C-reactive protein (CRP), interleukin-6 (IL-6), or tumor necrosis factor-alpha (TNF-α) to examine the association these biomarkers of systemic inflammation have with different socio-behavioural determinants. The mechanism of these associations has been hypothesized to be related to DNA methylation and gene expression, and hormones (Castagné et al., 2016). Matthews and Gallo (2011) critically examined the research investigating the link between lower socioeconomic status and inflammation. They assessed whether increased stressors across the socioeconomic gradient was the cause of differences in inflammation along the socioeconomic gradient and found little supporting evidence. They highlighted stronger evidence in favour of psychosocial resources as a mediator between socioeconomic status and inflammation. The resources include higher levels of reported resiliency, perceived control, and social supports in those of a higher socioeconomic status (Matthews & Gallo, 2011).

While CRP was not included in this analysis, it has been used extensively as a biomarker to illustrate the link between systemic inflammation in adulthood and social-behavioural factors, particularly adverse experiences and trauma in early childhood and socioeconomic status (Liu et al., 2017; Baumeister et al., 2016; Nazmi & Victora, 2007; Aiello & Kaplan, 2009; Loucks et al., 2006), however, limited similar work has focused on adolescents and young adults. Freeman et al. (2016) used data from the National Longitudinal Study of Adolescent to Adult Health to compare CRP levels with subjective social status between males and females aged 24 to 32. The association was found to be significant in males and not females. Chiang et al. (2015) found that over a 15 day period higher CRP levels in adolescents aged 14-20 were most

correlated with lower parental education, more negative social interactions, and higher BMI. The majority of these studies are conducted in the United States which highlights a gap in literature on this phenomenon in lower-resource settings and different cultures where perceptions of poverty are different and may affect the link between inflammation and socioeconomic factors.

Pietras and Goodman (2013) have criticized the use of CRP as a biomarker, and conducted one of few studies investigating the relationship of pro-inflammatory cytokines, IL-6 and TNF-α, with multiple indicators of socioeconomic status in adolescents (ages 12-19). They found an association between higher adolescent inflammation and lower parent education using both IL-6 and TNF-α as biomarkers. BMI was found to have a mediating role in this association.

This section has outlined previous research investigating the link between socio-behavioural factors and inflammation, much of which stems from explorations of cardiovascular disease risk in Western countries. In this study, we focused on this same relationship, in the context of understanding HIV acquisition risk among youth in South Africa. In this context, the relationship between immune activation and HIV risk is well established, but the causes of variation in the levels of immune activation are not always clear, particularly in the under-researched youth population (Naranbhai et al., 2012; Passmore, Jaspan, & Masson, 2016). Liebenberg et al. (2017) investigated an essential question for understanding the contributions of systemic inflammation to HIV risk: *How are systemic inflammation and genital tissue inflammation related when it comes to HIV acquisition?* This study, based in South Africa with women aged 20-25, found that a gradient of high mucosal levels of chemokines compared to lower levels of chemokines in plasma was associated with increased HIV acquisition risk, particularly for MIP-1β but also for IP-10. Elevated levels of IL-1β, TNF-α, and lower levels of IP-10 in plasma were also associated with increased risk in this study (Liebenberg et al., 2017). Socio-behavioural factors, such as hormonal contraception use, Herpes Simplex Virus-2 (HSV-2) status, age, and the number of sex acts and reported condom use during the study, were used to adjust models

but not analyzed so their role in the etiology of these inflammatory gradients cannot be assessed.

Liebenberg et al. (2017) have highlighted the important role of chemokines in trafficking susceptible cells to potential sites of HIV infection in acquisition risk. Studies in rhesus macaques found that the likelihood of infection upon exposure to HIV is dependent on the number of activated CD4+ T cells with a CCR5 receptor (CD4+CCR5+ T cells) present in the exposed mucosal tissue (Kaul et al., 2015). These cells have the ability to replicate more copies of the HIV virus intracellularly, thereby increasing their capacity for propagating the HIV infection. Persons with higher levels of these cells present in mucosal tissue are more susceptible to HIV infection per-exposure. Previous research has found that persons who have been exposed to HIV but remain seronegative have reduced systemic and mucosal immune activation leading to fewer susceptible cells present in exposed tissues (Chege et al., 2012; Lajoie et al., 2012; McLaren et al., 2010; Prodger et al., 2014; Yao et al., 2014; Kahle et al., 2014). On-going research is focused on identifying biological or clinical explanations for the elevated inflammatory state including co-infections, use of injectable progestin contraception, and genetic factors (Kaul et al., 2015).

Despite the greater focus on cytokine levels in genital tissues, systemic levels have also been associated with HIV acquisition. Kahle et al. (2014) and Naranbhai et al. (2012) found a direct association between increased systemic inflammation and HIV acquisition. As previously stated, Liebenberg et al. (2017) also found that some cytokines were associated with greater HIV acquisition risk but found the opposite direction of effect for IP-10 compared to Kahle et al.. Systemic levels of cytokines and inflammation are more subject to fluctuations based on multiple factors compared to genital tissue, making it more difficult to investigate a clear and consistent role in HIV acquisition research.

Furthering the understanding of associations between socio-behavioural and biological markers of HIV risk is essential to develop the knowledge base that guides the development of

HIV prevention policies and programs to address the HIV epidemic among youth in South Africa. This research is particularly lacking among youth populations, and variation in the developing biology and specifically, the immune system, and differences in the exposure to social and behavioural risk factors make age-specific research necessary.

2 OBJECTIVES AND HYPOTHESES

The purpose of this analysis is to contribute to reducing a gap in knowledge concerning the association between social and biological determinants of health contributing to the risk of HIV acquisition among South Africa's adolescents and young adults.

2.1 OBJECTIVES

Among a cohort of youth aged 16-24 years residing in Durban or Soweto, South Africa, this project had two objectives:

Objective 1: To measure and describe the profile of plasma cytokine and chemokine concentrations, as biological markers of systemic inflammation.

Objective 2: To assess the association between individual cytokine and chemokine concentrations and socio-behavioural characteristics.

2.2 HYPOTHESES

Hypothesis for Objective 1: Plasma cytokine concentrations will be within a biologically-plausible range.

Hypotheses for Objective 2: Socio-behavioural variables that have been previously linked to systemic inflammation such as sex, depression, obesity and socioeconomic status were hypothesized to be associated with higher levels of systemic pro-inflammatory cytokines.

3 METHODS

3.1 STUDY DESIGN AND SETTING

This exploratory analysis on biological markers of systemic inflammation used a sub-set of baseline data from adolescents and young adults (males and females) enrolled in a cohort study called AYAZAZI, a youth-centered, dual-site, community-based, prospective cohort study focused on understanding linked patterns of socio-behavioural and biomedical HIV risk among youth in South Africa. This study took place in two study sites in South Africa, Durban and Soweto, and enrolled a total of 425 youth aged 16-24 years (60% female) between November 2014 and April 2015 in Soweto and between September 2015 and April 2016 in Durban.

3.2 PARTICIPANTS

3.2.1 Recruitment and enrollment of cohort

All AYAZAZI cohort participants were recruited to participate in the study at either the Perinatal HIV Research Unit (PHRU) at Chris Hani Baragwanath Hospital in Soweto or at the Maternal, Adolescent, and Child Health (MatCH) Research Unit (MRU) Commercial City site in the Central Business District of Durban. Participants were also recruited through active community recruitment using posters, flyers, word-of-mouth, and face-to-face recruitment. Additionally, participants were recruited from the PHRU HIV voluntary counselling and testing clinic in Soweto and a public health sector clinic in Durban. Study activities occurred in a youth-friendly space set up to support the sexual, reproductive, and mental health needs of young people.

Eligibility criteria for inclusion in AYAZAZI included living in Soweto or Durban, being 16-24 years old, HIV-negative or unsure of HIV status, and willing to undergo regular HIV testing. All participants aged 18-24 provided voluntary informed consent. Individuals under 18 were required to get written voluntary informed consent from a parent or and the participant provided

voluntary informed assent. Participants were excluded from enrolling in the cohort if under the age of 18 and unable to provide consent to participate from a parent or guardian, if they had an obvious psychological disorder that would compromise the informed consent process, or if they were participating in another clinical or observational HIV study.

For this sub-study of biological markers of inflammation, participants who remained HIV-seronegative for the entire duration of the study (n=400/425) were assigned pseudo-random numbers using Microsoft Excel. Sixty-seven participants with the lowest random numbers were selected for analysis, maintaining an equal number of males and females as well as participants from Durban and Soweto. Of the 67 participants who were randomly selected, two did not have an available blood sample. The sample capacity of laboratory testing for this analysis was subsequently determined to be 64, so one participant was removed from the sample considering the distribution of sex, study site, and the largest assigned random number. Thus, the final sample size tested for cytokine concentrations was n=64.

3.3 STUDY PROCEDURES

At enrollment all AYAZAZI participants completed an interviewer-administered questionnaire, received pregnancy, HIV, and STI testing, and counselling for test results, contraception, and STI risk reduction. A clinical exam was also performed at enrollment, during which height and weight were recorded, peripheral blood samples were taken, and samples to test for STIs were taken including urine for males, and vaginal mucosa swabs for females. Study visits were completed at baseline and at 6, 12, and 18 (Soweto only) months after baseline. All participants had follow-up visits with a survey, clinical exam, and HIV testing and counselling every six months. Participants from the Durban study site attended a follow up visit after three months for an HIV test and a shorter version of the socio-behavioural questionnaire used at baseline.

3.3.1 Survey

Baseline survey data was collected at the time of enrollment using a questionnaire administered by youth interviewers using DataFAX™ software. The questionnaire could be completed and administered in English, isiZulu, or Sesotho depending on participant preference. A long-form questionnaire was used at baseline and at 12 months follow-up. A shortened version of the questionnaire was used at the 3-month (Durban only) and 6-month follow-up visit. The questionnaire was divided into sections that examined socio-demographics, sexual and reproductive history and behaviour, perceived risk of contracting HIV, use of health services, experiences with violence, substance use, mental health, willingness to participate in HIV vaccine trials, and use of technology.

