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SLEEP QUALITY AND IMMUNE CHANGES IN HIV POSITIVE PEOPLE IN THE FIRST SIX MONTHS OF STARTING HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART)

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This dissertation is submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg in fulfilment of requirements for a Master of Science in Medicine

Johannesburg, South Africa, 2016

The financial assistance of the National Research Foundation (NRF) and Belgian Technical Cooperation (BTC) have hereby been acknowledged. Opinions expressed and conclusions arrived at, are those of the author and are not necessarily to be attributed to the NRF or BTC.

DECLARATION

I, Kirsten Redman, declare that this Dissertation is my own, unaided work. It is being submitted for the Master of Science at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

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(Signature of candidate)

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ABSTRACT

Introduction: South Africa has the highest absolute number of persons living with HIV in the world. Previous research has shown that untreated and treated HIV positive patients have sleep disturbances. In a cross sectional pilot study I ran during honours, I showed that sleep disturbances were associated with increased current CD4+ T lymphocyte counts, which may point at a role for immune activation during CD4 reconstitution in the sleep disturbances of South African HIV positive patients. In this study, to better understand sleep disturbances in patients with HIV, I used a longitudinal design and investigated the changes in sleep quality, daytime sleepiness, and the risk of having sleep apnoea as well as the predictors of these changes in HAART-naïve HIV patients, up until at least six months on treatment.

Methods: 84 participants were originally enrolled into the study but only 23 (16 women, average age ± SD = 34.4 ± 7.8) came for 3 visits or more after treatment initiation. At all visits, the patients were asked to fill in questionnaires assessing sleep disruption (Pittsburgh Sleep Quality Index, PSQI), daytime sleepiness (Epworth Sleepiness Scale, ESS), risk of sleep apnoea (Berlin Questionnaire, BQ), depression (Beck Depression Inventory, BDI) and pain. In addition, CD4+ T lymphocyte counts, viral loads, body mass index and blood pressure were recorded. We used mixed models analyses in SAS for statistical analyses.

Results: The patients had low sleep disruption at baseline (PSQI average ± SD = 5.8 ± 3.3, 52% having a PSQI>5) and when adjusting for depression, sleep quality improved slightly over time ($p<0.01$). There was moderate daytime sleepiness at baseline (ESS average ± SD = 8.4 ± 4.9) which subsequently decreased over time with patients who had higher log viral loads (>2) showing lower daytime sleepiness than those with low viral loads throughout the study ($p<0.01$). Higher daytime sleepiness was also reported in patients with higher depression scores ($p<0.01$). Depression was high at baseline (average BDI score ± SD = 17.8 ± 11) and decreased at 3 months, thereafter with no significant change from baseline (<0.01). BMI was on average high at baseline (25.5± 7.1), in particular in women, and further increased over time ($p<0.01$), which was associated with a significant increase in the odds of having high risk for sleep apnoea ($p<0.01$).

Rating of pain in the past month decreased over time (p=0.02) and increased pain was associated with increased sleep disruption (p=0.02).

Conclusion: Overall, modifiers of sleep-related outcomes (PSQI, ESS, risk of sleep apnoea on the BQ) included depression, pain, viral loads and BMI. Further research would need to assess more metabolic developments in this cohort. Objective measurements of sleep such as polysomnography, and immune measurements such as cytokines will need to be conducted to better explain the underlying mechanisms at play.

DEDICATION

In memory of:

Eunice Redman

22 March 1943 - 12 July 2015

My beloved grandmother, friend, and cheerleader

Lendell Beaumont

13 April 1988 – 1 July 2016

My cousin, my brother, my friend.

Thank you two for your support up until your last days.

Thank you to my friends, my family, and to Alykhan who provided encouragement, food, and intellectual and emotional advice for the past three years. A special thanks goes to my friends and family members, who never quite understood exactly what I was doing, but who cheered me on anyway.

Finally, thank you to God, for my parents who carried me through on this journey, and supported me from the very beginning. Thank you, mum and dad for your unwavering support – this truly adds to the definition that a parent's love is unconditional – not only during the past three years, but throughout my entire life.

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Thank you to the School of Physiology, for their technical assistance, and backing when I needed to take more time to collect more data to improve the results of my study.

Thank you also to the staff at both the adult HIV clinic at Chris Hani Baragwanath Hospital, and at the Themba Lethu Right to Care clinic at Helen Joseph Hospital, who aided me in data collection where they could; for serving as interpreters where needed, and for providing assistance with any issues I may have had. Thank you to Professor Ian Sanne for the use of his clinic, and to Professor Alan Karstaedt for serving as a liaison at his clinic and as my supervisor.

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I extend my thanks also to the National Research Foundation and the Belgian Technical Cooperation (BTC) for their funding support during my degree.

List of Abbreviations

3TC – Lamivudine

ABC – Abacavir

AZT – Zidovudine

BDI – Beck's Depression Inventory

BMI – Body Mass Index

BQ – Berlin Questionnaire

CD – Cluster of differentiation

CHBAH – Chris Hani Baragwanath Academic Hospital

d4T – Stavudine

ddI – Didonasive

dsDNA – Double Stranded DNA

EFV – Efavirenz

ESS – Epworth Sleepiness Scale

FDC – Fixed dose combination

FTC - Emitricitabine

HIV – Human Immunodeficiency Virus

HJH – Helen Joseph Hospital

IRD – Immune Restoration Disease

IRIS – Immune Reconstitution Inflammatory Syndrome

LPV/r – Lopinavir with ritonavir booster

MSM – Men who have sex with men

NNRTI – Non-Nucleoside ReverseTranscriptase Inhibitor

NRTI – Nucleoside ReverseTranscriptase Inhibitor

NVP – Nevirapene

OSA – Obstructive Sleep Apnoea

PI – Protease Inhibitor

PSQI – Pittsburgh Sleep Quality Index

ssRNA – Single stranded RNA

TDF – Tenofovir

TABLE OF CONTENTS

ABSTRACT ..... **III**
INTRODUCTORY PARAGRAPH **1**
CHAPTER 1 - INTRODUCTION **4**
1.1.HIV in South Africa and the World **4**
1.1.1. Mechanism of action of the Human Immunodeficiency Virus 6
1.1.2. HIV entry 6
1.1.3. Integration and transcriptional events 7
1.1.4. Expression of viral genes, and assembly and budding of HIV 7
1.1.5. Antiretroviral Therapeutics that exist in South Africa, and the political hindrance of ARV therapy 10
HIV treatments that exist and those used in South Africa *10*
Political hindrance of ARV therapy in South Africa *15*
1.2. Sleep **17**
1.2.1. Sleep regulation 19
1.2.2. Measurements of sleep used in literature 21
Questionnaires *21*
Actigraphy *23*
Polysomnography *23*
1.2.3. Sleep and other physiological factors: 25
Sleep and pain *25*
Sleep and depression *25*
Sleep and immunity *26*
Sleep and non-communicable diseases *28*
1.3. Sleep in HIV **29**
1.3.1. Sleep disruption in untreated infection – the early studies 29
1.3.2. Sleep disruption in treated HIV and predictors thereof 31
Immune related factors: CD4+ T lymphocyte counts *32*
HIV proteins: effects on circadian rhythm *34*
Cytokine profiles related to sleep disruption in HIV *36*
Antiretroviral treatment with efavirenz *36*
Pain *38*
Depression, and socio-demographic factors *39*
Sleep and fatigue *40*
Sleep disordered breathing in HIV *41*
1.4. Gaps in the literature **51**
1.5. Pilot study run prior to masters' degree **52**
1.6. Aims **54**
CHAPTER 2 - METHODS **55**
2.1. Ethical Approval **55**
2.2. Recruitment **55**
2.2.1. Recruitment sites 55
2.2.2. Enrolment criteria and mechanisms of patient retention: 55
2.2.3. Study timeline 56
2.3. Longitudinal population retention **58**
2.4. Questionnaires **59**
2.4.1. General Information Questionnaire 59
2.4.2. Pittsburgh Sleep Quality Index (PSQI) 61
2.4.3. Berlin Questionnaire - sleep apnoea scale (BQ) 61
2.4.4. Epworth Sleepiness Scale (ESS) 62
2.4.5. Beck's Depression Inventory (BDI) 62

2.5. Data Pooling of time=0 and time=1 in the longitudinal data analysis 63
2.6. Data analysis 65
CHAPTER 3 – RESULTS 68
3.1. Pre-ARV treatment population characteristics for the initial 72 patients. 68
3.2 Pre-ARV treatment population characteristics for the 23 patients in the longitudinal study 69
3.3. Clinical evolution across time (disease markers and anthropometric data) 72
3.3.1. Evolution of HIV parameters across the study 72
3.3.2. Evolution of Body Mass Index, Mean Arterial Pressure and across the study 73
Evolution of Body Mass Index across time 73
Evolution of mean arterial pressure and hypertensive status across time 74
3.4. Sleep quality, daytime sleepiness, and sleep apnoea over time 74
3.4.1. Sleep quality across time 74
3.4.2. Daytime sleepiness over time 75
3.4.3. Evolution of the presence of sleep apnoea over time 76
3.5. Time effects on known predictors of sleep (pain and depression) 77
3.5.1 Time effects on the presence and rating of pain 77
Pain at the time of the visit 77
Pain in the past month over time: 77
3.5.2. Effects of time on depression severity 78
3.6. Multivariate predictors of sleep quality, daytime sleepiness, and the likelihood of reporting high risk for sleep apnoea 79
3.6.1. CD4+ T lymphocyte counts and log viral load effects on sleep quality, daytime sleepiness and the likelihood of reporting high risk for sleep apnoea 80
Effects of CD4+ T lymphocyte counts and log viral load on sleep quality 80
Effects of CD4+ T lymphocyte counts and log viral load on daytime sleepiness 80
Effects of CD4+ T lymphocyte counts and log viral load on scoring high risk of sleep apnoea 82
3.6.2. Depression and pain effects on sleep quality, daytime sleepiness and the likelihood of reporting high risk for sleep apnoea 83
Depression effects on sleep quality 83
Depression and pain effects on sleep apnoea 86
3.6.3 Relationship between BMI and risk of sleep apnoea 86
3.6.4 Multivariate predictors of non-communicable diseases 87
Multivariate predictors of body mass index 87
Multivariate predictors of mean arterial pressure 87
CHAPTER 4 - DISCUSSION 89
4.1. Summary of study design and findings: 89
4.2. Study Limitations 90
4.3. Factors that influence sleep and the changes across time 91
4.4. Changes in sleep variables across time and factors associated with these changes 93
Effects of CD4+ T lymphocyte count and viral load on being high risk for sleep apnoea 101
Effects of depression and pain on sleep apnoea 102
4.5. Impact of sleep disruption in HIV patients on risk of developing cardiovascular disorders and metabolic dysfunction across time 102
4.5.1. Body Mass Index 102
4.5.2. Mean Arterial Pressure and hypertensive status. 104
4.6. Conclusion: 104
4.7. Proposed further research: 105
LIST OF APPENDICES 107
REFERENCES 141

LIST OF TABLES AND FIGURES

	TABLES	
Table 1.1	Summary of studies on sleep and HIV and factors associated with sleep disturbances in HIV	43 - 50
Table 2.1.	Number of visits per participant	64
Table 3.1.	Demographics of 72 patients	69
Table 3.2.	Demographics of 23 patients	71
	FIGURES	
Figure 1.1	Progression of HIV infection	9
Figure 1.2	HIV entry and replication and targets for therapy	12
Figure 1.3	Normal sleep hypnogram	18
Figure 2.1	Study timeline	57
Figure 2.2	Population enrolment and loss to follow up	59
Figure 3.1	Evolution of HIV biomarkers over time	72
Figure 3.2	Average BMI over time	73
Figure 3.3	Average mean arterial pressure over time	74
Figure 3.4	Average PSQI score over time	75
Figure 3.5	Average ESS score over time	76
Figure 3.6	Frequency of sleep apnoea over time	76
Figure 3.7	Frequency of reporting pain	78
Figure 3.8	Mean BDI score over time	78
Figure 3.9	Mean ESS score over time in patients with high or low viral loads	82
Figure 3.10	Mean PSQI score over time in patients with or without pain	85

INTRODUCTORY PARAGRAPH

South Africa has the highest absolute number of HIV positive people in the world, and currently an estimated 48% are receiving combination drugs known as highly active antiretroviral therapy. Infection by HIV leads to a progressive drop in CD4+ T lymphocyte, which play a key role in adaptive immunity. Immune activation against the chronic infection leads to chronic fever, chronic fatigue and wasting. The drop in CD4+ T lymphocyte counts leads eventually to the development of an acquired immunodeficiency syndrome (AIDS), revealed by the development of opportunistic infections such as tuberculosis and/ or immunodeficiency-related neoplasias such as Kaposi's sarcoma and lymphomas. Treatment of HIV by highly active antiretroviral therapy (HAART) reverses these symptoms as viral loads decrease to undetectable levels and CD4+ T lymphocyte counts progressively normalise (I refer to this CD4 increase as immune reconstitution in this thesis). With HAART, HIV positive patients have been described to have life expectancies not much different from HIV negative controls. In South Africa, with WHO standards changing over the years, HAART initiation criteria have changed from only including HIV positive patients with CD4+ T lymphocyte counts <250/μL in 2008, to initiation as close to diagnosis as possible, regardless of CD4+ T lymphocyte count. These policy changes are reviewed in detail in Chapter 1 section 1.1. With the higher life expectancy of HIV positive patients under HAART, HIV medical care has slowly shifted focus towards the long-term quality of life and non-communicable diseases implications of such a chronic condition. As will be described in Chapter 1 Section 1.3, chronically disrupted sleep has been shown to have both psychiatric and medical consequences, leading to an increased risk of mental disorders such as depression, but also an increased risk of developing cardiovascular and metabolic diseases, such as obesity, and diabetes type II, which are already increased by ARV treatment alone. Therefore understanding the causes of chronic sleep disruption in HIV positive patients may help better guide the chronic medical care of controlled treated HIV patients. As will also be described in Chapter 1 Section 1.3, sleep disturbances are

common in chronic immune responses such as that seen in pre-treated HIV. Since the early 1990s, sleep changes in HIV positive patients have been investigated. HIV has indeed been shown to cause changes to sleep architecture early on in infection, and in untreated infection, there is an increased number of arousals, decreased total sleep time, decreased percentage of REM sleep and a shift of slow wave sleep to the later part of the night as compared to matched controls. Although a modification in sleep may be expected in the context of untreated HIV infection, studies have also shown that HIV positive patients complain of disrupted sleep even when their disease is controlled under treatment. Common predictors of sleep disturbances described in treated controlled HIV positive patients include lower CD4+ T lymphocyte counts, HIV itself through Tat, an HIV expressed protein, specific cytokine polymorphisms, ARV treatment including efavirenz, the presence of pain or depression and the presence of sleep disordered breathing. A review of sleep disturbances observed in HIV patients and the common predictors of these sleep disturbances will be presented in Chapter 1 section 1.3.

Although studies have reported sleep disruption in untreated and treated HIV cohorts, this has never been done in South Africa. During my Honours year, I therefore first conducted a pilot cross sectional study investigating the prevalence and predictors of sleep disruption in treated HIV patients of an urban community. In that study, I found that treated South African HIV patients also complained of sleep disturbances and that this was associated with the presence of pain, depression and more surprisingly, by a higher CD4+ T lymphocyte count, hinting at a potential relationship between immune reconstitution and sleep disturbances. This finding also helped formulate the questions addressed in this research report. Whereas the pilot study was cross sectional, this study's design was longitudinal in order to better of determine whether 1- sleep quality as measured by 3 questionnaires -the Pittsburgh Sleep Quality Index (PSQI, which assesses subjective sleep quality), the Epworth Sleepiness Scale (ESS, which assesses daytime sleepiness) and the Berlin Questionnaire (BQ, which assesses risk of sleep apnoea) - changes across time after ARV treatment is initiated, 2- sleep quality is predicted by CD4 / viral load changes when adjusting for known predictors of disrupted sleep such as age, sex, depression

and pain 3- modifications in sleep quality predict a higher non communicable disease risk profile through simple anthropometric measurements (BMI and high blood pressure).

CHAPTER 1 - INTRODUCTION

This chapter will first start with a description of the history and prevalence of HIV in South Africa, the mechanisms of action of HIV and the main drugs that target HIV.
A second section will cover the main aspects of sleep regulation (homeostatic and circadian) and the relationship between sleep and other physiological functions such as pain, depression, the immune system and cardio-metabolic disorders.
I will then do an overview of the main sleep disturbances described in the literature in HIV patients. Although this part will stay descriptive, I will also stress the main correlates of sleep disruption found in these studies. Finally I will detail the pilot study I ran in 2012, which gave me the ground to build the aims of my Masters study.

1.1.HIV in South Africa and the World

There are currently 37 million people living with HIV globally; 15 million (less than half) of which are receiving the treatment required to maintain adequate immune function (WHO, 2013). However, HIV testing reach is limited, and it is estimated that only 51% of all persons with HIV are aware of their status (WHO, 2013).

Sub-Saharan Africa is home to the largest number of HIV infected people, of which South Africa leads with an estimated 6.7 million people infected (WHO, 2013; Shisana, 2014). The latest overall estimate of HIV prevalence in South Africa conducted by statistics SA in 2013 revealed that 12.2% (95% CI: 11.4 – 13.1) of the population of 54 million people is infected with HIV. In terms of absolute numbers, this is a 1.2 million-person increase as compared to 2008 numbers (SA, 2013).

Previous studies carried out by the department of health in South Africa (Shisana, 2005, Shisana et al., 2009, Shisana and Simbayi, 2002) have shown that HIV in South Africa is disproportionately distributed by age, sex, locality, and province. The groups with the highest risk, according to these studies, are females of African ancestry, aged 20-34; males of African ancestry aged 25 – 29 years; and people living in informal settlements.

A survey conducted between 2007 and 2011, by the University of Cape Town, the Health Sciences Research Council and the Bill and Melinda Gates Foundation, revealed the HIV prevalence and incidence in South Africa in 2012. Within South Africa, KwaZulu-Natal has the highest HIV prevalence (16.9%), with a higher prevalence found in rural areas as compared to urban areas. This is followed by Mpumalanga (14.1%) and the Free State (14.0%); and finally, 5% in Gauteng – the lowest prevalence (Shisana, 2014).

Females have a higher prevalence than males with the highest risk age group being people between the ages of 15 and 49. Females in this age group have a higher risk of being HIV positive than men, (14.4% as compared to 9.9%). This trend of females having a higher HIV prevalence than males starts in younger age groups (15-19 years) and suggests that girls of a younger age are engaging in more frequent age disparate relationships. Males at this age have a prevalence of <1%, but females are at 5.6% in the same age group (Leclerc-Madlala, 2008).

Socioeconomic factors also played a factor, with people who lived in informal settlements being more at risk than people in formal settlements and urban areas (Shisana, 2014). Behavioural factors such as having multiple sexual partners, being in age-disparate relationships and a low usage of condoms also increases the risk of contracting HIV (Harling et al., 2014).

There has been an increase in the number of HIV infected individuals in South Africa, and as a result, South Africa currently has an estimated 6.3 million people living with HIV (WHO, 2013). South Africa has the highest number of HIV infected people, with Nigeria coming in second at an estimated 3.2 million people, and India, with 2.1 million people living with HIV.

With the increase of HIV positive people in South Africa, policies governing the distribution of antiretroviral therapy have been adapted throughout the years. Before I discuss the mechanisms of action of antiretroviral drugs, I first need to discuss how HIV functions.

1.1.1. Mechanism of action of the Human Immunodeficiency Virus

HIV is an encapsulated virus that contains two strands of positive sense RNA and a reverse transcriptase enzyme within the capsid. These RNA strands are what is inserted into the host cell and reverse transcribed into DNA by the enzyme reverse transcriptase.

1.1.2. HIV entry

In order for the virus to adequately carry out its infection, the capsid needs to bind fully to the host's target cell. The cells that HIV preferentially infects are CD4+ helper T-cells, which are lymphocytes that carry out immune functions (such as antigen presentation and release of cytokines), functions and other CD4 bearing cells such as macrophages or dendritic cells. Lymphocytes comprise of T-cells, B-cells, and natural killer (NK) cells. Helper T-cells (CD4+ T lymphocytes) form part of the adaptive immune response, and can be split into either Th1, or Th2 classes. T-cells respond to antigen presentation on the major histocompatibility complex type –II (MHC II) molecule on the surface of antigen presenting cells. Th1 cells respond primarily to infection by bacteria and viruses, and when activated, Th1 cells secrete the cytokines tumor necrosis factor (TNF), interferon gamma (IFN-γ), and interleukin-2 (IL-2). Th1 cytokines instruct the immune system (both the adaptive and innate immune system) to produce cells and antibodies to fight infection by bacteria and viruses. Th2 helper T-cells respond to infection by larger parasites such as a helminth or hookworm. When activated, Th2 helper T-cells secrete cytokines such as IL-4, IL-5, and IL-13. Th2 cytokines aid to fight infection against parasites or pathogenic bacteria that have invaded the digestive tracts (Janeway et al., 1997).

The CD4+ molecule is a protein on the surface of lymphocytes, dendritic cells, macrophages to which HIV binds through its surface glycoprotein, gp120. This fusion of gp120 to CD4 results in conformational changes that recruit virus specific chemokine receptors (CCR5 or CXCR4) that act as co-receptors, allowing firm attachment to the host cell. This results in fusion of the virus membrane to the host cell membrane, thus allowing the insertion of the viral RNA into the cell. Successful binding of the virus requires the presence of the transmembrane protein, chemokine receptor, CCR5 or CXCR4. Alterations in the conformation of these proteins result in unsuccessful

binding, and a decreased viral efficacy. A 32 base pair deletion in the genes that code for CCR5, known as delta-32, is associated with decreased HIV binding and increased HIV resistance. This occurs in 13% of the European population (Folwaczny et al., 2003, Novembre et al., 2005) and in 1.44% of the West African population (Kokkotou et al., 1997).

1.1.3. Integration and transcriptional events

After fusion occurs, and the viral capsid is inserted into the cell; the virion is uncoated. The first enzyme to be uncoated is reverse transcriptase. This enzyme reverse transcribes the viral single stranded (ss)RNA into double stranded (ds)DNA upon entry into the host cell. This uncoating and reverse transcribing allows for viral proteins to be formed. The RNA strands contain open reading frames, in which are the genes that code for the proteins *pol*, *vpu* and *env*. Effective reverse transcription produces the pre-integration complex, which is the combination of the dsDNA and other virus particles that are shuttled to the nucleus via microtubules within the cytoplasm, to the cell nucleus, where it crosses via the nuclear pore (Wyatt and Sodroski, 1998).

Once the viral dsDNA enters the nuclear pore, the viral protease forms viral proteins and the integrase enzyme integrates the viral dsDNA into the host cell genome, thus effectively hijacking the host cell's nucleus for its own purposes.

Gag, Pol and Env are structural genes, Tat and Rev are regulatory genes and accessory genes are Vpu, Vpr, Vir and Nef. Gag encodes for capsid proteins, these encapsulate the two positive sense single strands of RNA upon viral assembly within the host cell. Pol codes for reverse transcriptase, protease and integrase (Chan and Kim, 1998, Wyatt and Sodroski, 1998).

1.1.4. Expression of viral genes, and assembly and budding of HIV

The envelope gene, codes for the envelope protein, gp160 – which is heavily glycosylated in the Golgi body, and then cleaved by cellular protease to form gp120 and gp41. The primary function of

gp120 is to recognize the proteins on the surface of T-cells, namely CD4 and CCR5 or CXCR4 (Coakley et al., 2005).

Viral assembly occurs within the host cell at the plasma membrane. Viral proteins are shuttled to the cellular membrane; along with viral RNA. Membrane proteins, gp120 and gp41 are expressed on the cell surface and are available on the new, capsulated, virion particles as it buds out of the cell, which causes host cell death (Coakley et al., 2005).

Interaction with HIV usually occurs when the virus penetrates the vaginal or anal mucosa, and infects the helper T-cells of immune cells that exist on the surface of those tissues. These helper T-cells are what first come into contact with the virus. Once successful binding has been achieved, the viral DNA is transported to the nucleus of the cell where it is incorporated into the host cell's nucleus and can remain latent for an indefinite period of time. This phase is known as the latent phase of HIV, and is the second phase of viral pathogenesis. During this stage, the virus is clinically latent, still replicating at low levels and causing a steady decline in CD4+ T lymphocytes. This period can last anywhere between a few months, up to an indefinite amount of years, as seen in the figure below.

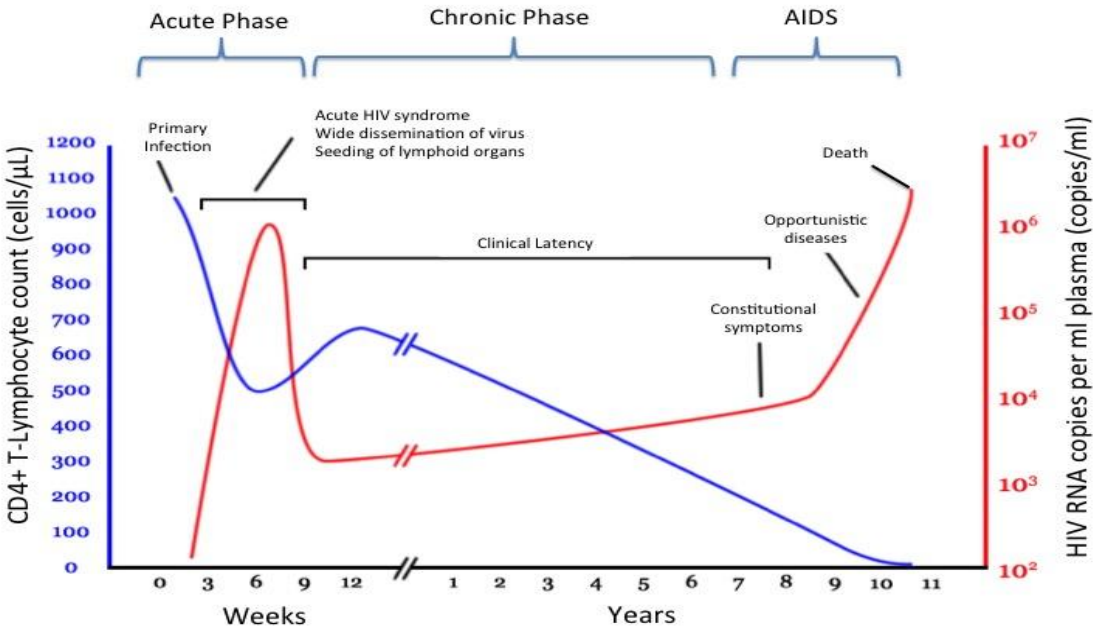


FIGURE 1.1: Graph depicting the progression of HIV infection from acute phase, to chronic phase and eventually AIDS. Adapted from Trends in Genetics (An and Winkler, 2010). After primary infection is established, an acute phase of infection occurs whereby there is a rapid increase in viral replication, and a rapid decline in CD4 T-cells. This is followed by the chronic phase of HIV infection, whereby the virus lays dormant in established reservoirs as a provirus, and a steady decline over the years of latency. The final phase is when CD4+ T lymphocyte counts drop to levels below 200cells/µL blood. This phase encompasses a high risk of opportunistic infections as a result of the low CD4+ T lymphocyte count. At this stage, there is a high chance of mortality, if untreated.

Recent works with Simian Immunodeficiency virus in rhesus monkeys – a model for HIV in humans – has shown that viral reservoirs are established very early on in infection (Whitney et al., 2014), before even the acute viraemia occurs. The study postulated that the first sites of viral replication were the intestinal mucosa and the lymphoid tissue as found previously (Haase, 2010). They confirmed this by testing for pro-viral DNA in these tissues after administering ARVs three days after infection, and found pro-viral DNA in the lymph nodes and in the gastrointestinal mucosa, but not in peripheral blood mononuclear cells (PBMCs) (Whitney et al., 2014). The

establishment of viral reservoirs in cells makes it extremely difficult to eradicate the virus as the pro-virus can remain latent indefinitely.

Once the reservoir is established, HIV can rebound, even after treatment. Central memory CD4+ T lymphocytes are severely affected by the viral infection, and experience a steady decline in numbers throughout infection. HIV specific memory T-cells are preferentially infected (Douek et al., 2002).

CD4+ T lymphocyte levels in the blood are measured, but the highest concentration of these cells are in the mucosa of the gut, where the highest amount of CD4+ T lymphocyte decline is noticed (Brenchley et al., 2004). At very low levels of CD4+ T lymphocyte counts (<200cells/μL blood), the immune system is no longer able to adequately protect the body against infective particles. When CD4+ T lymphocyte counts drop this low, there is a high level of opportunistic diseases, such as Pneumocystis carinii, and Kaposi Sarcoma. This is the final stage of infection and is known as Acquired Immune Deficiency Syndrome (AIDS).

Treatment of HIV infection increases chance of survival and inhibits the development of AIDS, but as soon as treatment is stopped, viral levels rebound and are resistant to the drugs previously used drugs.

1.1.5. Antiretroviral Therapeutics that exist in South Africa, and the political hindrance of ARV therapy

HIV treatments that exist and those used in South Africa

The treatment of HIV requires a combination of drugs (Feng et al., 2009). This is known as highly active antiretroviral therapy, or, HAART. Current research has shown that if HIV positive individuals take the treatment regularly as they should, life expectancies are comparable to uninfected individuals (Blanco et al., 2010, Johnson et al., 2013, Negin et al., 2012).

Over the years of ARV therapy, the WHO has prescribed ARV initiation guidelines depending on the CD4+ T lymphocyte count of an infected individual. In 2009, guidelines stipulated that patients with CD4+ T lymphocyte counts of <250 cells/μL blood could be initiated onto HAART. Studies

have shown that earlier initiation onto HAART is correlated with a better life expectancy (Johnson et al., 2013) and with a lower incidence of developing a sever complication of CD4 cell counts normalization, the immune reconstitution inflammatory syndrome (Hontelez et al., 2011). Immune reconstitution inflammatory syndrome (IRIS), also known as Immune Restoration Disease (IRD) is an exaggerated immune response that HIV positive people undergo soon after the administration of ARVs. If underlying tuberculosis is not treated before taking ARVs, or if there is a latent tuberculosis infection, when the immune system begins to reconstitute, the CD4 cells become hyperactive leading to an exaggerated immune response. This response causes inflammation at the site of the infection and resulting in lesions in the lung while attacking *M. tuberculosis* infection (Meintjes et al., 2008, Shelburne et al., 2005). Another infection that falls under IRIS is HIV co-infection with HCV. Soon after ARVs are administered, IRD occurs in the liver, causing liver enzyme elevation (Price et al., 2009). In 2012, the WHO guidelines changed to initiating people onto HAART once their CD4+ T lymphocyte counts dropped below 350-cells/μL blood (WHO, 2013). This number has since been increased to 500 cells/μL blood (Meintjes et al., 2015, WHO, 2013), and currently, the recommended treatment is to administer ARVs to whomever is infected, regardless of the CD4+ T lymphocyte count (Department of Health, 2016).

Antiretroviral therapies prevent the proliferation of HIV in various ways. There are currently 20 prescribed and allowed drugs in the world, of which d4T (stavudine) has been discouraged from use as it caused too many side effects (Brinkman et al., 1998, Gallant et al., 2012), in particular, painful peripheral neuropathy and lipid abnormalities, and has since been replaced by Tenofovir.

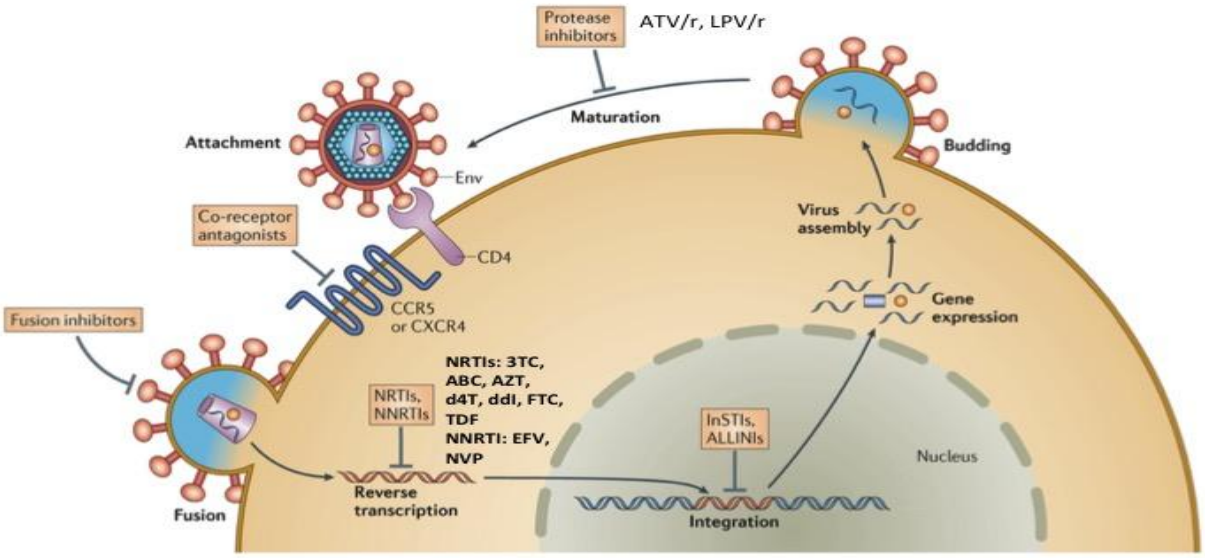


FIGURE 1.2: HIV entry and replication into host cell, as well as potential drug targets for antiretroviral therapies. Adapted from Laskey, 2014. Figure depicts mechanism of action of HIV when entering into a host cell, as well as at which points different antiretroviral drugs inhibit the action of the virus (Laskey and Siliciano, 2014). 3TC = Lamivudine, ABC = abacavir, AZT = zidovudine (AZT), d4T = stavudine, ddI = didonasine, FTC = emitricitabine, TDF = tenofovir (TDF), ATV/r = atazanavir with ritonavir booster, LPV/r = lopinavir with ritonavir booster,NNRTI = non nucleoside reverse transcriptase inhibitor, NRTI = nucleoside reverse transcriptase inhibitor.

Nucleoside reverse transcriptase inhibitors on the approved list in South Africa are Lamivudine (3TC), Abacavir (ABC), Zidovudine (AZT), stavudine (d4T), Didonasine (ddI), Emitricitabine (FTC) and Tenofovir (TDF). The combination drug, Atripla, comprises of a fixed dose combination (FDC) of TDF+FTC+EFV – two Nucleoside Reverse Transcriptase Inhibitors (NRTI) and one Protease Inhibitor (PI), and has been used as a first line drug since 2013. NRTIs act by competitive inhibition with the reverse transcription enzyme, interrupting the formation of the DNA chain, thus preventing the formation of the HIV-provirus (Arts and Hazuda, 2012). NRTIs are less potent that NNRTIs and thus are used in combination with the protease inhibitor and NNRTI.

Non-nucleoside reverse transcriptase inhibitors also block the reverse transcriptase enzyme. In South Africa, I use efavirenz (EFV), and nevirapine (NVP). NNRTIs inhibit reverse transcriptase by non-competitively binding to the enzyme, causing hydrophobic pockets to form. Hydrophobic

pockets result in conformational changes in the active binding site of RT, thus rendering it unable to bind to RNA and create viral DNA strands (Arts and Hazuda, 2012).

The HIV protease is responsible for the cleavage of *gag* and *gag-pol* polypeptide strands for the maturation of virion particles (Park and Morrow, 1993). Protease inhibitors bind directly to the protease, and inhibit further binding, thus preventing the cleavage of the polypeptides required for viral virulence and infection in mature virion particles (Arts and Hazuda, 2012). The approved protease inhibitors used in South Africa ATV (Atanazavir), or LPV (Lopinavir) boosted by RPV (Ritonavir) which is why these drugs are usually presented in tandem: LPV/r or ATV/r.

These drugs are administered on a basis of necessity dependent on the patient's needs. Guidelines stipulate that patients are eligible for first line regimen regardless of WHO stage or CD4+ T lymphocyte counts but those with less than 500-cells/μL blood take preference. If the patient has tuberculosis, tuberculosis treatment should be administered first, and ARV treatment is subsequently initiated. If the patient is pregnant, treatment should be administered regardless of CD4+ T lymphocyte count. Patients who have CD4+ T lymphocyte counts of less than 200 or with Stage 4 AIDS are to be fast tracked onto treatment (Meintjes et al., 2015).

Treatment efficacy is monitored by assessing viral loads – that is, the number of viral copies per milliliter of blood (copies/ml). In acute viraemia, these numbers are well into the hundreds of thousands (See Figure 1.1.). Patients with viral loads that remain below 400 copies/ml are to remain on treatment, if viral loads are between 400 and 1000, adherence needs to be revisited. If a patient's viral load is over 1000 twice (with a repeat two weeks after the initial viral load is taken) the patient is considered to be failing on regimen one and may need to be deferred to regimen two. A patient may become resistant to the drugs if treatment is halted for a period of time, but some drugs, such as efavirenz, have a long half-life, and thus can be re-administered once a patient is restarted on the treatment (SA guidelines, 2015).

Regimen two drugs include the addition of LPV/r and/or AZT, depending on the health status of the individual, and if they develop dyslipidemia on LPV/r, ATV/r replaces it.

If an individual experiences virologic failure on regimen two, as well as resistance to the protease inhibitor being administered, a review committee is put together to assess whether or not the individual should be deferred to regimen three. The protease inhibitors administered in regimen three are Darunavir/Ritonavir, as well as the integrase inhibitor Raltegravir and the NRTI etraviline, given on the previous treatment's NRTI backbone (EFV) even if there is resistance.

It costs more to supply, and, once the review committee has reached a decision, the medicine needs to be name-delivered for the individual (SA guidelines, 2015).

There are however side-effects of HIV treatment. For example, with treatment by efavirenz, various studies have found psychiatric side effects such as headaches, drowsiness, unusual dreams and trouble sleeping (Staszewski et al., 1999, Squibb, 1998).

With certain treatments of HIV, such as NNRTIs, there are also disruptions in lipid metabolism resulting in dyslipidemia and lipodystrophies (Carr et al., 1998, Pullinger et al., 2010). Indeed in 2005, 40 – 50% of treated HIV patients had lipid abnormalities (Sweet, 2005). These dyslipidemias are associated with an increase in the formation of atheroma, but also increased level of activation in the platelets (Gresele et al., 2012). This increased activation means there is an increased chance of aggregation, coupled with issues in lipid metabolism, increases the chances of atheroma formation, increasing the chances of developing cardiovascular disease; putting HIV positive people at high risk for developing cardiovascular disease (Guzmán-Fulgencio et al., 2011, Longo-Mbenza et al., 1998). A 6 year follow up of the Swiss HIV Cohort Study (SHCS) found that cardiovascular risk factors (including hypertension, smoking, dyslipidemia, diabetes, metabolic syndrome, and obesity) were elevated throughout the course of the study (Glass et al., 2006). This study also found that treated HIV positive patients were more at risk to develop cardiovascular disease than HAART naïve patients, and pre-treatment patients.

Diabetes is prevalent in the treated HIV positive community, and is associated with, but not restricted to, the use of HAART (Capeau et al., 2012). In HIV, the development of diabetes and cardiovascular disease is worsened by the high prevalence of metabolic syndrome in HIV (24.7%) which has also been attributed to NNRTI-related dyslipidemia (Alencastro et al., 2011).

Political hindrance of ARV therapy in South Africa

HIV was discovered in South Africa in the early 1980s. The first deaths attributed to HIV were in December 1981, and January 1982. Due to the political turmoil in the 1980s and early 1990s, not much was done about HIV and from a prevalence of 4% in 1982, to 12% by 1992, it reached epidemic proportions. Over the course of these two decades, there was significant political hindrance of drug administration to HIV infected people.

Nevirapine was rolled out to pregnant women in 2004 (Health, 2004), (and only) to pregnant woman with CD4+ T lymphocyte counts <200cells/μL, leaving thousands of infected people without treatment. In more recent years, with the current government, HIV treatment became mandatory, but only to people with low CD4+ T lymphocyte counts. This affected a large amount of people in poor areas, as the spread of HIV was not being curbed and people were still dying. As guidelines changed, the CD4+ T lymphocyte count cut off for government supplied ARVs increased, and some of the people who previously were unable to be treated were now able to receive this medication. Guidelines were only recently (SA Guidelines, 2016) changed to start HIV infected people on treatment, regardless of their CD4+ T lymphocyte count.

Shortly after the discovery of HIV in South Africa in 1985, the then apartheid government did not do much in terms of rolling out antiretroviral therapy to the public. In late 1987 HIV/AIDS got added to the South African list of communicable diseases. In 1992, as there was a closing to the apartheid regime, several members of the South African Parliament even suggested the use of HIV to reduce the numbers of homosexuals and black people in the country (Van der Vliet, 2004) .

With Nelson Mandela becoming president of South Africa in 1994, he brought Dr. Nksosazana Dlamini-Zuma as the minister of health. Of the projects that were put forth for the term of Mandela’s presidency, he added the HIV/AIDS and Sexually Transmitted Disease Advisory Group and the Committee of HIV/AIDS and Sexually Transmitted Diseases. The National Advisory Group (NACOSA), under the reform of Mandela, launched the National AIDS Plan. In 1997, Nkosazana Dlamini-Zuma was pushing to start public trials of the drug, Virodene, even though it had been proven to be toxic. Dr. Nkosazana Dlamini-Zuma also motivated prevention of HIV rather than

drug treatment. Mandela's goals were, as defined by the National AIDS plan for South Africa, were to increase public education campaigns, decrease transmission through appropriate care and to provide treatment support for those who were infected. Sadly, by the end of Mandela's presidency, the group had not met the targets they had set out to achieve (Butler, 2005).

Thabo Mbeki, the second post-apartheid president of South Africa, came into power after Mandela along with Dr. Manto Tshabala Msimang as the new Minister of Health. He firmly believed that HIV did not cause AIDS, and is known to have sent letters to world leaders asking them to reconsider their stance on the matter, motivating that AIDS was caused instead by socioeconomic factors (Schneider, 2002). The government supported a bid in 2001 to produce cheap, generic drugs in South Africa, against the pharmaceutical companies. This included the production of ARVs. In 2005, South Africa had five million cases of HIV positive people, making South Africa the country with the highest prevalence of HIV in the world. Dr. Manto Tshabalala Msimang suggested palliative care and nutrition rather than antiretroviral drugs. In 2004, President Mbeki rolled out Nevirapine to pregnant women, and thus started the mass treatment of HIV infection, even though there were still thousands of people who went untreated (McNeil, 2014).

Jacob Zuma became president in 2008, and appointed Dr. Aaron Motsoaledi as minister of health. Together, they made the most impact on the HIV/AIDS treatment campaign. Zuma's legislation made a commitment to test all children exposed to HIV, and provide ARVs to all HIV-positive children. By 2010, the National Strategic Plans (NSP's) goal was to provide HIV positive pregnant women with Nevirapine had reached 95% coverage.

There have since been great improvements with regards to the treatment of HIV, with Minister of Health Aaron Motsoaledi announcing at the end of 2011 that 11.9 million South Africans were being tested for HIV every year. On 1 December 2011, a third NSP was released with the following five goals: 1) Halve the number of new HIV infections; (2) Ensure that at least 80 percent of people eligible for HIV treatment are receiving it; (3) Halve the number of new tuberculosis infections and deaths from tuberculosis; (4) Ensure that the rights of people living with HIV are protected; and (5) Halve stigma related to HIV and tuberculosis (Motsoaledi A, 2011)This plan has

increased the budget allocations to the treatment of HIV, with 8 billion rands being spent on ARVs at the end of 2012 (Treasury, 2012).

By the end of 2014, there were a reported 2.7 million people on treatment, with the government spending over R1 billion per annum on ART. As life expectancy has extended in USA and Europe, secondary chronic comorbidities have come to light, in particular the increased risk for chronically treated HIV patients to develop non communicable diseases, such as cardiovascular disorders / metabolic syndrome and mental illnesses such as depression. A frequently reported complaint in studies of chronically treated people living with HIV is sleep disruption. In the past decade, a growing body of literature has pointed at the role of sleep disruption not only in the development and maintenance of depression but also in increasing the risks of developing metabolic syndrome/ cardiovascular disorders. Before discussing the impact of HIV and HIV therapies on sleep, we first need to understand how sleep works.

1.2. Sleep

Sleep is a naturally occurring, restorative process that is characterized by reduced interactions with the surrounding environment, an altered state of consciousness and a relaxation of voluntary movement. Humans typically sleep between 7 to 9 hours per day, usually in one consolidated bout (rather than interspersed throughout the 24-hour cycle). Sleep is divided into rapid eye movement (REM) sleep, and non-rapid eye movement (non-REM) sleep. The non-REM sleep is categorized into four stages. Using strategically placed electrodes on the scalp, chin and eyes, we are able to detect changes in the electrical activity of the brain during sleep. This process is called polysomnography, and allows one to categorize the different stages of sleep, as seen in the first polysomnograph studies done by Rechtschaffen and Kales in 1968. The first two stages of non-REM sleep are known as light sleep. The first stage of sleep is characterized by the extinction of alpha waves (a 10-13Hz rhythm that occurs when participants keep their eyes closed while being awake), which are then replaced by a mixed frequency EEG and slow eye movements; it marks the period between sleep and total wakefulness. Humans typically spend five to ten per cent of their total sleep time in this stage of sleep. The second stage of sleep is known as stage 2 sleep,

and is characterized by sleep spindles (a 13-16Hz rhythm) and the presence of K complexes (single electrical activity units characterized by a fast positive component followed by a slow negative component) (Rechtschaffen and Kales, 1968). The third and fourth (most recently combined to form “stage 3” sleep only) stages of sleep are known as slow wave sleep (SWS). Slow wave sleep is categorized by slow, delta waves.

REM sleep is the stage of sleep in which an individual reports vivid dreams, and the brain activity in this stage of sleep is similar to that of wake. During REM sleep, there is significantly decreased muscle tone (Rechtschaffen and Kales, 1968) and rapid eye movements.

Throughout the night, humans cycle through all stages of sleep, until sleep pressure dissipates until the end of the night. This can be seen in the hypnogram below:

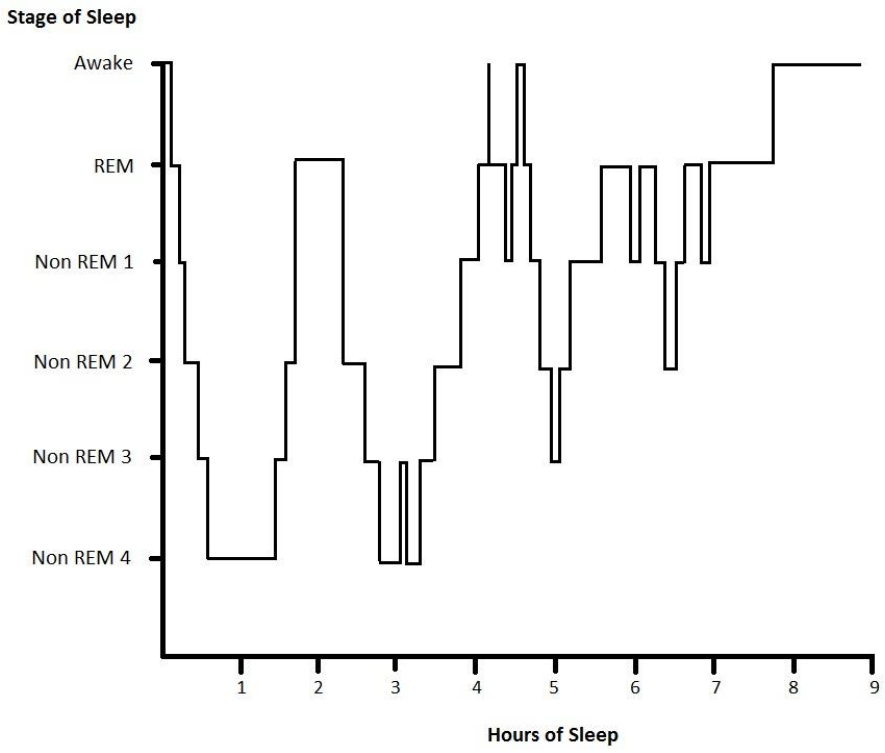


Figure 1.3: Normal sleep hypnogram in humans, showing the cycling of sleep stages throughout the night. The amount of slow wave sleep decreases throughout the night, until sleep pressure dissipates and wake occurs in the morning while the amount of REM sleep increases throughout the night reaching its peak just before wake time. Image constructed by Natasha_k (2012) – Wikipedia

Sleep is a natural, and complex process that is tightly regulated, and will be discussed in the following sections.

1.2.1. Sleep regulation

Sleep is regulated by two processes (Borbély, 1982). The first is a homeostatically controlled, sleep dependent process. In this process, with an increased time awake, there is an increase in sleep pressure accompanied by an increase in adenosine levels in the synaptic cleft. This increase in sleep pressure leads to an increase in sleepiness, which makes an individual tired and in need of sleep (Borbély, 1982).

This sleep pressure is only alleviated once a person goes to sleep, and throughout the night, the level of sleep pressure dissipates until they wake up the following morning.

The second process is a sleep independent, circadian rhythm, also known as a “biological clock”. This involves a nucleus in hypothalamus, the suprachiasmatic nucleus (SCN). Firing from the neurons of the SCN peaks at the end of the biological day and then dips again at night. These act against the increase in sleep pressure and provide a strong urge to stay awake during the biological day, even if the person has had insufficient sleep the night before (Borbély, 1982).

Just before bedtime, the firing of these nuclei decreases, causing a decrease in wakefulness pathways by removing the inhibitory effects of the wakefulness pathways on the sleep promoting nuclei, thus inducing sleep. This “sleep signal” increases throughout the biological night, maintaining sleep throughout the night (Saper et al., 2005).

In the middle of the biological day, the neurons of the SCN reach their firing peak (Saper et al., 2005). At this time, a person’s level of alertness is at its peak, this is also known as their “feeling best” peak. This circadian process controls core body temperature, endocrine and metabolic functions (Hastings et al., 2007); glucose and insulin levels (Van Cauter et al., 1992); the immune system (Scheiermann et al., 2013) and hormones (Hastings et al., 2007). The three markers of the circadian pace maker are core body temperature, melatonin and cortisol (Benloucif et al., 2008).

Core body temperature trough occurs a few hours before wake time and rises steadily throughout the day to reach a maximum a few hours before bedtime. Cortisol is at its peak in the biological morning, and levels dip throughout the day, while melatonin levels are at the lowest during the biological day. Melatonin is produced during the biological night, increasing throughout the night reaching a maximum in the middle of the biological night (Benloucif et al., 2008). With the increase of melatonin and the decrease of cortisol and core body temperature, the sleep-wake pathway is activated to favour the onset of sleep – thus making the person feel tired in preparation for the biological night. Melatonin levels increase throughout the biological night, and dip in the early morning, coinciding with the increase in cortisol and of core body temperature– in preparation for the biological day.

The ventrolateral preoptic nucleus (vlpo) and wake nuclei constantly fire throughout the day, and inhibit each other (Saper et al., 2005). The circadian system aids the wake nuclei in firing, even when a person has higher than normal sleep pressure during the biological day after not having slept the night before. This is why an individual can maintain wake even after a night of sleep deprivation. This explains the interaction between the circadian system and homeostatic control of sleep (Schwartz and Roth, 2008). In contrast to the homeostatic sleep process, circadian rhythms are not affected by sleep pressure,but rather are entrained by natural light exposure (Borbély, 1982). This process is known as light entrainment and is regulated by the N-methyl D-aspartate (NMDA) receptors – glutamate receptors in the SCN (Ebling, 1996). This strong sleep signal by melatonin is hindered by light exposure – specifically light on the blue spectrum (found in most mobile devices and screens) (Chellappa et al., 2011). The introduction of blue light during the biological night negatively affects an individual's ability to maintain sleep or fall asleep at all thereafter, by disrupting the timing of circadian rhythms by altering the release of melatonin hormone (Khalsa et al., 2003). Light exposure just after core body temperature trough or exogenous melatonin given before core body temperature trough leads to phase advances in the timing of circadian rhythms while light exposure received before core body temperature trough or exogenous melatonin given after bore body temperature trough leads to phase delays in the timing

of circadian rhythms. phase advances typically result in earlier timing of wake times and bedtimes while phase delays in the timing of circadian rhythms results in later timing of wake times and bedtimes.

Sleep is typically divided into non rapid eye movement sleep (NREM) and rapid eye movement sleep (REM). Dijk et al (1999) have shown that the timing of REM within the sleep episode follows a circadian pattern, whereby if the sleep episode is scheduled to start at the habitual bedtime (for example at 22:00), REM would mainly happen toward the end of the sleep episode (after core body temperature trough, i.e. after 04:00 – 05:00). However if the sleep episode were shifted with a bedtime occurring around 4 am and wake time scheduled around 12pm, with no change in the timing of the core body temperature trough, REM sleep would mainly occur from 4-5 am to 8 am and not be shifted towards the end of the sleep episode (Dijk et al., 1999). Increases in REM sleep latency can therefore be attributed to an earlier timing of the sleep cycle relative to a preserved timing of endogenous circadian rhythms or to a later timing of endogenous circadian rhythms relative to a preserved timing of the sleep cycle.

1.2.2. Measurements of sleep used in literature

Questionnaires

Questionnaires are the least invasive way of measuring sleep or problems with it. The questionnaires used most in the literature, pertaining to this study are listed, and explained in the following paragraphs.

The Pittsburgh Sleep Quality Index (PSQI) is a measurement developed by Buysse in 1989 to assess the level of sleep quality of an individual (Buysse et al., 1989). Made up of 21 questions, and broken down into seven components, the PSQI has the ability to tease apart areas of general sleep problems as well as give an overall view of subjective sleep quality. The PSQI asks individuals to rate their sleep over the past month, and answer a series of questions relating to

how often a factor disturbed their sleep – if at all – in that time period. The higher the PSQI global score (total), the higher the level of sleep disruption (or poor sleep quality) that the individual has.

The Epworth Sleepiness Scale (ESS) is a measure of daytime sleepiness. It gives the individual eight scenarios in which they have to rate their likeliness of falling asleep in. The higher the score, the higher the level of daytime sleepiness (Johns, 1991).

The Horne-Ostberg Morning-Eveningness Questionnaire relates more to circadian rhythm as it assesses an individual's daytime preference (Horne and Ostberg, 1975). This is thought to change with a person's level of health, especially in terms of HIV where the gp120 protein has been shown to alter the light entrainment pathway – which will be explored later on in this review.

Sleep apnoea is a condition whereby an individual spontaneously stops breathing (apnoea) or has episodes of decreased airflow (hypopneoas) in their sleep (Netzer et al., 1999). The severity of sleep apnoea is usually measured during an overnight polysomnography study and is expressed in terms of the apnoea/ hypopnoea index (AHI) with an AHI above 5 signalling mild sleep apnoea while an AHI>15 signals more severe sleep apnoea. The incidence of sleep apnoea increases when an individual is overweight (high BMI) or if they are known to snore. The Berlin Sleep Apnoea Scale is a measurement of the likeliness of an individual to have sleep apnoea. Sleep apnoea negatively affects sleep in that the apnoeas/ hypopnoeas themselves cause a person to wake up gasping for air, causing a disruption in sleep, and their oxygen saturation throughout the night is hindered – thus leaving them with sub-par sleep quality and tired (high level of daytime sleepiness) throughout the day (Ulasli et al., 2014). The presence of sleep apnoea in non-HIV positive populations is correlated with increased morbidity (including an increased risk of cardiovascular disorder and metabolic syndrome) and independently from other confounders (such as age, sex and BMI) is a predictor of mortality. Questionnaires have been used to assess the risk of sleep apnoea in an individual. The Berlin Questionnaire is a commonly used instrument to assess the risk of sleep apnoea with a reported sensitivity of ~80%. It is a useful tool to assess the likely prevalence of sleep apnoea in a cohort (Chiu et al., 2016).

Actigraphy

Actigraphs are devices which measure the activity of an individual. Some of those devices also include recording of ambient light exposure and are usually wrist-worn. The data shows the daily movement of an individual, its magnitude and periods of inactivity coinciding with lights off are their supposed time asleep (Chesson Jr et al., 2007). Actigraphy combined with information about patterns of ambient light exposure help determine bedtimes and wake times, sleep duration, sleep efficiency, wake after sleep onset for an individual on a day by day basis. It provides a more objective measurement of sleep than the questionnaires, but not as in-depth as polysomnography. Previous studies have used actigraphy to determine fatigue (Lee et al., 1999), as well as sleep quality, but generally in combination with sleep questionnaires or other sleep measurements.

Polysomnography

First conducted by Rechtschaffen and Kales in the late 1960s, polysomnography (PSG) has become the gold standard for sleep measurements. It involves the usage of electrodes placed on specific points on the scalp, that relate to specific areas in the brain, which, when read together, provide a visible log of electroencephalography allowing a print out of impulses on a sheet. The PSG monitors several different factors including brain (EEG), eye movements (EOG), skeletal muscle activity (EKG) and heart rate (ECG) (Rechtschaffen and Kales, 1968).

The sleep cycle was first divided into 5 stages – four non-REM stages, followed by one REM stage. Each stage is distinct from the other in the brainwaves present, as is shown by an EEG. The first of the non-REM stages is stage 1 whereby alpha waves cease and are replaced with mixed frequency EEG and slow eye movements. This is followed by stage 2 sleep where sleep spindles and K complexes are visible. Stage 1 and stage 2 are characterised as light sleep. During light sleep, the person is not yet fully asleep and can easily be woken up if the environment surrounding them is not conducive to sleep, or by the use of an alarm clock. If a person is woken up during stage 2 sleep, they experience sleep inertia, which can result in performance impairment (Hofer-Tinguely et al., 2005). After light sleep, comes slow wave sleep, or “deep sleep.” This stage

of sleep is characterized by a typical EEG pattern of slow (0.5-4 Hz) large amplitude (75 mV or more) waves occupying 20% or more of a 30-second epoch. During these stages of sleep (stage 3 and 4), memory is consolidated, daily actions are processed and it is very important for learning and memory. If an individual takes a nap and is woken up at any stage during slow-wave sleep, they will have a high level of sleep-inertia, i.e. they experience high sleep pressure and a desire to go back to sleep. They also have low cognitive function and appear dazed or droopy. This time period is approximately 30 – 90 minutes long (Rechtschaffen and Kales, 1968).

After deep sleep comes REM sleep, or rapid-eye-movement sleep. This stage of sleep is characterized by brain activity very close to that seen while the person is awake (mixed frequency EEG). It is during REM sleep that vivid dreaming is reported. In this stage of sleep, the body undergoes sleep paralysis, and the person is unable to move. Dreams in this stage are very vivid, and if a person was able to move freely, they may cause harm to themselves or to the people around them. One complete REM cycle (stage 1 – REM) usually lasts about 90 minutes, and an individual typically has 5 or 6 REM cycles a night.

Polysomnography allows a researcher to see all these stages of sleep, and detect sleep disorders such as sleep apnoea, periodic limb movement disorder, REM behaviour disorder, and parasomnias such as sleep-walking. For instance, one uses polysomnography to detect episodes when the blood oxygen saturation drops below 80% due to the restricted air-flow, which corresponds to apnoea/hypopnoea. When the apnoea-hypopnoea index (AHI) is above 5 events per hour, an individual is considered as having sleep apnoea. It becomes a moderate /severe apnoea when the AHI reaches >15 events per hour.

Questionnaires, actigraphy, and polysomnography-combined paint a picture of subjective versus objective sleep quality, and serve as insight into the overall sleep of an individual. Normal sleep implies therefore a seven to nine hour period of uninterrupted sleep. A decrease in the duration of sleep, multiple interruptions of sleep represent sleep disturbances. Studies have shown a clear

association between sleep disturbances and different pathophysiological processes, which I will discuss below.

1.2.3. Sleep and other physiological factors:

Sleep and pain

Sleep and pain have a relationship with each other whereby studies have shown that disrupted sleep increases pain sensitivity by inducing generalized hyperalgesia (a pain response to non-noxious stimuli) (Schuh-Hofer et al., 2013). Studies have also shown that sleep disruption disrupts specific types of pain sensitivity, such as increasing mechanical pain sensitivity both peripherally and centrally (Onen et al., 2001) and increased sensitivity to temperature induced pain (Kundermann et al., 2004). Sleep disruption causes increased pain sensitivity, and the presence of pain has an impact on sleep quality. Studies have shown that the presence and intensity of pain in affects sleep whereby people who have pain have disrupted sleep (Robbins et al., 2004, Sandoval et al., 2013, Vosvick et al., 2004).

Sleep and depression

Poor sleep has a negative effect on overall quality of life. Coupled with the increase in daytime sleepiness, there is a decrease in daytime functioning (Irwin et al., 2013); increased level of depression and decreased quality of life (Payne et al., 2013, Poudel-Tandukar et al., 2014). Although widely reported, the mechanism of sleep induced depression or depression induced sleep deprivation isn't very well understood.

Sleep disruption and depression have a well reported relationship, and although there are many papers that cite this issue, the mechanisms that give rise to either sleep disruption first, or depression first then sleep disruption are poorly understood. Three quarters of persons with depression are diagnosed with insomnia (trouble falling asleep, trouble staying asleep, etc.) (Nutt et al., 2008).

Four out of ten young adults, and one out of ten older adults with depression report some form of hypersomnia (increase in total sleep above normal). In adults, there is a strong relationship between excessive daytime sleepiness and mood disorders (Bixler et al., 2005). And in an epidemiological study conducted by Ohayon et al. in 2003 on 14790 people showed that problems with sleep occur at the same time (>22%) or before (>40%) the mood disorders (Ohayon and Roth, 2003). This study seems to suggest that sleep disorders may precede mood disorders, therefore preventing mood disorders may be to tackle sleep disorders as soon as they present.

A review conducted by Luca et al. in 2013 went so far as to caution against diagnosing depression in the absence of sleep disturbances (Luca et al., 2013). How this theory was tested was using the DSM-IV screening tool for clinical insomnia, 41% of people who tested positive for insomnia also had mood disorders. That may not seem like a lot, but in the same study 96% of people without insomnia also did not have depression, giving credence to the caution of diagnosing depression in the absence of sleep disorders, and proving that there is a strong relationship between depression and sleep disturbances.

Sleep and immunity

Sleep and circadian rhythms have been shown to be involved in the regulation of the immune system and conversely, immune changes have been shown to be associated with changes in sleep architecture. In a normal, non-immune challenged individual, some immune cells (such as T-cells) and products (such as cytokines) exhibit a diurnal, or circadian, rhythm. Regulatory T-cells, for example, are at their highest levels during the biological night, and at their lowest levels during the biological day (Bollinger et al., 2009) . Interleukin-6 (IL-6) has also been shown to have a diurnal rhythm, similar to that of regulatory T-cells, whereby levels increase during sleep (the biological night), and are at nadir levels during wake time – the biological day (Vgontzas et al., 2005).

Cytokines have also been shown to play a role in sleep regulation, and sleep modulation. Activation of macrophages by pathogens leads to the secretion of several cytokines, in particular

tumor necrosis factor alpha (TNF-α) and interleukin-1β (IL-1β), which have been shown to modify sleep. These two cytokines, and their corresponding receptors are found throughout the brain, including the hypothalamus, and have been shown to have effects on sleep-wake behaviour. In rats, after administration of either IL-1β or TNF-α, there are increases in slow wave sleep (Yoshida and Caruso, 2002). These changes only occurred in the cerebral hemisphere in which the cytokine was injected. IL-1β has also been shown to decrease the firing rate of the wake-nuclei located in the preoptic nucleus and basal forebrain in rats by an average of 50% (Alam et al., 2007), meaning that the stimulus of wakefulness was dampened by the administration of exogenous IL-1β. Blockades of TNF-α and IL-1β receptors interfere with non-REM sleep, by interactions with the serotonin pathway. In humans administered with IL-1β or TNF-α, there is an increase of symptoms associated with sleep deprivation such as fatigue, daytime sleepiness, increased pain sensitivity, and decreased cognitive performance in spite of being associated with increased slow wave sleep (Krueger et al., 1998). Thus, acute increases in IL-1β or TNF-α lead to increases in non-REM sleep, and substances that interfere with the secretion or reception of IL-1β or TNF-α, lead to inhibition of non-REM sleep (Opp and Toth, 2003).

Cytokines which have been shown to either increase or decrease non-REM sleep are labelled sleep modulatory cytokines. Such examples are interferon gamma (IFN-γ), and the following interleukins: IL-2, IL-4, IL-6, IL-8, IL-10, IL-13, IL-15, IL-18. All these cytokines modulate sleep by increasing non-REM sleep, except for IL-4 and IL-10, which inhibit the synthesis of IL-1β and TNF-α thus decreasing non-REM sleep (Vgontzas et al., 2005). IL-6 has been shown to have a circadian rhythm (Vgontzas et al., 2005) and is able to modulate certain aspects of sickness behaviour – in humans and in animals, and responses to IL-1β and TNF-α. Sickness behaviour is a collection of behavioural responses to infections which include fatigue, fever, anorexia, lethargy and malaise (Dantzer and Kelley, 1989, Dantzer and Kelley, 2007). Acute subcutaneous injections of IL-6 increase slow wave sleep, and decrease REM in humans (th-Schwalbe et al., 1998), and sleep ailments such as sleep apnoea and chronic insomnia have been associated with increased levels of circulating IL-6 (Entzian et al., 1996, Vgontzas et al., 2005). Interestingly,

although increased concentrations of those sleep modulatory cytokines lead to increased slow wave sleep, the subjective assessment of patients is that they don't sleep well and feel drowsy/ tired, therefore not assessing their sleep as normal, which may reflect that any departure from normal sleep architecture may be perceived and may actually be non-restorative sleep. These modifications in sleep due to acute immune activation may not be exactly reproducible in the context of chronic infections or inflammation. In untreated HIV infection, there is chronic activation of the immune system, which may be also be involved in modifications in sleep architecture. This aspect will be discussed in the section reviewing the literature on sleep and HIV.

Sleep and non-communicable diseases

Sleep is important for the maintenance of hormones that control hunger and glucose regulation. These hormones include the anorexogenic hormone, leptin, and the hunger-inducing hormone, ghrelin. When there are low levels of leptin, ghrelin production increases to stimulate appetite. Once an individual is satiated, leptin levels increase and they are no longer hungry. Sleep disruption leads to hyperphagia (over eating) (Knutson et al., 2007). Sleep disruption leads to a decrease in leptin and an increase in ghrelin, thus inducing hyperphagia, which leads to weight gain, and ultimately, obesity (Beccuti and Pannain, 2011). Weight gain is associated with insulin resistance, and thus can lead to an increased chance of developing Type-2 diabetes (Spiegel et al., 2005). Being overweight leads to sleep problems such as sleep apnoea, and coupled with diabetes, is also a major contributor to developing metabolic syndrome. The presence of metabolic syndrome among treated HIV positive people has been increasing over the past few years (Blanco et al., 2010, Knutson et al., 2007, Pullinger et al., 2010) and even in non-HIV positive populations, is worsened by the presence of poor sleep.

The regulation of glucose is also affected by sleep deprivation. One night of total sleep deprivation leads to an increased plasma glucose concentration (reduced glucose uptake) in and low insulin levels in healthy subjects (Van Cauter et al., 1992). In addition, sleep restriction, to four hours, caused insulin resistance in healthy men (Buxton et al., 2010). This imbalance of insulin and

glucose metabolism leads to type 2 diabetes, and in 2005, Spiegel et al. classified sleep disruption as an independent risk factor for the development of type 2 diabetes (Spiegel et al., 2005).

Further studies supported this finding (Buxton et al., 2010, Gangwisch et al., 2007, Stamatakis and Punjabi, 2010). Thus, sleep disruption alone is an independent risk factor for the development of diabetes, and thus can be seen as a contributing factor for the development of the multifactorial metabolic syndrome. Thus, in sleep-deprived people, there is a high risk of developing diabetes, obesity, metabolic syndrome, and cardiac disease. This risk may further be increased in HIV infection, where, as seen in the previous Section, HAART itself has been implicated in dyslipidemias, increased risk of diabetes type II and cardiovascular disorders. Thus treating sleep disturbances in HIV positive people may help prevent the advent of these non-communicable diseases.

1.3. Sleep in HIV

1.3.1. Sleep disruption in untreated infection – the early studies

HIV has been shown to cause sleep disturbances very early on in infection (Norman et al., 1992, Norman et al., 1990) and has been hypothesized to be associated with the rapid and hyperactive immune response soon after infection (Norman et al., 1990). In addition, in the late 1980s, Grant discovered that HIV had the ability to cross the blood-brain-barrier and directly affect the glial cells – resulting in sleep alterations (Grant et al., 1987). During primary HIV infection, HIV crosses the blood brain barrier and infects (to name a few) microglial cells, astroglia and macrophages. Among the products which these activated cells produce are IL-1, IL-6, and TNFα (Merrill and Chen, 1991). These cytokines have already been shown to cause alterations in sleep (Shoham et al., 1987, Späth-Schwalbe et al., 1998, Yoshida and Caruso, 2002).

Initial studies investigating changes in sleep during HIV infection were conducted on these two groups of men; men who had sex with men (MSM) and intravenous drug users (IDU). Norman et al., in the early 1990s, conducted polysomnography studies on 10 HIV positive in MSM and 5 seronegative controls. They found that there was an increase in slow wave sleep (stage 3 and 4)

and an increase in time spent in non-REM sleep, towards the later part of the night (Norman et al., 1990).

In normal sleep, the largest amount of slow wave sleep occurs during the first half of the night, with more bouts of REM sleep occurring in the latter part of the night, until wake occurs in the morning, once sleep pressure is dissipated (Rechtschaffen and Kales, 1968).