3.3.2 Clinical

A research nurse collected clinical data at baseline using a questionnaire and a clinical exam was conducted at every study visit to identify any health issues. Height and weight was also measured. Peripheral blood was drawn for all new and existing HIV-positive diagnoses at each study visit. Peripheral blood was drawn for those with a negative HIV test result only at the baseline and 6-month follow-up study visits. Peripheral blood drawn from HIV-negative participants at baseline was used to conduct laboratory procedures for this analysis.

3.3.3 Laboratory

All plasma samples were stored in secure freezer storage at the HIV Pathogenesis Programme at the University of KwaZulu-Natal in Durban. For this analysis, multiplex immunoassays were performed to assess the concentrations of cytokines. Plasma samples sourced from the peripheral blood of sample participants (n=64) collected at baseline were tested for the concentration of twelve cytokines.

3.4 MEASURES

3.4.1 Survey

Survey data from the demographics, substance use, and mental health sections was included in this analysis. Specifically, age, race, number of children, number of financial dependents, level of education, average monthly income, food insecurity, substance use, and several self-reported mental health scales measuring anxiety, depression, and perceived stress were included in this analysis. Food insecurity, average monthly income, anxiety, and depression, perceived stress data have all been summarized as categorical variables. Anxiety, depression, and perceived stress were also analyzed in their original continuous form when possible to retain statistical power.

Substance use variables were included for cigarette smoking, drug use, and alcohol use. Cigarette smoking was measured using the number of cigarettes smoked per day in the past 30 days. *Cigarette smoking* was categorized into non-smokers, less than 1 to 5 cigarettes per day, and more than 5 cigarettes per day (coded as 0, 1, and 2, respectively). *Drug use* was measured for many different drugs but for this analysis, the variable was limited to three categories. The categories of drug use were those who had never used drugs, those who had ever used drugs but had not used any within the past 30 days, and those who reported using any drug within the past 30 days (coded as 0, 1, and 2, respectively). *Alcohol use* was measured using the number of days of alcohol consumption within the past 30 days. Those who reported no days of alcohol consumption in the past 30 days were coded as 0, those who reported having between 1 and 4 drinks any number of days within the past 30 days were coded as 1, and those who reported having 5 or more drinks any days within the past 30 days were coded as 2. Five or more drinks in a single day was considered binge drinking. Alcohol and cigarette use questions were sourced from the CDC Standard High School Youth Risk Behaviour Survey (2013) but the responses were structured differently. Drug use questions

were developed for this study but informed by a guide from the Youth Risk Behaviour Survey (CDC, 2016).

Food insecurity was measured using the six-question Household Hunger Scale (HHS) (Ballard, Coates, Swindale, & Deitchler, 2011). This scale measures whether participants had any one of three defined experiences of hunger in their household, and how frequently they experienced that hunger. For each experience, those who had not had that experience within the past 30 days were coded 0, those who had experienced it rarely or sometimes (1-2, or 3-10 times within 30 days) were coded as 1, and those who had experienced it often (more than 10 times within 30 days) were coded as 2. All of the coded responses are summed to form the Household Hunger Scale indicator. Scores are categorized into 'little to no hunger' (0-1 total), 'moderate hunger' (2-3 total), or 'severe hunger' (4-6).

Perceived stress was measured using a 10-item scale (PSS) inquiring about emotional responses to stressors experienced in the past 30 days (Cohen, Kamarck, & Mermelstein, 1983). Responses were structured as a 5-point Likert scale (Never, 1-2 days, 3-10 days, 11-20 days, More than 20 days). Perceived stress levels were analyzed as both a categorical and a continuous variable and were scored from 0 to a maximum of 40 and divided into low (0-13), medium (14-26), and high (27-40) stress levels for reporting.

Depression was measured using the Center for Epidemiologic Studies Short Depression 10-item scale (CES-D 10) inquiring about emotional responses to stressors experienced in the past 7 days (Bradley, Bagnell, & Brannen, 2010; Radloff, 1977). Responses were structured as a 4-point Likert scale (Less than 1 day, 1-2 days, 3-4 days, 5-7 days). Depression was analyzed as both a categorical and a continuous variable. The possible range of values was from 0 to a maximum of 30, with values of 10 or greater categorized as high depression symptomology.

Anxiety was measured using the American Psychiatric Association 3-item scale inquiring about feelings of anxiety and its effects on their ability to conduct normal activities in the past 14 days (American Psychiatric Association, 2013). Responses were structured as a 5-point Likert

scale (Not at all, 1-2 days, 2-7 days, 8-12 days, 13-14 days). Anxiety was analyzed as both a categorical and a continuous variable. The possible range of values was from 0 to a maximum of 12, with higher values indicating greater anxiety. A response of 2-7, 8-12, or 13-14 days in response to any of the three items was sufficient to warrant follow up from a clinical professional for anxiety (American Psychiatric Association, 2013). This was coded as Probably Anxiety in the analysis. This means that any participants who experienced any of the indicators more than one in fourteen days experienced enough anxiety to be considered a probable clinically anxious individual.

3.4.2 Clinical

For this analysis, we included laboratory-confirmed STI diagnoses and Body Mass Index (BMI) for each participant based on measures of height and weight. BMI for each participant was categorized based on clinical guidelines defining underweight (< 18.5), normal weight (18.5-24.9), and overweight ranges (25-29.9) (South Africa Department of Health, 2016). Blood drawn at the same time as clinical data collection was used to perform the multiplex immunoassay.

3.5 MULTIPLEX IMMUNOASSAY

Multiplex testing was performed on plasma specimens collected from baseline at the HIV Pathogenesis Programme (HPP) laboratory in Durban using a Milliplex® Human Cytokine/Chemokine Magnetic Bead Panel (EMD Millipore Corporation, Billerica, USA). Inflammatory markers examined in this testing included 8 pro-inflammatory cytokines (IFN-α2, IFN-γ, IL-1α, IL-1β, IL-6, IL-12 (p70), IL-12 (p40), and TNF-α), 2 anti-inflammatory cytokines (IL-4 and IL-10), and 2 chemokines (IP-10, MIP-1β). Plasma samples of approximately 50mL were stored in a secure freezer at -80°C after collection and prior to testing. Laboratory procedures were performed according to the manufacturer's procedures, but the concentrations of all

reagents were halved to double the capacity of samples to be analyzed. This procedure was validated internally prior to this analysis. Observed concentrations of each cytokine were estimated with Milliplex® Analyst version 5.1 using standard curves with four to seven points depending on the cytokine. All samples were analyzed in pairs and averages were taken as the observed concentration value for each sample. Values below the range of detection were corrected as 0.25 times the minimum recorded value for each cytokine. Only one value was below the range of detection in this sample.

The results from one of the 96-well plates, containing the samples of 25 participants, had a high proportion of undetectable or values estimated outside of the range of the standard curve (24%), indicating either very high, or very low values, or an issue with the quality of the test results. These results have not yet been confirmed, however, due to concerns about data quality, all 25 participants were excluded from this analysis. Thus, the resulting sample size was 39 participants. The majority of the samples included on this plate were from the Soweto study site, making this sub-population underrepresented in this analysis.

3.6 DATA ANALYSIS

3.6.1 Sample characteristics

Socio-behavioural variables were summarized using descriptive statistics. Continuous variables, including mental health scales (perceived stress, anxiety, and depression) and BMI were categorized as described above and summarized along with categorical variables using counts and percentages. The distribution between categories of variables was compared in the sample used for this analysis (n=39) and the overall AYAZAZI cohort (n=425).

3.6.2 Cytokine profile

Cytokine concentration data was analyzed using descriptive statistics to define the median and distribution of each cytokine. Cytokine concentrations (pg/mL) were estimated based on

standard curves of four to seven standard points for each cytokine. Normality of the data both before and after Log_{10} transformation was evaluated using kernel density plots as well as Shapiro-Wilk tests. While the distribution of some cytokines was normal, others retained skew despite transformation, so nonparametric methods were used to evaluate statistical findings. Descriptive statistics including median and the interquartile range (IQR) were calculated. Correlation between cytokines was assessed using Spearman's rank correlation coefficient, rho.

3.6.3 Bivariable analyses

Bivariable analyses were conducted to investigate associations between the concentrations of cytokines and socio-behavioural variables.

The Kruskal-Wallis H test was used to test for differences in the distribution of cytokine concentrations between categorical groups of socio-behavioural variables. This test was performed for all cytokines in this analysis compared to indicators for gender, age, income, number of children, number of financial dependents, food insecurity, substance use, self-reported mental health, STI diagnosis, and BMI. This test was also performed on the same data stratified by study site.

To assess the relationship between the distribution of each cytokine and the potentially explanatory variables non-parametric testing methods were used. Spearman rank correlation coefficients and testing was used to evaluate the associations between cytokine concentrations and socio-behavioural variables. To maximize statistical power, continuous self-reported mental health scales were analyzed separately against each cytokine. This analysis compared three linear bivariate models, one using the complete data set, one stratified by BMI category, and one stratified by study site.

All statistical analyses were completed using R software version 3.3.2.

3.7 ETHICS

Ethics approval was granted by Simon Fraser University in Canada, and by the University of KwaZulu-Natal and the University of Witwatersrand in South Africa.