The initial study by Norman was repeated with a larger sample, with 24 men – 14 HIV positive and 10 seronegative men. It was again found that there was an increase in slow wave sleep in the later part of the night, along with an increase in non-REM sleep. The authors hypothesized that these modifications may be caused by the chronic immune activation (Norman et al., 1992). Ferini-Strambi in 1995 ran a similar study to Norman's and similarly found decreased slow wave sleep in the first third of the night, but, contrary to Norman's studies, did not find an increased time spent in slow wave sleep in the second and third parts of the night (Ferini-Strambi et al., 1995). This study hypothesized that these changes in REM latency and slow wave sleep may be due to increased levels of interferon-alpha (IFN-α); a cytokine that was found elevated in untreated HIV positive patients.

In another study, 14 HIV positive patients and 14 HIV negative controls had polysomnography done for 1 night (plus one adaptation night). It was found that HIV positive patients had an overall impairment of nocturnal sleep (Wiegand et al., 1991). This sleep impairment was characterized by increased sleep onset latency and decreased total sleep time. There was also increased time spent awake, and more time spent in stage 1 sleep, coupled with decreased time spent in stage 2 (characterized by decreased sleep spindles) (Wiegand et al., 1991) and shifts in the pattern of slow wave sleep were found even in asymptomatic men (Ferini-Strambi et al., 1995).

Based on the early results from Norman (1992) and White (1995), more research was done to look at the prevalence and incidence of sleep disturbances and fatigue in HIV positive people (Norman et al., 1992, White et al., 1995). A case-control study on 45 HIV+ and 45 matched HIV- people revealed that being HIV positive alone, was significantly associated with an increased severity of

sleep disturbances (Wheatley and Smith, 1994). Five items from the sleep section of the Wheatley Stress Profile were used in this study (Wheatley, 1990).

Darko et al. (1995) assessed fatigue in 112 MSM – 62 HIV positive and 50 HIV negative men in a longitudinal study, and found that patients with lower CD4+ T lymphocyte counts had higher levels of fatigue, and that they required more sleep in the night (Darko et al., 1995). Fatigue was measured by the Profile of Mood States (POMS) (McNair et al., 1981) and this allowed a separation between a non-fatigue and fatigue group. The fatigue group had a lower mean CD4+ T lymphocyte count (330 ± 227 cells/μL) as compared to the non-fatigue group with higher CD4+ T lymphocyte counts (681 ±377cells/μL) (Darko et al., 1995). Similarly, White et al. (1995) conducted a case-control study, taking daytime and nighttime EEG, ECG and EMG. They found that higher CD4+ T lymphocyte counts (>400cells/μL) were associated with increased stage 3 and 4 sleep (as found previously by Norman et al.) and non-REM sleep in the second half of the night (White et al., 1995).

These early studies were on a limited sample size, focusing on HIV effects on sleep architecture. The studies mentioned before laid the foundation of sleep in HIV infected people, it became known that being HIV positive was a contributor to sleep disruption. In subsequent studies, factors associated with, or predicting sleep disruption – in treated individuals – were explored.

1.3.2. Sleep disruption in treated HIV and predictors thereof

One of the early studies (Wheatley et al, 1994) investigating the effect of HIV treatment on sleep changes in an HIV positive cohort found that there was no significant difference between sleep quality in treated versus untreated HIV infection. Higher sleep onset latency and higher levels of sleep disruption were found in patients with HIV, when compared to matched controls – irrespective of whether or not they were on treatment (zidovudine, dideoxyinosine, or acyclovir) (Wheatley, 1994). The Wheatley et al. (1994) study showed that the only factor that differentiated good sleepers from poor sleepers was their HIV status, and it did not matter whether or not they were on antiretroviral treatment. A more recent study done by Low et al. in 2012 showed that in patients meeting the DSM-IV clinical definition of insomnia, HAART treated HIV positive

insomniacs had a decreased sleep efficiency, decreased time spent in REM sleep, and increased sleep onset latency, when compared to matched HIV negative controls (Low, 2012). This section examines sleep disturbances in treated HIV positive patients. Past studies have investigated predictors of those sleep disturbances so this section is organised by main predicting factors of the sleep disturbances.

Treated HIV positive patients have more disturbed sleep than matched healthy controls. The prevalence of insomnia in the general population ranges between 10 and 40% in clinical studies (Mellinger et al., 1985, Rosekind, 1992, Simon and VonKorff, 1997). In HIV positive populations, the prevalence of insomnia ranges from 40 – 60% (Reid and Dwyer, 2005). A review of 29 HIV/sleep studies conducted by Reid and Dwyer in 2005 investigated insomnia in HIV. They defined insomnia using studies that used self-reported insomnia using polysomnography, the sleep section of the Wheatley Stress Profile (Wheatley, 1990) and studies that used PSQI>5. This review revealed that insomnia in HIV is one of the highest reported issues in treated HIV, but that the underlying cause or associated factors at this point in time (2005) were not as widely investigated and understood.

The overall message from this review was that sleep was most disrupted by psychological morbidity such as AIDS-related dementia. This review also stated that the role of the immune system and adverse drug effects remained unclear (Reid and Dwyer, 2005). In the following paragraphs I will describe factors associated with disrupted sleep in HIV, including immune related factors, HIV proteins that affect circadian rhythm, pain, depression, and antiretroviral treatment with efavirenz.

Immune related factors: CD4+ T lymphocyte counts

A cross sectional study conducted by Lee in 2001 investigated the incidence of sleep disruption on women living with HIV, using the PSQI, actigraphy, and the general sleep disturbance scale (Lee, 1992). On average, there was a high level of sleep disruption (mean PSQI = 9.0 (± 4.4)) reported, which was unrelated to CD4+ T lymphocyte counts . The population was separated into high

fatigue and low fatigue groups, and patients in the high fatigue group, when using actigraphy, had increased night arousals and increased difficulty falling asleep (Lee et al., 2001). This study, similar to the work done by Darko et al. in 1992 on untreated HIV + patients, separated the high fatigue and low fatigue groups (Darko et al., 1992). However, whereas Darko et al. found that there were lower CD4+ T lymphocyte counts in the high-fatigue group, and higher CD4+ T lymphocyte counts in the low-fatigue group, no similar association was found in this study.

Seay et al. in 2013 investigated sleep disturbances in low-income HIV positive women of ethnic minorities in the USA (African American, Hispanic and “other”). They used the PSQI to determine sleep quality and measured CD4+ T lymphocyte counts (mean = 484.5 (±303.0) cells /μL), and urine dopamine. Lower dopamine levels have been found in people with sleep disturbances such as restless legs syndrome (Cohrs et al., 2004). Decreased CD4+ T lymphocyte counts and decreased urine dopamine were associated with increased sleep disruption, and it was concluded that sleep disruption was independently correlated with immune status and dopamine levels in women living with HIV (Seay et al., 2013) .

A cross sectional study investigated sleep in a convenience sample of 58 HIV positive people, whereby increased sleep disruption was associated with cigarette, caffeine, and marijuana use; as well as increased clinical symptom severity on the HIV assessment tool (Nokes et al., 1994), daytime sleepiness and functional status (Nokes and Kendrew, 2001). There was no relationship found between PSQI score, and disease factors such as CD4+ T lymphocyte counts, viral load, and AIDS status. The data for this study was collected in the pre-HAART era, (1997 – 1998) on unemployed people of colour, and 72% of the sample was treated. A cross-sectional study conducted by Lee et al. in 2012 used the PSQI, sleep diaries, actigraphy and self-reported sleep onset latency to assess sleep quality and sleep architecture on a mixed (70% treated and 30% untreated) HIV cohort of 290 persons living with HIV. The mean PSQI score of 7.7 (±3.7) indicated sleep disruption (PSQI>5). Shorter sleep duration (<6h) was associated with being African American, lower education and lower CD4+ T lymphocyte counts (Lee et al., 2012). The cohort was separated into four groups: one group of good sleepers, one group with difficulty falling asleep,

one group with difficulty maintaining sleep, and the final group who reported difficulty both falling asleep and staying asleep. Difficulty falling asleep and difficulty maintaining sleep was associated with being African American, being less likely to be employed and having a lower household income – when compared to good sleepers. These patients also had the lowest CD4+ T lymphocyte counts, and were less likely to be on ARVs .

In total, some studies have shown that poor sleep quality are associated with lower CD4+ T lymphocyte counts and another showed that there was no relationship. Thus there may be other disease related factors, or factors associated with the immune system or HIV infection, that may come in to play, which are affecting the sleep and sleep quality of treated HIV positive people.

HIV proteins: effects on circadian rhythm

Early in infection, there is a rapid immune response to HIV infection, which causes an increase in pro-inflammatory cytokines such as TNFα and IL1-β. As mentioned in my section on the interaction between sleep and the immune activation, TNFα is known to induce increases in slow wave sleep in animals (Terao et al., 1998), and the same in humans (Krueger et al., 1998, Shoham et al., 1987).

Early studies had found increased REM sleep latency in patients with HIV (White et al., 1995, Wiegand et al., 1991). REM sleep latency is the time taken to reach REM sleep after falling asleep. As described in my section on sleep regulation, REM sleep is strongly under circadian regulation. As mentioned before, factors that interfere with circadian control of sleep would affect REM sleep latency (Dijk et al., 1999). Therefore, increased REM latency in HIV as described in the Wiegand and White studies may suggest an effect of HIV on circadian rhythms.

Indeed, a few studies have shown that diurnal biological rhythms are modified during HIV infection, which I will explore in these paragraphs. Patients infected with HIV show clinically significant changes in diurnal rhythms in the early stages of infection, and throughout the progression of the disease (Bourin et al., 1993, Burudi and Fox, 2001). In uninfected individuals, natural killer (NK) cells, granulocytes, macrophages, CD8+ T-cells, and γδ T-cells increase during the biological day,

and T-cells, B-cells, αβ T-cells and CD4+ T lymphocytes increase during the biological night (Suzuki et al., 1997). As HIV infection progresses, it also causes a gradual eradication of the diurnal rhythms of B-lymphocytes ($CD3^-$, $CD16^+$) and T-lymphocytes ($CD2^+$, $CD3^+$), but HIV had no effect on the circadian rhythm of NK cells ($CD3^-$, $CD57^+$), which were similar to uninfected individuals (Bourin et al., 1993).

A mechanism to explain this effect of HIV on circadian rhythms has been found in murine studies: HIV secretes the transactivator of transcription (Tat), which resets the murine circadian rhythm. Tat is an important factor in HIV pathogenesis as it enhances the efficacy of viral protein transcription, and is secreted by infected cells (Costin, 2007) such as macrophages and astrocytes. The Tat protein of HIV affects the Glutamate receptors, N-methyl D-aspartate (NMDA) receptors, on the nuclei of the SCN and results in the resetting of these receptors, and the misfiring of signals for the circadian control of the body's rhythms (Clark et al., 2005).

Tat was proven to cause phase shifts in a murine model, both in vivo and in vitro. These shifts occurred at clinically significant levels of Tat, and affected the entrainment of the suprachiasmatic nucleus (SCN) during the biological night by the modulation of glutamate released by the SCN. Glutamate has been shown to cause phase delays in the SCN in the same way that light exposure does (Low et al., 2012, Patton et al., 2000) .This modification of circadian rhythms directly through Tat seen in mice may also explain the modification of circadian rhythms seen in humans (Wang et al., 2014).

A study published by Wang et al in 2014 showed that Tat interferes with the circadian rhythm by increasing the amount of circulating melatonin in the morning (melatonin was taken between 07:00 and 08:00 in the morning) – increased levels of which are normally found in the biological night. In addition to this effect on the timing of endogenous biological rhythms, Tat protein induces TNFα production (Chen et al., 1997), which is associated with sleepiness, as shown before , (Krueger et al., 1998, Opp and Toth, 2003). A study by Wang et al., (2014) investigated the relationship between Tat protein levels in HIV positive patients, melatonin levels taken between 07:00 and 08:00 in the morning, and subjective sleep quality evaluated by the PSQI. In this study, they

showed that with higher melatonin measured between seven and eight in the morning was associated with higher Tat protein measured in the cerebrospinal fluid. This finding may result from a phase delay in the endogenous circadian rhythm of melatonin or an effect on melatonin rhythm amplitude. This study also described a negative correlation between PSQI score and Tat levels whereby at higher levels of Tat, patients slept better. This study was conducted on treated HIV positive patients who had an average CD4+ T lymphocyte count of <200cells/μL blood, which corresponds to advanced immunosuppression. Subjects in the Wang et al. (2014) study were all considered to have "advanced AIDS" as per their own definition, but no information was given about how long they had been treated, or whether or not the participants were treatment resistant at the time of the study. Their finding, though novel, is in contrast to studies done on patients with advanced immunosuppression, which suggest that patients at this late stage of disease sleep poorly.

Cytokine profiles related to sleep disruption in HIV

The above studies have attributed sleep changes in HIV to factors within the immune system such as pro-inflammatory cytokines, Tat, and inflammation. In more recent years it has been found that, in HIV, cytokine polymorphisms have been associated with increased fatigue (Lee et al., 2014); and increased sleep onset latency (Gay et al., 2015). Single nucleotide polymorphisms (SNPs) of interleukins IL-1β, IL-4 and tumor necrosis factor alpha (TNFα), were associated with high fatigue in a mixed (treated and non-treated) sample of HIV positive people (Lee et al., 2014). Similarly, increased (>30 minutes) sleep onset latency was associated with increased IL-13 levels, as well as SNPs of IL1-β, IL-6, IL-13, NF-KB1 (nuclear factor kappa-light-chain-enhancer of activated B-cells), and TNFα (Gay et al., 2015). These findings suggest a role of the immune system in the alterations of sleep in HIV positive patients.

Antiretroviral treatment with efavirenz

The Wheatley (1994) study mentioned previously found no difference in the sleep in treated or untreated HIV infected patients (Wheatley and Smith, 1994). This was very early on in the

treatment strategy of HIV, and years later, efavirenz was introduced to the treatment regimen and is part of the antiretroviral agents used since 1999.

Efavirenz, a non-nucleoside reverse transcriptase inhibitor, is an effective means of controlling HIV viral loads and it has been shown to reduce viral multiplication with a daily dose (Feng et al., 2009). It has been reported that treatment with Efavirenz causes an increased sleep onset latency (time taken to fall asleep), increased sleep fragmentation, nightmares (Clark et al., 2005, Gallego et al., 2004, Salahuddin et al., 2009, Taibi, 2013, Wibbeler et al., 2012). Increased plasma efavirenz levels have been associated with increased sleep disturbances (Gallego et al., 2004, Núñez et al., 2001).

Two studies done in Spain assessed the effects of efavirenz using questionnaires, and using ambulatory polysomnography. The first study, assessing insomnia, conducted by Nunez in 2001, had 15 HIV positive persons complaining of insomnia and 36 matched controls. A clinical evaluation of insomnia was performed, and plasma efavirenz levels >3.5μL/mL was associated with increased self-reported insomnia (Núñez et al., 2001). The second study was conducted on 18 efavirenz-treated HIV positive patients – 13 with insomnia and 5 without insomnia, and 13-matched controls. This study used the Pittsburgh Sleep Quality Index (PSQI) and ambulatory polysomnography. They found that on efavirenz, there was an increased sleep onset latency, decreased sleep duration and decreased duration of slow wave sleep, when compared to non-efavirenz treated patients, and to controls (Gallego et al., 2004). In 2006, a single arm prospective study using polysomnography was performed on ten HIV positive men in the first three months of initiating antiretroviral therapy containing efavirenz. The changes in sleep architecture that they found was an increase in deep sleep (stage 4 sleep) and a moderate increase in REM sleep (Moyle et al., 2006). Most of these changes were seen in the first two weeks of treatment, and had partially resolved by week 12 of treatment.

Another study using PSQI was done in Nigeria, by Oshinaike et al. in 2014. This cross sectional study had 300 HIV positive patients, and separated good sleepers (PSQI ≤ 5) from poor sleepers (PSQI > 5). It was found that poor sleep was associated with CD4+ T lymphocytes <200 cells/μL,

duration of HIV disease (in univariate analysis, not in multivariate analysis) and being treated with efavirenz. Multivariate analysis revealed that efavirenz-treated patients had higher odds of being poor sleepers than non-efavirenz treated patients (Oshinaike et al., 2014).

Contradictory to the findings that mentioned efavirenz negatively impacted sleep, Crum-Cianflone in 2012 found no effect on sleep quality when being treated with efavirenz (Crum-Cianflone et al., 2012). Allavena et al. in 2016 showed that efavirenz actually had a protective effect from sleep disruption, whereby being on either efavirenz or nevirapine decreased the odds of reporting sleep disruption in multivariate analysis (Allavena et al., 2016).

The role of efavirenz in sleep disruption is unclear, and using a longitudinal design may allow one to assess how efavirenz effects sleep disruption in treated HIV positive patients.

Pain

A systematic review of pain in persons living with HIV was conducted by Parker et al. in 2014. This review assessed 61 studies, and found that pain has been is present in 54-83% of treated HIV positive cohort (Parker et al., 2014). This pain has been shown to impact regular function, has been reported as being undertreated, and has not diminished over the course of the 30 years that these studies spanned. An earlier review done by Hewitt et al. in 1997 assessed the types of pain experienced in persons living with HIV (PLWH) and found that polyneuropathy was experienced by 28% of PLWH. Headaches, joint pain, and muscle pain was experienced by 46%, 31% and 27% of PLWH respectively (Hewitt et al., 1997). The presence and intensity of pain have a negative effect on sleep quality, as people who are in pain are unable to sleep (Robbins et al., 2004, Sandoval et al., 2013, Vosvick et al., 2004). And conversely, disrupted sleep increases mechanical pain sensitivity both peripherally and centrally (Onen et al., 2001), induces generalized hyperalgesia (increased sensitivity to pain) (Schuh-Hofer et al., 2013) and increased sensitivity to temperature induced pain (Kundermann et al., 2004).

In treated HIV patients, pain is a common complaint (Namisango et al., 2012), usually due to sensory neuropathy. This neuropathy has been reported in 40-60% of ambulatory HIV positive

patients (Mphahlele et al., 2012, Wadley et al., 2011). In untreated HIV infection, gp120 causes HIV sensory neuropathy (Herzberg, 2001). It usually starts as a burning at the tips of extremities such as the toes and feet, and in extreme cases progresses to the fingers and hands as well. Distal sensory neuropathy is also associated with the use of the nucleoside reverse transcriptase inhibitor (NRTI), stavudine (d4T) (Wadley et al., 2011). Replacing stavudine with tenofovir has since decreased the incidence of neuropathy in treated HIV patients (Sandoval et al., 2013, Wadley et al., 2011).

Pain, sleep and HIV are not as well studied as other effects of chronic HIV and sleep. Upon investigating the body of literature mentioned in Table.1, only two papers had found pain in HIV affecting sleep. These were Crum-Cianflone in 2012, who cited that having a history of peripheral neuropathy symptoms were associated with increased odds of reporting PSQI > 5; and a study by Vosvick et al which found that increased pain was associated with increased sleep disturbance (Crum-Cianflone et al., 2012, Vosvick et al., 2004). We know the effects of pain and sleep, of HIV on sleep, but the effects of pain in HIV on sleep have not been as widely explored as I would like.

Depression, and socio-demographic factors

Poor sleep has a negative effect on overall quality of life. Coupled with the increase in daytime sleepiness, there is a decrease in daytime functioning (Irwin et al., 2013), increased level of depression and decreased quality of life (Payne et al., 2013, Poudel-Tandukar et al., 2014). High levels of depression are found in HIV patients, both untreated and treated (Keltner et al., 2012, Low et al., 2012, Poupard et al., 2007). Depressive patients are also known to have altered sleep patterns compared to non-depressive patients (Kupfer et al., 1984, Oshinaike et al., 2014) and this negatively impacts sleep quality.

As mentioned above, Crum-Cianflone investigated the correlates of sleep disruption in a treated HIV cohort, and found that reporting PSQI>5 was associated with increased anthropometric measurements, pain, and depression. Depression is well documented throughout HIV-cohorts (Crum-Cianflone et al., 2012, Salahuddin et al., 2009, Wibbeler et al., 2012, Barroso et al., 2015).

In Germany, in a cohort of 180 HIV-positive adults and 120 age- and sex-matched controls Wibbeler et al. described, increased sleep disruption (as per PSQI), increased daytime sleepiness (as per ESS), and increased depression (as per BDI) scores in patients with HIV compared to controls. The higher depression that this study found was associated with increased daytime sleepiness, and with increased sleep disruption (Wibbeler et al., 2012). In the same year, a cross sectional study in the United States was performed with 193 HIV positive and 50 HIV negative war veterans, using the PSQI and BDI-II. This study found that increased sleep disruption was associated with having depression (reporting BDI>10). Decreased odds of reporting sleep disruption was found in participants with higher military rank, and higher education (Crum-Cianflone et al., 2012).

In 2015, an observational study done on 128 HIV positive patients – 82% of which were on treatment, used the HIV-related fatigue scale and the BDI-II. This study found that fatigue was associated with anxiety and depression, and that the fatigue did not dissipate over the three-year duration of the study (Barroso et al., 2015).

Sleep and fatigue

Another study investigating fatigue in HIV was done in 2009 by Salahuddin et al. used the same HIV-related fatigue scale, the PSQI and ESS. They found an overall high amount of sleep disruption present in the cohort (PSQI = 9.4 (±4.4). This sleep disruption was correlated with fatigue intensity, daytime sleepiness and fatigue related impairment in function (Salahuddin et al., 2009).

To summarize, these studies have found that higher PSQI scores are associated with increased fatigue intensity (Salahuddin et al., 2009), depression (Wibbeler et al., 2012, Barroso et al., 2015, Crum et al., 2006), anxiety (Barroso et al., 2015), daytime sleepiness (Salahuddin et al., 2009, Wibbeler et al., 2012) and fatigue-related impairment in function (Salahuddin et al., 2009). Many of these studies gave snapshots and painted a picture of sleep disruption in HIV, next I will be discussing the long-term effects of both sleep disruption in HIV.

Sleep disordered breathing in HIV

With the advent of life-long ARVs, there is a decrease in sleep disruption caused by disease progression to AIDS, but disrupted sleep is still present. Disrupted sleep has been associated with an increased daytime sleepiness, decreased daytime alertness, decreased daytime functioning, and an overall level of tiredness throughout the day (Irwin et al., 2013, Payne et al., 2013). During phases of tiredness, humans increase their caloric intake, causing weight gain and the increased chance of developing diabetes. Being overweight itself leads to sleep problems such as sleep apnoea, and coupled with diabetes, is also a major contributor to developing metabolic syndrome (Beccuti and Pannain, 2011).

Weight gain or an increase in BMI even in non-HIV populations is a risk factor for the development of sleep apnoea (Beccuti and Pannain, 2011). Sleep apnoea is found mostly in obese persons, or people with an increased neck circumference. An apnoea is defined as the cessation of breathing during sleep, and an individual that experiences an apnoea wakes up during a sleep episode gasping for air (Beccuti and Pannain, 2011). Factors that lead to obesity or the development of metabolic syndrome increase the risk of sleep apnoea. For this reason, when screening for obstructive sleep apnoea (OSA), body mass index (BMI) is an important factor to assess.

In treated HIV cohorts, the presence of metabolic syndrome among treated HIV positive people has been increasing over the past few years (Blanco et al., 2010, Knutson et al., 2007, Pullinger et al., 2010) and in a non-HIV infected population, the risk of developing metabolic syndrome is increased by chronic sleep disturbances.

In a recent observational cohort study on veterans, Kunisaki et al. investigated the prevalence and correlates of obstructive sleep apnoea in patients with or without HIV. In patients with obstructive sleep apnoea (OSA), the patients with HIV were younger, had lower BMI, and were less likely to be hypertensive than non-HIV infected OSA counterparts. However, HIV-positive patients with obstructive sleep apnoea had higher BMI, were more likely to be smokers, and have medical comorbidities, such as diabetes and hypertension compared to HIV patients who did not have

OSA – these factors are known correlates of obstructive sleep apnoea. When adjusting for the aforementioned factors, being HIV positive was associated with decreased odds of having OSA (Kunisaki et al., 2015). The PSQI and ESS were used in this study.

The study conducted by Kunisaki et al. in 2015 is contradictory to a study done the previous year, by Patil, et al., who found that in men who have sex with men (MSM), sleep disordered breathing was associated with being HIV positive, even with lower BMI (Patil et al., 2014). This sleep disordered breathing was associated with daytime sleepiness, and not fatigue. Crum-Cianflone et al. found that patients who had increased BMI, were more likely to report PSQI>5 (Crum-Cianflone et al., 2012). This study did not use a specific instrument to measure sleep apnoea however this association between sleep disturbances and BMI may have been caused by sleep apnoea in the HIV+ population.

With the modulating effects on pain, metabolic profile, depression and quality of life, sleep quality is an important factor in the assessment of HIV positive patients.

Table 1.1. Summary of studies on sleep and HIV and factors associated with sleep disturbances in HIV

Author(s)	Year	Location of study	Study design	Demographic	Mean (±SD) /Median (IQR) CD4 count of HIV+ patients	Methods used	Summary	Factors associated with sleep changes
Changes in sleep associated with being HIV positive, in untreated and treated HIV								
Untreated HIV								
Norman, S.E., Chediak, A.D., Kiel, M., et al.	1990	USA	Case-control	10 HIV seropositive males and 5 HIV seronegative matched controls	Not defined	Polysomnography	Increased percentage of slow wave sleep and increased slow wave sleep in the later part of the night in HIV positive men when compared to controls	Being HIV positive associated with changes in sleep architecture.
Norman, S.E., Chediak, A.D., Freeman, C., et al.	1992	USA	Case-Control	14 HIV seropositive and 10 HIV seronegative matched controls	Not defined	PSQI, BDI, and polysomnography	Slow wave sleep shifted to the later part of the night when compared to controls>, PSQI in HIV positive men: 3.5 (±1.9); control: 2.4 (±1.2) (p=0.06).	Being HIV positive associated with changes in sleep architecture and trend increased subjective assessment of sleep disturbances
Ferini-Strambi, L., Oldani, A., Tirloni, G., et al.	1995	Italy	Case-Control	9 HIV seropositive asymptomatic men (untreated, early infection) and 9 matched controls	Mean CD4: 750 (± 130) cells/ µL	Polysomnography	Reduced percentage of slow wave sleep in the first third of the night and no difference in the second half of the night when compared to controls. Suggested that interferon alpha may be the reason.	Factors not investigated.
Wiegand, M., Möller, A. Arnulf, A., et al.	1991	Germany	Case-control	14 HIV positive patients and 14 HIV negative controls	Not given	Polysomnography	Patients with HIV had an increased sleep onset latency, reduced total sleep time, increased night arousals, increased REM latency and more time in stage 2 sleep than healthy controls	Factors not investigated.
Darko, D.F. McCutchan, J.A. Kripke, D.F. et al.	1992	USA	Cross sectional	112 men who have sex with men (62+ & 50-ve)	(Mean ±SD) Fatigue group 330±222 cells/µL Non-fatigue group 681±377 cells/µL	Fatigue measured by: Profile of Mood States “POMS”	Patients with lower CD4+ T lymphocyte counts had higher levels of fatigue and patients with more severe CDC/ WHO status reported higher sleep duration than healthy controls	CD4+ T lymphocyte counts negatively correlated with sleep duration (r=-0.34, p<0.0009)

Author(s)	Year	Location of study	Study design	Demographic	Mean (±SD) /Median (IQR) CD4 count of HIV+ patients	Methods used	Summary	Factors associated with sleep changes
Treated HIV								
Wheatley, D. Smith, K.	1994	United Kingdom	Case-Control	45 HIV positive (42 males, 3 females, 20 on ARvs) and 45 HIV negative	Not done	5 item questionnaire assessing sleep onset, nocturnal awakenings, early morning awakenings, duration of sleep and well-being on final awakening	HIV patients had significantly higher levels of sleep disturbance than their matched controls (higher sleep onset latency, earlier final awakening).	Being HIV positive had an effect on sleep disruption, irrespective of whether or not subjects were treated. Trends with duration of disease (p=0.055) and not being treated for HIV (higher sleep disturbances, p=0.052)
Low, Y. Goforth, H.W. Omonuwa, T. et al.	2012	USA	Case-control observational study	18 HIV+ insomniacs and 18 age-, sex-matched HIV- insomniacs, as determined by the DSM-IV-TR clinical criteria for the diagnosis of insomnia.	Not done	PSG	High prevalence of insomnia in HIV positive people, associated with poorer disease outcomes, cognitive impairment and HIV dementia. Adjusted, HIV positive patients with insomnia have significantly worse sleep than patients. . HIV positive insomniacs had 8% decreased sleep efficiency, 8-10% decreased time spent in REM sleep and longer sleep onset latency than non-HIV positive insomniacs.	Insomnia associated with poorer disease outcomes. No other factors investigated.
Factors Predicting Sleep disruption in treated HIV – CD4+ T lymphocyte counts								
White, J.L. Darko, D.F.	1995	USA	Cross sectional	23 HIV positive (15 treated) and 13 HIV negative men	Mean CD4 count in HIV+ patients = 387 cells/µL CD4>400 cells/µL (N=11) CD4<400 cells/µL (N=12)	Day and nighttime EEG, EMG and ECG	HIV positive patients had an increased REM latency and were less accurate in their cognitive performance testing compared to controls. HIV positive patients with CD4>400 cells/µL had an increase in SWS % in the later part of the night, and decreased awakenings compared to HIV negative controls. HIV positive patients (p-0.004). Cognitive performance not correlated to sleep measurements. .	There was an increase in slow wave sleep in the later part of the night, compared to controls in patients with CD4+ T lymphocyte counts>400 cells/µL, and decreased number of awakenings associated with HIV patients who had CD4+ T lymphocyte counts >400cells/ µL compared to controls (no difference between HIV patients <400 CD4/ul compare dot controls)

Author(s)	Year	Location of study	Study design	Demographic	Mean (±SD) /Median (IQR) CD4 count of HIV+ patients	Methods used	Summary	Factors associated with sleep changes
Seay, J. S. McIntosh, R. Fekete, E.M. et al	2013	USA	Cross sectional	139 low income women living with HIV, ethnic minority i.e. African American	Mean CD4 = 484.5 (±303.0) cells/ µL	PSQI, urine dopamine measured	Mean global PSQI: 6.9 ± 4.2. Sleep disturbance was associated with decreased CD4 count and increased urine dopamine concentration. CD4+ T lymphocyte count negatively associated with overall sleep quality ($p<0.05$) and PSQI score ($p<0.05$).	Decreased CD4+ T lymphocyte count ($p=0.03$) and decreased urine dopamine concentration ($p=0.5$) were associated with increased sleep disruption. Conclude that sleep disturbance is independently correlated with immune status and dopamine levels in women living with HIV.
Lee, K.A. Gay, C. Portillo, R.N. Aouizerat, B.E.	2012	USA	Cross-sectional	290 adults, 22-77 years old 70% on treatment, 12.1 years treated.	Mean CD4: 450(±267) cells/µL	PSQI, sleep diaries and self-reported sleep onset latency. Actigraph watches were used to determine sleep patterns. MSAS (memorial symptom assessment scale) used to determine symptom experience.	Global PSQI score: 7.4(±3.7). Most participants slept less than six hours a night, with no impact on daytime function, which was associated with more severe experience. Sleep onset latency > 30 minutes were found in 34% of the population, 56% had WASO >15%. Four groups: good sleepers, troubles falling asleep, troubles staying asleep and troubles both falling asleep and staying asleep. Troubles falling asleep and troubles staying asleep (two groups) were associated with being African American, less likely to be employed and having lower household income than good sleepers. Patients in these two groups also had higher symptom severity (anxiety and depression) than good sleepers (one group).	Participants with sleep fragmentation reported low sleep disturbance, and sleep fragmentation was associated with socio-demographic factors and slightly lower CD4+ T lymphocyte counts. Patients who reported trouble falling and staying asleep had the lowest CD4+ T lymphocyte counts, and were less likely to be on ARVs than good sleepers.

Author(s)	Year	Location of study	Study design	Demographic	Mean (±SD) /Median (IQR) CD4 count of HIV+ patients	Methods used	Summary	Factors associated with sleep changes
Oshinaike, O., Akinbami, A., Ojelabi, O., et al.	2014	Lagos, Nigeria	Cross sectional	300 HIV positive patients	Mean CD4 count: 334.2 (± 215.4) cells/ µL	PSQI	PSQI used to separate poor sleepers (PSQI>5) and good sleepers (PSQI≤5). Poor sleepers had mean PSQI = 9.21 (±3.3) and good sleepers had PSQI = 1.30 (±1.4).	Poor sleep was associated with CD4+ T lymphocyte counts <200 cells/ µL (adjusted p value <0.001), duration of HIV disease (in univariate analysis, not significant in multivariate analysis), and ARV treatment (efavirenz) treated patients had higher odds of being poor sleepers than those who did not receive EFV, even in multivariate analysis, adjusted p value <0.001).
Factors Predicting Sleep disruption in treated HIV – Other immune factors								
Wang, T., Jiang, Z., et al.	2014	China	Cross sectional	98 Treated HIV positive	Mean CD4 = 89.16 ± 114.24 cells/µL	PSQI, MESOR	HIV Tat protein was inversely associated with PSQI, but positively associated with melatonin concentration. Mean global PSQI score: 11.97 (± 4.87)	Sleep disturbance inversely related to Tat protein concentration.
Lee, K.A., Gay, C.L., Lerdal, A., et al.	2014	USA	Cross sectional	224 adults living with HIV. 151 men, 55 women and 18 transgender. 69% not on treatment, 31% on treatment.	CD4 < 200 = 18% CD4 ≥ 200 = 82%	Four item version of the Lee fatigue scale	High fatigue patters associated with single nucleotide polymorphisms of IL1-B (rs1071676 and rs1143627), TNF-a (rs1800683 and rs1041981), and IL-4 (rs2243274).	High fatigue was associated with inflammation, and SNPs of IL-1B, TNF alpha and IL-4.
Gay, C.L., Zak, R.S., Lerdal, A., et al.	2015	USA	Cross sectional	307 adults living with HIV. 212 men, 72 women and 23 transgender. 28% not on treatment, 72% on treatment.	CD4 < 200 = 17% CD4 ≥ 200 = 83%	Sleep onset latency (time taken to fall asleep) item in the PSQI	Increased sleep onset latency (>30 minutes) associated with increased IL-13 levels, as well as single nucleotide polymorphisms IL1B rs1143642 and rs1143623, IL6 rs4719714, IL13 rs1295686, NFKB1 rs4648110, and TNFA rs2857602.	Association between sleep disturbance, and inflammation, and SNPs of IL-1B, IL-6, IL-13, NF-KB1 and TNF alpha.

Author(s)	Year	Location of study	Study design	Demographic	Mean (±SD) /Median (IQR) CD4 count of HIV+ patients	Methods used	Summary	Factors associated with sleep changes
Factors Predicting Sleep disruption in treated HIV – treatment with efavirenz								
Nuñez, M. de Requena, D.G. Gallego, L. Et al.	2001	Spain	Case-Control	15HIV positive persons complaining of insomnia and 36 matched controls	None taken	Clinical evaluation with insomnia grading from 1 to 4 (4=severe insomnia)	Increased plasma EFV levels (>3.5μL/ml) was associated with increased self-reported insomnia	EFV levels >3.5 associated with worse self reported sleep disturbances
Gallego, L. Barreiro, P. del Rio, R. et al.	2004	Spain	Case-control	18 EFV treated, HIV positive people – 13 with insomnia and 5 without, and 13 healthy controls.	Mean CD4: 420.3 (± 172.4) cells/ μL	PSQI Ambulatory EEG (PSG?)	On efavirenz (EFV) there was an increase in SOL, decreased sleep duration and decreased duration of slow wave sleep	Increased EFV concentrations associated with increased sleep disturbances
Allavena, C., Guimard, T., Billaud, E., et al.	2016	France	Cross sectional	1354 HIV positive adults; treated for a median of 10-years	Median CD4 (IQR): 604 (434–784) cells/ μL	PSQI, BDI-II	Multivariate analysis revealed that male participants had better sleep quality than females. Being unemployed, smoking, having depression (BDI-II>19) were associated with increased sleep disruption. Comorbidities, CD4+ T lymphocyte counts, viral loads, age, had no effect on sleep disruption. Being treated with efavirenz or nevirapine decreased the odds of reporting sleep disruption.	Factors associated with increased sleep disruption were being female, a smoker, being unemployed, or depressed, as well as being treated for HIV longer than ten years. Being treated with efavirenz or nevirapine associated with decreased sleep disruption, thus, a protective effect of efavirenz treatment.

Author(s)	Year	Location of study	Study design	Demographic	Mean (±SD) /Median (IQR) CD4 count of HIV+ patients	Methods used	Summary	Factors associated with sleep changes
Factors Predicting Sleep disruption in treated HIV – pain								
Vosvick, M. Gore-Felton, C. Ashton, E. et al.	2004	USA	Cross-sectional	N = 146 HIV positive people. 57% male, 47% Caucasian, 26% African American, 9% Latino and 12% other ethnicity. 77% of males identified as homosexual.	Mean CD4 = 384 (±443) cells/ µL	Sleep related items from the Medical Outcome Study (MOS). Assessed difficulty getting to sleep and difficulty falling asleep.	Increased understanding from friends associated with decreased sleep disturbance. Men were significantly older, reported higher education, higher household income and had more pain than women in this cohort. Women were more likely to report understanding from friends than men were. No demographic factors were associated with sleep disturbance, only household income in univariate analysis. In multivariate analysis, pain, and understanding and assistance from friends were significantly associated with increased or decreased sleep disturbance.	Pain and increased assistance from friends associated with increased sleep disturbance, whereas Increased understanding from friends associated with decreased sleep disturbance.
Crum-Cianflone, N. F., Roedinger, M.P., Moore, D. J., et al.	2012	USA	Cross sectional	HIV+ n = 193, mean age = 35.9 (± 8.6) years HIV- n = 50; mean age = 35.1 (± 9.2) years	Current mean CD4+ T lymphocyte count: 586.8 (± 230.1)	PSQI, BDI-II	In univariate analysis, factors associated with increased odds of reporting sleep disruption (PSQI>5), were BMI>30kg/m^2, metabolic syndrome, increased waist size, lipidystrophy, being a smoker, having a prior head injury, depression (BDI>10) and having a history of peripheral neuropathy. Factors associated with decreased odds of reporting PSQI >5 were increased education and higher military rank. In multivariate analysis, Odds of PSQI>5 associated with depression (BDI >20), waist size, lower education, and current illicit drug use.	No association (p=0.46) between long term EFV usage and insomnia (PSQI >5). Increased anthropometric measurements, depression and pain were associated with increased risk of reporting PSQI >5.

Author(s)	Year	Location of study	Study design	Demographic	Mean (±SD) /Median (IQR) CD4 count of HIV+ patients	Methods used	Summary	Factors associated with sleep changes
Factors Predicting Sleep disruption in treated HIV – depression								
Wibbeler, T. Reichelt, D. Husstedt, I-W. et al.	2012	Germany	Cross-sectional	180 HIV positive adults with 120 age- and sex-matched controls. Caucasian	Mean CD4 = 404 (±288) cells/μL	PSQI, ESS and BDI	Increased PSQI, ESS and BDI scores in patients with HIV as compared to non-infected controls. PSQI = 8.0 (±4.4); ESS = 9.4(±4.9); BDI = 13.9 (±10.7) (in HIV positive group).	Higher depression (BDI) score associated with increased sleep disruption and daytime sleepiness.
Barroso, J. Leserman, J. Harmon, J.L. et al.	2015	USA	Observational cohort study	128	517 cells/μL	HIV-related fatigue scale; BDI-II	Fatigue as a result of stressful life events was associated with anxiety and depression, which did not dissipate over the three-year course of the study.	Fatigue associated with anxiety and depression.
Factors Predicting Sleep disruption in treated HIV – Others								
Lee, K.A. Portillo, C.J. Miramontes, H.	2001	USA	Cross sectional	100 women	CD4+ range: 2-974 cells/μL 35% < 200 cells/μL	Subscale of PSQI General sleep disturbance scale Actigraphy	PSQI = 9.0 ±4.4 (high level of sleep disruption). Fatigue and depression were unrelated to CD4+ T lymphocyte counts counts. The high fatigue group had increased difficulty falling asleep, increased night-time awakenings and decreased daytime function.	Unrelated to CD4+ T lymphocyte counts. Patients in the high fatigue group had increased difficulty falling asleep and increased night arousals.
Nokes, K.M. Kendrew, J.	2001	USA	Cross sectional	N = 58 Mean age = 46 HIV + Unemployed People of colour; 61% black, 19% white, 19% Hispanic 72% of subjects were treated, study from 1997-1998; pre-ART era.	CD4= 276(±191) cells/μL	PSQI Mean PSQI score: 10 (±5)	Factors associated with PSQI score: Symptom severity, depressive symptoms, daytime sleepiness, functional status. employment status, general well being and trait anxiety, as well as sleeping alone, separate bed for sleeping and noisy bedroom. Cigarette use, caffeine use and marijuana use also associated with PSQI score. No relationship between PSQI and disease factors such as CD4+ T lymphocyte counts, AIDS status and viral load	Better sleep quality was found when a person was not working, had a positive general well-being, a less anxious personality, less daytime sleepiness, less depressive symptoms and less symptom severity. Sleeping in a noisy room, having impaired functional status and living longer with HIV was associated with worse sleep quality.

Author(s)	Year	Location of study	Study design	Demographic	Mean (±SD) /Median (IQR) CD4 count of HIV+ patients	Methods used	Summary	Factors associated with sleep changes
Salahudin, N. Barroso, J. Leserman, J. et al.	2009	USA	Longitudinal	128 HIV positive people, 65.6% African American, 30.9% white, 3.9% Hispanic. Median age = 44 years.	Mean CD4=517 cells/μL	PSQI, ESS, HIV-related fatigue scale	PSQI = 9.4 (±4.4). In multivariate analysis, one model of fatigue intensity found that stress and nighttime sleep quality (PSQI) were predictors of fatigue intensity. In another model, stress, daytime sleepiness (ESS) and nighttime sleep quality were predictors of fatigue-related impairment of functioning. All models adjust for monthly income, years since diagnosis, CD4+ T lymphocyte count, viral load, and ART status.	PSQI correlated with fatigue intensity ($r = 0.46$, $p < 0.05$), daytime sleepiness ($r=0.2$, $p<0.05$) and fatigue related impairment in function ($p < 0.05$).
Sleep apnoea in HIV								
Kunisaki, K.M. Akgün, K.M. Fiellin, D.A. et al.	2015	USA Veterans cohort	Obsservational cohort study	HIV+ = 3683; mean age = 48.3 HIV - = 3641; mean age = 50.5	Median: 366 (210, 553) cells/μL HIV	PSQI, ESS	In patients with OSA, those with HIV were younger, had low BMI and less likely to be hypertensive. Having HIV was associated with increased odds of OSA diagnosis. HIV positive patients with OSA had higher BMI compared to HIV patients without OSA, were more likely to be smokers, have medical morbidities (diabetes, hypertension, etc.)	Patients with HIV were more likely to report symptoms associated with OSA (obstructive sleep apnoea). In adjusted and adjusted models, there were decreased odds of having OSA associated with positive HIV diagnosis.
Patil, S.P., Brown, T.T., Jacobson, L.P., et al.	2014	USA Baltimore; Pittsburgh	Cross-sectional	Men who had sex with men: HIV positive, on HAART: (N=58), HIV positive, not on HAART: N=41; HIV negative: N=60	Median (IQR) 531 (449 – 686) cells/uL	PSG	Sleep disordered breathing prevalence was 86.7% in HIV seronegative participants, 70.7% in treated HIV positive participant and 73.2% in untreated HIV positive participants, despite a lower BMI.	Sleep disordered breathing associated with being HIV positive, even with lower BMI scores. Sleep disordered breathing associated with daytime sleepiness, not fatigue.

MSM: men who have sex with men; OSA: obstructive sleep apnoea; EFV: efavirenz; BDI: Beck's depression inventory; PSQI: Pittsburgh sleep quality index; ESS: Epworth sleepiness scale

1.4. Gaps in the literature

I have now covered the evolution of HIV infection, current antiretroviral treatment in South Africa, how HIV infection and its treatment can affect sleep, and possible underlying mechanisms affecting the sleep of people infected with HIV.

We know that HIV associated sleep disorders are a common complaint in treated and untreated HIV positive cohorts. Each study mentioned above has in some way, assessed either sleep quality alone, or factors associated with sleep quality.

South Africa keeps up with the world standard of administering HAART to infected patients, but only once CD4+ T-lymphocyte levels drop to the prescribed amount of <500cells/μL blood. This only came into effect recently (2015) (Shisana, 2014). Prior to this, the cut off for HAART administration was ≤200cells/μL – when an individual already has AIDS – before 2013, and ≤350cells/μL between 2013 and 2015. Due to the apprehension that people have to get tested in South Africa, HAART initiation usually occurs at much lower CD4+ T-lymphocyte counts.

Many of the studies described study participants with nadir CD4+ T-lymphocyte counts above 400 cells/μL, which may differ greatly from the studies done on people living with HIV in South Africa (who typically start ARV treatment below 200 cells/μL) (Grimsrud et al., 2015). Lower CD4+ T-lymphocyte counts at HAART initiation has been associated with poorer disease outcomes (Maskew et al., 2007), increased chance of developing immune reconstitution inflammatory syndrome and increased mortality (Crum et al., 2006, Jain et al., 2003). Not much is reported on the starting CD4+ T-lymphocyte counts of treated HIV patients onto HAART, nor how that affects their sleep. As far as I am aware, there hasn't been a longitudinal study on sleep in people living with HIV.

In South Africa, a majority of people living with HIV is from African ancestry (over 6 million infected). The prevalence of HIV in South Africa is also significantly higher in females as compared to males in the 30-35 year age group, at 36.0% versus 32.7% respectively. This is in contrast to the different populations that the previously mentioned studies have been conducted in

Caucasian males, men who have sex with men, and in China (Lee et al., 2012, Lee et al., 2001, Poudel-Tandukar et al., 2014, Wang et al., 2014). Therefore, findings on HIV and sleep from other studies may not be generalizable to the South African population.

Mood changes have been shown to occur in HIV populations, including high levels of depression (Junqueira et al., 2008, Keltner et al., 2012, Poudel-Tandukar et al., 2014) and decreased quality of life (Foster et al., 2012, Gallego et al., 2004). Depression, as a common complaint of living with HIV, negatively impacts sleep – the relationship has been proven but is to this day, poorly understood. It is unclear as to whether or not depression in a treated cohort gets better or worse over time. The impact that sleep has on the metabolic and cardiovascular system have been discussed, as well as how antiretrovirals also increase cardiometabolic risk. In HIV positive people with chronic sleep disturbances, there may be an increased chance of developing metabolic syndrome, and cardiovascular disease. Though it is well known that people living with HIV have a poor sleep quality, the evolution of this sleep quality is unknown, in particular in South Africa. In this country, the demographic is different to most of the other studies conducted in that we have a largely female cohort, almost exclusively of African ancestry, that time to initiate ARVs are longer because of the guidelines having lower CD4+ T-lymphocyte counts to start ARVs.

1.5. Pilot study run prior to masters' degree

In 2012, I conducted a pilot study investigating the possibility of sleep disruption in a treated HIV positive cohort. 153 HIV positive patients from the adult HIV clinic at the Chris Hani Baragwanath Hospital and asked to fill out questionnaires. The questionnaires used were the Pittsburgh Sleep Quality Index to assess sleep quality, the Epworth Sleepiness Scale to assess daytime sleepiness, a pain rating scale, and Beck's Depression Inventory to assess mood. I also collected disease information, such as CD4+ T-lymphocyte counts, viral loads, time on treatment and type of treatment. I found an overall high level of sleep disruption (mean PSQI (± SD)= 7.2 (± 5.0)), moderate to high depression scores: mean BDI (± SD)= 17.5 (± 12.6), and at the time of the interview, 35% of patients had CD4+ T-lymphocyte counts < 250 cells/μL, indicative of AIDS. In

univariate analysis, I found that sleep disruption, as defined by PSQI>5, was associated with higher depression (i.e. higher BDI scores), and increased daytime sleepiness (i.e. higher ESS scores). In multivariate analyses, I found an association between sleep disruption (high global PSQI score) and high CD4+ T lymphocyte counts. There was also an interaction between sleep, pain and CD4+ T lymphocyte counts whereby in patients without pain, the main predictor of their sleep disruption was the high CD4+ T lymphocyte counts (Redman, 2013). Increased CD4+ T lymphocyte counts being associated with increased sleep disruption is contradictory to Seay's finding that decreased CD4+ T lymphocyte counts were associated with increased sleep disruption. Seay et al. (2013) may have found a difference because at the time of the study, the enrolled participants already had lower CD+ T lymphocyte counts. Lower CD+ T lymphocyte counts are associated with increased disease burden and thus a higher chance of HIV-related fatigue and sleep disruption. The reason why they found a difference to my pilot study, in my opinion, is because the change from a state of low-CD4 to high-CD+ T lymphocyte counts was more drastic, and the sleep disruption could have been a side effect of the reconstituting immune system This contradiction could also be attributed to socio-demographic factors such as ethnicity, gender, (as my population were predominantly females of African descent); CD4+ T lymphocyte counts at time of initiation onto antiretroviral therapy, and timing of treatment.

Studies reviewed above, including the pilot study, were performed in a cross-sectional manner, which showed associations of HIV-related sleep disruption with pain, depression, cytokines, HIV itself but could not determine causality. In an attempt to better tease apart which factors lead to sleep disruption and which factors resulted from sleep disruption, as well as to better understand the relationship between sleep disruption in HIV and CD4+ T lymphocyte reconstitution, I designed my masters' research project to follow HIV patients longitudinally, from the start of antiretroviral treatment up to at least six months on treatment.

1.6. Aims

The aims of this longitudinal study were to:

1) assess through validated questionnaires measuring different aspects of sleep the evolution of subjective sleep quality (PSQI), daytime sleepiness (ESS) and risk of sleep apnoea (Berlin Questionnaire) in a treated HIV-population, from the visit that precedes ARV initiation up until at least six months on treatment,
2) investigate the evolution across time of known predictors of sleep disturbances, pain and depression.
3) investigate how immune reconstitution (i.e. increased CD4+ T lymphocyte counts) and / or decreasing viral loads impacted these sleep measurements, when adjusting for known predictors of sleep disturbances such as age, sex, depression and pain.
4) investigate how changes in sleep (as measured through the PSQI, ESS and Berlin Questionnaire) may participate in the development of non-communicable diseases, using a simple monitoring of BMI and blood pressure.

CHAPTER 2 - METHODS

2.1. Ethical Approval

Ethics for this study were applied for and approved by the Human Research Ethics Committee (Medical); by the University of the Witwatersrand. Ethics number: M120411 with an amendment to include longitudinal measurements in 2013 (see appendix 2)

2.2. Recruitment

I aimed to recruit 100 participants into this study, expecting a dropout of 50% by the end of the study.

2.2.1. Recruitment sites

I recruited patients from 2 adult HIV clinics. The first one was Chris Hani Baragwanath Academic Hospital (CHBAH), adult HIV clinic. The second clinic was at the Helen Joseph Hospital, Right To Care Centre. Recruitment at CHBAH ran from 13 Oct 2013 and data was collected up until April 2016; and recruitment at Right to Care site started in May 2014, and data was collected up until January 2016.

2.2.2. Enrolment criteria and mechanisms of patient retention:

HIV positive subjects over the age of 18, visiting an HIV clinic (any one of the ones above) were invited to participate in the study – provided that the patients were treatment naïve, or patients on treatment for up to one month, but could accept up to three months on treatment.

Towards the end of the study, I included patients who had a change in regimen of ARVs. At Helen Joseph, interested subjects would come after their adherence class – given by the counsellors – to a separate room where I would inform them about the study. Similarly, at the adult HIV clinic at Chris Hani Baragwanath, interested participants were taken to a different room within the clinic. I obtained consent from interested individuals prior to enrolment. The study was explained to them

and in cases where an interpreter was required; one of the counsellors on site would assist. I invited any HIV positive person who fitted the inclusion criteria to participate.

Included in the longitudinal analyses are subjects who had completed three or more time points in the study, regardless of whether or not the time points attended were consecutive. .
Over the course of the study, efforts were made to retain the recruited patients. Visit reimbursement from R50 for any extra visits was increased to R150. Phone-calls were made from the laboratory phone – the number for which does not appear on a cellphone (a disadvantage of this is that the patients may not know who was calling them and could decide not to take those calls). A cell-phone was also purchased and used to contact participants and WhatsApp messages were also sent. When patients would come to the clinic at CHBAH, it was recorded on the hospital system (as it was at Helen Joseph). At Helen Joseph returning patients did not necessarily have to see the counsellors every time they came in, and would go straight to the pharmacy. I was stationed at the counsellors work-stations upstairs, and if I had not gotten hold of them via their mobile phones I wouldn't know whether or not they were there. At CHBAH all the patients for the day are written down in a log book every morning, so I could see who was there on that day, making it easier for me to maintain follow up visits.

2.2.3. Study timeline

Initially, the timeline was meant to be from 0 – 6 months. With the low recruitment and high loss to follow up in the first 12 months of the study, more subject numbers were acquired but a few patients who had been enrolled previously were still being seen for follow up visits. Several patients also missed 3-month or 6-month follow-ups and were only seen again at 12 months or 18 months, therefore instead of limiting my analysis to the baseline, 3-month and 6-month visits, I included all patients who had a minimum of 3 visits in total, some of them had follow up data up to 18 months after starting antiretroviral treatment. Patients were retained in the study until the end of the study, where possible. As seen in figure 2.1 below, the study timeline and what was done at each visit.

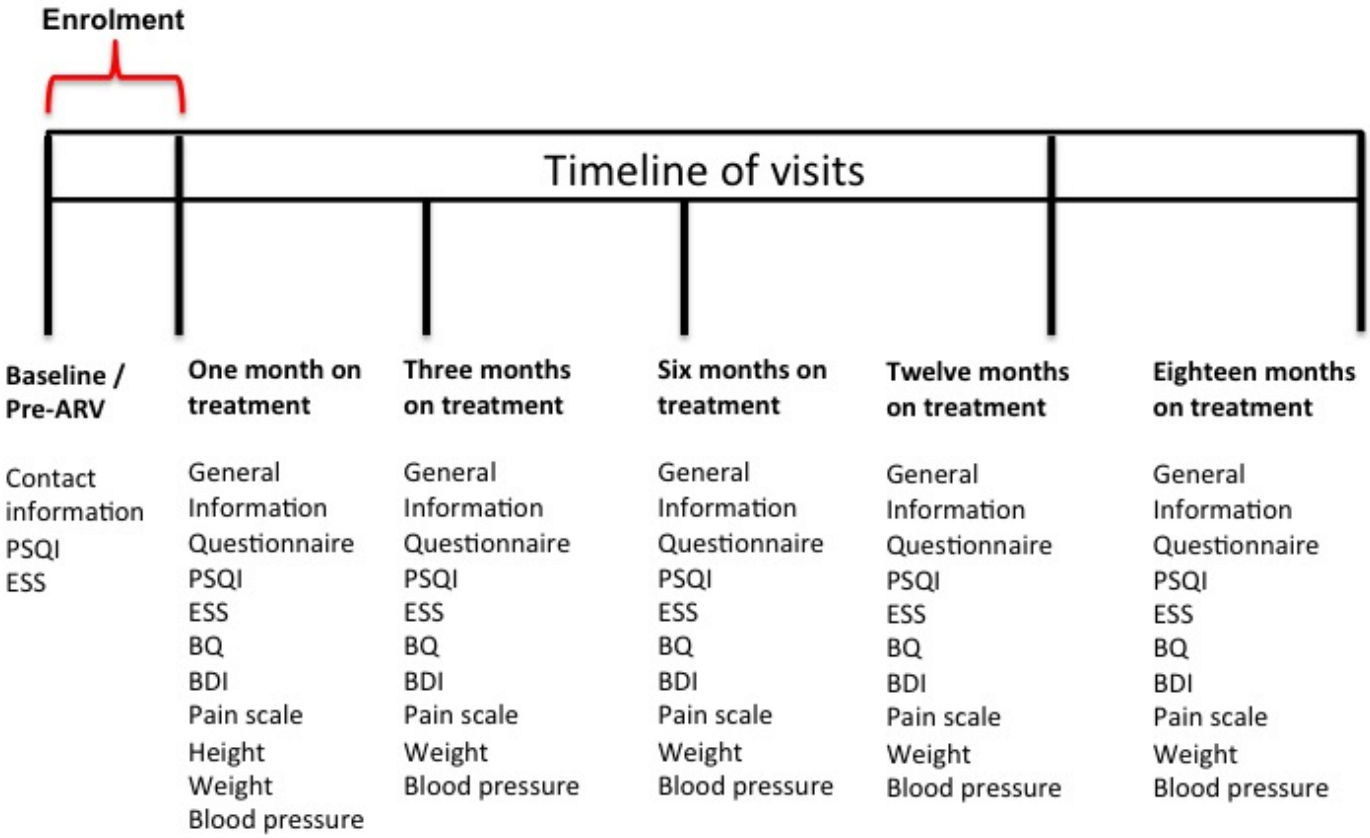


Figure 2.1. Study timeline. Subjects were enrolled at either pre-ARV or one month on treatment. A short questionnaire containing only the PSQI and ESS was given at the pre-ARV visit to lower the patient burden. The full general information questionnaire, pain scale, PSQI, ESS, BQ and BDI were given at each time point on treatment and anthropometric measurements were taken as well. For any visit after one year, the same questionnaires were administered and the same anthropometric measurements were taken.

At their first visit, patients had either just been diagnosed with HIV or had been living with it for some time and just enrolling onto treatment for the first time, were fatigued, and usually overwhelmed by the diagnosis. They needed to start treatment and to understand the consequences of the disease for their everyday life so the aim was to limit the amount of time spent filling out questionnaires. Therefore, once they had been enrolled and had consented to participate in the study, I limited the questionnaire set on that first visit to collecting basic contact information and asking the patients to fill in the Pittsburgh Sleep Quality Index (PSQI) and the Epworth Sleepiness Scale (ESS).

Subsequently the following visit, between 2-4 weeks later, would be more comprehensive. Patients would get the full general information questionnaire, as well as the PSQI, ESS, Berlin

Questionnaire (BQ), and the Beck Depression Inventory (BDI). Each visit also had measures for body mass index (height and weight) performed and blood pressure was taken.

Population in the longitudinal part of the study

A total of 85 people were enrolled for the study (see figure 2.2 below). Seventy-two were enrolled at baseline, i.e. before starting their ARV treatment, 12 were enrolled at 2-4 weeks on treatment and one person was enrolled at 3 months on treatment – as per his own request.

2.3. Longitudinal population retention

Subjects were asked to provide contact numbers, and were contacted about when they could come back for follow up visits. Unfortunately, although I had accounted for a 50% loss to follow up, I had a 73% loss to follow up instead. Some patients had given incorrect phone numbers, had moved away, or simply would schedule appointments and not turn up. At the end of 2014 (12 months into the study), preliminary analyses were performed on the data I had collected up until that point. Less than ten patients fit the eligible criteria of three or more visits, and thus I decided to recruit more people into the study.

Ten additional patients were recruited at Chris Hani Baragwanath Academic Hospital (CHBAH), as it was found that patients from this hospital appeared more likely to remain in contact with us. Thus, there are some patients who have completed the study to (and some over) 18 months, but the newer patients were at 12 months when analyses were conducted. Figure 2.2 details the patient attrition and retention throughout the course of the study.

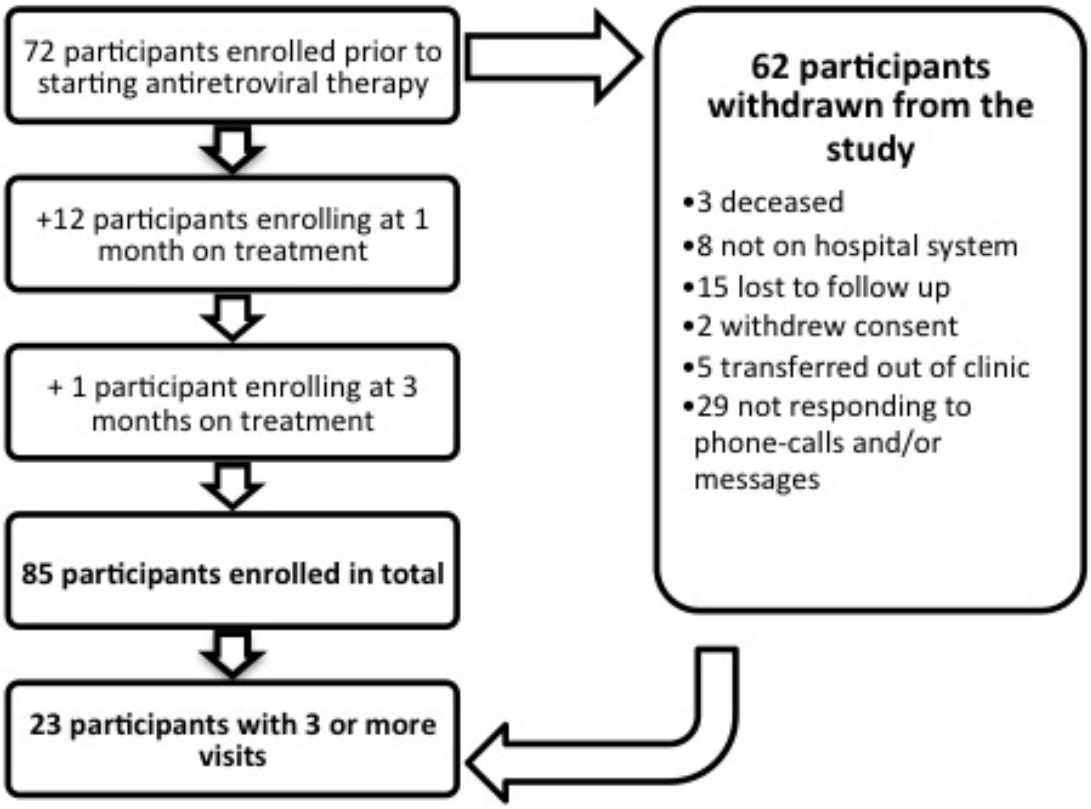


Figure 2.2. – **Population enrolment and loss to follow up during the course of the study.**

2.4. Questionnaires

The questionnaires I used in this study can all be found in the Appendix from page 19 to 27.

2.4.1. General Information Questionnaire

This collects general information about the individual, including demographics, specific HIV-related information, medical history and a basic health impact screening, as well as a modified version of the Wisconsin Brief Pain Questionnaire. This questionnaire asks an individual to indicate where pain is on the body using a diagram of the human body, and to rate how severe the pain is on a scale of 1-10; one being no pain and 10 being the highest pain ever felt. Below are the main sections of the general information questionnaire:

i) General information. Name, contact details, age, sex, education, employment, whether or not they sleep in their own room, and possible reasons that could disturb their sleep at night. WHO stage was also collected.

ii) Educational background: highest level of education received and whether or not the participant is studying for a degree/certificate/diploma at the time of questionnaires being filled in.

iii) Information about their disease. These questions included when they found out they were HIV positive, whether they had told their families/friends; and if they had ever been admitted to the hospital as a complication of HIV. Subjects were asked to list ALL their current medication, as well as their CD4+ T lymphocyte counts and viral loads.

iv) Past medical history, unrelated or related to HIV. Subjects were asked if they had, or ever had the following diseases: tuberculosis, cryptococcal meningitis, cerebral toxoplasmosis, cancer – and if cancer was present, to define which kind of cancer they had. Anthropometric measurements height, weight and blood pressure were collected

v) Information about pain. I asked participants to give information on pain at the time of their visit ("Do you have pain ***now***?"), as well as in the past month (Did you have pain **in the past month**). Study participants were asked to indicate whether they experienced pain at the time of that visit (yes/no) and if yes, they had to indicate on a diagram where the pain was felt and using a 10-point likert scale, to rate the severity of the pain. The scale was rated from one to ten, where "one" was the anchor for "no pain" and "ten" was the anchor for "worst pain ever felt. Participants were also asked to report whether they had had pain in the past month, to also indicate on the body diagram where that pain had been felt, and to indicate the highest pain level experienced in the past month on the same 10-point Likert scale.

If disease information was missing or could not be provided at the time of the interview, with consent, the information was obtained from the patient files or from the system that the hospital uses to track patient information.

Tests to acquire disease information such as CD4+ T lymphocyte counts were performed for every patient as per the request for each clinic. CD4+ T lymphocyte counts were obtained by using a

routine haematology analyser to first assess absolute white cell counts and flow cytometry using the PanLeucogating method for CD4+ T lymphocyte counts enumeration (Glencross et al., 2002). Quantitation of Human Immunodeficiency Virus Type 1 (HIV-1) RNA in human plasma (Viral load) was determined using the COBAS® AmpliPrep Instrument for automated specimen processing and the AmpliPrep/COBAS® TaqMan® HIV-1 Test, version 2.0 (v2.0). The test can quantitate HIV-1 RNA over the range of 20 - 10,000,000 copies/mL.

Data were either received from the patient at the time of the questionnaire, or from the patient system at the clinic. CHBAH has an onsite laboratory service and viral loads are done routinely, whereas at HJH, viral loads are only performed if there is a specific purpose, and are usually not done at all

2.4.2. Pittsburgh Sleep Quality Index (PSQI)

The PSQI is a validated questionnaire to evaluate sleep disruption and it has been used in many studies investigating the relationship between sleep and HIV (Crum-Cianflone et al., 2012, Lee et al., 2012, Lee et al., 2001, Lee et al., 2014, Nokes and Kendrew, 2001, Wibbeler et al., 2012) PSQI was the main outcome variable in the pilot study and has remained as such in this one.

It is comprised of 18 questions, broken down into 7 components of which the highest possible total – referred to as the global PSQI score – is 21. Questions about how much sleep an individual gets as compared to their time in bed known as sleep efficiency, as well as how long they take to fall asleep – sleep onset latency, are also asked. Global PSQI cores of five or lower indicate a normal level of sleep disruption, that is, a normal sleep quality. Scores greater than five indicate the presence of sleep disruption (Buysse et al., 1989). Internal consistency and construct validity was run to use these questionnaires in a South African cohort, details of which are found in Appendix 13. Cronbach's alpha for the PSQI was 0.78. This will be the main outcome variable of the study.

2.4.3. Berlin Questionnaire - sleep apnoea scale (BQ)

The Berlin Sleep Apnoea Questionnaire is an assessment of the risk that an individual has for having sleep apnoea.

It collects information about the subject's sex, snoring levels, sleepiness, body mass index and blood pressure. This questionnaire is broken down into ten questions relating to sleep, fatigue during the day, fatigue after wake up, snoring, if breathing stops during sleep (having an apnoea) and height, weight, (for BMI) and blood pressure. These questions form three categories. If there are two or more positive categories, this is an indication of a high likelihood of sleep-disordered breathing (Netzer et al., 1999). I used the BQ as the second of the three sleep outcome variables of the study.

2.4.4. Epworth Sleepiness Scale (ESS)

The Epworth Sleepiness Scale (Johns, 1991) is an assessment of daytime sleepiness, commonly used in conjunction with the PSQI. Fatigue in treated HIV positive patients is well reported (Aouizerat et al., 2013, Barroso et al., 2015, Corless et al., 2008, Darko et al., 1995, Lee et al., 1999)

The Epworth sleepiness scale is comprised of 8 scenarios, on which a person is asked to rate the likeliness that they would fall asleep in a given scenario. The ratings range from never falling asleep, to there being a high chance that they would fall asleep in a given scenario. Similarly to the PSQI, levels of five and lower are considered normal levels of daytime sleepiness, but anything over five is indicative of higher than normal daytime sleepiness. A score above 10 signals usually an underlying sleep disorder. An increase in daytime sleepiness has been shown to have a negative impact on quality of life, and thus is assessed in this study. For the purposes of defining daytime sleepiness, I used scores that were anything over ten for the ESS. Internal consistency and construct validity was run to use these questionnaires in a South African cohort, details of which are found in Appendix 13. Cronbach's alpha for the ESS was 0.76. I used the ESS as the third measurement of sleep outcome.

2.4.5. Beck's Depression Inventory (BDI)

There is a known relationship between disrupted sleep and depression (Kupfer et al., 1984) as well as depression being well characterized in both treated and untreated HIV cohorts (Poupard et

al., 2007). Disrupted sleep increases the risk of depression (Irwin et al., 2013), but depression also conversely is associated with disrupted sleep or hypersomnia. These relationships were investigated in this study, using this validated questionnaire.

Beck's Depression Inventory is a measure of mood or depression. Scores of 10 and below are normal, that is, "normal" levels of depression. Scores over ten indicate that depression may be present, and scores over 17 indicate the presence of moderate-severe depression, which may call for treatment. There are 21 questions with four possible answers each, and the person is asked to indicate which answer most applies to them. It is important to note that this is not a clinical diagnosis of depression, and further tests do have to be conducted to assess the severity of the mood disturbance (Beck et al., 1988). Internal consistency and construct validity was run to use these questionnaires in a South African cohort, details of which are found in Appendix 13. Cronbach's alpha for the BDI was 0.9. I used the BDI instead of BDI-II as at the time the study was started, there was no funding available and the BDI is freely available.

2.5. Data Pooling of time=0 and time=1 in the longitudinal data analysis

Out of the 72 for whom I have pre-ARV treatment information, only 18 stayed on for 3 or more visits and therefore are included in the longitudinal part of the study. Four out of the 12 who were enrolled at 2-4 weeks also completed a minimum of 3 visits in total; and finally the person who had been enrolled at 3 months also completed 3 visits. For the longitudinal data study, I therefore have in total 23 patients who have had a minimum of 3 visits (see table 2.1. below, where I detail the number of patients seen at each visit).

Table 2.1: Table of visits per enrolled participant.