4 RESULTS

4.1 SOCIO-BEHAVIOURAL VARIABLES

The sample had a nearly equal sex distribution (20 males, 19 females) with a majority of samples from the Durban study site (32 from Durban, 7 from Soweto) (**Table 2**), compared to 52% of participants from Durban in the source cohort. All participants in this sample identified as heterosexual and cis-gender. Ninety-five percent of individuals in this sample identified as black, the remainder identified as coloured (n=2). Most individuals did not have children (n=34), those who did were female (n=5). Males reported having financial dependents who were not their children. Average monthly income was reported to be less than 400 ZAR for 41% of individuals, between 401 and 1600 ZAR for 46%, and greater than 1600 ZAR for 13%. Most individuals lived in formal housing (62%), other housing was less stable. Most individuals reported experiencing little to no hunger in their households, 18% reported moderate hunger in their household and none reported severe hunger. Half of the individuals reported symptoms indicative of depression. Most reported feelings of anxiety (59%) and moderate-to-high levels of stress (72%). 24% of individuals in this sample were diagnosed with a STI other than HIV at baseline. Nearly half (47%) of all participants in the source cohort reported binge drinking within the past 30 days, compared to 8% in the sample used in this analysis. Also in the analysis sample, 64% of participants never drank alcohol.

4.2 CYTOKINES

Transformed to the Log10 scale, concentrations of cytokines spanned a range from the lowest value of 0 pg/mL of IL-6 detected, to the highest value of 2.85 pg/mL of IP-10. **Figure 2** shows the variation in the range of detected concentrations between cytokines and summarizes the exact values describing the distribution of cytokine concentrations in this analysis. IL-10, and IL-6 are the lowest two medians with a broad range of values extending beyond the lower whiskers. The broadest range of cytokine concentrations is IL-10 which has values varying between 0.243 and 1.818 pg/mL. The narrowest range of values was IL-1a, which varied between 2.232 and 1.979 pg/mL.

The cytokine concentrations within this sample were highly correlated. All correlations were positive and highly statistically significant (p <0.001) (**Figure 1**). The correlations, excluding IP-10, ranged from 0.72 to 0.97. IP-10 was positively correlated with all other cytokines, however, with a weaker magnitude (range: 0.27-0.60).

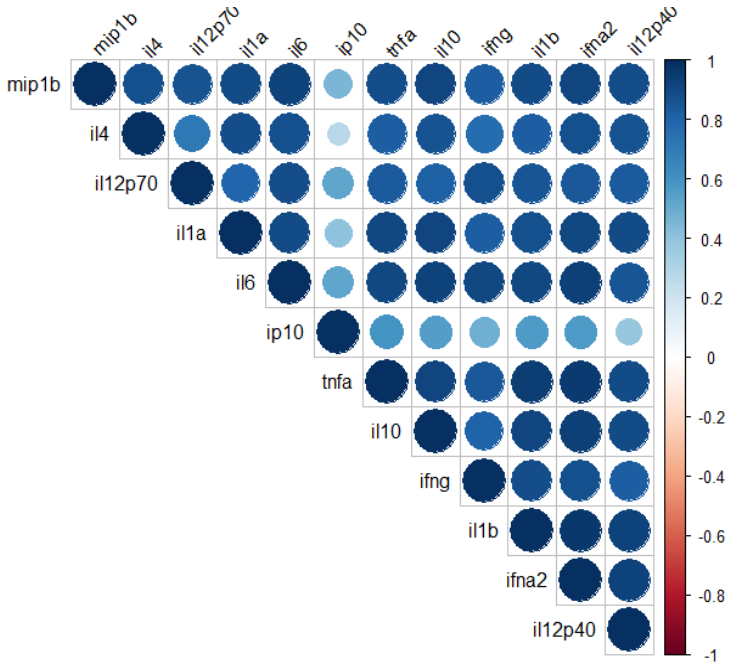


Figure 1: Spearman correlation matrix comparing correlations between analyzed cytokine concentrations (log10 pg/mL).

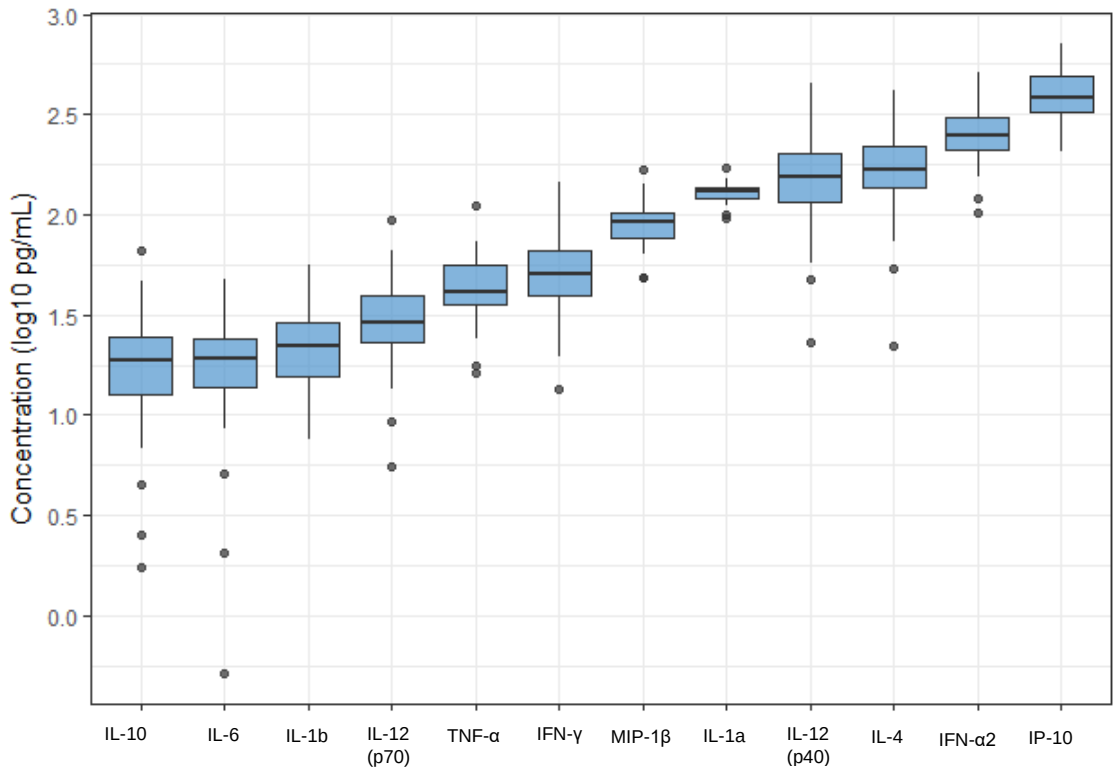
Note: All correlations in this matrix were statistically significant at p< 0.05. Dark blue indicates a very strong positive correlation while dark red indicates a very strong negative correlation

Table 2: Comparison of the distribution of socio-behavioural factors in sample and source cohort, ***sample sizes are noted for variables with missing values, otherwise n=39***

Variable	Categories	Sample (n=39)		Cohort (n=425)	
		n	%	n	%
Study Site	Soweto	7	18	205	48
	Durban	32	82	220	52
Age	16-17	12	31	106	25
	18-21	25	64	256	60
	22-26	2	5	63	15
Number of Children	None	34	87	330	78
	≥1	5	13	95	22
Average Monthly Income (ZAR)	0-400	16	41	124	29
	401-1600	18	46	204	48
	1601 +	5	13	97	23
# of Financial Dependents	None	30	76	303	72
	≥1	9	23	120	28
Housing	Formal Housing	24	62	301	71
	RDP Housing	11	28	78	18
	Shack/Informal Settlement	2	5	38	9
	Other	2	5	8	2
Household Hunger	Low/No Hunger	32	82	364	86
	Moderate Hunger	7	18	55	13
	Severe Hunger	0	0	6	1
Smoke Cigarettes Past Month	None	31	79	305	72
	1-5 cigarettes per day	5	13	92	22
	6+ cigarettes per day	3	8	26	6
Used Drugs Past Month	Never Use	20	51	237	56
	Have Used	11	28	145	34
	Used past month	8	21	43	10
Binge Drink Past Month	Does not drink	25	64	167	39
	Yes drinking	11	28	57	13
	Yes binge drink 30 days	3	8	201	47
Perceived Stress Levels	Low (0-13)	11	28	115	27
	Moderate (14-26)	26	67	269	64
	High (27-40)	2	5	37	9
Depression Levels (sample n=38)	Symptoms Unlikely (0-9)	19	50	239	57
	Significant Symptoms (>10)	19	50	179	43
Anxiety Levels	Anxiety Unlikely	16	41	157	37
	Probable Anxiety	23	59	268	63
Any STI (sample n=38)	No	29	76	243	59
	Yes	9	24	171	41
BMI (sample n=36)	Underweight	6	17	59	14
	Normal Weight	26	72	260	63
	Overweight	4	11	91	22

Figure 2: Box plots of Log10 concentrations (pg/mL) of IFN-α2, IFN-γ, IL-10, IL-12 (p40), IL-12 (p70), IL-1a, IL-1b, IL-4, IL-6, IP-10, MIP-1β, and TNF-α from n=39 participants.

Note: The middle of the box is the median, the upper edge is the 75th quantile, and the lower edge is the 25th quantile. Dots represent outliers, defined as any value beyond the Tukey method whiskers, which are the length of 1.5 times the nearest quantile.