	0 months	1 month	3 months	6 months	12 months	18 months	Total visits per study participant	
13HS001		■		■	■	■	4	
13HS003		■	■	■	■	■	5	
13HS004	■	■				■	3	
13HS007	■	■	■				3	
13HS011	■	■	■	■	■		5	
13HS012	■	■	■	■		■	5	
13HS015		■			■	■	3	
13HS017	■			■	■	■	4	
13HS021	■	■		■	■		4	
13HS022	■	■	■	■		■	5	
13HS023	■	■	■	■	■	■	6	
13HS024		■	■	■			3	
13HS028	■	■		■	■		4	
13HS030	■	■		■			3	
13HS032	■	■	■				3	
13HS033	■				■	■	3	
13HS039	■	■	■				3	
13HS041	■	■	■	■			4	
13HS056	■	■			■		3	
13HS079	■	■		■	■	■	5	
13HS081	■	■	■	■	■		5	
13HS083	■	■	■	■	■		5	
13HS085			■	■	■		3	
Total visits per time point	18	20	13	16	14	10	Median	4

Black coloured blocks indicate visits attended, white coloured blocks indicate visits missed and blocks coloured grey indicate visits that had not yet taken place.

For patients who were enrolled at the pre ARV visit and also came for a 2-4 weeks visit, they only had one CD4+ T lymphocyte count or one viral load for that whole period. Then the 4 others enrolled at 1 month only had one value as well.

Using a Student's paired t-test, I tested the difference in sleep quality, as defined by Pittsburgh Sleep Quality Index (PSQI) score and daytime sleepiness, as defined by the Epworth Sleepiness Scale (ESS) score of subjects at Time=0 (pre-ARV) and Time=1 (2-4 weeks). I found that there was no statistical difference between the two time points for the PSQI and ESS. Data was thus pooled into Time=1, and in cases where there were two values at the two time points, the average of the two values was taken and used as the value of the variable for Time=1. Four patients only

came at the 0 month visit. I only have their ESS score, PSQI score, and CD4+ T lymphocyte count or viral load, and not their BDI, MEQ, pain evaluation or anthropometric data. So at time =1 for these variables I only have n=19. Although the analysis was not specifically designed to test for the sleep side effects of efavirenz, there was no significant difference between pre-ART baseline and week 2-4 on all the sleep measurements (PSQI and ESS).

2.6. Data analysis

I examined 3 outcome variables in univariate and subsequent multivariate analyses: the PSQI global score, ESS global score and the BQ. PSQI and ESS were used as continuous variables. However, when exploring the relationship between PSQI score and pain, I also dichotomized PSQI into PSQI>5 (evidence of disrupted sleep) and PSQI≤ 5 (normal sleep) to try and better capture the relationship between sleep and pain.

First, I tested the relationship of these variables with time, then in univariate analyses with predictors of interest.

Preliminary data were analysed using Microsoft Excel software. This included tests for normality, calculating means, and standard deviations for each variable of interest. Non-normal data were log-transformed for further analyses. Only one variable had to be log-transformed, viral load. The log transformed viral load met the criteria for a normal distribution.

Univariate analyses were done on SAS ® 9.1.3. Due to the large loss to follow up, I decided to separately analyse the very first initial enrolment visit (pre-ARV start) where I had 72 patients but only collected age, sex, CD4+ T lymphocyte counts, viral loads, PSQI and ESS to test the hypothesis that sleep quality / daytime sleepiness were associated with markers of immunity/ disease at ART initiation. This was an exploratory analysis, not planned in the original study design but trying to use the larger cohort collected at ART initiation to explore the relationship between sleep quality and HIV itself and CD4+ T lymphocyte counts as indirect markers of immune function. I ran simple Pearson's (for normally distributed data) or Spearman's (for data not following the normal distribution) correlation analysis examining the relationship between

continuous variables and unpaired t-tests when comparing a continuous variable between 2 groups (women vs. men).

Twenty-three patients were followed up for at least 3 visits and up to 5 (18 months after start of ARVs). For this longitudinal data analysis, I used mixed model analysis in SAS. In SAS, missingness can be overlooked by using mixed model analysis (PROC MIXED) with the random intercept statement (SAS, 2011). I used the PROC MIXED procedure for continuous outcome variables such as PSQI and ESS, and the PROC GENMOD procedure for categorical outcome variables like the BQ.

Before I tackled the analysis addressing my specific aims for this study, I first decided to examine the clinical evolution of the patients in my cohort overtime to understand whether they had followed an expected clinical evolution of improvement of CD4+ T lymphocyte counts/ decrease in viral loads as they took their treatment. The markers of clinical evolution included specific markers of HIV (CD4+ T lymphocyte counts and viral load) as well as anthropometric variables (BMI, mean arterial pressure, hypertensive status). For this, I performed a simple univariate analysis of the effect of time on CD4+ T lymphocyte counts, viral loads, BMI, mean arterial pressure and hypertensive status.

To answer the first specific aim (effects of time on sleep measurements) and specific aim 2 (effects of time on classical predictors of sleep, such as depression and pain), I analysed the univariate effect of time, first on sleep variables (PSQI, ESS and BQ) and secondly on known predictors of sleep quality: BDI score and pain status/ ratings.

I then focused my analysis on understanding the predictors of sleep quality, examining PSQI, then ESS then BQ as outcome variables.

I first assessed the effect of age and sex on these three outcome variables of interest. I then tested specifically for the effect of CD4+ T lymphocyte counts and viral loads in unadjusted and adjusted analyses (first adjusting for age and sex, then adjusting for the effect of time). Finally, I tested for the effect of two more classical predictors of sleep disturbances, BDI score and pain

status (presence or absence of pain) or pain rating (as per the 1-10 Likert scale), again in unadjusted then adjusted analyses (again first adjusting for age and sex, then for the effect of time).

To better understand whether sleep quality may participate in the building of non-communicable disease, I also analysed the impact of PSQI, then ESS, then BQ on BMI, mean arterial pressure and hypertension status, in unadjusted and adjusted analyses (again adjusting for age and sex, then time). Any variable that came up as significant predictors of sleep quality in the pilot study, and variables that had a significance of p=0.2 or lower, or variables that are known predictors of sleep quality (such as age and sex) from literature, were systematically added to the model.

Collinearity and confounding was monitored throughout the process and the model was adjusted accordingly. Each time the model was run, and as the model was being built, I monitored the standard errors given out by SAS, and if there was too great a change (>10%) in one variable from a previous model, I could test for collinearity. Collinear variables were not included in the model, whereas confounding variables were included in the model.

For the effect of time, if I assumed non-linear effects of time, I used time as a categorical variable (time included in the class statement in the mixed model), and if linear effects of time were assumed, I used time as a continuous variable (time not included in the class statement in the mixed model). I used time as a categorical variable because I was comparing other variables to each other at different time points. Using time as a categorical variable also meant that I would use random intercepts to determine the best model rather than calculating random slope because I was not using time as a linear component (which would then require calculating the slope).

CHAPTER 3 – RESULTS

Because I collected data on 72 patients at baseline before they started ARV treatment, I decided to present the results from this very first meeting first. The longitudinal analyses were then conducted on the 23 study participants (27% of the initially enrolled 85) who came back for three or more questionnaires at different time points.

3.1. Pre-ARV treatment population characteristics for the initial 72 patients.

As mentioned before, because the patients were often very ill before starting ARV treatment, I had decided to only collect at this time point data on date of birth, sex, CD4+ T lymphocyte counts and viral loads, the PSQI and the ESS. The demographics of the patients at baseline (Time=0) are shown in Table3.1 below2. The majority of the population were female, around 36 years of age, with 73% with CD4+ T lymphocyte counts <250/µl (including 38% with CD4+ T lymphocyte counts <100/µl) and high viral loads. They reported relatively low sleep disruption with less than a third (27.8%) having a PSQI > 5. However they reported high levels of daytime sleepiness with 36 (50 %) reporting an ESS>10, which is the cut-off for diagnosing an underlying sleep disorder.

Table 3.1. Baseline demographic and clinical characteristics of 72 participants enrolled at baseline (Before starting antiretroviral treatment). The only questionnaires given (and presented below) at this time point were the ESS and PSQI.

Demographic and clinical characteristics	
Number of people (N)	72
Age: mean (±SD)	36.05 (±8.39)
Sex: N (%)	46 F (63.8%)
	26 M (36.2%)
CD4 count/µl (N=53): median [IQR]	146 [72-282]
Log viral load (N=29): mean (±SD)	3.58 (±1.58)
PSQI score: mean (±SD)	6.04 (±3.25)
ESS score: mean (±SD)	9.38 (±6.25)

Baseline analyses revealed that there was no statistical association between PSQI score and CD4+ T lymphocyte counts (Spearman's r=0.00007, p=0.9), viral loads (Pearson's r= -0.22, p=0.44), ESS score (Pearson's, r=0.08, p=0.5), age (Univariate testing in SAS, p=0.15) and sex (unpaired t-test, p=0.2). I also ran correlation analysis with ESS score and the factors mentioned above (CD4+ T lymphocytes, viral loads, age), and found that there was no correlation between ESS score and any of the factors above. Using an unpaired t-test, I found that there was also no difference between the ESS scores in men as compared to women.

3.2 Pre-ARV treatment population characteristics for the 23 patients in the longitudinal study

Table 3.2 shows the population characteristics at the pooled baseline. Again more than two thirds of the cohort were female, in their mid-30s, had an average CD4+ T lymphocyte count <250 cells/µL, and an average log viral load >4. All patients were on the fixed dose combination therapy (regimen one) including efavirenz, and one person was on regimen two treatment. The average BMI of the cohort was above 25 kg/m^2, which places them in the overweight category of BMI, with

28.6% having BMI > 25 kg/m^2. One third of the patients were hypertensive. I define hypertensive as having either or both systolic BP ≥140mmHg, diastolic BP≥90mmHg, or being treated for hypertension.

The average PSQI score was indicative of mild sleep disruption (52.2% had a PSQI >5), there was higher than normal daytime sleepiness as per the ESS score and 26% of patients (5 out of 19) reported high risk for reporting sleep apnoea on the Berlin Questionnaire. The BDI score indicates an overall high level of depression. Eight participants out of the initial 23 had BDI scores of 17 or higher, indicative of clinical depression.

I asked questions about the presence of past and/or current comorbities related or non-related specifically to HIV (as seen on page 4 and 12 of the Appendix). For HIV associated comorbidities, one person reported cytomegalovirus infection causing her to have lost sight in her eye, three patients reported having Kaposi Sarcoma, and one person had had cryptococcal meningitis. In terms of non-HIV associated comorbidities, seven reported hypertension (as discussed in more detail later on), two patients reported renal insufficiency, two people reported having depression, one person reported migraines, and nobody reported diabetes.

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Table 3.2. Patient demographics at baseline of the 23 subjects who remained in the study.

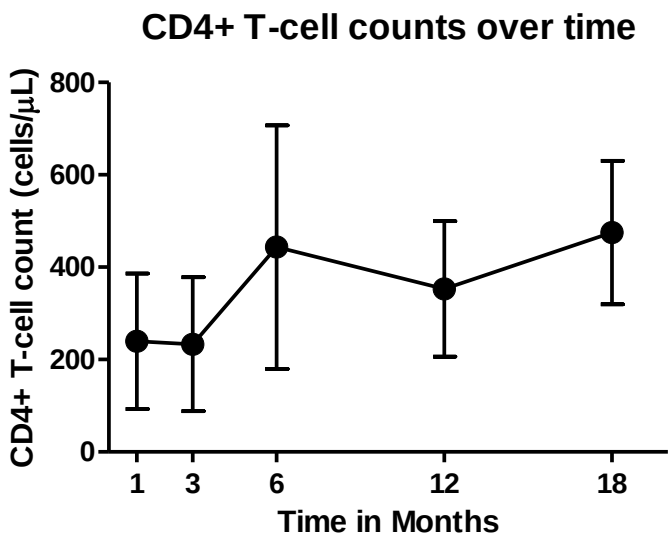
Population characteristics	
Demographic	
Number of people: N	23
Age: mean (±SD)	34.4(±7.8)
Females N (%)	16 (70%)
HIV	
Number of people on regimen 2	1
CD4 count cells/µL: mean (±SD) ,	239 (± 146)
median [IQR]	216 [138-337]
Log viral load: mean (±SD)	4.5 (±1.9)
Anthropometric	
BMI (kg/m2) (N=13): mean (±SD)	25.5 (±7.1)
MAP (mm Hg): mean (±SD)	95.1 (±19.4)
Hypertensives: N (%)	7 (32%)
Sleep, sleepiness and risk of sleep apnoea	
PSQI score: mean (±SD)	5.8 (±3.3)
ESS score: mean (±SD)	8.4 (±4.9)
High-risk sleep apnoea (BQ) N (%)	5 (35.7%)
BDI score: mean (±SD)	17.8 (±11)
Pain characteristics	
Reported presence of pain in the past month, N (%)	11 (57.8%)
Mean rating of pain, mean (±SD) of those who reported pain in the past month (scale 1-10, 1=no pain 10=worse pain ever)	6.5 (±2.2)
Reported presence of pain at the time of the visit, N (%)	7 (36,8%)
Mean rating of pain, mean (±SD), of those who reported pain at the time of the visit (scale 1-10, 1=no pain 10=worse pain ever)	5.4 (±2.6)

3.3. Clinical evolution across time (disease markers and anthropometric data)

3.3.1. Evolution of HIV parameters across the study

There was an increase in CD4+ T lymphocyte counts over time, as depicted in figure 3.1., panel A. This was statistically ($p<0.01$) and biologically significant. There was a decrease over time in viral load, as seen in figure 4, panel B ($p<0.01$).

Panel A



Panel B

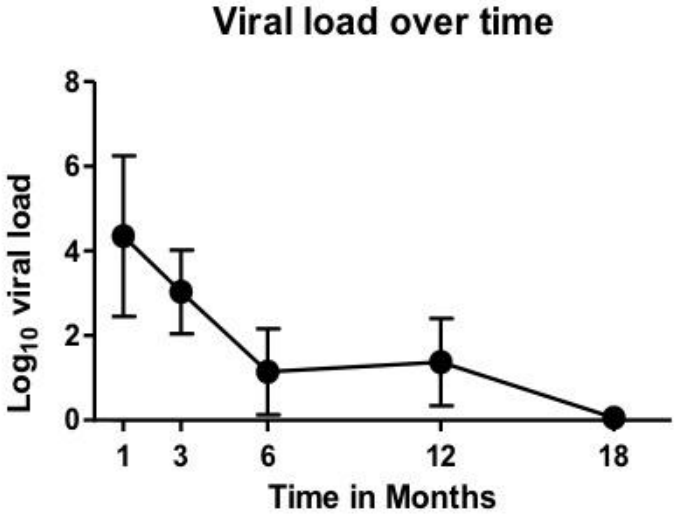


FIGURE 3.1. Evolution of HIV biomarkers over time. **Panel A:** Mean ±SD CD4+ T lymphocyte counts over time ($p<0.01$). **Panel B** Mean ±SD log viral loads over time ($p<0.01$).

3.3.2. Evolution of Body Mass Index, Mean Arterial Pressure and across the study

Evolution of Body Mass Index across time

Figures 3.2 below shows the evolution of BMI at each time point of the study. There was a significant main effect of time on BMI ($p<0.01$), whereby BMI increased across the study. At the beginning of the study, 28.6% (4 out of 13) had BMI>25 kg/m^2. At 6 months, it increased to 50%, and at 18 months it was 80%. Using Proc GenMod in SAS to assess the odds of reporting BMI>25 kg/m^2 I found that for each one month on treatment, the odds of reporting BMI>25 were 1.16 (Wald CI [1.05 – 1.27], $p<0.01$).

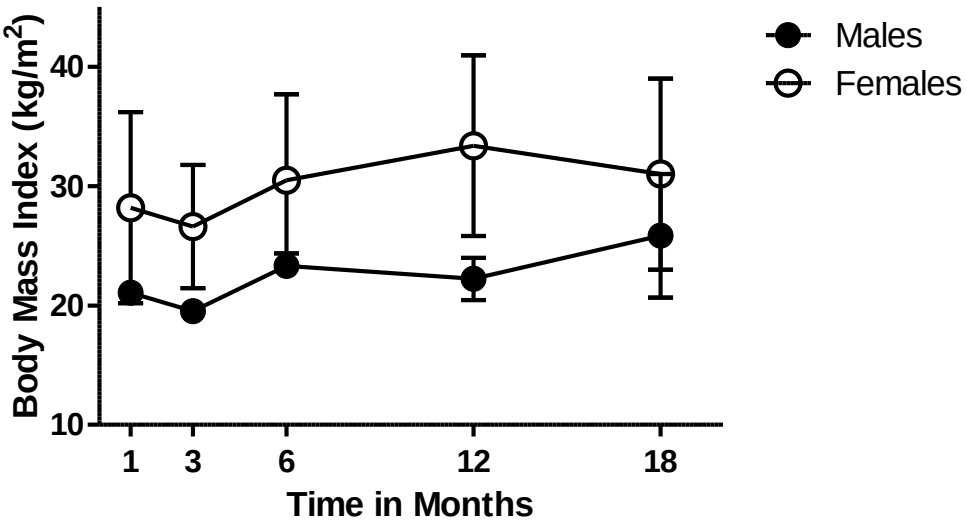


FIGURE 3.2: Average ± SD BMI over time

From a baseline of BMI=25.5 kg/m^2, the average reached 29.9kg/m^2 (±7.5) at the end of the study. There was a significant increase of 0.25kg/m^2 in BMI with each additional month on treatment ($p<0.01$). I also found a main effect of sex on BMI, when adjusting for time, whereby females had a higher BMI than males ($\beta=7$, $p<0.01$, see figure 3.3).

Evolution of mean arterial pressure and hypertensive status across time

I investigated the effects of time on mean arterial pressure, as seen in figure 3.3 below. There was no change in mean arterial pressure over time (p=0.12).

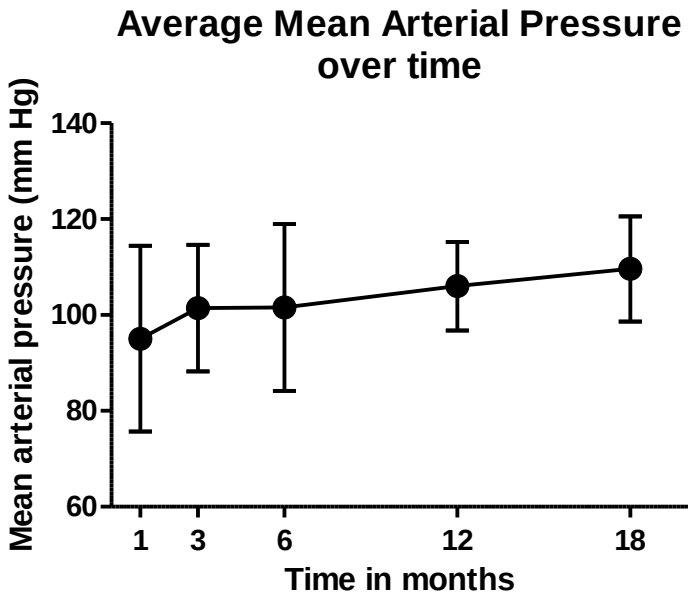


Figure 3.3: Mean ± SD mean arterial pressure (MAP) over time.

The percentage of people at each time point who were hypertensive (including treated controlled hypertensives) went from 32% prevalence in the first month, (6 out of 19 who had available measurements), and up to 60% in month 18 (or 6 out of 10 who had available measurements). This increase was not statistically significant (95% CI [-0.019 – 0.15], p=0.13).

3.4. Sleep quality, daytime sleepiness, and sleep apnoea over time

3.4.1. Sleep quality across time

Sleep Quality as measured by global PSQI score was the main outcome variable of the project. PSQI global score has a cut off of five out of a possible 21 points. Scores of higher than five indicate poor sleep quality/ disrupted sleep. Using Proc Mixed I found no significant effect of time (as a continuous variable) on PSQI score (p=0.057). For each month on treatment, there was a trend effect of time whereby PSQI score decreased by an average of 0.09 points. The average PSQI global score over time is given in figure 3.4, below.

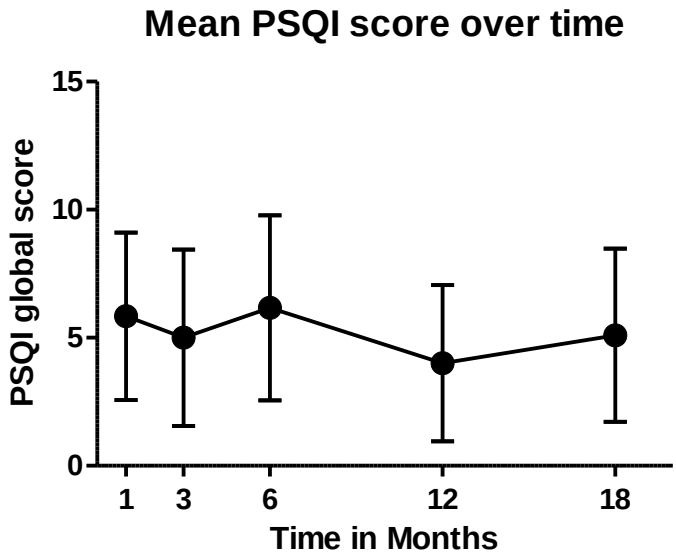


FIGURE 3.4: Mean ±SD PSQI score over time

Subsections of the PSQI include sleep efficiency, which is percentage of time spent asleep out of total time spent in bed; and sleep onset latency, which is how long one takes to fall asleep after going to bed. Both self assessed sleep efficiency and sleep onset latency are components of the PSQI global score. Self-assessed sleep efficiency at the beginning of the study was 89%, and decreased to 79% over the 18-month period, but this was not significant (p=0.2). Sleep onset latency is the time taken to fall asleep, and is the second question on the PSQI "How long (in minutes) do you take to fall asleep?". The mean time taken to fall asleep at the beginning of the study was 30.1 minutes, which decreased to 15.0 minutes after 18 months but this did not represent a significant change over time (p=0.2).

3.4.2. Daytime sleepiness over time

Daytime sleepiness measured by the Epworth Sleepiness Scale (ESS). I found a main effect of time (as a categorical variable) on ESS score (p=0.03) whereby in post-hoc testing at 6 months, average ESS score was 2.55 points lower as compared to the first month (post-hoc analysis with Tukey adjustment p = 0.04), see figure 3.5 below.

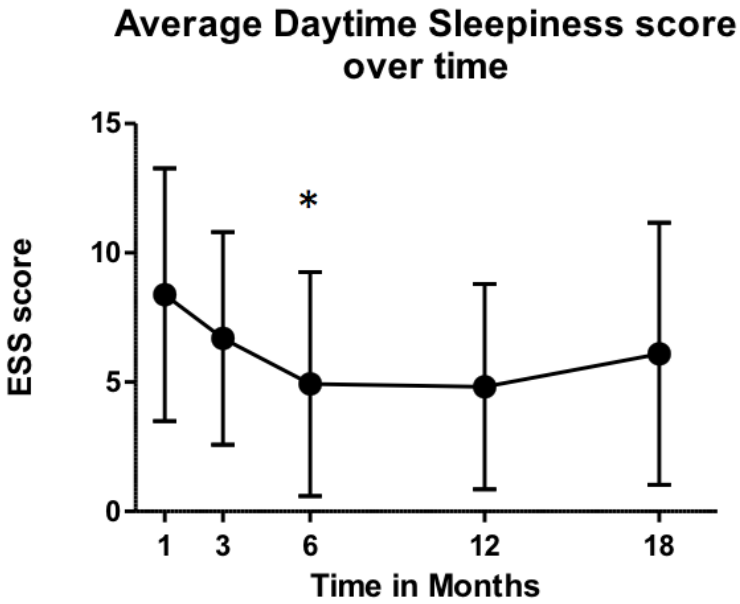


FIGURE 3.5: ESS score Mean ±SD of over time. There was a significant main effect of time. The (*) indicates a post hoc significant difference with ESS score at baseline.

3.4.3. Evolution of the presence of sleep apnoea over time

The third questionnaire used to measure sleep was the Berlin questionnaire (BQ). Figure 3.6 below shows the percentage of people who scored high risk of sleep apnoea on the BQ across the study. There was a non-significant main effect of time on the odds of scoring high risk of sleep apnoea (Proc GenMod p=0.99).

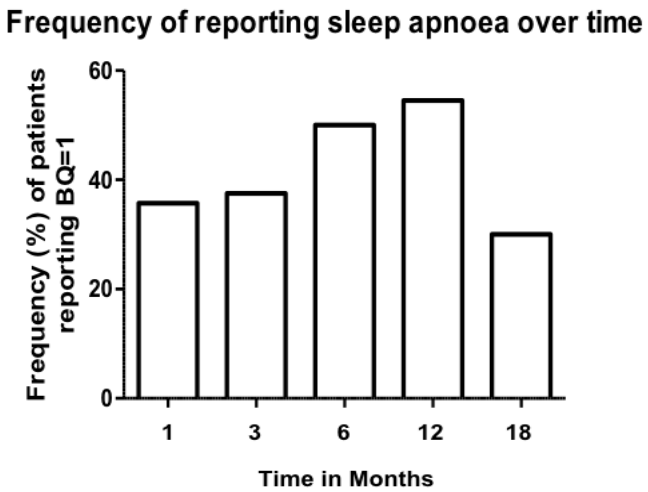


FIGURE 3.6: Percentage of people scoring high risk of sleep apnoea on the Berlin Questionnaire (BQ=1) over time (p=0.99).

3.5. Time effects on known predictors of sleep (pain and depression)

3.5.1 Time effects on the presence and rating of pain

The presence of pain was assessed at each time point – both pain at the time of the visit ("Do you have pain **now**?") and pain in the month preceding the visit ("Did you have pain **in the past month**?").

Pain at the time of the visit

At the beginning of the study, the prevalence of pain at the time of the visit was 37.8% (7 out of 19), and by the end of the study, the prevalence was 10% (1 out of 10). This decrease was not statistically significant (p=0.24). The rating of pain at those two time points was 2.5 (±3.3); and 0.8 (±2.5), respectively. The change in ratings of pain was not statistically significant (p=0.51).

Pain in the past month over time:

The frequency of patients reporting the presence of pain in the past month did not change significantly over time. It went from 57.9% in the first month (11 out of 19), to 27.3% (3 out of 11) at 18 months. This change across time was not statistically significant (p=0.32).

There was a statistically significant decrease in the rating of pain in the past month, as seen in figure 3.7, panel B (p=0.02), whereby for each month on treatment, the rating of pain in the past month decreased by 0.15 points. The mean (±SD) rating of pain at baseline and 18 months was 4.2 (±3.6) and 0.8 (±1.3), respectively.

Panel A **Panel B**

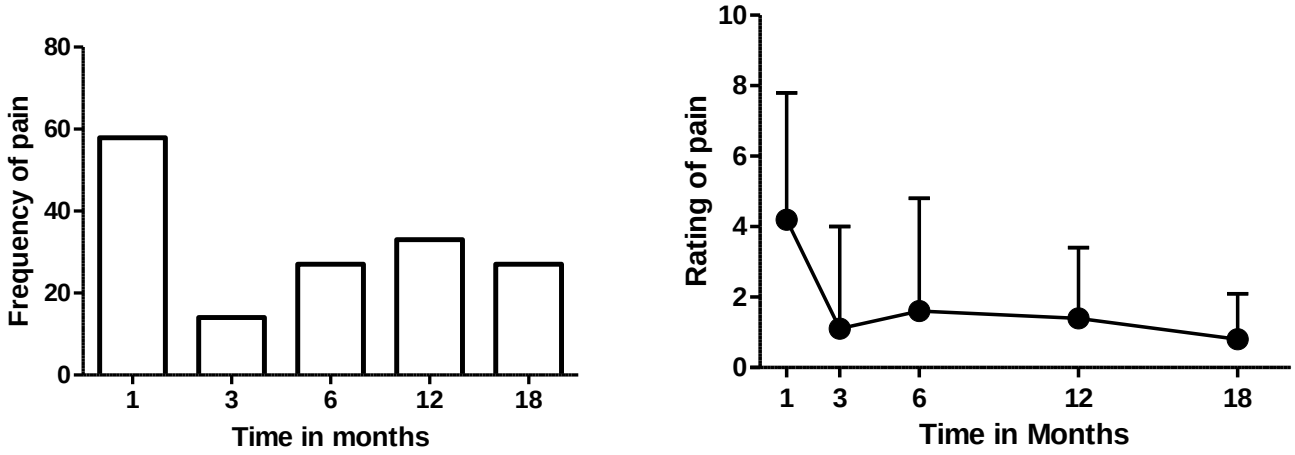


Figure 3.7: Frequency (Panel A) and rating (Panel B) of pain in the past month over time.

There was no significant effect of time on the frequency of reporting pain in the past month (p=0.32). There was a significant time effect on the rating of pain in the past month (p=0.02).

3.5.2. Effects of time on depression severity

Beck's Depression Inventory (BDI) was used as an assessment of depression severity. There was a significant change in depression severity over time (p<0.01), as seen in figure 3.8 below. Post-hoc analyses with Tukey adjustment showed a 7-point drop in depression severity from baseline to 3 months (p<0.01), and then a levelling out to approximately 11 points but with no longer significant differences compared to baseline in post hoc comparisons.

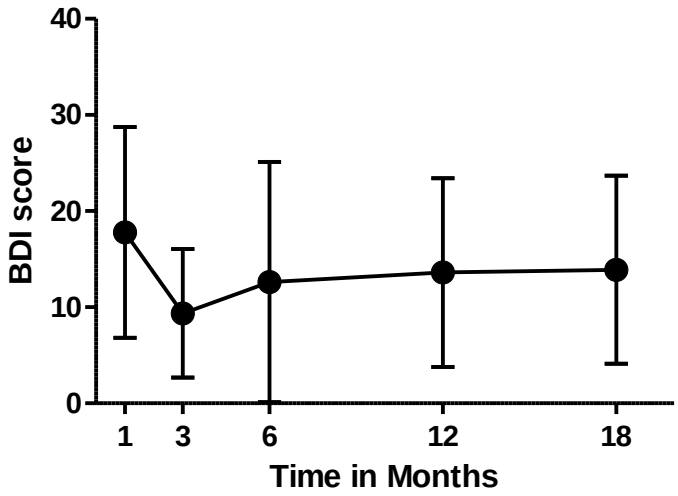


Figure 3.8: Mean ±SD BDI score over time

3.6. Multivariate predictors of sleep quality, daytime sleepiness, and the likelihood of reporting high risk for sleep apnoea

Our next aim was to identify predictors of sleep disruption, as measured by PSQI, ESS and BQ in my cohort over time. The known predictors of sleep disruption are age and sex, whereby females do not sleep as well as males (Buysse et al., 2005, Duffy and Czeisler, 2002) and an increase in age is associated with higher sleep disruption (Dijk et al., 1999, Duffy and Czeisler, 2002). I found no unadjusted significant effect of age or sex on any of those outcome variables (PSQI, ESS and BQ) nor when I adjusted for time. However, for all subsequent testing of predictors, to test for potential confounding effect, age and sex were systematically added as covariates in the models. Based on the preliminary results from my pilot study, I had specifically designed this study to investigate how immune reconstitution could affect the sleep quality in this cohort. The following section investigates the effects of CD4+ T lymphocyte counts and viral loads on sleep quality, daytime sleepiness and being at high risk of sleep apnoea, in unadjusted analyses, then adjusted for age and sex and finally adjusting for time.

The second section explores the relationship between these same outcome variables (PSQI, ESS and BQ) and more classical predictors of sleep disruption, i.e. depression as measured by the BDI and pain, again in unadjusted then adjusted analyses (age and sex first, then time, as described above). Finally the last section explores more the potential cardiometabolic consequences of sleep disruption in HIV infection, specifically investigating the relationship between BMI and PSQI, ESS and the Berlin questionnaire when adjusting for time, age and sex, then the relationship between mean arterial pressure and PSQI, ESS and the Berlin questionnaire when adjusting for time, age and sex.

3.6.1. CD4+ T lymphocyte counts and log viral load effects on sleep quality, daytime sleepiness and the likelihood of reporting high risk for sleep apnoea

Effects of CD4+ T lymphocyte counts and log viral load on sleep quality

a) Unadjusted and adjusted effects of CD4+ T lymphocyte counts on PSQI

As described previously, I had found no significant main effect of time on PSQI (p=0.057).

I first tested unadjusted CD4+ T lymphocyte counts and log viral loads effect. I then adjusted for age and sex, and then removed age and sex, and added time as a covariate to the model.

Mixed model analysis revealed that there was no main effect of CD4+ T lymphocyte counts on PSQI score (p=0.95). When adjusting for age and sex, there was no significant effect of CD4+ T lymphocyte counts on PSQI score (p=0.91). Since I found no significant effect of age and sex, I removed them from the model, and adjusted for time. Adjusting for time, there was a non-significant effect of CD4+ T lymphocyte counts on PSQI score (p=0.37).

b) Unadjusted and adjusted effects of Log viral load on PSQI

In unadjusted and adjusted analyses (sex and age, then time), I found no significant effect of log viral load on PSQI score (unadjusted: p=0.48, adjusted for age and sex, p=0.48; adjusted for time p=0.91).

Effects of CD4+ T lymphocyte counts and log viral load on daytime sleepiness

a) Unadjusted and adjusted effects of CD4+ T lymphocyte counts on daytime sleepiness

As described previously I had found a significant effect of time on ESS score.

In unadjusted and adjusted analyses (sex and age, then time), I found no significant effect of CD4+ T lymphocyte count on ESS score (unadjusted: p=0.11, adjusted for age and sex, p=0.11; adjusted for time p=0.45) but there remained a time effect on ESS score (p=0.03).

b) Unadjusted and adjusted effects of Log viral load on ESS

The unadjusted effects of log viral load on ESS score was non-significant (p=0.13). Then, when adjusting for age and sex, log viral load effect on ESS score was non-significant (p=0.15), and

when adjusting for time (removing age and sex), there was no main effect of log viral load. In the model of ESS in function of time and log viral load, there was a main effect of time (p=0.03), no main effect of log viral load (p=0.15) but I found a significant interaction between time and log viral load (p=0.04). The interaction was such that at time=1 and time=12, increased log viral loads were associated with lower ESS scores; at time=3 and time=6, increased log viral loads were associated with higher ESS scores.

To better explain this interaction, I categorized log viral load into two categories: highVL as patients who had log viral loads greater than a log of 2 (viral load > 100 copies/μL), and lowVL as patients who log viral loads less than or equal to 2 (viral load ≤ 100 copies/μL). Viral loads are considered to be at undetectable levels when less than 100 copies, and was the cut off point for separation between “high” and “low” viral load for the purposes of this analysis. This allowed for the construction of figure 3.9 (seen below).

Prevalence of highVL was 93% at 1 month, and decreased to 0% at 18 months (as seen in Figure 3.9). I then ran a model of ESS as a function of high viral load (highVL) vs low viral load (lowVL), time and the interaction between time and high vs. low viral load. I found a main effect of highVL versus lowVL whereby patients who had low viral loads had significantly higher ESS scores than those with high viral loads (β=6.15; p<0.01). I found a significant effect of time (p=0.01). Using a Tukey adjustment for post-hoc analysis, I found significant differences between time 1 and 3 (β= -7.5; p=0.02), 1 and 6 (β= -8; p<0.01), and 1 and 12 months (β= -9; p<0.01) across both groups. I also found an interaction between high viral load and time effect on daytime sleepiness (p<0.01). Using a Tukey adjustment for post-hoc analysis, at time=1 and at time=12, in the **low** viral load group, there was even **higher** daytime sleepiness than expected from just the high versus low viral load group main effect (β=12, p<0.01 and β=6.15; p<0.01, respectively).

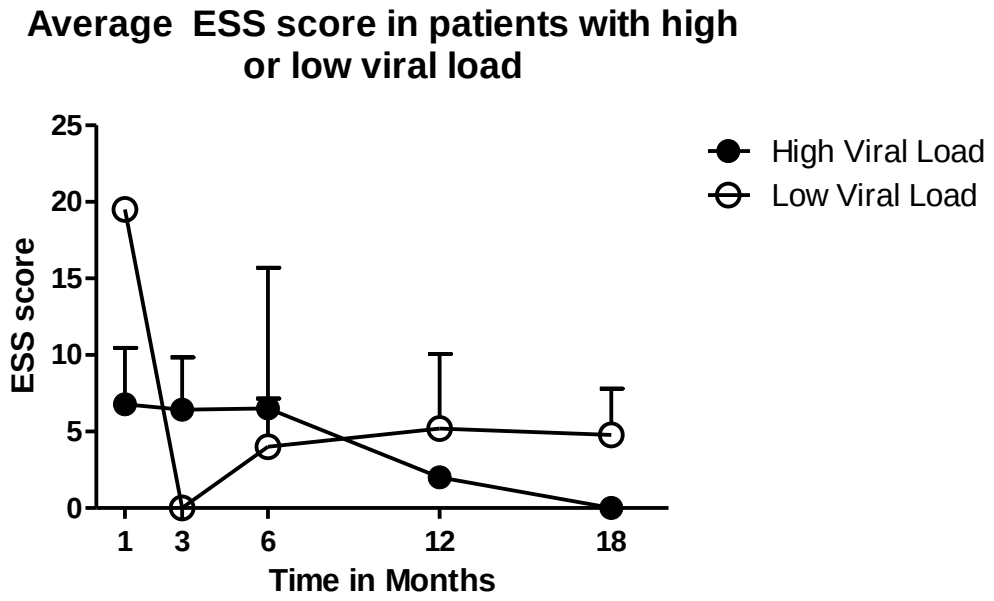


Figure 3.9. Mean ± SD ESS score in patients with high (>2) or low (≤2) log viral loads

Effects of CD4+ T lymphocyte counts and log viral load on scoring high risk of sleep apnoea

a) Unadjusted and adjusted effects of CD4+ T lymphocyte counts on the Berlin Questionnaire (BQ)

To test the risk of being high risk for sleep apnoea using the BQ, I used logistic regression with repeated measures (proc GENMOD in SAS) modelling the odds of scoring high risk of sleep apnoea on the Berlin Questionnaire. I found a non significant trend effect of CD4+ T lymphocyte on BQ whereby increased CD4+ T lymphocyte counts were associated with lower odds to score high risk of sleep apnoea on the Berlin Questionnaire (OR= 0.99 (Wald 95% CI [0.99 – 1.0001]; p=0.057). When adjusting for age and sex, I found again a non-significant trend whereby higher CD4+ T lymphocyte counts were associated with lower odds to score high risk on the Berlin Questionnaire (p=0.059). In addition I found a non significant trend whereby females were at higher odds of scoring high risk of sleep apnoea on the BQ compared to males (OR=6.11, Wald CI [0.95-39], p=0.055) and a non significant effect of age (p=0.89).

When adjusting for time, an increase in CD4+ T lymphocyte counts by 100 was associated with significantly lower odds of scoring high risk of sleep apnoea (OR=0.52 [0.28-0.98], p=0.04) and there was a non significant effect of time (p=0.12).

In addition, for risk of sleep apnoea, I also controlled for the effect of BMI (a known predictor of sleep apnoea) on the odds of scoring high risk of sleep apnoea on the BQ. When adding BMI to the model with CD4+ T lymphocyte count and time, I found that this did not modify the beta parameter of CD4 (no change, therefore no confounding effect). However it led to a substantial increase in the standard error associated with CD4+ T lymphocyte count. This indicated collinearity and no confounding effect of BMI on the relationship between CD4+ T lymphocyte count and BMI. I therefore decided not to consider it in the model examining the effects of CD4+ T lymphocyte count on the risk of scoring high risk of sleep apnoea on the BQ.

b) Unadjusted and adjusted effects of log viral load on BQ

In the unadjusted analysis of the effects of log viral load on BQ, log viral load was not significantly associated with the risk of scoring high risk of sleep apnoea on the BQ (p=0.54). When adjusting for age and sex, there was no significant effect of log viral load or age, however sex became a significant predictor, whereby being female increased the odds of scoring high risk of sleep apnoea on the BQ (OR=12.8, Wald 95% CI: [2-84]; p=0.006). When adjusting for time, there was no significant effect of log viral load or time on scoring high risk of sleep apnoea on the BQ (p=0.72 and p=0.97, respectively).

3.6.2. Depression and pain effects on sleep quality, daytime sleepiness and the likelihood of reporting high risk for sleep apnoea

Depression effects on sleep quality

In the unadjusted analysis, BDI was not a significant predictor of PSQI score (p=0.17), nor was it a significant predictor of PSQI score in the adjusted analysis with age and sex (all p values >0.2). Using time as a continuous variable, I found no main effect of BDI but a main effect of time whereby each month on treatment was associated with a decrease in PSQI score (β= -0.25,

p<0.01) whereby for an increase in time by 1-month, there was a decrease in PSQI score by 0.25 points. I also found a significant interaction between BDI and time, whereby 10 points of BDI gained over 1 month were associated with an increased PSQI score (β= 0.11, p=0.03).

Pain effects on sleep quality

*a) Pain at the time of the visit (**"Pain now"**)*

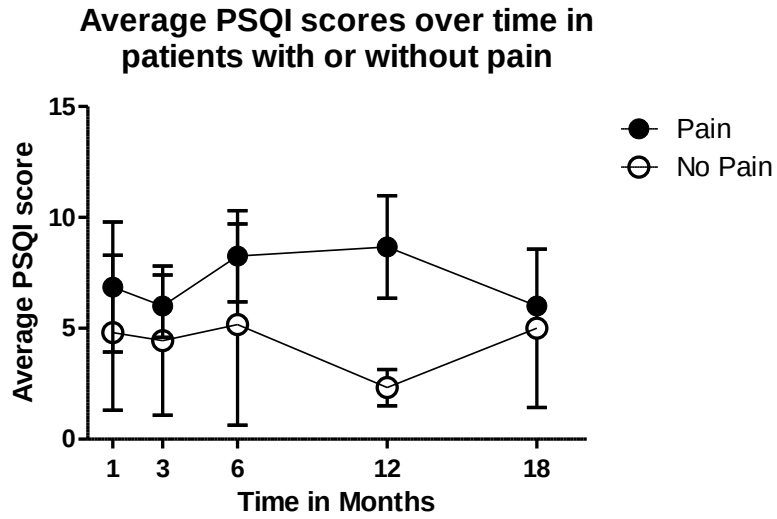
I found no significant effect of reporting pain at the time of the visit on PSQI scores, in unadjusted and adjusted (age and sex, then time) analyses. I also found no significant effect of the rating of pain at the time of the visit on PSQI scores, in unadjusted and adjusted (for age and sex, then time) analyses.

However, I also assessed the likelihood of reporting PSQI scores >5 in patients reporting pain versus those not reporting pain at the time of the visit. In both unadjusted and adjusted analyses (adjusted for age and sex, then time), I found that patients reporting pain at the time of the visit would have a significantly higher odds of reporting PSQI scores >5 (unadjusted and adjusted OR=9; Wald 95% CL=[2.2 - 36.6]; p=0.002). If there was a 1-point increase reported in the rating of pain at the visit, there would be an increased odds of reporting PSQI score>5 (OR=1.3; Wald 95% CL=[1-1.7] p=0.03).

b) Pain in the past month

I then investigated the effect of reporting pain in the past month on PSQI score. In unadjusted analyses, reporting pain in the past month was associated with higher PSQI scores (β=2.03; p<0.01). This was still true when adjusting for age and sex (β=2.04; p=<0.01) then time (β=1.85; p=0.02), with age, sex and time being non-significant covariates. Increased rating of pain in the past month was associated with increased PSQI scores (β=0.27; p=0.02) in unadjusted and adjusted analysis (age and sex), then lost significance when adjusting for time (p=0.07). I also investigated these effects on the likelihood of reporting PSQI >5 in adjusted and unadjusted analyses, and found that reporting pain and having an increased rating of pain were associated with a higher likelihood of reporting a PSQI>5.

Panel A)



Panel B)

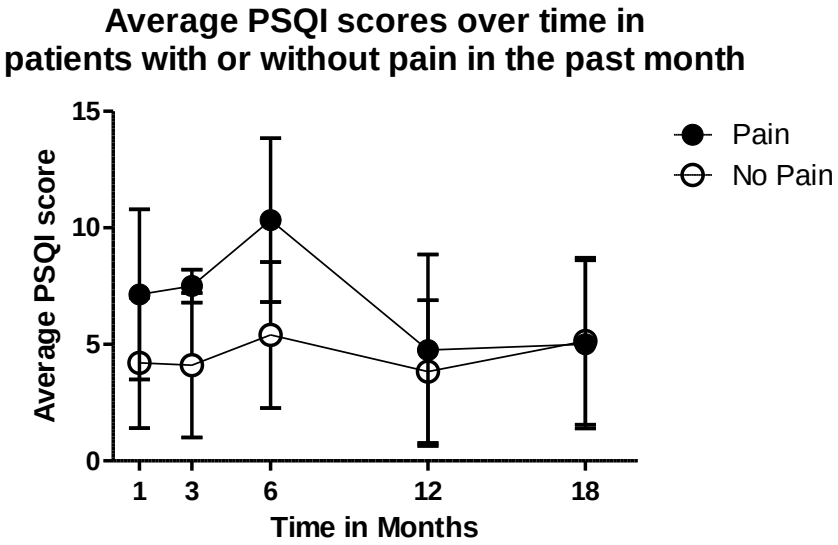


FIGURE 3.10: Mean ±SD of PSQI scores in patients with or without pain. **Panel A)** Patients with or without pain at the time of the interview, and **Panel B)** Patients with or without pain in the past month.

Effects of depression on daytime sleepiness

In the unadjusted and adjusted (age and sex, then categorical time) analyses, there was no significant effect of BDI on ESS. Using time as a continuous variable, I modelled the effect of BDI, time, and an interaction between BDI and time, and found that when adjusting for time, increased

BDI scores were associated with higher ESS scores (β=0.14; p=0.047). There was also a significant BDI by time interaction (β= -0.014, p=0.02).

Effects of pain on daytime sleepiness

a) Pain at the time of the visit

In unadjusted and adjusted (for age and sex, then time) analyses, I found that there was no significant effect of both reporting pain at the time of the visit, or the rating of pain at the time of the visit, on ESS score.

b) Pain in the past month

In unadjusted and adjusted (for age and sex, then time) analyses, I found that there was no significant effect of both reporting pain in the past month, or the rating of pain in the past month on ESS score.

Depression and pain effects on sleep apnoea

Depression effects on reporting high risk of sleep apnoea on the Berlin Questionnaire (BQ).

When testing for the effects of BDI, pain at the time of the visit, rating of pain at the time of the visit, pain in the past month and rating of pain in the past month in the unadjusted and adjusted (for age and sex, then categorical time) analyses, there was no significant effect of BDI on BQ.

3.6.3 Relationship between BMI and risk of sleep apnoea

BMI is a known risk factor for developing sleep apnoea in non-HIV positive patients. I saw a trend effect of time on BMI whereby participants started off in the overweight category of BMI (>25kg/m2), and increased over time. BMI had a non-significant increase over time (as seen in 3.2), so I modelled the effect of time, BMI and an interaction between BMI and time, on BQ. BMI came out as a predictor of BQ, whereby an increase by one point in BMI lead to a 2.54 odds of reporting high risk for sleep apnoea (p<0.01; 95% Wald CI = 1.9 – 5.01). An increase in time by one-month lead to a 3.4 odds of reporting high risk for sleep apnoea (p=0.02; 95% CI: 1.2-8.2).

The interaction between time and BMI had an effect, whereby if you increase BMI across time, there was a 0.95 odds of reporting high risk for sleep apnoea (p=0.02; 95% CI: 0.92-1).

3.6.4 Multivariate predictors of non-communicable diseases

The last specific aim was to investigate whether any of the sleep assessments could predict higher risk of developing cardiometabolic disorders. The outcome variables were BMI, mean arterial pressure and hypertensive status across the study .In addition, anthropometric measurements, such as BMI, are known to influence mean arterial pressure, and thus I also assessed the effect of BMI on mean arterial pressure. First, I modelled BMI and time as separate variables, with MAP as the outcome variable, and found that there was a main effect of time on MAP whereby for every one month increase in time, MAP increased by 0.65mmHg, when adjusting for BMI ($p<0.05$).

Multivariate predictors of body mass index

I assessed the effects of sleep quality; daytime sleepiness and reporting high risk for sleep apnoea on the Berlin Questionnaire on body mass index. I inserted each factor in a model that systematically included age, sex, and time. I found that there was no significant effect on body mass index by sleep quality (p=0.09), daytime sleepiness, (p=0.87) and reporting high risk for sleep apnoea on the Berlin Questionnaire (p=0.95). Age did not have any significant effect on BMI (p=0.95). The only factors that remained significant predictors of body mass index were time ($\beta=0.2$; $p<0.01$) and being female ($\beta=6.2$; p=0.01). Thus, the only factors that influenced BMI in a mixed model, was time elapsed since starting antiretroviral treatment and being female.

Multivariate predictors of mean arterial pressure

I assessed the effects of sleep quality, daytime sleepiness and reporting high risk for sleep apnoea on the Berlin Questionnaire on mean arterial pressure. I inserted each factor in a model that systematically included age, gender, and time. I found that there was no significant effect on mean arterial pressure of daytime sleepiness (p=0.93), reporting high risk for sleep apnoea on the Berlin Questionnaire (p=0.69) and found a non-significant trend of sleep quality (p=0.08). The only

factors that remained significant predictors of mean arterial pressure were age and BMI, whereby with an increase of 1 year in age, there was an increase of 0.668 mm Hg in MAP ($p<0.01$), and an increase of 1kg/m^2 in BMI caused a 0.77 mm Hg increase in MAP ($p<0.05$).

CHAPTER 4 - DISCUSSION

4.1. Summary of study design and findings

This study was intended to be a 6-month protocol, but turned out to be an 18-month protocol. Study participants were enrolled and maintained across time, answering questions whenever they came into the clinic.

This study was a follow up from a previous cross sectional study, where I had found a significant relationship between increased CD4+ T lymphocyte counts and increased sleep disruption (Redman, 2013). The aims were to determine whether or not sleep disruption was present in patients with HIV before ARV treatment, to track the evolution and development of sleep disruption from start of ARV treatment to at least 6 months of treatment, and isolate factors associated with sleep disruption, in particular those related to immune reconstitution (i.e. CD4 increase under HAART).

In this longitudinal study, I used three measurements of sleep: sleep quality, as per the Pittsburgh Sleep Quality Index (PSQI); daytime sleepiness, as per the Epworth Sleepiness Scale (ESS), and the risk of sleep apnoea using the Berlin Questionnaire.

To prove treatment efficacy, although it wasn't one of the specific aims, I first assessed the evolution of HIV disease markers across time on treatment. There was an increase in CD4+ T lymphocyte counts and a decrease in viral loads across time. Next, to satisfy the first specific aim of monitoring the evolution of the sleep variables (sleep quality, daytime sleepiness, and risk of sleep apnoea) across time, I looked at the effects of time on these variables. I found that sleep quality improved across time when adjusting for depression. I found that there was an effect of time on daytime sleepiness, and that the risk of sleep apnoea as per the Berlin Questionnaire did not vary across time.

The second specific aim was to assess the evolution across time of known factors that influence sleep in treated HIV cohorts. This included the presence and rating of pain at the time of the visit, and in the month preceding the visit, and depression severity (as per the Beck's Depression

Inventory). I found that there was no change in the prevalence or the rating of pain at the time of the visit across the study. For pain in the month preceding the visit, I found that there was no change across time, but that there was a decrease in the rating of pain (when pain was present) across time. Depression scores improved with time.

The third specific aim was to investigate how known markers of immune status (CD4+ T lymphocyte counts and viral load levels) affected sleep variables. Although I found that there was no effect of CD4+ T lymphocyte counts or viral loads on sleep quality, or of CD4+ T lymphocyte counts on daytime sleepiness, I did find that there was an effect of viral loads on daytime sleepiness whereby patients with low viral loads in the pre-ART stage had more daytime sleepiness than patients with high viral loads. I also found that low CD4+ T lymphocyte counts were associated with a decreased risk of sleep apnoea, and viral loads had no effect on sleep apnoea risk.

The fourth specific aim was to assess the effects that sleep variables had on the anthropometric measurements I took – body mass index (BMI) and mean arterial pressure (MAP). Although I didn't find a direct effect of sleep variables on those measurements, I did find that BMI increased over time and that this was associated with a higher risk of sleep apnoea. This increase in BMI was also associated with age and sex (being female). Finally, BMI increase predicted MAP increase across time.

4.2. Study Limitations

The high loss to follow up has been seen in various other studies conducted in Johannesburg – results of which are still on-going in our school and will be addressed at a later stage as to why. On more than one occasion, when contacting a person, I would just be told "this is too much [work] for me" and "I don't want to do this anymore, it's too much" and they would be withdrawn from the study. This high loss to follow up caused a final small sample size, which could be the reason that some of the results were "trends" rather than absolute significance. A retrospective study by Grimsrud et al. (2015) found that in a treated HIV cohort, people starting ARVs at CD4+ T lymphocyte counts less than 100 and greater than 300 cells/μL were more likely to be lost to follow

up over a 24 month period than people with CD4+ T lymphocyte counts from 150-199cells/ μL. In my study, 43% of the people initiated at baseline, and 46% of people enrolled in the follow up study fell into the category of either CD4 less than 100 cells/μL, or more than 300cells/μL (Grimsrud et al., 2015).

All of my questionnaires were conducted in English, which is not the first language of most of the people included in the study. Although there were people available to translate for participants when required, it is possible that the message in the questionnaires may have been misinterpreted. However I've shown that the PSQI, ESS and BDI had good internal consistency and construct validity when tested in my cross sectional pilot study and therefore this effect should be limited. There were also more women in my study, and as discussed, women were less likely to be employed than men – so it is possible that men were working instead of coming to the clinic and/or volunteering for the study. There was also only one person collecting data, at different locations and it was not possible to be in multiple places at once. More people recruiting may have allowed for increased subject retention, and more people in the final analyses would have given increased power, and thus given more significance to the trends in relationships seen above.

That being said, even with the low numbers, I was still able to analyse my data and come up with the results which will be discussed below and answer the afore mentioned specific aims.

4.3. Factors that influence sleep and the changes across time

4.3.1. CD4+ T lymphocyte count and viral load change across time

Our analysed cohort was on average 34 years old, mostly female, with an average CD4+ T lymphocyte count <250 cells/μL, and average log viral load of >4 (which is a viral load of >10 000 copies/mL) at the beginning of the study. Across in time in the study (and therefore on ARV treatment), as expected in patients with HIV who are taking their ARV treatment, there was an increase in CD4+ T lymphocyte counts, and a decrease in viral loads. Although this was not one of the specific aims, this proved that my participants observed their treatment and that the treatment was effective. CD4+ T lymphocyte counts are a measure of overall immune status and higher counts equate to better disease outcomes (Johnson et al., 2013). With the change in WHO

guidelines over the course of the study (from 2013 to 2015) with regards to CD4+ T lymphocyte counts and ARV administration, HIV positive persons were administered ARVs at higher levels of CD4+ T lymphocyte count, as compared to patients having to wait until getting to counts below 250 cells/μL before qualifying to receive government sponsored ARVs (WHO, 2015).

Three participants enrolled in the study had been initiated onto ARVs in the late 2000s, when the WHO guidelines applied in South Africa were still only allowing ARVs to patients with CD4+ T lymphocyte counts<250cells/μl. Those three participants were enrolled onto regimen 2 after becoming resistant to a previous line of medication, or developing problems with the ARVs regimen they were on. Only one of these three was met the 3-visit criteria to be included in the final data analysis. For those participants who started ARVs in 2013, the guidelines of receiving treatment had changed to starting treatment when CD4+ T lymphocyte counts went lower than 350cells/μL. In 2015, the guidelines were further changed to initiation at <500cellsμl, and at the time of writing up, ARV treatment is to be given to all those who test positive for HIV (WHO, 2015). There is an increased chance of treatment efficacy and favourable disease outcome when treatment is started at higher CD4+ T lymphocyte counts (Johnson et al., 2013). The initiation of people onto ARVs sooner will positively impact the lives of persons living with HIV. The reduction in viral loads over time is also a marker of treatment efficacy. By the end of the study, all of the 23 patients had reverted to the “low viral load” group of log viral load <2 (viral load <100 copies/mL). With high CD4+ T lymphocyte counts and low viral load, the clinic is ensuring that these participants are managing their disease well.

4.3.2. Evolution of known predictors of sleep disruption: depression and pain

The third specific aim was to assess the evolution of known predictors of sleep quality: depression and pain. Analyses revealed that there was a decrease in depression in the first 3 months of treatment but after that it levelled off. Depression was found in other HIV cohorts, and in this current study, the overall depression (BDI score) at the beginning of the study was 11.07 (±4.07) which is indicative of a mood disorder.

In tying with the third specific aim, I measured the prevalence of pain in this cohort, in the hopes that I would be able to track the incidence of pain across time. In the beginning of the study, 57.8% of participants reported pain at the time of the visit with a mean rating of pain of 6.6 (±2.2), and 36.8% of participants reported pain in the past month with a mean pain rating of 5.4(±2.6). I found that there was not an increase in the presence of pain (at the time of the interview or in the past month) across time. I did however find that when pain was present in the past month, the rating of that pain across time in the study decreased. In untreated HIV, with the decline in health there is an increase in pain. In treated HIV, the prevalence of pain is 54-83% in some studies (Parker, 2014). This current study used a 10-point likert scale, which is different to the 11-point likert scale used in the Mphahlele et al. papers. This 10-point scale is the same scale used in the pilot study, and in the pilot study, there were higher levels of pain at the time of the visit. When pain was present at the time of the interviw, or pain in the past month, the rating of pain this pain was moderate. Pain has a negative impact on daytime performance, on a person's quality of life, and later on in this chapter, we'll explore the impact that pain has on the sleep variables tested in this study: sleep quality, daytime sleepiness, and the risk of sleep apnoea.

4.4. Changes in sleep variables across time and factors associated with these changes

The next three sections will discuss the evolution of the sleep variables across time (sleep quality, daytime sleepiness, and risk of sleep apnoea on the Berlin Questionnaire). In each of the sections, I will discuss how the sleep variables evolved across time, and factors that influenced these sleep variables. The factors discussed are the traditional factors which influence sleep – such as depression and pain, as well as disease related factors that came up as predictors in the pilot study (like CD4+ T lymphocyte counts) and viral loads.

4.4.1. Sleep quality in treated HIV positive patients and changes across time

Poor sleep quality is well documented in untreated (Norman et al., 1990; Wiegand et al., 1991; Wheatley and Smith 1994; Darko et al., 1995) and in treated HIV cohorts (Kunisaki et al., 2015, Lee et al., 2001, Nokes and Kendrew, 1996, Nokes and Kendrew, 2001, Taibi, 2013) and this has

been found to be related to fatigue (Barroso et al., 2015, Corless et al., 2008, Lee et al., 1999, Payne et al., 2013, Pence et al., 2008), daytime sleepiness (Lee et al., 2001, Nokes and Kendrew, 2001, Salahuddin et al., 2009, Wibbeler et al., 2012), depression (Low et al., 2012, Nokes and Kendrew, 2001, Wibbeler et al., 2012), and treatment (Gallego et al., 2004, Lee et al., 2014, Poupard et al., 2007). At baseline, and across the study, subjective sleep disruption assessed by global PSQI score was not very high.

As compared to other studies, the average PSQI of 6.04 (±3.25) at baseline in my study was lower: Salahuddin et al. (2009) had an average PSQI of 9.4; Wibbeler et al. (2012) had an average PSQI score of 8.0 (±4.4); Lee et al. (2012) had an average PSQI score of 7.4(±3.7); and Wang et al. (2014) in China had an average PSQI of 11.97(±4.75). Using subscales of the PSQI, average sleep efficiency was overall below 90%, which is similar to that found in age-and sex-matched healthy individuals. However I did not have an age- and sex- matched control group to contrast the sleep efficiency found in my HIV patients. Lower sleep efficiency has been found in HIV patients in previous studies where on average, HIV positive people slept less than age-sex matched controls (Wibbeler et al., 2012). I also investigated self-reported sleep onset latency – time taken to fall asleep – from the PSQI subscale, the norm of which is between 15 and 20 minutes. I found that there was an increased sleep onset latency (about 30 minutes) in this cohort. This is comparable to previous studies (Clark et al., 2005, Gallego et al., 2004, Gay et al., 2015, Wiegand et al., 1991) which found increased sleep onset latencies not only using questionnaires but also actigraphy and polysomnography.

Effect of treatment with efavirenz on sleep quality

Although all my participants were on efavirenz (and therefore I could not compare patients with efavirenz to those not taking efavirenz), the fact that I found no difference in sleep quality in the first month of treatment is interesting. Indeed, studies performed in the early 2000s had found that efavirenz causes sleep disturbances in the first month of treatment (Clark, 2005; Moyle, 2006). The PSQI assesses sleep quality in the past month, so the 2-4 week period between the pre ARV visit and one month on ARVs may have been too short to detect the sleep issues previously

reported in the first few weeks of taking efavirenz (Gallego et al., 2004). Another possibility is that indeed efavirenz does not cause as much sleep disturbances as originally thought, especially after a few years on treatment. Indeed, more recent studies have shown that treatment with efavirenz had either no effect on sleep quality (Crum-Cianflone et al., 2012) or that treatment with efavirenz has a protective effect against sleep disruption (Allavena et al., 2016) in patients treated for four years or more.

Effects of CD4+ T lymphocyte count on sleep quality

In my pilot study I found a significant effect of CD4+ T lymphocyte counts on sleep quality whereby increased CD4+ T lymphocyte counts were associated with higher levels of sleep disruption (Redman, 2013). In this study, I found no effects of CD4+ T lymphocyte counts on PSQI score. In the pilot study, mean PSQI scores were 8.20 (± 4.91) and in this study, PSQI scores at baseline were a lot lower at 6.04 (±3.25) – indicating mild sleep disruption. In the pilot study, at baseline, 71% of people had CD4+ T lymphocyte counts <250cells/μL and in my current study, this was true for 48% of the cohort.

In the pilot study, study participants had been on ARVs for an average of 4.5 years at the time of the study (2012). This dates back to when qualifying for government subsidised ARVs, CD4+ T lymphocyte counts had to be very low (less than 200 cells/μL) – as per WHO guidelines at the time of initiation (2008). Thus CD4+ T lymphocyte counts would have been very low at the time of initiation in that cohort. In contrast, when I started my masters data collection, the threshold of qualification of ARVs was a CD4+ T lymphocyte count of less than 350cells/μL and by the end of the study the guidelines were changed to whosoever tested positive for HIV (test-to-treat method) (WHO, 2015), allowing people to receive treatment at much higher baseline CD4+ T lymphocyte counts.

An immune reconstitution inflammatory syndrome (IRIS) has been described in HIV patients starting ARV treatment: it is due to the hyperactivation of CD4 T-cells leading to a chronic pro-inflammatory state. The risk of developing IRIS increases when HAART is initiated at low CD4+ T lymphocyte counts (Murdoch et al., 2009). In a study by Murdoch et al (2009), patients who had

developed IRIS had started HAART at significantly lower CD4+ T lymphocyte counts (<100/ μL) compared to those who never developed IRIS (Murdoch et al., 2009) suggesting that patients starting at low CD4+ T lymphocyte counts would subsequently be in a chronic pro-inflammatory state. An indeed, in treated HIV patients who develop IRIS, there is increased CD4 activation, shown by increased expression of known CD4 activation markers, such as Ki-67, PD-1, HLA-DR, and CD38 (Antonelli et al., 2010).

As mentioned earlier, the average at baseline (when being initiated onto HAART) CD4+ T lymphocyte counts in this current cohort was higher than 200/μL while in my pilot study, patients had been initiated onto HAART at CD4+ T lymphocyte counts <100/μL. As described in Chapter 1, HIV preferentially infects CD4 memory T-cells, and establishes a viral reservoir in these cells (Douek et al., 2002). Earlier treatment with HAART (at higher CD4+ T lymphocyte counts) has been shown to reduce the viral reservoir, so that when patients reconstitute their CD4 pool under ARV, there are less infected CD4 memory T-cells to become hyperactivated (Group, 2015, Okulicz et al., 2015).

Since IRIS mainly develops when patients are initiated at low CD4+ T lymphocyte counts, the fact that the CD4+ T lymphocyte counts were above 200/μL in this cohort may have protected against IRIS. As shown in Chapter 1, increased activation of the immune system is associated with increased sleep disturbances. Thus lower immune reconstitution inflammatory syndrome would be expected to result in lower sleep disruption, as seen in my patients. It is also possible that PSQI might not be as sensitive a tool for detecting the effects that immune reconstitution may have had in this cohort, and other measurements of sleep will have to be investigated.

Effects of viral load on sleep quality

I systematically tested the effects of viral loads on sleep quality, and found no significant effect of viral load on sleep quality. In a study conducted in China by Wang in 2014, it was found that there was an association between the viral protein, transactivator of transcription (Tat) and PSQI scores whereby increased levels in Tat (a reflection of higher viral loads as well) were associated with decreased PSQI scores (Wang et al., 2014). It appears as though there is almost a protective

effect of high viral load from sleep disruption whereby sleep disruption is lessened with an increased viral load. I did not find any relationship between PSQI and viral loads. However I did find that higher daytime sleepiness was associated with lower viral loads, as discussed below.

Effects of depression and pain on sleep quality

Depression in HIV is well documented (Junqueira et al., 2008, Penzak et al., 2000, Poudel-Tandukar et al., 2014), and depression is known to affect sleep (Kupfer et al., 1984). In Chapter 1, I reviewed studies that showed how depression and pain were predictors of sleep disturbances in patients living with HIV. In my pilot study, I had also found that there was a significant correlation between depression and sleep quality and daytime sleepiness, as well as a significant effect of pain on sleep quality (Redman, 2013).

Effects of depression on sleep quality

When assessing the relationship between depression and sleep quality, when adjusting for depression, sleep quality improved with time. Disrupted sleep increases the risk of depression (Irwin et al., 2013), and depression increases the chances of reporting sleep disruption (Nutt et al., 2008), or problems with sleep. Depression is a well-known complaint in HIV cohorts, so it follows that sleep disruption would be associated with increased depression. The reported relationship between depression and sleep quality in this cohort ties in well with other studies that have found depression impacting sleep quality (Crum-Cianflone et al., 2012, Salahuddin et al., 2009, Wibbeler et al., 2012). Sleep and depression have a bidirectional relationship, and if sleep is treated, then the depression may improve. All my participants were taking efavirenz, which has known neuropsychiatric effects and could impact in depression as is shown in other studies. Post hoc analyses revealed that the greatest difference in the BDI scores were between time=1 and time=3. A study done by Clifford et al. in 2005 revealed that in some cases, the neuropsychological effects associated with treatment with efavirenz only go away after one month on treatment (Clifford et al., 2005). Which may have been the case in my group whereby there was a difference between time=1 and time=3. Even though it is not clear in the data as depression levelled off for the duration of the study – but still remained higher than normal (>10 on the BDI).

Effects of pain on sleep quality

The presence of pain at the time of the interview and in the past month increased sleep disruption, as expected, as did the rating of the pain at the time of the interview and in the past month. The presence of pain at the time of the interview and in the past month also increased the odds of reporting sleep disruption (PSQI>5), as did the rating of pain at the time of the interview, but not pain in the past month.

This is true in non-HIV cohorts as well, whereby when decreased sleep quality is associated with increased pain sensitivity (Busch et al., 2012, Chhangani et al., 2009, Schuh-Hofer et al., 2013). Disrupted sleep has been shown to increase mechanical pain sensitivity both peripherally and centrally (Onen et al., 2001), and induce generalized hyperalgesia (Schuh-Hofer et al., 2013) and increased sensitivity to temperature-induced pain (Kundermann et al., 2004). Conversely the presence of pain, regardless of the severity of the pain, affects sleep quality. Pain in HIV cohorts is well documented (Namisango et al., 2012, Vosvick et al., 2004), and this is true in South African cohorts as well (Mphahlele et al., 2012, Wadley et al., 2011), as seen in Chapter 1. The impact of pain on sleep quality in this cohort was maintained across all time points. Subjects were asked to rate the severity of their pain, and when testing rating of pain and presence of pain in the same model, pain severity lost significance. I also found that some participants who had not previously reported pain, had started reporting pain towards the end of the study. This could mean that there is a window period of pain development while on treatment. This was not tested, and was just an observation as the numbers of people who reported pain remained relatively the same across all time points. The study also aimed to assess sleep quality, and pain was one of the covariates, not an outcome variable. A study investigating the relationship purely between the evolution of pain and sleep in this cohort will have to be conducted in future. As in other HIV cohorts, pain is well documented, and in my study, it was a predictor of worse sleep quality.

4.4.2. Daytime sleepiness and changes across time

The second measurement of sleep used was the Epworth Sleepiness Scale, to measure daytime sleepiness. I found high daytime sleepiness in the patients at ARV start. Cross-sectional studies

have found that daytime sleepiness is present in HIV-positive cohorts (Beccuti and Pannain, 2011, Lee et al., 2012, Patil et al., 2014, Salahuddin et al., 2009, Wibbeler et al., 2012), including at ARV start (Salahuddin et al., 2009). In my study, I found that daytime sleepiness decreased over time in this cohort. Below, I discuss the factors I found associated with daytime sleepiness.

Effects of CD4+ T lymphocyte count and viral load on daytime sleepiness

Because I found an association between increased CD4+ T lymphocyte counts and worse sleep quality in my pilot study, I systematically tested the effects of CD4+ T lymphocyte counts and viral loads on all sleep variables, not just PSQI score. I found no significant effect of CD4+ T lymphocyte counts on daytime sleepiness in both unadjusted and adjusted analyses. However, I did find that when adjusting for time, there was a significant effect of viral load on ESS score whereby patients with low viral loads overall had higher levels of daytime sleepiness as compared to those with high viral loads. When adjusting for time I found that there was an even more pronounced difference in daytime sleepiness between the high viral load and the low viral group, whereby there was a greater level of daytime sleepiness across time in the low viral load group – even more so than just explained by the group effect. This is, as far as I know, a novel finding.