Cytokine	IL-10	IL-6	IL-1b	IL-12(p70)	TNF-α	IFN-γ	MIP-1β	IL-1a	IL-12(p40)	IL-4	IFN-α2	IP-10
Max	1.818	1.681	1.746	1.96	2.045	2.158	2.222	2.232	2.651	2.617	2.713	2.850
75	1.387	1.380	1.461	1.600	1.749	1.819	2.008	2.132	2.310	2.345	2.488	2.692
Median	1.272	1.285	1.346	1.458	1.610	1.708	1.963	2.114	2.192	2.222	2.395	2.587
25	1.102	1.138	1.193	1.359	1.553	1.593	1.882	2.083	2.061	2.136	2.324	2.513
Min	0.243	0	0.880	0.744	1.207	1.129	1.683	1.979	1.362	1.348	2.012	2.312
IQR	0.285	0.242	0.268	0.241	0.196	0.226	0.126	0.049	0.249	0.209	0.164	0.179
Range	1.575	1.681	0.866	1.216	0.838	1.029	0.539	0.253	1.289	1.269	0.701	0.538

4.3 ASSOCIATIONS BETWEEN INFLAMMATORY MARKERS AND SOCIO-BEHAVIOURAL VARIABLES

Three groups of socio-behavioural variables showed statistically significant correlations with multiple cytokines when stratified by study site, Durban or Soweto: depression and anxiety, BMI, and some socio-economic indicators of deprivation. These results are shown in **Table 3, Figure 3, and Figure 4**.

Kruskal-Wallis testing revealed significant differences in the distribution of each significantly correlated cytokine between Durban and Soweto sites, categories of depression symptoms, and groups of underweight, normal weight, and overweight. There were two exceptions to the consistency of this finding; there were no significant differences in the distribution of IFN-α2 between study sites, nor IP-10 between underweight, normal weight and overweight individuals. This, combined with additional evidence that study site and BMI were associated based on Fisher's Exact testing (p-value= 0.015), informed the decision to perform stratification of the sample based on study site.

Overall correlation between BMI categories and cytokines was significant for IL-1α, IL-1β, IL-6, IL-10, IL-12(p70), TNF-α, IFN-α2, IFN-γ, and IP-10. When stratified by study site and analyzed on a continuous scale of BMI values, participants in Soweto had strong correlations between increasing BMI and decreasing cytokine levels, most of which were significant, and participants in Durban had no observable correlation between BMI and any of the cytokines. Participants in Soweto had significant negative correlations between continuous BMI values and IFN-α2, TNF-α, IL-12(p70), IL-12(p40), IL-10, IL-6, IL-1α, and IL-1β, indicating lower concentrations of these cytokines with increasing BMI.

Overall sample correlation between depression and elevated cytokine concentration was significant for IL-1α, IL-4, IL-6, IL-10, IL-12 (p40), MIP-1β, and IFN-α2. When stratified by study site, the correlation did not remain significant for participants based in Soweto with any of the cytokines. In the Durban strata, correlations between the categorical probable diagnosis of

depression and IL-4, IL-12(p40), and MIP-1β remained significant, and showed stronger positive correlations than the overall sample. Participants in Durban had a consistently positive and statistically significant correlation between continuously increasing levels of reported depression symptoms and IL-4, IFN-γ, IL-6, IL-10, IL-12(p70), MIP-1β, and TNF-α (**Figure 3**).

Anxiety, which using the categorical diagnosis threshold did not have a significant correlation with any cytokines, was found to be significantly correlated with IFN-γ, IL-4, IL-12(p70), and MIP-1β as a continuous variable. Participants in Durban had a statistically significant correlation between continuous increases in reported anxiety and increased levels of IFN-γ, IL-4, IL-12(p70), and MIP-1β (**Figure 4**). In Soweto, there appeared to be no correlation between anxiety and any cytokine.

A group of socioeconomic indicators were significantly correlated with certain cytokines. Levels of cytokines were found to be correlated with the number of financial dependents in Soweto not Durban, and correlated with both higher income and food insecurity in Durban but not Soweto. In Soweto, a greater number of financial dependents was significantly correlated with IL-10, both IL-12 variants, and MIP-1β. In Durban, increased average monthly income was significantly correlated with lower IL-1α, IL-4, IL-6, and IL-10. In Durban, more food insecurity was consistently correlated with lower levels of all of the cytokines. Although, only the association between IL-10 and food insecurity had a significant p-value, TNF-α, IFN-α2, and IL-6 were all close to significance (p=0.06).

In Durban, the association between cytokine concentrations and a STI diagnosis at baseline hovers around null. In Soweto, the results were more varied, with most hovering between 0.15 on either side of zero. In Soweto, lower levels of IFN-α2 was significantly correlated with the diagnosis of an STI at baseline.

Table 3: Spearman correlation coefficients (rho) of the relationship between Log10 concentrations of analyzed cytokines and categorical socio-behavioural variables

		IL-1α	IL-1β	IL-4	IL-6	IL-10	IL-12(p40)	IL-12(p70)	MIP-1β	TNF-α	IFN-α2	IFN-γ	IP-10
BMI[1]	All	**-0.371***	**-0.448⁺**	-0.287	**-0.369***	**-0.407***	-0.356	**-0.366***	-0.283	**-0.445+**	**-0.357***	**-0.398***	**-0.341***
	Soweto	**-0.866***	**-0.86***	-0.722	**-0.866***	**-0.866***	**-0.866⁺**	**-0.866***	-0.722	**-0.866***	**-0.866***	-0.577	-0.433
	Durban	-0.005	-0.039	-0.003	-0.027	-0.074	-0.030	-0.120	0.005	-0.113	-0.027	-0.051	-0.142
Depression (yes/no)[2]	All	**0.401***	0.286	**0.410***	**0.324***	**0.336***	**0.338***	0.378	**0.403***	0.314	**0.326***	0.276	0.127
	Soweto	0.433	0	0.144	0.289	0.433	0	0.289	0.289	0.433	-0.158	0	0.577
	Durban	0.337	0.289	**0.463⁺**	0.322	0.310	**0.352***	0.271	**0.494⁺**	0.276	-0.055	0.343	0.037
Anxiety (yes/no)	All	0.162	0.065	0.148	0.051	0.072	0.190	0.134	0.151	0.097	0.107	0.139	-0.148
	Soweto	0	-0.289	-0.144	-0.144	0	-0.144	0	-0.144	0	-0.289	-0.433	0.144
	Durban	0.210	0.169	0.238	0.162	0.124	0.272	0.210	0.268	0.141	0.189	0.300	-0.210
Income[3]	All	-0.255	-0.130	-0.299	-0.264	-0.254	-0.171	-0.183	-0.171	-0.169	-0.188	-0.123	0.065
	Soweto	0.158	0.316	0.158	0.316	0.316	0.158	0.316	0.632	0.158	0.474	0.474	0.474
	Durban	**-0.355***	-0.225	**-0.382***	**-0.443***	**-0.355***	-0.225	-0.314	-0.321	-0.249	0.290	-0.276	-0.061
Food insecurity	All	-0.122	-0.243	-0.145	-0.237	-0.282	-0.279	-0.264	-0.267	-0.243	-0.294	-0.166	-0.220
	Soweto	0.408	0	0.204	0.204	0.204	0	0	-0.204	0.408	0	0	0
	Durban	-0.195	-0.251	-0.178	-0.388	**-0.360***	-0.295	-0.260	-0.269	-0.338	-0.334	-0.199	-0.269
Financial Dependents	All	0.111	0.191	0.113	-0.095	0.236	0.091	0.098	0.0166	0.167	0.167	0.150	0.175
	Soweto	0.458	0.697	0.239	0	**0.797***	**0.797***	**0.777***	**0.797***	0.578	0.697	0.020	0.697
	Durban	-0.221	-0.238	-0.215	-0.226	-0.087	-0.215	-0.226	-0.163	-0.197	-0.203	-0.192	-0.017
STI Diagnosis (yes/no)	All	-0.054	-0.082	0.020	0.008	0.025	-0.133	-0.014	0.068	-0.124	-0.051	-0.133	0.093
	Soweto	-0.158	-0.316	-0.158	0	-0.158	-0.316	0.158	0.158	-0.316	**-0.866***	0.158	-0.158
	Durban	-0.054	-0.067	0.006	0.003	0.052	-0.080	-0.027	0.065	-0.091	-0.027	-0.171	0.025

P value from Kruskal-Wallis H-testing and Spearman Correlation Testing: * 0.05-0.01, + <0.01

[1] BMI is categorized into underweight (1), normal weight (2), and overweight-obese (3)

[2] Depression and anxiety "yes" cases are those that reach a probable diagnosis based on a severity of self-reported symptoms

[3] Income is categorized into ZAR 0-400(1), 401-1600(2), 1600+(3), thus a negative correlation describes a relationship between increasing income and decreasing cytokine levels

[4] Food insecurity is based on the Household Hunger Scale with low values being little to no hunger and high values being severe hunger

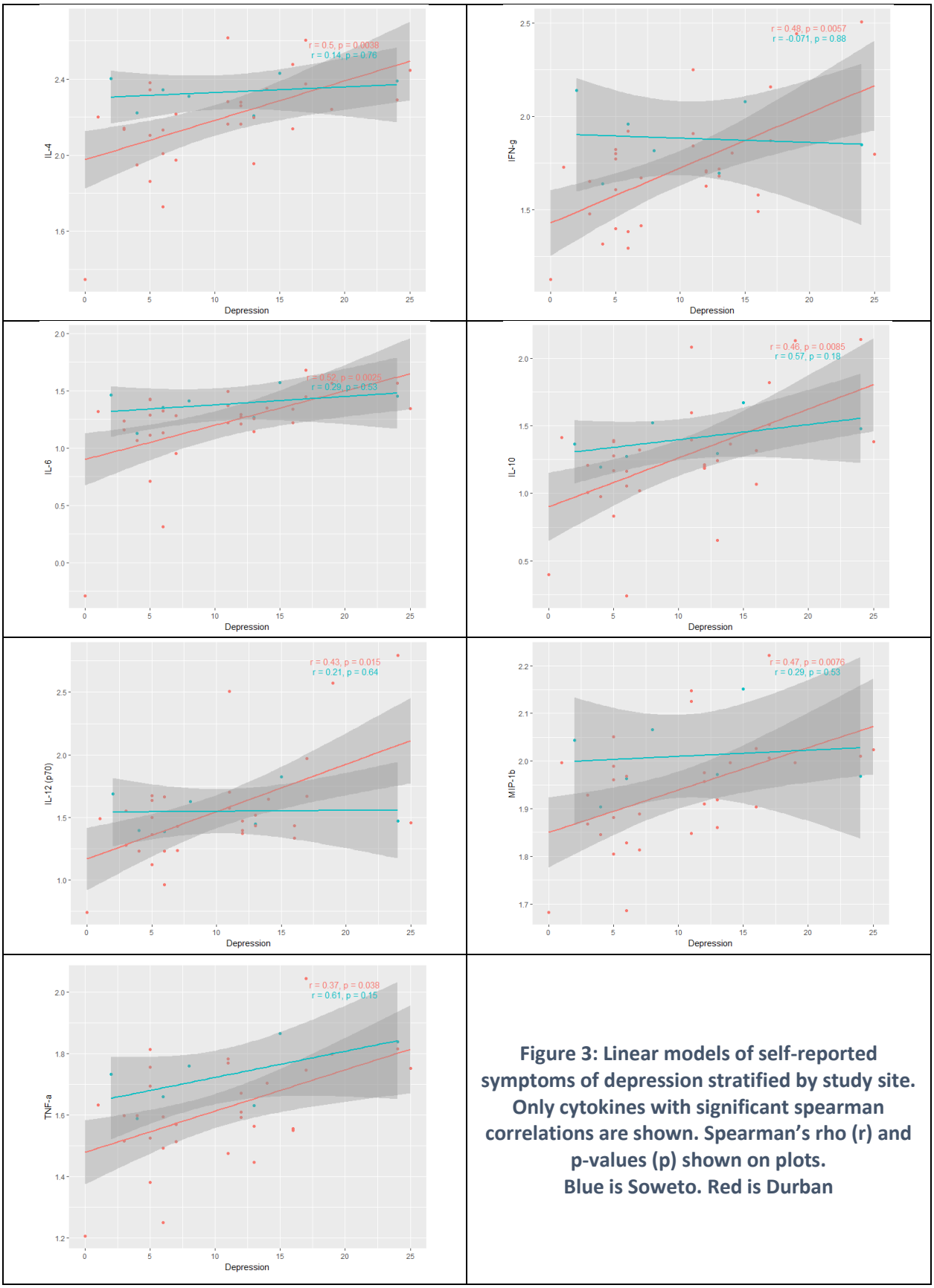


Figure 3: Linear models of self-reported symptoms of depression stratified by study site. Only cytokines with significant spearman correlations are shown. Spearman's rho (r) and p-values (p) shown on plots.
Blue is Soweto. Red is Durban

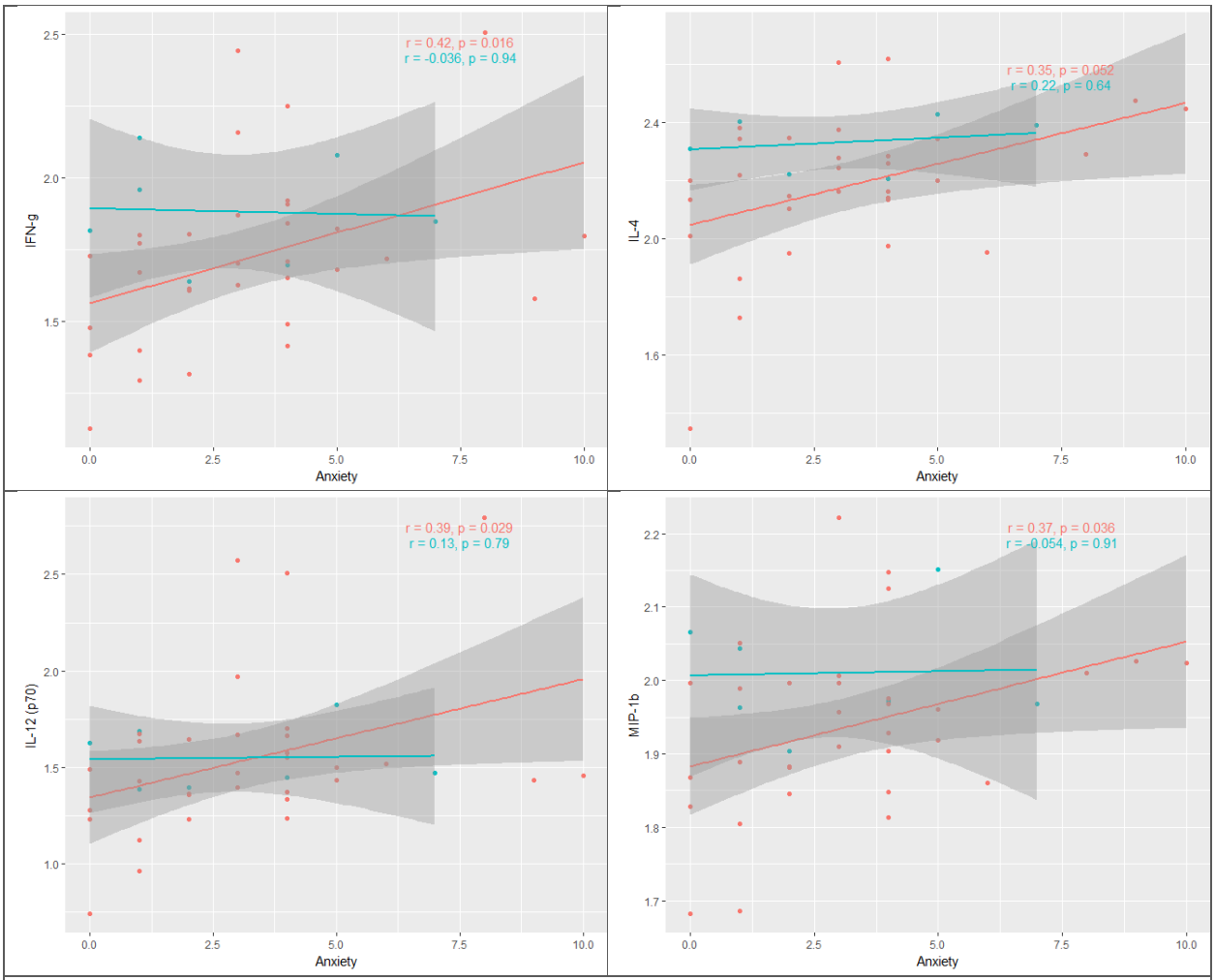


Figure 4: Linear models of self-reported symptoms of anxiety stratified by study site. Only cytokines with significant spearman correlations are shown. Spearman's rho (r) and p-values (p) shown on plots.
Blue is Soweto. Red is Durban

5 DISCUSSION

In this exploratory analysis of the distribution of cytokine levels and socio-behavioural factors among 39 South African youth, the median and range of plasma levels of all 12 inflammatory biomarkers were within a biologically plausible range, and significant associations were found between levels of these biomarkers in plasma and three groups of socio-behavioural variables: depression and anxiety, BMI, and some socioeconomic indicators. Increased depression correlated with higher levels of cytokines was expected, but only observed in Durban. Higher BMI has previously been found to be associated with higher levels of certain cytokines, however, in Soweto, the opposite direction of effect was observed. Socioeconomic associations with elevated levels of certain cytokines have previously been reported, these associations were observed to be different based on study site; low income and more food security in Durban, and more financial dependents in Soweto. Participants in Soweto also had a surprising association with lower levels of IFN-α2 and a STI diagnosis.

None of the socio-behavioural factors were found to be significantly associated with all 12 cytokines included in the analysis. However, patterns of association were observed for some socio-behavioural factors showing a consistent direction of effect. Low BMI was consistently associated with higher levels of all cytokines. Experiencing more symptoms of depression was consistently associated with higher levels of all cytokines. The same was true for anxiety, except for the Soweto strata which had an inconsistent effect across all cytokines. Both low income and food security were consistently associated with higher levels of cytokines in Durban and lower levels in Soweto. Financial responsibility for more dependents was the opposite of income with consistently lower levels of cytokines in Durban and higher levels in Soweto. Interestingly, all associations with STI diagnoses were low, hovering around null, with the exception of the observed significant association between lower levels of IFN-α2 and a STI diagnosis in Soweto.

5.1 STUDY SITE

Study site was a challenging variable in this analysis. The original sample was structured to sample from both study sites equally but 24% of all observed concentration values in one of the two plates were out of the detectable range or extrapolated beyond the standard concentration curve, making the data from this plate less reliable. Most participants whose samples were being analyzed on that plate were from Soweto, ultimately shrinking the Soweto sample from 32 to 7. The 7 individuals from Soweto included in this analysis made up 18% of the sample.

Median cytokine concentrations were higher for Soweto, and lower for Durban. The cytokines involved are strongly pro-inflammatory with the exception of IL-4. These site-associated pro-inflammatory cytokines include IL-1β, IFN-α2, IFN-γ, and TNF-α and the median values in Soweto for these cytokines were 1.46, 2.49, 1.85, and 1.73 log10 pg/mL respectively. The variables most significantly associated with these increased concentrations were the number of financial dependents ($p<0.001$) and BMI ($p=0.005$). The greater number of financial dependents at the Soweto site is also present in the source cohort. Nearly 42% of participants in Soweto reported having financial dependents compared to 16% in Durban.

There are three possible explanations for the differences observed between the study sites. The first possible explanation for the differences observed between study sites is that the smaller sample size was not representative and contained individuals with cytokine concentrations that altered the distribution that might normally be observed in the Soweto population. There is no known or obvious reason to assume that the cytokine profile in Soweto is different from that in Durban. The second possible explanation is that the blood samples were treated differently in the different study site clinics. Parkitny et al. (2013) investigated the effects of blood collection tubes and freezing then thawing samples. They found differences in the values obtained from each, except for MIP-1β which was able to tolerate slight differences in handling well. Although both study sites shared and adhered to the same protocol even slight

variations in handling could result in divergent results. The third possible option is that there is an association due to differences in variables present in the population in Soweto that contribute to an altered cytokine profile. Further investigation would be required, both to confirm that the results obtained were not by chance by expanding the sample size from Soweto, and to investigate the cause of the difference between the two study site populations.