I hypothesize that at the first month of treatment in the low viral load group, there is a high amount of HIV suppression by the immune system. With that immune activity, there is a high amount of cytokines with sleep effects such as TNFα and IL-6 (Darko et al., 1995), which are released during HIV infection. As shown in Chapter 1, elevation in these cytokines has been associated with increased daytime sleepiness (Vgontzas et al., 1997). In a recent study by Espindola et al. (2016), levels of cytokines such as TNFα, IL-1β, IL-6, and IFN-γ in untreated HIV patients were higher than in treated patients (Espíndola et al., 2015). Prior to taking ARVs, there is more immune activity in an attempt to clear the virus, as shown by the far lower cytokine levels in treated HIV patients (Espíndola et al., 2015). The Espindola et al. study did not have any sleep measurements. I hypothesize that when taking ARVs, the need for the immune system to be as active as it was without ARVs is lessened, thus the release of cytokines that have an effect on sleep may be lower

– creating the lower daytime sleepiness across time on treatment. In participants who had high viral loads at the beginning of the study, as compared to those with low viral loads, they had lower daytime sleepiness thus the immune response must not have been as high. In order to investigate this, blood plasma samples will need to be assessed to see which cytokines are upregulated at different time points, and how they relate to daytime sleepiness at the time of the interview.

Effects of depression and pain on daytime sleepiness

After systematically testing the effects of depression and pain on sleep quality, I tested their effects on daytime sleepiness as well. I found that that there was an effect of depression on daytime sleepiness when adjusting for time, whereby though there was a decrease in daytime sleepiness over time, in the presence of depression, daytime sleepiness increased. This is in congruence with previous studies in HIV negative cohorts which have found an increased daytime sleepiness in the presence of depression (Claghorn et al., 1981). Conversely, patients with excessive daytime sleepiness usually associated with a positive diagnosis for a mood disorder (Bixler et al., 2005, Ohayon and Roth, 2003). In a treated HIV cohort, we've already established that depression is an issue. If the depression is treated, there may be a lessening of daytime sleepiness.

4.4.3. Risk of sleep apnoea in treated HIV positive patients over time

Throughout the study, about 20% of my patients scored high risk of sleep apnoea. There was no change in the risk of sleep apnoea over time. To my knowledge, there is no study that assesses the risk of sleep apnoea across time in treated HIV-positive people in South Africa,. Sleep apnoea, along with obesity and metabolic syndrome, has been considered a pro-inflammatory state (Alam, 2004). Being on HAART alters the immune profile, and as seen above, increases BMI across time. And thus I tested other variables' effects on the risk of sleep apnoea across time, and will be discussed further on in this section. The first specific aim was to assess the evolution of sleep variables across time, and in the risk of sleep apnoea, there was no change across time.

Effects of CD4+ T lymphocyte count and viral load on being high risk for sleep apnoea

I found that the increase in CD4+ T lymphocyte counts across time, when adjusting for time, was significantly associated with a decrease in being at high risk for sleep apnoea but I found no effect of viral loads risk for sleep apnoea. Upon researching the association between CD4+ T lymphocyte counts and obstructive sleep apnoea (OSA), there is no known relationship between CD4+ T lymphocyte counts in HIV and obstructive sleep apnoea – hence this finding is novel. At best, this finding shows that there is a marginally decreased risk of sleep apnoea as CD4+ T lymphocyte counts were reconstituting over time. However I also found that my HIV-positive patients gained weight while on treatment. BMI increased over time in this cohort – especially in females, and is seen as an independent risk factor for developing sleep apnoea. I attempted to assess which factor – either CD4+ T lymphocyte count or BMI score, affected sleep apnoea by inserting them into a multivariate linear regression model, but found that these two factors were collinear and could not be placed in the same model. Independently, however I found that BMI predicted increased risk of sleep apnoea over time.

The current knowledge is that with increased inflammation, or if a person is in a pro-inflammatory state such as obesity or metabolic syndrome, there is an increased risk of having sleep apnoea (Alam et al., 2007). Cytokines associated with this increased risk of sleep apnoea are both IL-6 and TNFα (Huiguo et al., 2000). IL-6 and TNFα also have important functions for lipid and glucose metabolism (Ciftci et al., 2004) on top of being pro-inflammatory and sleep modulatory cytokines. As seen in Chapter 1, elevated TNFα and IL-6 are associated with increased sleep disturbances, and in patients with sleep apnoea have been associated with increased neck circumference and more apnoeas or hypopnoeas during sleep (Ciftci et al., 2004). Finally, previous studies have shown relationships between increased levels of cytokines and an increased risk of sleep apnoea in an HIV positive cohort (Entzian et al., 1996, Vgontzas et al., 2005).

The fact that there were collinear effects between BMI and CD4+ T lymphocyte counts may suggest that there may be a decreased inflammatory reaction occurring while on treatment: as

seen in Espindola et al 2015, the HIV–associated immune response decreases as viral load decreases under ARVs. Increased CD4+ T lymphocyte counts over time in this case would therefore also follow the decreased inflammatory response (Espíndola et al., 2015). The decreased inflammatory response may mitigate the increase in BMI in the first year after ARV initiation on risk of sleep apnoea, resulting in the overall stable risk of having sleep apnoea over time. Further studies should further investigate risk of sleep apnoea / presence of sleep apnoea in relation to CD4+ T lymphocyte counts and underlying immune activation (while measuring cytokine concentrations) to better explain this relationship.

Effects of depression and pain on sleep apnoea

I found no relationship between depression and the risk of sleep apnoea or between pain and the risk of sleep apnoea in this cohort. There is no known definitive mechanism linking depression and the risk of sleep apnoea, but a review article done by Ejaz et al. in 2011 stated that although the mechanism is poorly understood, there is a relationship between sleep apnoea and depression. In most patients with obstructive sleep apnoea, depression is a common complaint, and in patients with depression, there is a higher prevalence of obstructive sleep apnoea (Ejaz et al., 2011). It is not known which develops first – depression or sleep apnoea, but in treated HIV cohorts, depression may have to be monitored as a risk factor for the development of sleep apnoea.

4.5. Impact of sleep disruption in HIV patients on risk of developing cardiovascular disorders and metabolic dysfunction across time

The final specific aim was to assess how sleep variables influence the development of basic anthropometric measurements body mass index (BMI) and mean arterial pressure (MAP) both markers of increased risk of cardiovascular diseases and metabolic dysfunction .

4.5.1. Body Mass Index

At the beginning of the study, the average BMI was greater than 25kg/m^2, indicating that the cohort before starting ARV treatment was already in the “overweight” class of the body mass index scale. This study supports recent studies that have found average BMI in HIV positive people

greater than 25kg/m2 (Crum-Cianflone et al., 2012, Kunisaki et al., 2015). There was a significant increase in overall body mass index (BMI) over time, whereby for each one month on treatment, there was an increase in BMI. Upon further analysis, it was found that this increase was true for both males and females, but that females had on average higher BMI scores than males throughout the entire study. my population consisted largely of women of African ancestry. A recent study conducted in South Africa found that 56.6% of women in this country were overweight as compared to 29.2% in males. The average BMI in women was 27.1kg/m^2 and 22.9kg/m^2 in men (Puoane et al., 2002). This dichotomy is mirrored in my study, and is maintained throughout the course of the study.

Our study is in agreement with the finding that there is an increase in BMI after the initiation of ARVs (Pullinger et al., 2010). The Pullinger study in 2010 found that there was an increased risk of developing metabolic syndrome when viral loads were suppressed. As mentioned above, viral loads were suppressed and there was an increase in BMI across time, so there may be an increased risk for developing metabolic syndrome. However, I did not investigate other markers of metabolic syndrome, such as the development of diabetes (Pullinger et al., 2010).

No sleep variables affected body mass index, which is contrary to other studies which have shown relationships between poor sleep quality and increased weight gain. This study did not start off with a high level of sleep disruption, and that did not fluctuate much over the course of the study. The effect of being female was significantly associated with an increase in BMI. Two patients, 13HS022 and 13HS017 who started treatment and then had an increase in weight: 10.5kgs and 12.1kgs respectively from time=1 and time=18 months, increasing their BMI and thus increasing their risk of sleep apnoea. I found that when BMI increased across time, there were even greater odds of reporting high risk for sleep apnoea on the Berlin Questionnaire. According to analyses, significant predictors of BMI in this cohort was gender and time, and as described previously, females carried the greatest change in BMI across time. my sample population had started out with BMIs that put them in the “overweight” category (BMI>25kg/m^2), and by the end of the study their BMIs had increased.

4.5.2. Mean Arterial Pressure and hypertensive status

I found a non-significant effect of time on mean arterial pressure. There was also a non-significant increase of hypertensives from 32% in the first month to 60% prevalence at the end of the study. Both older age and higher BMI predicted higher mean arterial pressure, which is in accordance with other studies such as (Pinto, 2007). None of the sleep variables (PSQI, ESS or Berlin Questionnaire) were significant predictors of sleep apnoea. However, BMI affected mean arterial pressure, and BMI was shown to increase across time. Scoring high risk of sleep apnoea also increased with higher BMI. Sleep apnoea is a known independent risk factor for hypertension due to the hypoxia-induced over-activation of the sympathetic system (Kario, 2009). This suggests that with the increase in BMI across time, there could also be an increase in MAP, both through a direct effect of BMI on MAP and an indirect effect through the increase in sleep apnoea. However I may not have had the power to show this relationship in my cohort.

4.6. Conclusion

To summarize, this has been one of the first studies assessing the relationship between sleep and HIV in sub-Saharan-Africa in a longitudinal setting. I have shown treatment efficacy and patient adherence throughout the course of the study, by the increase in CD4+ T lymphocyte counts and decrease in viral loads. I've also shown that before starting treatment, HIV patients had mild sleep disruption and that the level of sleep disruption as measured by the PSQI did not change much over the course of the study, but when adjusting for other factors such as depression, sleep quality improved throughout the study. I have shown that daytime sleepiness was high initially and improved across time, and that depression was also associated with higher daytime sleepiness. This suggests that detecting and treating depression may be important to improve sleep quality and daytime sleepiness in those patients.

I also found that participants with low viral loads had higher daytime sleepiness compared to those with high viral load, which was unexpected as higher viral loads are associated with higher disease burden /fatigue. I hypothesized that it could be due to increased immune activation in

those with better viral load control, which could in itself be leading to an increased daytime sleepiness.

Several studies have shown that, even treated, HIV-infected patients were more likely to nap, feel drowsy and have decreased alertness during the day (Darko et al., 1992, Lee et al., 2009, Lee et al., 2001). As a result of this sleep disruption, HIV-infected workers are at risk of falling asleep at work, which is considered a form of misconduct in South African Labour Law. Therefore better controlling sleep quality and the daytime sleepiness in treated HIV patients may lead to an improved overall quality of life.

The risk for sleep apnoea did not change across time. However increased BMI was associated with higher risk of being at high risk of sleep apnoea while improved CD4+ T lymphocyte counts under treatment were protective against risk of sleep apnoea.

BMI and MAP increased over time, and combined, pose the greatest risk for metabolic syndrome.

In patients with HIV who are being treated, the risk of developing lipid disorders is high (Bernasconi et al., 2002, Lichtenstein et al., 2001). Even though the immunologicial status of patients in this cohort is improving, treatment is potentially increasing the risk of developing non-communicable diseases such as obesity and hypertension, thus increasing their risk of cardiovascular disease, independent of their sleep status. This study was conducted for a short period of time on a low number of people, and thus the finding should be treated with reserve. There is a concern, however, as I am already seeing significant metabolic changes this early on in treatment – especially because HIV positive people will have to be on treatment for the rest of their lives in order to adequately control viral replication and maintain their advantageous high CD4+ T lymphocyte counts.

4.7. Proposed further research

Further research will have to be done to assess the expression of cytokines associated with sleepiness (TNF-α, IL-1 β, IL-6) and the relation to viral loads, and daytime sleepiness of patients. The immune activation on treatment will have to be assessed, and possibly in relation to the

aforementioned sleep quality, daytime sleepiness and sleep apnoea questionnaires, to see if any relationships can be found. I also propose assessing markers of inflammation such as C-reactive protein and the aforementioned cytokines to assess whether or not inflammation has a role in the risk of sleep apnoea in this cohort. A longer longitudinal observational study would be better at assessing not only the risk of metabolic syndrome, but also track the development and incidence of factors associated with metabolic syndrome such as BMI (especially in females) in this cohort.

LIST OF APPENDICES

		Page number
APPENDIX 1	Ethics Clearance	108
APPENDIX 2	Ethics Amendment	109
APPENDIX 3	Sleep 2013 Abstract	110 - 111
APPENDIX 4	Information Document	112 - 114
APPENDIX 5	Consent Form	115
APPENDIX 6	Long General Information Questionnaire for first follow up visit	116 - 122
APPENDIX 7	Long General Information Questionnaire for subsequent visits	123 - 127
APPENDIX 8	Pittsburgh Sleep Quality Index	128
APPENDIX 9	Epworth Sleepiness Scale	129
APPENDIX 10	Berlin Questionnaire	130
APPENDIX 11	Morningness-Eveningness Questionnaire	131 - 132
APPENDIX 12	Beck's Depression Inventory	133 - 135
APPENDIX 13	Internal consistency for Pittsburgh Sleep Quality Index, Beck's Depression Inventory and Epworth Sleepiness Scale	136 - 141

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Dr Karine Scheuermaier

CLEARANCE CERTIFICATE	**M120411**
PROJECT	Sleep, Mood and Morningness-Eveningness Preference in Treated and Untreated HIV Patients
INVESTIGATORS	Dr Karine Scheuermaier.
DEPARTMENT	School of Physiology
DATE CONSIDERED	04/05/2012
+DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 17/05/2012 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor :

DECLARATION OF INVESTIGATOR(S)
To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

APPENDIX 2

Human Research Ethics Committee (Medical)
(formerly Committee for Research on Human Subjects (Medical)
Secretariat: Research Office, Room SH10005, 10th floor, Senate House • Telephone: +27 11 717-1234 • Fax: +27 11 339-5708
Private Bag 3, Wits 2050, South Africa

University of the Witwatersrand, Johannesburg

25 august 2013

Dr Karine Scheuermaier
Department of Physiology
Medical School
University

Sent by email to: Karine.scheuermaier@wits.ac.za

Dear Dr Scheuermaier

RE: **Protocol: M120411: 'Sleep, Mood and Morningness Eveningness in Treated and Untreated HIV Positive Patients'**
Adding Longitudinal CD4 Activaton/CD4 Regulatory T Cell Measurements Using Flow Cytometry and Objective Sleep Measurement

This letter serves to confirm that the Chairman of the Human Research Ethics Committee (Medical) has reviewed and approved the following amendments on the abovementioned protocol as detailed in your letter dated 27 August 2013:

- New study objectives 1-4 (as detailed in your letter)
- Modification of the longitudinal questionnaire (as detailed)
- Revised general information questionnaire (as detailed)
- Add the Berlin Questionnaire (as detailed)
- Add a follow-up general information questionnaire (as detailed)
- Add blood draw (as detailed)

Thank you for keeping us informed and updated.

Yours sincerely,

Anisa Keshav
Administrator
Human Research Ethics Committee (Medical)

TITLE: Increased CD4 Cell Counts and Pain Predict Higher Sleep Disruption in 152 South African HIV-infected Patients on Highly Active Antiretroviral Treatment .

AUTHORS (FIRST NAME, LAST NAME): Kirsten Redman[1], Alan Karstaedt[3, 2], Karine Scheuermaier[1]

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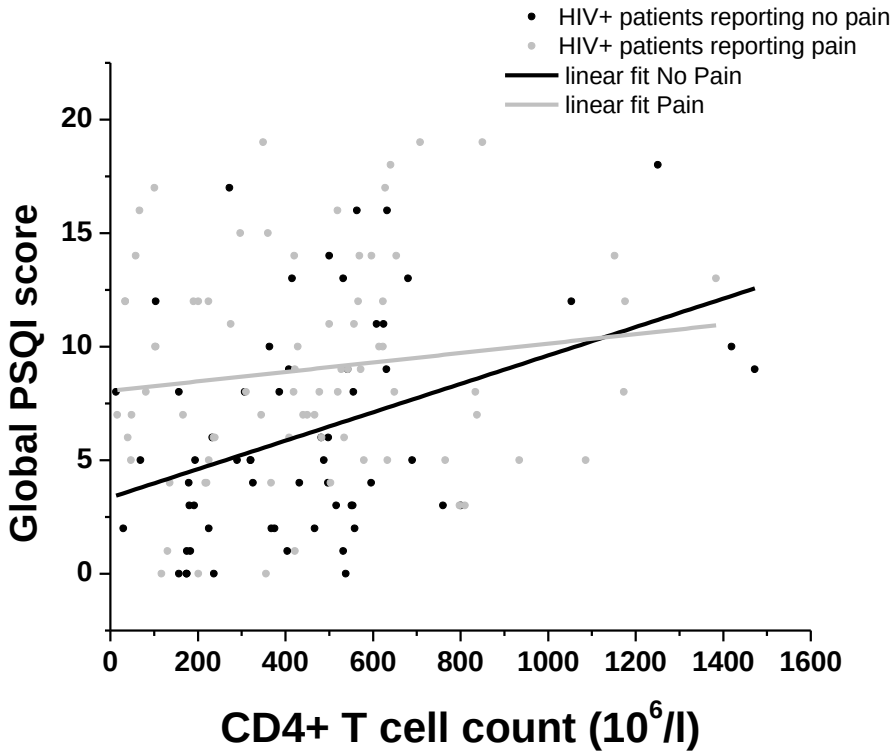
ABSTRACT BODY:

Introduction : Ten percent of the total South African population lives with chronic HIV infection. Highly Active Antiretroviral Treatment (HAART) has increased life expectancy of HIV-positive individuals to a near-normal level however little is known in South Africa on how treated and stabilized chronic HIV infection affects the patients' daytime functioning and sleep quality. This cross-sectional study investigates the prevalence and predictors of sleep disruption in treated South African HIV-positive patients.

Methods : 152 HIV-positive adult patients (average age ± SD= 43 ± 9; females = 120 (79%)) were recruited from Chris Hani Baragwanath Teaching Hospital in Soweto, Johannesburg, South Africa from May to August 2012. They were asked to fill in questionnaires assessing sleep quality (PSQI, Pittsburgh Sleep Quality Index), daytime sleepiness (ESS, Epworth Sleepiness Scale), depression (BDI, Beck Depression Inventory) and pain (from the Wisconsin Brief Pain Questionnaire). We also collected demographic and environmental information, baseline and current CD4 cell counts, viral load and treatment.

Results : Poor sleep quality (PSQI score>5) was reported by 69.7% of the patients. Average ± SD for PSQI score was 8.2 ± 4.9, for ESS: 8.0 ± 5.9 and for BDI: 17.4 ± 12.6. In univariate analyses, higher depression scores ($p=0.006$), longer time on treatment ($p=0.0086$), higher current CD4 counts ($p=0.0003$) and presence of pain ($p<0.0001$) were significantly associated with increased sleep disruption. Age and sex were not significant predictors. In a multivariate linear regression, the only remaining significant predictors of sleep disruption were CD4 counts ($p<0.0001$) and the presence of pain ($p=0.0003$), with increased CD4 counts and presence of pain being associated with a significant increase in sleep disruption. There was also an interaction between CD4 counts and presence of pain whereby the effect of pain on sleep disruption was lower with higher CD4 counts ($p=0.0075$).

Conclusion: We found a high prevalence of sleep disruption in this treated HIV population, which matches that found in other treated HIV cohorts in the USA. As expected, the presence of pain was a predictor of increased sleep disruption. However, more surprisingly, higher CD4 counts also predicted increased sleep disruption. Further studies should try and focus on the relationship between immune reconstitution, sleep and pain in treated HIV positive patients.



Appendix 3, Figure 1: Scatter plot and linear fit of the relationship between sleep disruption (as measured by the Global PSQI score) and CD4 counts in HIV positive patients with pain (grey dots and grey line) and those without pain (black dots and black line). There was a significant main effect of CD4 counts whereby increased CD4 counts were associated with increased PSQI scores ($p=0.0003$), a main effect of pain whereby patients with pain had increased sleep disruption than patients without pain ($p<0.0001$) and a pain-by-CD4 count interaction whereby patients without pain had a higher increase in sleep disruption as their CD4 counts increased than those with pain ($p=0.0075$).

INFORMATION DOCUMENT

Study title: Sleep, mood and morningness-eveningness preference in treated and untreated HIV positive patients

Greeting:

Introduction:
We, Kirsten Redman (MSc student), Dr Karine Scheuermaier and Dr Alan Karstaedt, are doing research on how HIV infection and its treatment affect your sleep, your mood and your activities during the day. Research is just the process to learn the answer to a question. In this study we want to learn if HIV itself, the impact it has on your CD4 counts, and the treatment of HIV have an effect on your sleep and your mood.

Invitation to participate: We are inviting you to take part in this research study because you attend the adult HIV clinic at Chris Hani Baragwanath hospital today.

What is involved in the study

In this study, we would like you to please fill out the questionnaires which we will give to you. These questionnaires will ask you detailed questions about your sleep and your general motivation to get things done during the day. They will also ask you when you feel at your best during the day and if you get sleepy during the day.

Because the following can have an effect on your sleep and mood, we will also ask you how long you have been taking your treatment (if you are treated for HIV), if you are taking other treatments, what is your last CD4 count, what is your last viral load and if you have another infection (like tuberculosis) or condition (like cancer, high blood pressure etc) that you are treated for. We will also collect your height, weight and blood pressure. Because pain may influence your sleep and mood, we will also ask you if you have any regular pain and how intense it is.

We also ask you to please allow us to check in your file if there is information that you don't remember (for example: when did you start your treatment? – when were you diagnosed for the first time with HIV? - your last CD4 count - your last viral load).
We will only look for the information that we need to complete your questionnaire.

Since you will come back to the Nthabiseng clinic several times during the time we run the study, we will ask you to fill in the questionnaires at each of your visits. This will give us an idea of how HIV or your treatment may affect your sleep, mood and activities during the day over a period of time. However your participation in this study is completely voluntary and if you don't wish to fill in these questionnaires, you can say so at any time.

Filling in these questionnaires should typically take 30 minutes but can take up to one hour. A person from the study is here and you can ask them to help you understand the questions if they are not clear.
The aim of the study is to characterize exactly how much your sleep changes while you are taking treatment, in order for us to do so, you will need to answer the questionnaires every time you come back to the clinic. The visit schedule is described in the table below:

In addition to the questionnaires, we will ask you to give blood (10mL or two teaspoons) at all visits (Visits 2, 3, 4 and 5). This is so that we can look at exactly how your immune system is changing while you are taking the treatment, and understand if changes in your immune system affect your sleep.

When you have your usual blood samples taken by the nurses at the clinic, they will just take two additional 5mL tubes of your blood that will be used for the study. Even if you are not scheduled to get blood drawn at one specific visit to the clinic, we will ask you to give the two teaspoons of blood for the study.

Your participation in this study is voluntary; you are free to withdraw at any time.

Visit	Activity
1 – first visit at the clinic, before you start treatment	Short questionnaire (General info, PSQI and ESS)
2 – less than one month on treatment	Full questionnaire – all questions Blood draw Optional: actigraphy recording/sleep diary for 2 weeks Optional: sleep recording at the Wits Sleep Laboratory*
3 – three months on treatment	Full questionnaire Blood draw Optional: actigraphy recording/sleep diary for 2 weeks Optional: sleep recording at the Wits Sleep Laboratory*
4 – 6 months on treatment	Full questionnaire Blood draw Optional: actigraphy recording/sleep diary for 2 weeks Optional: sleep recording at the Wits Sleep Laboratory*
5 – 1 year on treatment	Full questionnaire Blood draw Optional: actigraphy recording/sleep diary for 2 weeks Optional: sleep recording at the Wits Sleep Laboratory*

The Wits Sleep Laboratory is also a sleep-over facility within the School of Physiology. It has 3 bedrooms with shower, bathtub and toilets and a kitchenette which is equipped with a fridge, microwave oven, kettle and toaster. Each bedroom is private. The bathroom facilities are separated between men and women's bathrooms. We will serve breakfast on the morning after the sleep recording.

Optional
We may ask you to wear an actigraphy watch for two weeks at a time at Visits 2, 3, 4 and 5. This watch measures your daily light exposure and activity levels during the day and the night. In addition to this, we will also ask you to keep a sleep diary during these two weeks. You will be paid R25 per week for wearing the watches and keeping the diary, and we will pay you once you return the actigraphy watch.

Optional:
If you participate in the part of the study where we ask you to wear the actigraphy watches and keep the sleep diary, we may also ask you to come and record your sleep overnight at the Wits University Sleep Laboratory at Visits 2, 3, 4 and 5. This involves typically spending two nights in the sleep laboratory at each visit (so by the time you finish Visit 5, you would have had 8 sleep recordings or 8 nights spent in the sleep laboratory). We will time these overnight studies so they happen at the end of the actigraphy recording/sleep diary keeping.
At each visit, the first overnight study is so that you can get used to sleeping in the laboratory. The second overnight study is when we do the sleep measurements which we will use for the study. For each overnight study done at the Sleep Laboratory, we will ask you be at the Wits Sleep Laboratory a few hours before your habitual bedtime. We will then prepare you for the recording of your sleep. To record your sleep, we record your brain waves with small sensors. Before we apply each of these small sensors, we will wash several places on your head and face with special soaps and clean your skin with alcohol. Then, we will apply 15

small sensors with glue (8 on the scalp where there is hair) or with tape (7 on the face where there is no hair). In addition, you will have two small sensors taped to your chest so we can monitor your heart rate during your sleep. We may also ask you to wear a small detector which looks like a microphone in front of your mouth to analyse the air you breathe during the night. We may also ask you to wear a large elastic band around your chest to monitor your breathing during the night. While you are in bed, we will ask you not to get up to go to the bathroom but instead we will bring you a bedpan or a urinal.
In the morning of each of these overnight studies, the study investigator will wake you up, remove the sensors. You will then be given a breakfast and will be able to shower. You will be compensated ZAR 50 per night spent at the laboratory and for transport.

If you agree to participate in the optional actigraphy/ sleep diary recording and optional sleep recording parts, the investigators will call you back to organize those as we cannot guarantee that we will be able to run these parts of the study at this stage.

Risks of being involved in the study: there should not be any risk. You may not want to answer these questions because you may find the questionnaires too long or not interesting. At any point, you can decide not to participate in the study and stop filling in the questionnaires.
You may also feel uncomfortable when answering sensitive, personal questions about you. You can choose to skip these questions and not answer them. You can also decide not to participate in the study.

Benefits of being in the study: we are hoping to learn more on how HIV and its treatment influence sleep quality, your mood and your daily activities. If you feel your sleep is not good enough, we may be able to find solutions for improving it through this study.
You may also attend the information sessions about sleep that we will organize throughout this study.

There are no alternatives to this study but you may withdraw from the study at any time. There is no particular way of withdrawing: just tell us at any time you would rather not be part of the study.

As soon as we have results from this study, we will post in the waiting room a copy of these results and of any discovery we have made that could help with your sleep or mood.

Participation is voluntary: it is your right to refuse to participate in this study. If you have started the study, you can withdraw from the study at any time. This will not change the way your doctors take care of you or your access to treatment in this hospital or any other hospital you decide to go to.

Reimbursements for "out of pocket" expenses: such as taxi fare to the Sleep Laboratory at Wits University, for wearing the watches – R25 a week, and for nights spent at the Sleep Lab – R50 a night. .

Confidentiality: Efforts will be made to keep personal information confidential. Absolute confidentiality cannot be guaranteed. Personal information may be disclosed if required by law. Organizations that may inspect and/or copy your research records for quality assurance and data analysis include groups such as the Research Ethics Committee and the Medicines Control Council. If results are published, we will use a code that we only know how to identify. Therefore, your results will stay anonymous.

Contact details of researcher/s – for further information / reporting of study related adverse events, you can contact Dr Karine Scheuermaier on 011 717 2453 or by email Karine.scheuermaier@wits.ac.za, or Kirsten Redman on 073 239 2892 or by email: kirsten.redman@students.wits.ac.za

Contact details of REC administrator and chair – for reporting of complaints / problems with this study, you may contact Professor Cleaton-Jones through Ms Anisa Keshav on 011 717 1234 or anisa.keshav@wits.ac.za

UNIVERSITY OF THE WITWATERSRAND
School of Physiology
CONSENT TO ACT AS A STUDY PARTICIPANT IN RESEARCH

I, _____, being 18 years or older,
consent to participate/take part in a research project entitled : "**Sleep, mood and morningness-eveningness preference in treated and untreated HIV positive patients"**

The questionnaires have been explained to me and I understand and appreciate their purpose, any risks involved, and the extent of my involvement, I have read and understand the attached information leaflet.

I understand that the procedures form part of a research project and may not provide any direct benefit to me.

I understand that all experimental procedures have been sanctioned by the Human Research Ethics Committee, University of the Witwatersrand, Johannesburg.

I understand that my participation is voluntary and that I am free to withdraw from the project at any time without prejudice.

_____ _____
Subject NAME and SIGNATURE DATE

_____ _____
Investigator NAME and SIGNATURE DATE

OPTIONAL ACTIGRAPHY AND SLEEP DIARY RECORDING

I agree to participate in the actigraphy and sleep diary recording part of the study:
YES ☐ **NO** ☐
SIGNATURE: …….

OPTIONAL SLEEP RECORDING

I agree to participate in the part of the study which involves sleep recording two nights at the Wits Sleep Laboratory:
YES ☐ **NO** ☐
SIGNATURE: …….

APPENDIX 6

General information questionnaire 2013

CODE *(study member will fill this in- leave blank)* :_____

Name: _____

Surname: _____

Date of birth: _____

Hospital number: _____

Home telephone number: _____

Cell phone number: _____

Residential address: _____

_____

_____

General information questionnaire 2013

CODE *(study member will fill this in- leave blank)* :_____

GENERAL INFORMATION ABOUT YOU AND YOUR SLEEP

Age: _____

Gender: Female ☐ Male ☐

Are you currently studying? YES ☐ NO ☐

If YES, for which degree?_____

Do you work?: YES ☐ NO ☐

If YES, what do you do?

If YES: do you ever work at night (shiftwork)?

Do you sleep alone in your room?

YES ☐ NO ☐

If No, do you have a bed partner?

If No, how many people in addition to you sleep in the same room?

If you wake up during sleep, what is likely to wake you up?

Having to go to the bathroom ☐

Noise ☐

Too hot ☐

Too cold ☐

Pain ☐

Bad dreams ☐

Other ☐

WHO stage (will be filled out by researcher)_____

General information questionnaire 2013

CODE *(study member will fill this in- leave blank)* :_____

EDUCATIONAL BACKGROUND

Have you received any formal education in school or college?

YES ☐ NO ☐

If yes, what is the highest level of education obtained (ie. Primary school, Secondary school. College, University?)

_____

INFORMATION ABOUT YOUR DISEASE:

When did you find out you were HIV positive?_____(month/year)

Have you told your family and /or friends that you are HIV positive? YES ☐ NO☐

When did you start taking HIV treatment?_____

Have you ever been admitted to the hospital due to a complication of HIV? YES ☐ NO☐

If YES, when was your last hospitalization? _____ (month/year)

Current treatment (please give a list of ALL medications you take):

Are you on tuberculosis (TB) treatment? YES ☐ NO☐

If YES, please list your medication:

Are you on Chemotherapy? YES ☐ NO☐

If YES, for which type of cancer? Kaposi ☐

Lymphoma ☐

Other ☐_____

Baseline CD4 (when first put on antiretroviral therapy)_____

Baseline viral load:_____

Current CD4 count:_____

Current viral load:_____

General information questionnaire 2013

CODE *(study member will fill this in- leave blank)* :_____

Past medical history related to HIV

Have you had **in the past** or do you **presently** have any of the following diseases?

(if you cannot fill in this information, we can check your medical file)

	past	YEAR?	current
tuberculosis	☐	_____	☐
cryptococcal meningitis	☐	_____	☐
cerebral toxoplasmosis	☐	_____	☐
pneumocystis carinii	☐	_____	☐
cancer (Kaposi, Lymphoma)	☐	_____	☐
other	☐	_____	☐

Past medical history: (Check YES or NO and all that apply)

1. From which of the following medical conditions do you suffer?	**YES**	**NO**
• Diabetes	☐	☐
• Hypothyroidism	☐	☐
• Asthma	☐	☐
☐ at night only ☐ allergic ☐ Other		
• Hypertension	☐	☐
• Psychiatric disorder	☐	☐
☐ Depression ☐ Anxiety ☐ Panic Disorder ☐ Schizophrenia ☐ Bipolar disorder (manic-depression) ☐ Other		
• Heart disease	☐	☐
☐ Coronary artery disease (heart attack / angina) ☐ Congestive Heart Failure ☐ Irregular heart beat ☐ Pacemaker ☐ Other		

General information questionnaire 2013

CODE *(study member will fill this in- leave blank)* :_____

- Neurologic disorder ☐ ☐

  ☐ Stroke
  ☐ Seizures
  ☐ Migraine headaches
  ☐ Brain infection (meningitis, abcess, present or past)
  ☐ Other
- Kidney disease ☐ ☐

  ☐ Dialysis
  ☐ Renal insufficiency
  ☐ Other
- Gastrointestinal or Liver disease ☐ ☐
- Arthritis ☐ ☐

Measurements: (filled in by researcher)

Height (cm): _____

Weight (kg): _____

Blood pressure:

Systolic: _____ mm Hg

Diastolic: _____ mm Hg

General information questionnaire 2013

CODE *(study member will fill this in- leave blank)* :_____

INFORMATION ABOUT ANY PAIN YOU MAY FEEL NOW

Do you have pain **now**? YES☐ NO ☐

If YES:

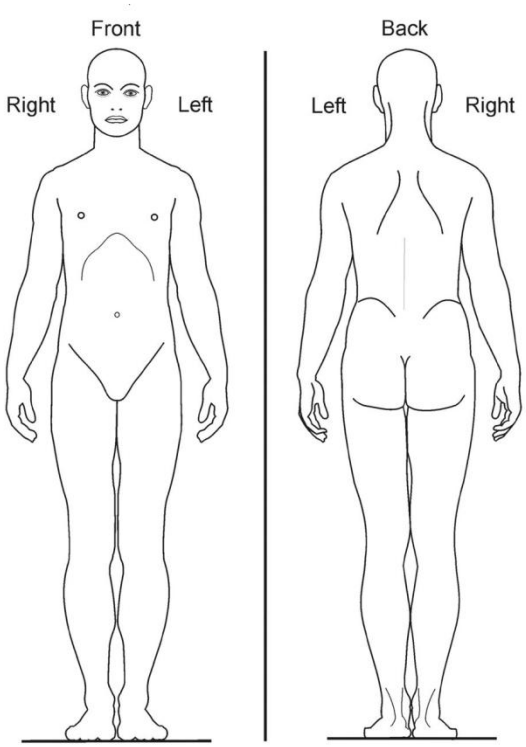
Can you please rate your pain level by picking **one** number that best describes your pain **now**?

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

1 2 3 4 5 6 7 8 9 10

No pain — mild pain — moderate pain — severe pain — worst pain ever felt

Can you please show on this picture **where** it hurts? (colour the areas where it hurts)



General information questionnaire 2013

CODE *(study member will fill this in- leave blank)* :_____

INFORMATION ABOUT ANY PAIN YOU HAVE FELT IN THE PAST MONTH

Did you have pain **in the past month**? YES☐ NO ☐

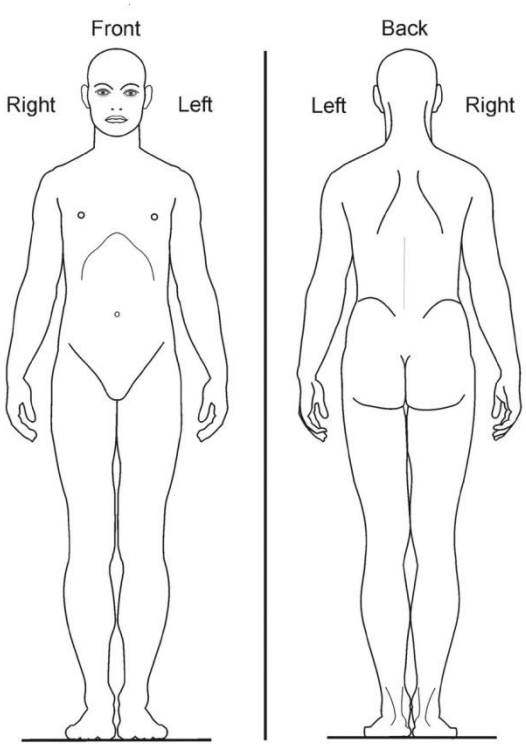
If YES: what was your **highest pain level** in the **past month**?

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

1 2 3 4 5 6 7 8 9 10

No pain — mild pain — moderate pain — severe pain — worst pain ever felt

Can you please show on this picture **where** it was hurting when you felt this highest pain level? (colour the areas where you felt your worst pain)



APPENDIX 7

General information questionnaire 2013

CODE *(study member will fill this in- leave blank)* :_____

Name: _____

Surname: _____

Date of birth: _____

Hospital number: _____

Home telephone number: _____

Cell phone number: _____

Residential address: _____

_____

_____

General information questionnaire 2013

CODE *(study member will fill this in- leave blank)* :_____

GENERAL INFORMATION ABOUT YOU AND YOUR SLEEP

Age: _____

Gender: Female ☐ Male ☐

Are you currently studying? YES ☐ NO ☐

If YES, for which degree?_____

Do you work?: YES ☐ NO ☐

If YES, what do you do?

If YES: do you ever work at night (shiftwork)?

Do you sleep alone in your room?

YES ☐ NO ☐

If No, do you have a bed partner?

If No, how many people in addition to you sleep in the same room?

If you wake up during sleep, what is likely to wake you up?

Having to go to the bathroom ☐

Noise ☐

Too hot ☐

Too cold ☐

Pain ☐

Bad dreams ☐

Other ☐

WHO stage (will be filled out by researcher)

General information questionnaire 2013

CODE *(study member will fill this in- leave blank)* :_____

INFORMATION ABOUT YOUR DISEASE:

Have you told your family and /or friends that you are HIV positive? YES ☐ NO☐

Since your last visit, have you been admitted to the hospital? YES ☐ NO☐

If YES, when was your last hospitalization? _____ (month/year)

If YES, where were you hospitalized? _____

If YES, reason for hospitalization:

Current treatment (please give a list of ALL medications you take):

Are you on tuberculosis (TB) treatment? YES ☐ NO☐

If YES, please list your medication:

Are you on Chemotherapy? YES ☐ NO☐

If YES, for which type of cancer? Kaposi ☐

Lymphoma ☐

Other ☐_____

Current CD4 count:_____

Current viral load:_____

Measurements: (filled in by researcher)

Weight (kg): _____

Blood pressure:

Systolic: _____ mm Hg

Diastolic: _____ mm Hg

General information questionnaire 2013

CODE *(study member will fill this in- leave blank)* :_____

INFORMATION ABOUT ANY PAIN YOU MAY FEEL NOW

Do you have pain **now**? YES☐ NO ☐

If YES:

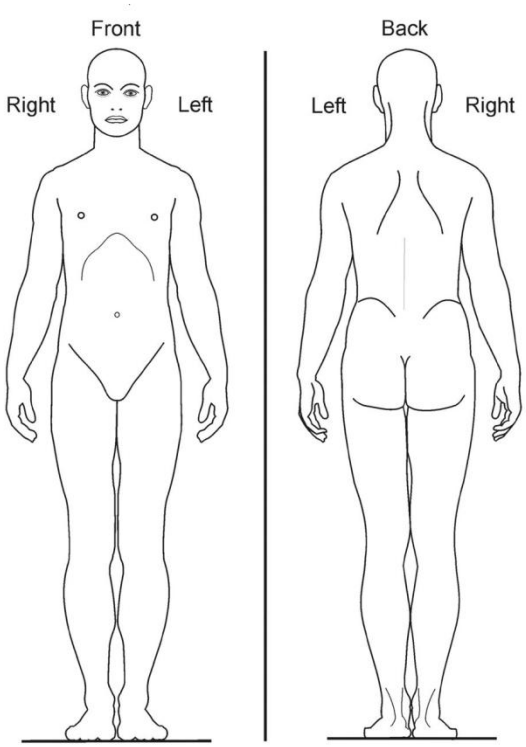
Can you please rate your pain level by picking **one** number that best describes your pain **now**?

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

1 2 3 4 5 6 7 8 9 10

No pain — mild pain — moderate pain — severe pain — worst pain ever felt

Can you please show on this picture **where** it hurts? (colour the areas where it hurts)



General information questionnaire 2013

CODE *(study member will fill this in- leave blank)* :_____

INFORMATION ABOUT ANY PAIN YOU HAVE FELT IN THE PAST MONTH

Did you have pain **in the past month**? YES☐ NO ☐

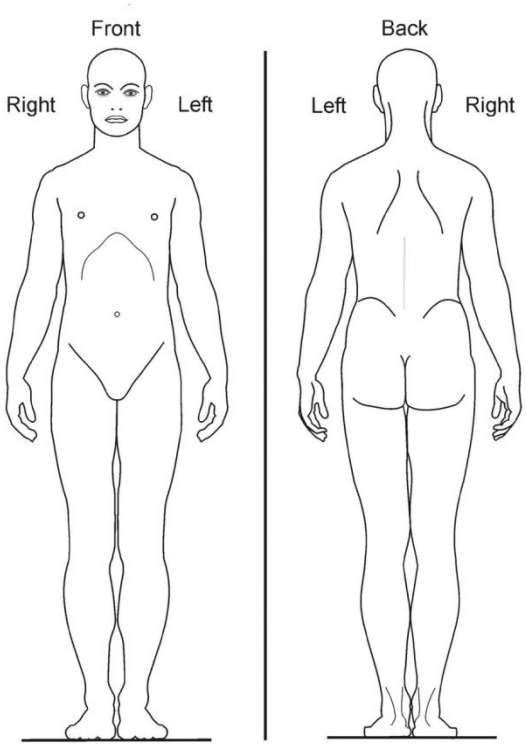
If YES: what was your **highest pain level** in the **past month**?

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

1 2 3 4 5 6 7 8 9 10

No pain — mild pain — moderate pain — severe pain — worst pain ever felt

Can you please show on this picture **where** it was hurting when you felt this highest pain level? (colour the areas where you felt your worst pain)



APPENDIX 8

Subject's Name _____ Date
Score_____

PITTSBURGH SLEEP QUALITY INDEX

INSTRUCTIONS: *The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. Please answer all questions.*

During the past month,

1. When have you usually gone to bed? _____

2. How long (in minutes) has it taken you to fall asleep each night? _____

3. When have you usually gotten up in the morning? _____

4. How many hours of actual sleep did you get that night? (This may be different than the number of hours you spend in bed.) _____

5. During the past month, how often have you had trouble sleeping because you . . .	Not during the past month	Less than once a week	Once or twice a week	Three or more times a week
a. Cannot get to sleep within 30 minutes				
b. Wake up in the middle of the night or early morning				
c. Have to get up to use the bathroom				
d. Cannot breathe comfortably				
e. Cough or snore loudly				
f).Feel too cold				
g. Feel too hot				
h. Had bad dreams				
i. Have pain				
j. Other reason(s), please describe, including how often you have had trouble sleeping because of this reason(s):				
6. During the past month, how often have you taken medicine (prescribed or "over the counter") to help you sleep?				
7. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?				
8. During the past month, how much of a problem has it been for you to keep up enthusiasm to get things done?				
	Very good	Fairly good	Fairly bad	Very bad
9. During the past month, how would you rate your sleep quality overall?				

Reprinted from: Buysse DJ, Reynolds CF, Monk TH, Berman SR, Kupfer DJ: Journal of Psychiatry Research, 28:193-213, 1989.

DATE:_____ SCORE:_____

APPENDIX 9

DAYTIME ACTIVITY (Epworth Scale)

1. In the last 30 days, how likely are you to dose off or fall asleep in the following situations (in contrast to feeling just tired)?	HIGH CHANCE	MODERATE CHANCE	SLIGHT CHANCE	NEVER DOZE
• Sitting and reading.	☐	☐	☐	☐
• Watching TV.	☐	☐	☐	☐
• Sitting inactive in a public place (e.g. theater, Church)	☐	☐	☐	☐
• As a passenger in a car for an hour without a break.	☐	☐	☐	☐
• Lying down to rest in the afternoon when circumstances permit.	☐	☐	☐	☐
• Sitting and talking to someone.	☐	☐	☐	☐
• Sitting quietly after lunch without alcohol.	☐	☐	☐	☐
• In a car while stopped for a few minutes in traffic.	☐	☐	☐	☐

Berlin questionnaire

Name _____
Address _____
_____

SLEEP EVALUATION

1 Complete the following:

height _____ age _____
weight _____ male/female _____

CATEGORY 1

2 Do you snore?

- ☐ yes
- ☐ no
- ☐ don't know

If you snore:

3 Your snoring is?

- ☐ slightly louder than breathing
- ☐ as loud as talking
- ☐ louder than talking
- ☐ very loud. Can be heard in adjacent rooms.

4 How often do you snore?

- ☐ nearly every day
- ☐ 3-4 times a week
- ☐ 1-2 times a week
- ☐ 1-2 times a month
- ☐ never or nearly never

5 Has your snoring ever bothered other people?

- ☐ yes
- ☐ no

6 Has anyone noticed that you quit breathing during your sleep?

- ☐ nearly every day
- ☐ 3-4 times a week
- ☐ 1-2 times a week
- ☐ 1-2 times a month
- ☐ never or nearly never

CATEGORY 2

7 How often do you feel tired or fatigued after your sleep?

- ☐ nearly every day
- ☐ 3-4 times a week
- ☐ 1-2 times a week
- ☐ 1-2 times a month
- ☐ never or nearly never

8 During your wake time, do you feel tired, fatigued or not wake up to par?

- ☐ nearly every day
- ☐ 3-4 times a week
- ☐ 1-2 times a week
- ☐ 1-2 times a month
- ☐ never or nearly never

9 Have you ever nodded off or fallen asleep while driving a vehicle?

- ☐ yes
- ☐ no

If yes, how often does it occur?

- ☐ nearly every day
- ☐ 3-4 times a week
- ☐ 1-2 times a week
- ☐ 1-2 times a month
- ☐ never or nearly never

CATEGORY 3

10 Do you have high blood pressure?

- ☐ yes
- ☐ no
- ☐ don't know

BMI =

Scoring Questions: Any answer within box outline is a positive response.

Scoring Categories:
- Category 1 is positive with 2 or more positive responses to questions 2-6 ☐
- Category 2 is positive with 2 or more positive responses to questions 7-9 ☐
- Category 3 is positive with 1 or more positive responses and/or a BMI>30 ☐

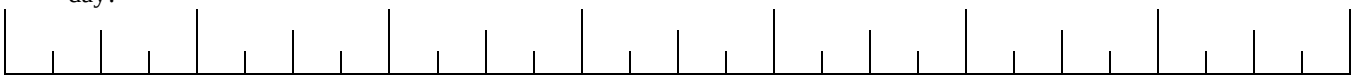
Final Results: 2 or more positive categories indicates a high likelihood of sleep disordered breathing.

EDWARD

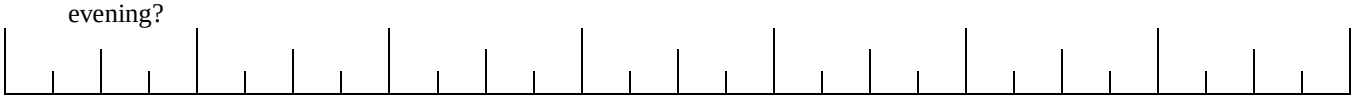
REFERRAL COPY

1. Please read each question very carefully before answering. **Subject Name:**
2. Answer all questions
3. Answer questions in numerical order. **Score**_____ **Initials** _____ **Date**
4. Each question should be answered independently of others. Do NOT go back and check your answers.
5. All questions have a selection of answers. For each question place a cross alongside ONE answer only. Some questions have a scale instead of a selection of answers. Place a cross at the appropriate point along the scale.

1. Considering only your own "feeling best" rhythm, at what time would you get up if your were entirely free to plan your day?



2. Considering only your own "feeling best" rhythm, at what time would you go to bed if you were entirely free to plan your evening?



3. If there is a specific time at which you have to get up in the morning, to what extent are you dependent on being woken up by an alarm clock?

- Not at all dependent ❑
- Slightly dependent ❑
- Fairly dependent ❑
- Very dependent ❑

4. Assuming adequate environmental conditions, how easy do you find getting up in the morning?

- Not at all easy ❑
- Not very easy ❑
- Fairly easy ❑
- Very easy ❑

5. How alert do you feel during the first half-hour after having woken in the mornings?

- Not at all alert ❑
- Slightly alert ❑
- Fairly alert ❑
- Very alert ❑

6. How is your appetite during the first half-hour after having woken in the mornings?

- Very poor ❑
- Fairly poor ❑
- Fairly good ❑
- Very good ❑

7. During the first half-hour after having woken in the morning, how tired do you feel?

- Very tired ❑
- Fairly tired ❑
- Fairly refreshed ❑
- Very refreshed ❑

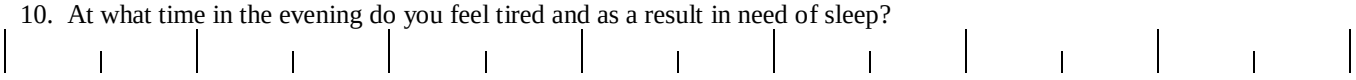
8. When you have no commitments the next day, at what time do you go to bed compared to your usual bedtime?

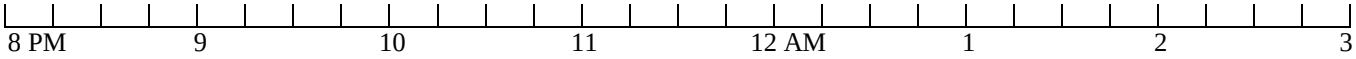
- Seldom or never later ❑
- Less than one hour later ❑
- 1 - 2 hours later ❑
- More than two hours later ❑

9. You have decided to engage in some physical exercise. A friend suggests that you do this one hour twice a week and the best time for him is between 7:00 - 8:00 AM. Bearing in mind nothing else but your own "feeling best" rhythm how do you think you would perform?

- Would be in good form ❑
- Would be in reasonable form ❑
- Would find it difficult ❑
- Would find it very difficult ❑

10. At what time in the evening do you feel tired and as a result in need of sleep?





11. You wish to be at your peak performance for a test which you know is going to be mentally exhausting and lasting for two hours. You are entirely free to plan your day and considering your "feeling best" rhythm which ONE of these four testing times would you choose?

- 8:00 - 10:00 AM ❑
- 11:00 AM - 1:00 PM ❑
- 3:00 - 5:00 PM ❑
- 7:00 - 9:00 PM ❑

12. If you went to bed at 11:00 PM at what level of tiredness would you be?

- Not at all tired ❑
- A little tired ❑
- Fairly tired ❑
- Very tired ❑

13. For some reason you have gone to bed several hours later than usual, but there is no need to get up at any particular time the next morning. Which ONE of the following events are you most likely to experience?

- Will wake up at usual time and will NOT fall asleep ❑
- Will wake up at usual time and will doze thereafter ❑
- Will wake up at usual time but will fall asleep again ❑
- Will NOT wake up until later than usual ❑

14. One night you have to remain awake between 4:00 - 6:00 AM in order to carry out a night watch. You have no commitments the next day. Which ONE of the following alternatives will suit you best?

- Would NOT go to bed until after watch was over ❑
- Would take a nap before and sleep after ❑
- Would take a good sleep before and nap after ❑
- Would take ALL sleep before watch ❑

15. You have to do two hours of hard physical work. You are entirely free to plan your day and considering only your own "Feeling best" rhythm which ONE of the following times would you choose?

- 8:00 - 10:00 AM ❑
- 11:00 AM - 1:00 PM ❑
- 3:00 - 5:00 PM ❑
- 7:00 - 9:00 PM ❑

16. You have decided to engage in hard physical exercise. A friend suggests that you do this for one hour twice a week and the best time for him is between 10:00 - 11:00 PM. Bearing in mind nothing else but your "feeling best" rhythm how well do you think you would perform?

- Would be in good form ❑
- Would be in reasonable form ❑
- Would find it difficult ❑
- Would find it very difficult ❑

17. Suppose that you can choose your own work hours. Assume that you worked a FIVE-hour day (including breaks) and that your job was interesting and paid by results. Which FIVE CONSECUTIVE HOURS would you select?

12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12

MIDNIGHT NOON MIDNIGHT

18. At what time of the day do you think that you reach your "feeling best" peak?

12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12

MIDNIGHT NOON MIDNIGHT

19. One hears about "morning" and "evening" types of people. Which ONE of these types do you consider yourself to be?

- Definitely a "morning" type? ❑
- Rather more a "morning" than an "evening" type? ❑
- Rather more an "evening" than a "morning" type? ❑
- Definitely an "evening" type? ❑

Beck's Depression Inventory

This depression inventory can be self-scored. The scoring scale is at the end of the questionnaire.

1.
- 0 I do not feel sad.
- 1 I feel sad
- 2 I am sad all the time and I can't snap out of it.
- 3 I am so sad and unhappy that I can't stand it.

2.
- 0 I am not particularly discouraged about the future.
- 1 I feel discouraged about the future.
- 2 I feel I have nothing to look forward to.
- 3 I feel the future is hopeless and that things cannot improve.

3.
- 0 I do not feel like a failure.
- 1 I feel I have failed more than the average person.
- 2 As I look back on my life, all I can see is a lot of failures.
- 3 I feel I am a complete failure as a person.

4.
- 0 I get as much satisfaction out of things as I used to.
- 1 I don't enjoy things the way I used to.
- 2 I don't get real satisfaction out of anything anymore.
- 3 I am dissatisfied or bored with everything.

5.
- 0 I don't feel particularly guilty
- 1 I feel guilty a good part of the time.
- 2 I feel quite guilty most of the time.
- 3 I feel guilty all of the time.

6.
- 0 I don't feel I am being punished.
- 1 I feel I may be punished.
- 2 I expect to be punished.
- 3 I feel I am being punished.

7.
- 0 I don't feel disappointed in myself.
- 1 I am disappointed in myself.
- 2 I am disgusted with myself.
- 3 I hate myself.

8.
- 0 I don't feel I am any worse than anybody else.
- 1 I am critical of myself for my weaknesses or mistakes.
- 2 I blame myself all the time for my faults.
- 3 I blame myself for everything bad that happens.

9.
- 0 I don't have any thoughts of killing myself.
- 1 I have thoughts of killing myself, but I would not carry them out.
- 2 I would like to kill myself.
- 3 I would kill myself if I had the chance.

10.
- 0 I don't cry any more than usual.
- 1 I cry more now than I used to.

2 I cry all the time now.

3 I used to be able to cry, but now I can't cry even though I want to.

0 I am no more irritated by things than I ever was.

1 I am slightly more irritated now than usual.

2 I am quite annoyed or irritated a good deal of the time.

3 I feel irritated all the time.

12.

0 I have not lost interest in other people.

1 I am less interested in other people than I used to be.

2 I have lost most of my interest in other people.

3 I have lost all of my interest in other people.

13.

0 I make decisions about as well as I ever could.

1 I put off making decisions more than I used to.

2 I have greater difficulty in making decisions more than I used to.

3 I can't make decisions at all anymore.

14.

0 I don't feel that I look any worse than I used to.

1 I am worried that I am looking old or unattractive.

2 I feel there are permanent changes in my appearance that make me look unattractive

3 I believe that I look ugly.

15.

0 I can work about as well as before.

1 It takes an extra effort to get started at doing something.

2 I have to push myself very hard to do anything.

3 I can't do any work at all.

0 I can sleep as well as usual.

1 I don't sleep as well as I used to.

2 I wake up 1-2 hours earlier than usual and find it hard to get back to sleep

3 I wake up several hours earlier than I used to and cannot get back to sleep

0 I don't get more tired than usual.

1 I get tired more easily than I used to.

2 I get tired from doing almost anything.

3 I am too tired to do anything.

18.

0 My appetite is no worse than usual.

1 My appetite is not as good as it used to be.

2 My appetite is much worse now.

3 I have no appetite at all anymore.

19.

0 I haven't lost much weight, if any, lately.

1 I have lost more than five pounds.

2 I have lost more than ten pounds.

3 I have lost more than fifteen pounds.

20.

0 I am no more worried about my health than usual.

1 I am worried about physical problems like aches, pains, upset stomach, or constipation.

2 I am very worried about physical problems and it's hard to think of much else.

3 I am so worried about my physical problems that I cannot think of anything else.

21.

0 I have not noticed any recent change in my interest in sex.

1 I am less interested in sex than I used to be.

2 I have almost no interest in sex.

3 I have lost interest in sex completely.

Reliability and Validity of the English Version of the PSQI When Completed by Second Language English Speakers

One hundred and fifty two patients that were second-language English speakers completed the English version of the PSQI. Factor analysis revealed that the questionnaire had a three-factor structure (appendix 13, figure 1). Factor 1 included the sleep quality components (component 1: overall estimate of sleep quality, component 2 which evaluates sleep onset latency, component 5 for causes of night awakenings). Factor 2 included the sleep duration items (component 3 for sleep duration and component 4 for sleep efficiency), and Factor 3 included the life impacting components (component 6 for sleeping aids and component 7 for interference of sleep with daytime activities/ mood). The eigenvalues of the three factors were 2.16 for factor 1, 0.59 for factor 2 and 0.18 for Factor 3. The three factors explained 48% of the variance; factor loadings are shown in appendix 13, figure 1.

The Cronbach alpha for each component separately was above 0.7 (see appendix 13, table 2). Omission of any of the items in each of the subscales did not negatively affect the underlying construct of the subscales (appendix 13, table 2).

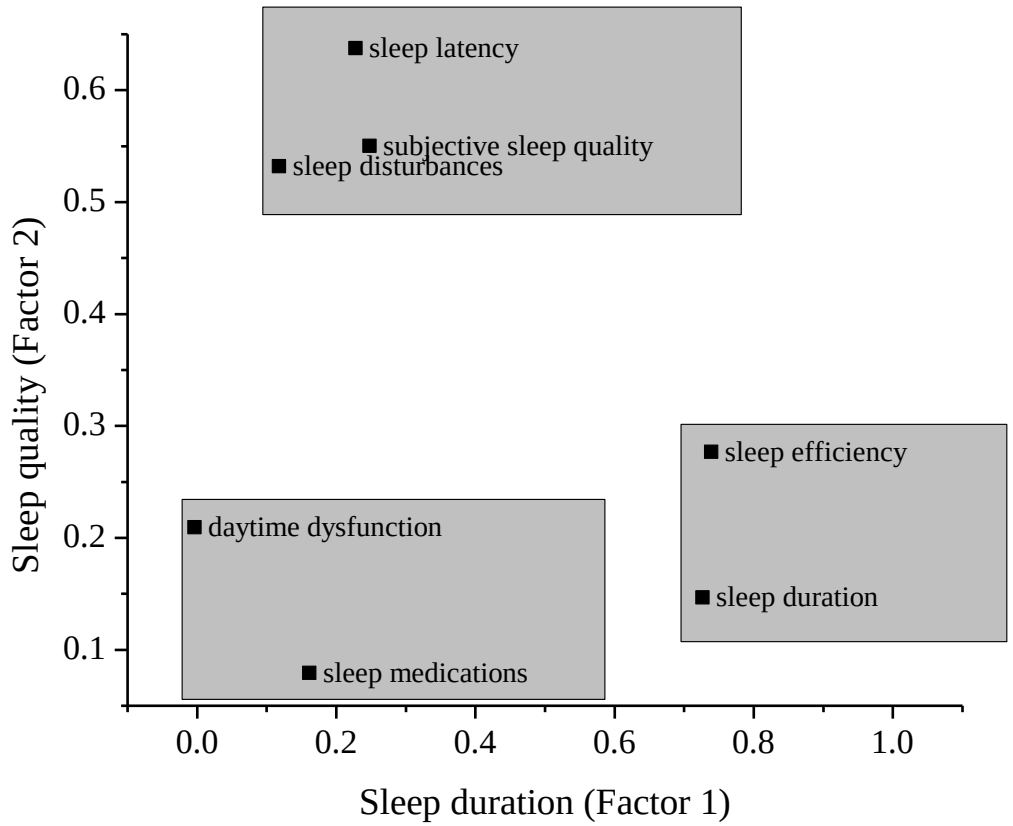
For the ESS, the Cronbach's alpha was 0.76. Loading of the 8 items comprising the questionnaire followed 3 main factors: a 'sleepiness while physically inactive' factor (Questions 1 to 3 and Question 7) with an eigenvalue of 2.5, a 'sleepiness when passive' factor (Questions 4 and 8) with an eigenvalue of 0.54 and a 'sleepiness while engaged' factor (Questions 5 and 6) with an eigenvalue of 0.25. These 3 factors represented 33% of the variance. Factor loadings are shown in Appendix 13, figure 2.

For Beck's Depression Inventory, the Cronbach's alpha (both raw and standardized) was 0.90. Omission of one of the 21 questions did not affect the questionnaire's construct with Cronbach's alpha staying at 0.9 (see Appendix 13, table 3). As observed in the original BDI validation study and subsequent validation studies of translated versions of the BDI, loading followed 2 main factors, a cognitive –affective factor (Factor 1, with an eigenvalue of 6.76) and a somatic factor (Factor 2, with an eigenvalue of 1.16) representing 43% of the variance. Factor loadings are represented in Appendix 13, figure 3.

Appendix 13, Table 1: Cronbach's coefficient Alpha with deleted variable for the Pittsburgh Sleep Quality Index Scale – English as a second language in Johannesburg, South Africa.

	Raw variables		Standardized variables	
	Correlation with total	Alpha	Correlation with total	Alpha
component 1: Subjective sleep quality	0.60	0.71	0.62	0.72
component 2: Sleep latency	0.55	0.73	0.57	0.73
component 3: Sleep duration	0.53	0.73	0.51	0.75
component 4: Sleep efficiency	0.53	0.73	0.51	0.75
component 5: Sleep disturbances	0.59	0.73	0.61	0.73
component 6: Sleep medications	0.32	0.77	0.33	0.78
component 7: Daytime dysfunction	0.34	0.77.	0.36	0.77
Cronbach's alpha		**0.77**		**0.78**

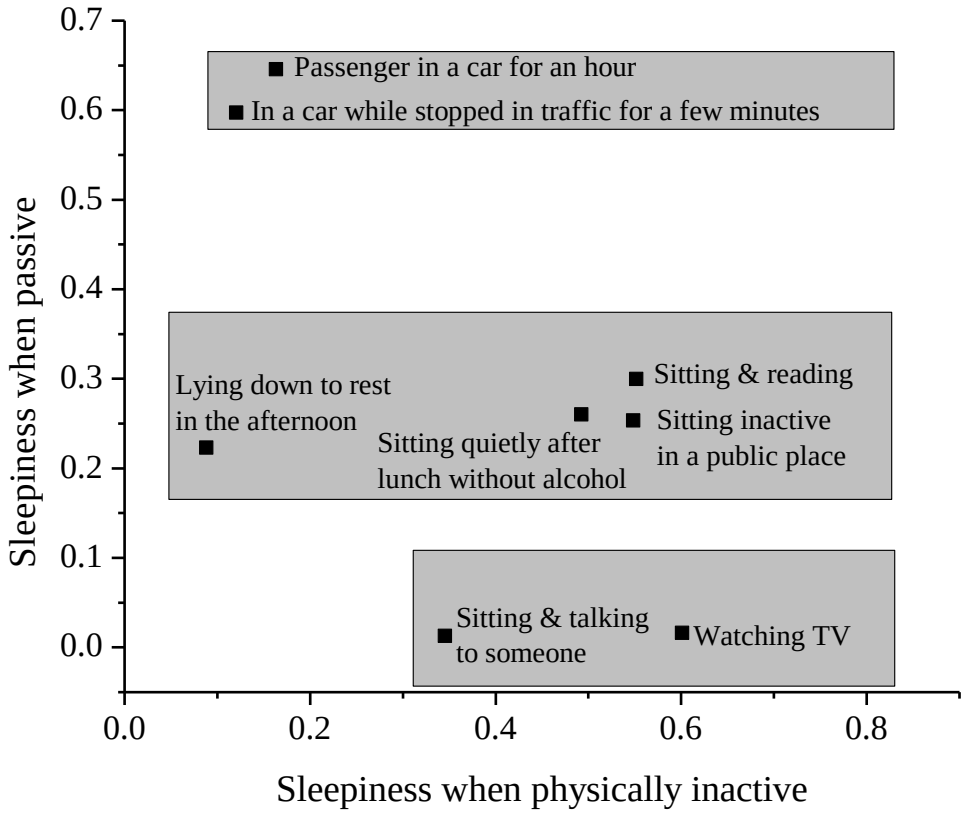
Appendix 13, figure 1



Appendix 13, Figure 1: Representation of the loading of the 7 PSQI components on the 2 main factors (sleep duration and sleep quality) in the rotated matrix of the factor analysis.

Appendix 13, Table 2: Cronbach's coefficient Alpha with deleted variable for the Epworth Sleepiness Scale – English as a second language in Johannesburg, South Africa.

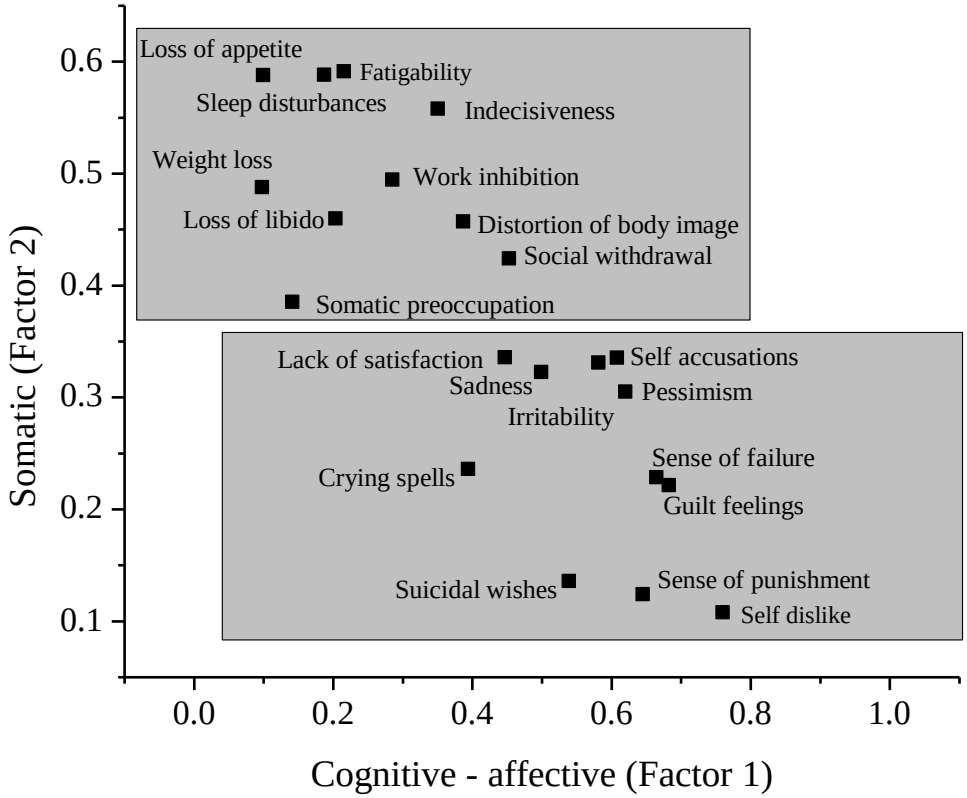
Deleted Variable (*In the last 30 days, how likely are you to doze off or fall asleep in the following situations?)*	Raw variables		Standardized variables	
	Correlation with total	Alpha	Correlation with total	Alpha
Q1 Sitting and reading	0.49	0.74	0.49	0.73
Q2 Watching TV	0.40	0.75	0.40	0.75
Q3 Sitting inactive in a public place	0.55	0.73	0.55	0.72
Q4 As a passenger in a car for an hour without a break	0.55	0.72	0.55	0.72
Q5 Lying down to rest in the afternoon	0.36	0.76	0.35	0.76
Q6 Sitting and talking to someone	0.39	0.75	0.39	0.75
Q7 Sitting quietly after lunch without alcohol	0.62	0.71	0.62	0.71
Q8 In a car while stopped for a few minutes in traffic	0.35	0.76	0.35	0.76
Cronbach's alpha		**0.77**		**0.76**



Appendix 13, Figure 2: Representation of the loading of the 8 questions comprising the ESS on the 2 main factors ('Sleepiness when physically inactive' and 'Sleepiness when passive') in the rotated matrix of the factor analysis.

Appendix 13, Table 3: Cronbach's coefficient Alpha with deleted variable for the Beck Depression Inventory – English as a second language in Johannesburg, South Africa.

Deleted Variable	Raw variables		Standardized variables	
	Correlation with total	Alpha	Correlation with total	Alpha
Sadness	0.55	0.90	0.55	0.90
Pessimism	0.62	0.89	0.62	0.90
Sense of failure	0.62	0.90	0.62	0.90
Lack of satisfaction	0.53	0.90	0.52	0.90
Guilt feelings	0.62	0.89	0.62	0.90
Sense of punishment	0.54	0.90	0.53	0.90
Self dislike	0.60	0.90	0.60	0.90
Self accusations	0.65	0.89	0.65	0.90
Suicidal wishes	0.47	0.90	0.46	0.90
Crying spells	0.44	0.90	0.43	0.90
Irritability	0.61	0.90	0.61	0.90
Social withdrawal	0.58	0.90	0.58	0.90
Indecisiveness	0.59	0.90	0.59	0.90
Distortion of body image	0.56	0.90	0.56	0.90
Work inhibition	0.51	0.90	0.51	0.90
Sleep disturbance	0.49	0.90	0.49	0.90
Fatigability	0.51	0.90	0.52	0.90
Loss of appetite	0.43	0.90	0.43	0.90
Weight loss	0.37	0.90	0.37	0.90
Somatic preoccupation	0.34	0.90	0.34	0.90
Loss of libido	0.43	0.90	0.43	0.90
Cronbach's alpha		**0.90**		**0.90**



Appendix 13, Figure 3: Representation of the loading of the 21 BDI questions on the 2 main factors (cognitive affective and somatic) in the rotated matrix of the factor analysis.

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