5.2 BODY MASS INDEX

Finding significant associations between cytokine concentrations and BMI was not a surprise. However, the discovery of a strong association between high BMI and lower cytokine concentrations was a surprise as high BMI is most often associated with elevated levels of inflammation and inflammatory cytokines. Many studies have investigated the association of body weight and systemic inflammation in adults, although there is some debate about whether BMI is the best measure of weight, most studies find some correlation with IL-6 (Stepanikova, Oates, & Bateman, 2017; AVENA Study group, 2006). Wärnberg et al. (2007) reviewed the relationship in adolescents and found consistent correlations with increased IL-6 and obesity, and a gap in research on other markers of inflammation. Herder et al. (2007) attempted to describe the markers of chronic low-level inflammation with obesity in adolescents. They found that IL-6, and IP-10 were associated with BMI.

BMI of Soweto participants was found to be significantly associated with IL-6, IL-1a and IL-1β, IFN-α2, TNF-α, IL-10, IL-4, and IL-12 both p40 and p70. The correlations were consistently negative, indicating that underweight individuals had higher concentrations of the significantly correlated cytokines than normal weight individuals. There were no overweight individuals represented in the Soweto stratum. This is essential to understanding this finding. Rather than describing higher levels of inflammatory cytokines in overweight and obese individuals, this finding describes higher levels in underweight individuals within a small population that does not include overweight or obese individuals. However, this finding is still counter to established

research that in many cases has shown an increase in pro-inflammatory cytokines with increasing BMI, a positive correlation. Further investigation is necessary to determine the factors driving inflammation in these lower BMI youth.

Chi-square testing revealed significant associations between BMI, and both study site and financial dependents. The unexpected values observed to be associated with BMI in Soweto are likely due to extreme values within the small sample.

5.3 Depression and Anxiety

Cytokine concentrations were found to be higher among participants in Durban with higher depression and anxiety symptoms. This relationship between depression and inflammatory cytokines has also been investigated previously. Gabbay et al. (2008) investigated the link in an adolescent population aged 12 to 19. They found that adolescents diagnosed with Major Depressive Disorder (MDD) had increased IFN-γ and a ratio of IFN-γ to IL-4 that favoured a pro-inflammatory state. Dahl et al. (2014) found that IL-1β, IL-10, IFN-γ, and IL-6 were significantly higher in depressed cases compared to controls.

The link to anxiety was a surprise, but collinearity cannot necessarily be ruled out. Anxiety was significantly associated with depression in chi-square testing and all other variables significantly associated with anxiety are also significantly associated with depression. Moriarty, McArthur, Ellman, Coe, Abramson, and Alloy (2018) described a link between systemic inflammation and anxiety in youth (mean age 16.5). Their analysis was specifically structured to show that systemic inflammation, as represented by IL-6 levels, could mediate the relationship between anxiety symptoms and depression symptoms.

5.4 DEPRIVATION

The negative correlations with income among Durban based study participants agree with published literature showing that lower socioeconomic status is associated with increased non-specific inflammation. The most common markers of inflammation used in these studies are CRP and IL-6. The correlation with income and IL-6 in this study was -0.443 with a p-value of less than 0.05. There are also significant negative correlations observed with strong anti-inflammatory cytokines, IL-4 and IL-10.

Many studies have previously compared levels of CRP to socioeconomic indicators. Nazmi, Oliveira, Horta, Gigante, and Victora (2010) analyzed CRP in young adults; similar to the results of this analysis, their results were inconsistent across different indicators of socioeconomic status such as maternal education, and family income. Again, similar to the results of this study they found strong associations with measures of body weight, in their case adiposity. The negative correlation with income and food insecurity variables found here is not the expected direction of effect.

Significant correlations with income were balanced between pro-inflammatory cytokines, IL-6 and MIP-1β, and anti-inflammatory cytokines IL-4, and IL-10. Further analysis would be required to determine which inflammatory profile is dominant. However, an anti-inflammatory profile would indicate that increases in income were associated with potentially protective inflammatory factors.

Significant correlations with food insecurity were limited to the strong anti-inflammatory cytokine, IL-10. This indicates that higher food insecurity is associated with lower concentrations of IL-10 and vice-versa. It should be noted that in this sample, there were no individuals who reported experiencing severe household hunger.

The association with financial dependents was briefly discussed with study site in section 5.1 as a possible explanatory variable for the association between study site and some cytokine

concentrations. There is a large difference between the study sites in the number of financial dependents being supported by adolescents and young adults. Research investigating the nature of this difference would help to inform further inquiry and broaden understandings of the social challenges faced by youth in Soweto. The anti-inflammatory, IL-10 was significantly positively associated with increasing number of financial dependents. Both IL-12 p40 and p70 had a significant positive correlation with increasing financial dependents. Without individual ratios comparing the concentrations of these complementary cytokines it is unknown which one dominates the functional balance. IL-12 p40 and p70 also share their sub units with IL-23 and IL-17 (Dembic, 2015). It is possible that the presence of either of these cytokines could interfere with the accuracy of measurements.

The observed associations with income, food insecurity, and financial dependents were a bit of a surprise because early stage exploration of the data did not show strong relationships with any of the variables that relate to socioeconomic status. Previous research supported a correlation between lower income and higher inflammatory cytokine concentration. However, the identity of the cytokines with a relationship to income tends toward anti-inflammatory. The range of incomes in this analysis might be more difficult to differentiate between than in other research. The income range per month used for participants classifies them as less than 400ZAR (approximately 39.50CAD), greater than 1600ZAR (approximately 158.50 CAD) and in between. In a high-income country, it would be unlikely to find a person living on less than 40CAD per month. This small range of potential incomes may make it difficult to detect small effects within this limited scale. It also limits the applicability of the existing, comparatively high-income, research to this context.

5.5 STI DIAGNOSIS

This finding, was perhaps the most surprising and counter-intuitive, given that the role of IFN-α2 in the viral immune response is well described (Zhou, Fragala, McElhaney, & Kuchel,

2010). Based on the concentrations of IFN-α2 within that strata it seems that this association is driven more by high values among participants who were not diagnosed with an STI. Due to the very small sample size in this analysis from the Soweto site this finding would require further investigation to be considered reliable.

5.6 STRENGTHS AND LIMITATIONS

There are both strengths and limitations to this exploratory analysis. Even prior to the elimination of one of the lab plates the sample size would have been a limitation. The small sample size of participants from Soweto is particularly problematic. The observations and associations described in this paper would benefit from a larger sample size to contribute to the triangulation of a more precise estimate of the associations of sub-clinical inflammation. Comparisons of specific inflammatory markers with socio-behavioural variables are fairly uncommon. More uncommon still are investigations into these processes in adolescents and young adults in low- and middle-income countries. While that underlies the importance of performing this research, it also means that methodologies are less established for this type of data.

Multiplex immunoassays are a complex and sensitive methodology. While this means that researchers are able to detect and quantify biomarkers more quickly and with greater accuracy than ever before, it also means that slight alterations can result in unreliable data. This also limits the reproducibility of this method, which is a central tenet of the scientific method. Cytokine concentrations themselves are very sensitive to slight differences in physiological processes. Acute stress, exercise, or sub-clinical tissue injury or infection at the time of sample collection would be undetectable to this research and with the potential to skew results. The analytical methods used in this preliminary analysis had limited ability to distinguish between the naturally highly correlated biomarkers. While this is not an unusual result, some have used

methodological strategies, such as decision-tree analysis to improve the ability to differentiate between these highly correlated biomarkers (Liebenberg et al., 2017).

6 CONCLUSION

These preliminary results have accomplished two important goals; they have shown us how much we don't know about the inflammatory processes of this key group and further informed the biopsychosocial intersection within the HIV epidemic among South African youth. This study is one of a comparatively small number investigating inflammation in adolescents, particularly adolescents in countries that are not considered high-income.

These results show that some youth experience more risk factors for HIV than others. They also show that some risk factors are correlated with other risk factors, leading to compounded risk for acquiring HIV. As a cross-sectional analysis, it cannot determine that socio-behavioural factors cause increases in inflammation, only that the two are linked and may lead to increased risk for HIV. This provides biological evidence in addition to existing epidemiological studies that document that youth living with more financial struggles may be more at risk for HIV acquisition.

This study, as with all research studies outside of laboratory conditions, is not without its weaknesses. Some of the significant associations observed in this analysis follow consistent patterns with existing literature including positive correlations with several cytokines and both depression and anxiety. While the findings associated with BMI were at a glance contrary to the established literature, they speak more to elevated inflammation in a small population of underweight individuals compared to normal weight individuals.

This area of research seeks to directly link the social and behavioural environments that are inequitably distributed across South African society to biological risk and outcomes. Research of this nature contributes further support for the strong role of the social determinants of health by establishing plausible biological mechanisms. This research supports upstream approaches to addressing determinants of risk for adolescents and young adults in South Africa.

7 CRITICAL REFLECTION

I am very glad that this is the project that I ended up with. I have both loved and hated immunology since the first time I received a lecture on it. The complexity of social and immunological processes are endlessly interesting and overwhelming. This project was similar in its complexity, and research that further investigates this intersection of immunological and social processes will continue to be marred by the complexity of these phenomena. This project embodies the Faculty of Health Sciences' Cell-to-Society approach by exploring the direct association between cellular processes and social and behavioural dynamics.

This project was challenging in many ways. The data collection process during my practicum was difficult for several reasons including the location of the placement which was centered in a virological and immunological academic laboratory setting. But the quality of the facilities, and the kindness, and intelligence of my peers in that setting was incredibly affecting. The administration wasn't sure how best to involve and support a student of public health in the laboratory setting on an ongoing basis. Travelling to the site in Durban was also difficult without regular access to a vehicle. The experience in South Africa was an invaluable immersion in the culture and nuances of some of the contexts in which participants of the project experience.

The scientific and statistical technicality of this project was also challenging. The scientific element set my project apart from other members of the cohort, limiting my ability to engage them and the concentration head for support in activities organized by the department. The statistical element of this project was the most significant challenge. During my undergraduate thesis experience, I performed a meta-analysis. My supervisor for that project would often tell me to complete a statistical action without explaining the choice to me or considering the full implications of that action. As a result, I found myself experiencing a large amount of anxiety that I was not knowledgeable enough about my own project to defend it, nor satisfied enough by its quality to pursue further publication or investigation. During my practicum in the early stages of data exploration, with no prior experience in that sort of investigation, I found myself similarly

performing statistical treatments and queries without fully investigating for myself that this was what I thought to be the best course of action. As a result, during the initial stages of writing this project, I found myself unable to go on without fully investigating, and understanding the statistics presented in this paper. This led to delays, and considerable extra work as well as stress. However, I find myself more confident and proud of the product and prepared for my new position as a data analyst with the First Nations Health Authority's Surveys and Data Secretariat.

8 REFERENCES

Aiello, A. E., & Kaplan, G. A. (2009). Socioeconomic position and inflammatory and immune biomarkers of cardiovascular disease: applications to the Panel Study of Income Dynamics. *Biodemography and Social Biology*, *55*(2), 178-205.

Albert, M. A., Glynn, R. J., Buring, J., & Ridker, P. M. (2006). Impact of traditional and novel risk factors on the relationship between socioeconomic status and incident cardiovascular events. *Circulation*, *114*(24), 2619-2626.

American Psychiatric Association. DSM-5 self-rated level 1 cross-cutting symptom measure—child age 11–17. *Arlington: American Psychiatric Association* (2013).

AVENA Study group. (2006). Inflammatory proteins are related to total and abdominal adiposity in a healthy adolescent population: the AVENA Study, *The American Journal of Clinical Nutrition*, *84*(3), 505–512. https://doi.org/10.1093/ajcn/84.3.505

Ballard, T., Coates, J., Swindale, A., Deitchler, M. (2011). Household Hunger Scale: Indicator Definition and Measurement Guide. In: Food and Nutrition http://www.fantaproject.org/sites/default/files/resources/HHS-Indicator-Guide-Aug2011.pdf

Baumeister, D., Akhtar, R., Ciufolini, S., Pariante, C. M., & Mondelli, V. (2016). Childhood trauma and adulthood inflammation: a meta-analysis of peripheral C-reactive protein, interleukin-6 and tumour necrosis factor-α. *Molecular Psychiatry*, *21*(5), 642.

Bradley, K.L., Bagnell, A.L., Brannen, C.L. (2010). Factorial validity of the Center for Epidemiological Studies Depression 10 in adolescents. *Issues in Mental Health Nursing, 31,* 408-412.

Bunyasi, E. W., & Coetzee, D. J. (2017). Relationship between socioeconomic status and HIV infection: findings from a survey in the Free State and Western Cape Provinces of South Africa. *BMJ open*, *7*(11), e016232.

Castagné, R., Kelly-Irving, M., Campanella, G., Guida, F., Krogh, V., Palli, D., ... De Kok, T. (2016). Biological marks of early-life socioeconomic experience is detected in the adult inflammatory transcriptome. *Scientific Reports*, *6*, 38705.

Chege, D., Chai, Y., Huibner, S., Kain, T., Wachihi, C., Kimani, M., ... Jaoko, W. (2012). Blunted IL17/IL22 and pro-inflammatory cytokine responses in the genital tract and blood of HIV-exposed, seronegative female sex workers in Kenya. *PloS one*, *7*(8), e43670.

Chen, E., McLean, K. C., & Miller, G. E. (2015). Shift-and-persist strategies: associations with socioeconomic status and the regulation of inflammation among adolescents and their parents. *Psychosomatic Medicine*, *77*(4), 371.

Chiang, J. J., Bower, J. E., Almeida, D. M., Irwin, M. R., Seeman, T. E., & Fuligni, A. J. (2015). Socioeconomic status, daily affective and social experiences, and inflammation during adolescence. *Psychosomatic Medicine*, *77*(3), 256.

Cluver, L. D., Orkin, F. M., Meinck, F., Boyes, M. E., & Sherr, L. (2016). Structural drivers and social protection: mechanisms of HIV risk and HIV prevention for South African adolescents. *Journal of the International AIDS Society*, *19*(1), 20646.

Cohen S, Kamarck T, Mermelstein R (1983) A Global Measure of Perceived Stress. *Journal of Health and Social Behavior*, *24,* 385-396.

Dahl, J., Ormstad, H., Aass, H. C. D., Malt, U. F., Bendz, L. T., Sandvik, L., ... Andreassen, O. A. (2014). The plasma levels of various cytokines are increased during ongoing depression and are reduced to normal levels after recovery. *Psychoneuroendocrinology*, *45*, 77-86.

Decker, M. L., Gotta, V., Wellmann, S., & Ritz, N. (2017). Cytokine profiling in healthy children shows association of age with cytokine concentrations. *Scientific Reports*, *7*(1), 17842.

Dembic, Z. (2015). *The cytokines of the immune system: The role of cytokines in disease related to immune response*. Retrieved from http://ebookcentral.proquest.com/lib/sfu-ebooks/detail.action?docID=2056908

Elliot, A. J., & Chapman, B. P. (2016). Socioeconomic status, psychological resources, and inflammatory markers: Results from the MIDUS study. *Health Psychology*, *35*(11), 1205.

Elliot, A. J., Turiano, N. A., & Chapman, B. P. (2016). Socioeconomic status interacts with conscientiousness and neuroticism to predict circulating concentrations of inflammatory markers. *Annals of Behavioral Medicine*, *51*(2), 240-250.

Foo, Y. Z., Nakagawa, S., Rhodes, G., & Simmons, L. W. (2017). The effects of sex hormones on immune function: a meta-analysis. *Biological Reviews*, *92*(1), 551-571.

Fraga, S., Marques-Vidal, P., Vollenweider, P., Waeber, G., Guessous, I., Paccaud, F., ... Stringhini, S. (2015). Association of socioeconomic status with inflammatory markers: a two cohort comparison. *Preventive Medicine*, *71*, 12-19.

Freeman, J. A., Bauldry, S., Volpe, V. V., Shanahan, M. J., & Shanahan, L. (2016). Sex differences in associations between subjective social status and C-reactive protein in young adults. *Psychosomatic Medicine*, *78*(5), 542.

Gabbay, V., Klein, R. G., Alonso, C. M., Babb, J. S., Nishawala, M., De Jesus, G., ... Gonzalez, C. J. (2009). Immune system dysregulation in adolescent major depressive disorder. *Journal of Affective Disorders*, *115*(1-2), 177-182.

Gimeno, D., Brunner, E. J., Lowe, G. D., Rumley, A., Marmot, M. G., & Ferrie, J. E. (2007). Adult socioeconomic position, C-reactive protein and interleukin-6 in the Whitehall II prospective study. *European Journal of Epidemiology*, *22*(10), 675-683.

Harrison, A., Colvin, C. J., Kuo, C., Swartz, A., & Lurie, M. (2015). Sustained high HIV incidence in young women in Southern Africa: social, behavioral, and structural factors and emerging intervention approaches. *Current HIV/AIDS Reports*, *12*(2), 207-215.

Herder, C., Schneitler, S., Rathmann, W., Haastert, B., Schneitler, H., Winkler, H., ... Martin, S. (2007). Low-grade inflammation, obesity, and insulin resistance in adolescents. *The Journal of Clinical Endocrinology & Metabolism*, *92*(12), 4569-4574.

Huijbregts, R. P., Helton, E. S., Michel, K. G., Sabbaj, S., Richter, H. E., Goepfert, P. A., & Hel, Z. (2013). Hormonal contraception and HIV-1 infection: medroxyprogesterone acetate suppresses innate and adaptive immune mechanisms. *Endocrinology*, *154*(3), 1282-1295.

Kahle, E. M., Bolton, M., Hughes, J. P., Donnell, D., Celum, C., Lingappa, J. R. … Mugo, N. R. (2014). Plasma cytokine levels and risk of HIV type 1 (HIV-1) transmission and acquisition: a nested case-control study among HIV-1–serodiscordant couples. *The Journal of infectious diseases*, *211*(9), 1451-1460.

Kaul, R., Prodger, J., Joag, V., Shannon, B., Yegorov, S., Galiwango, R., & McKinnon, L. (2015). Inflammation and HIV transmission in sub-Saharan Africa. *Current HIV/AIDS Reports*, *12*(2), 216-222.

Kelly, J. D., Richardson, E. T., Drasher, M., Barrie, M. B., Karku, S., Kamara, M., ... Farmer, P. E. (2018). Food Insecurity as a Risk Factor for Outcomes Related to Ebola Virus Disease in Kono District, Sierra Leone: A Cross-Sectional Study. *American Journal of Tropical Medicine and Hygiene, 98*(5), 1484-1488.

Kyongo, J. K., Crucitti, T., Menten, J., Hardy, L., Cools, P., Michiels, J., ... Fichorova, R. (2015). A cross-sectional analysis of selected genital tract immunological markers and molecular vaginal microbiota in Sub-Saharan African women with relevance to HIV risk and prevention. *Clinical and Vaccine Immunology*, CVI-00762.

Lajoie, J., Mwangi, L., & Fowke, K. R. (2017). Preventing HIV infection without targeting the virus: how reducing HIV target cells at the genital tract is a new approach to HIV prevention. *AIDS research and therapy*, *14*(1), 46.

Lajoie, J., Juno, J., Burgener, A., Rahman, S., Mogk, K., Wachihi, C., ... & Fowke, K. R. (2012). A distinct cytokine and chemokine profile at the genital mucosa is associated with HIV-1 protection among HIV-exposed seronegative commercial sex workers. *Mucosal immunology*, *5*(3), 277.

Liebenberg, L. J., Masson, L., Arnold, K. B., Mckinnon, L. R., Werner, L., Proctor, E., ... Karim, S. S. A. (2017). Genital—Systemic Chemokine Gradients and the Risk of HIV Acquisition in Women. *Journal of Acquired Immune Deficiency Syndromes*, *74*(3), 318.

Liu, R. S., Aiello, A. E., Mensah, F. K., Gasser, C. E., Rueb, K., Cordell, B., ... Burgner, D. P. (2017). Socioeconomic status in childhood and C reactive protein in adulthood: a systematic review and meta-analysis. *Journal of Epidemiology and Community Health*, *71*(8), 817-826.

Loucks, E. B., Sullivan, L. M., Hayes, L. J., D'Agostino Sr, R. B., Larson, M. G., Vasan, R. S., ... Berkman, L. F. (2006). Association of educational level with inflammatory markers in the Framingham Offspring Study. *American Journal of Epidemiology*, *163*(7), 622-628.

Louw-du Toit, R., Hapgood, J. P., & Africander, D. (2014). Medroxyprogesterone acetate differentially regulates interleukin (IL)-12 and IL-10 in a human ectocervical epithelial cell line in a glucocorticoid receptor (GR)-dependent manner. *Journal of Biological Chemistry*, jbc-M114.

Matthews, K. A., & Gallo, L. C. (2011). Psychological perspectives on pathways linking socioeconomic status and physical health. *Annual Review of Psychology*, *62*, 501-530.

McLaren, P. J., Blake Ball, T., Wachihi, C., Jaoko, W., Kelvin, D. J., Danesh, A., ... Fowke, K. R. (2010). HIV-exposed seronegative commercial sex workers show a quiescent phenotype in the CD4+ T cell compartment and reduced expression of HIV-dependent host factors. *The Journal of infectious diseases*, *202*(S3), S339-S344.

Mitchell, R. H., & Goldstein, B. I. (2014). Inflammation in children and adolescents with neuropsychiatric disorders: a systematic review. *Journal of the American Academy of Child & Adolescent Psychiatry*, *53*(3), 274-296.

Monastero, R. N., & Pentyala, S. (2017). Cytokines as biomarkers and their respective clinical cutoff levels. *International Journal of Inflammation*, *2017*.

Naranbhai, V., Abdool Karim, S. S., Altfeld, M., Samsunder, N., Durgiah, R., Sibeko, S., ... & CAPRISA004 TRAPS team. (2012). Innate immune activation enhances HIV acquisition in women, diminishing the effectiveness of tenofovir microbicide gel. *The Journal of infectious diseases*, *206*(7), 993-1001.

Naswa, S., & Marfatia, Y. S. (2010). Adolescent HIV/AIDS: Issues and challenges. *Indian Journal of Sexually Transmitted Diseases*, *31*(1), 1–10. http://doi.org/10.4103/0253-7184.68993

Nazmi, A., Oliveira, I. O., Horta, B. L., Gigante, D. P., & Victora, C. G. (2010). Lifecourse socioeconomic trajectories and C-reactive protein levels in young adults: findings from a Brazilian birth cohort. *Social Science & Medicine*, *70*(8), 1229-1236.

Nazmi, A., & Victora, C. G. (2007). Socioeconomic and racial/ethnic differentials of C-reactive protein levels: a systematic review of population-based studies. *BMC public health*, *7*(1), 212.

Nur Atiqah, A., Norazmir, M. N., Khairil Anuar, M. I., Mohd Fahmi, M., & Norazlanshah, H. (2015). Food insecurity status: It's association with inflammatory marker and lipid profile among young adult. *International Food Research Journal*, *22*(5).

O'reilly, D. S. J., Upton, M. N., Caslake, M. J., Robertson, M., Norrie, J., McConnachie, A., ... Packard, C. J. (2006). Plasma C reactive protein concentration indicates a direct relation between systemic inflammation and social deprivation. *Heart*, *92*(4), 533-535.

Parkitny, L., McAuley, J. H., Kelly, P. J., Di Pietro, F., Cameron, B., & Moseley, G. L. (2013). Multiplex cytokine concentration measurement: how much do the medium and handling matter? *Mediators of Inflammation*, *2013*.

Passmore, J. A. S., Jaspan, H. B., & Masson, L. (2016). Genital inflammation, immune activation and risk of sexual HIV acquisition. *Current Opinion in HIV and AIDS*, *11*(2), 156-162.

Pietras, S. A., & Goodman, E. (2013). Socioeconomic status gradients in inflammation in adolescence. *Psychosomatic Medicine*, *75*(5), 442.

Prodger, J. L., Hirbod, T., Kigozi, G., Nalugoda, F., Reynolds, S. J., Galiwango, R., ... & Kaul, R. (2014). Immune correlates of HIV exposure without infection in foreskins of men from Rakai, Uganda. *Mucosal Immunology*, *7*(3), 634.

Radloff, L. S. (1977). The CES-D scale: A self-report depression scale for research in the general population. *Applied Psychological Measurement*, *1*(3), 385-401.

Radloff, L. S. (1991). The use of the Center for Epidemiologic Studies Depression Scale in adolescents and young adults. *Journal of Youth and Adolescence*, *20*(2), 149-166.

Richman, A. D. (2017). Concurrent Social Disadvantages and Chronic Inflammation: The Intersection of Race and Ethnicity, Gender, and Socioeconomic Status. *Journal of Racial and Ethnic Health Disparities*, 1-11.

Roff, S. R., Song, E. N., & Yamamoto, J. K. (2014). The significance of interferon-γ in HIV-1 pathogenesis, therapy, and prophylaxis. *Frontiers in immunology*, *4*, 498.

Rohleder, N. (2014). Stimulation of systemic low-grade inflammation by psychosocial stress. *Psychosomatic Medicine*, *76*(3), 181-189.

Schmeer, K. K., & Yoon, A. (2016). Socioeconomic status inequalities in low-grade inflammation during childhood. *Archives of Disease in Childhood*, *101*(11), 1043-1047.

Schreier, H. M., Roy, L. B., Frimer, L. T., & Chen, E. (2014). Family chaos and adolescent inflammatory profiles: The moderating role of socioeconomic status. *Psychosomatic Medicine*, *76*(6), 460-467.

South Africa Department of Health. (2016). *Strategy for the prevention and control of obesity in South Africa: 2015-2020*. Retrieved from http://www.health.gov.za/index.php/2014-03-17-09-09-38/policies-and-guidelines/category/327-2017po?download=1832:strategy-for-the-prevention-and-control-of-obesity-in-south-africa

Spangenberg, J. J., & Henderson, K. (2001). Stress and coping in Black South African adolescents. *Studia Psychologica*, *43*(2), 77-90.

Statistics South Africa. (2017). Mid-year Population Estimates. [Statistical Release Number P0302]. Retrieved from https://www.statssa.gov.za/publications/P0302/P03022017.pdf

Steinert, J. I., Cluver, L., Melendez-Torres, G. J., & Herrero Romero, R. (2017). Relationships between poverty and AIDS Illness in South Africa: an investigation of urban and rural households in KwaZulu-Natal. *Global Public Health*, *12*(9), 1183-1199.

Stepanikova, I., Oates, G. R., & Bateman, L. B. (2017). Does one size fit all? The role of body mass index and waist circumference in systemic inflammation in midlife by race and gender. *Ethnicity & Health*, *22*(2), 169-183.

UNAIDS (2010) Global Report: UNAIDS report on the global AIDS epidemic, 2010. Geneva, Switzerland

UNICEF. (2017). Adolescent HIV Prevention. Retrieved from https://data.unicef.org/topic/hivaids/adolescents-young-people/

Wärnberg, J., Nova, E., Romeo, J., Moreno, L. A., Sjöström, M., & Marcos, A. (2007). Lifestyle-related determinants of inflammation in adolescence. *British Journal of Nutrition*, *98*(S1), S116-S120.

Welsh, P., Woodward, M., Rumley, A., & Lowe, G. (2008). Associations of plasma pro-inflammatory cytokines, fibrinogen, viscosity and C-reactive protein with cardiovascular risk factors and social deprivation: the fourth Glasgow MONICA study. *British Journal of Haematology*, *141*(6), 852-861.

Wessels, J. M., Felker, A. M., Dupont, H. A., & Kaushic, C. (2018). The relationship between sex hormones, the vaginal microbiome and immunity in HIV-1 susceptibility in women. *Disease models & mechanisms*, *11*(9).

Yao, X. D., Omange, R. W., Henrick, B. M., Lester, R. T., Kimani, J., Ball, T. B., ... Rosenthal, K. L. (2014). Acting locally: innate mucosal immunity in resistance to HIV-1 infection in Kenyan commercial sex workers. *Mucosal Immunology*, *7*(2), 268.

Yi, T. J., Shannon, B., Prodger, J., McKinnon, L., & Kaul, R. (2013). Genital immunology and HIV susceptibility in young women. *American Journal of Reproductive Immunology*, *69*, 74-79.

Ziegler, S. M., & Altfeld, M. (2017). Human immunodeficiency virus 1 and Type i interferons—where Sex Makes a Difference. *Frontiers in immunology*, *8*, 1224.

Zhou, X., Fragala, M. S., McElhaney, J. E., & Kuchel, G. A. (2010). Conceptual and methodological issues relevant to cytokine and inflammatory marker measurements in clinical research. *Current Opinion in Clinical Nutrition and Metabolic Care*, *13*(5), 541.