Long-term virological and clinical outcomes of treating Human Immunodeficiency Virus infection in an urban setting in Ghana

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy

By

Giovanni Villa

June 2019

This work is dedicated to all mothers and women of Kumasi living with HIV,

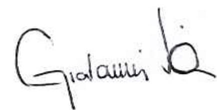
and to the memory of my mother

DECLARATION OF AUTHORSHIP

I hereby certify that this dissertation constitutes my own product, that where the language of others is set forth, quotation marks so indicate, and that appropriate credit is given where I have used the language, ideas, expressions or writings of another.

I declare that the dissertation describes original work that has not previously been presented for the award of any other degree of any institution.

Giovanni Villa



June 2019

ACKNOWLEDGEMENTS

This work was accomplished thanks to the guidance and support of many.

I am most thankful to my principal supervisor, Professor Anna Maria Geretti, for her excellent scientific guidance and meticulous review of my work during the course of my Ph.D. studies, and to my co-supervisor Dr Colette Smith at UCL for her extensive teaching and precise advice in statistics and research methods.

I have learnt a great deal in laboratory techniques over the years from members of Professor Geretti's research group at the Institute of Infection and Global Health of the University of Liverpool. I would like to thank here Dr Apostolos Beloukas, Dr Alex Stockdale, Mr Athanasios Papadimitropoulus, Mr Adam Abdullahi, and Mr Harrison Austin. I would also like to thank Mrs Rachel Dagnall and Mr Andrew McCallum for their work.

This research was realized thanks to the precious support of Professor Richard Phillips and Dr Dorcas Owusu of the Kwame Nkrumah University of Science and Technology, in Kumasi, Ghana. I would also like to thank my colleagues and friends at the Komfo Anokye Teaching Hospital (KATH) in Kumasi: Ms Marilyn Azumah, Ms Maria Agyei-Frimpong, and all the doctors, nurses, laboratory technicians and translators at KATH that made these studies possible. I am and always will be very thankful for the kindness of the patients of the HIV clinics, who eagerly took part in these studies.

I am grateful for the patience and precise work of Dr Suzannah Phillips of the Department of Biochemistry of the Royal Liverpool and Broadgreen University Hospital.

Old friends have always been my backbone over the years, and I thank them all for making me feel supported and loved. A special thank goes here to my friends and fellow researchers Dr Alessandra Ruggiero, Ms Loreley Hahn, and Dr Sophie Jullien. I am very grateful to Dr Simone De Cia for always coping with me during the hardest moments.

I thank the support of the members of my family, of my sister Ms Giulia Villa above all, and the neat work of my father's assistants and carers, lead by Ms Andreea Licsandru.

Finally, my deepest gratitude, love and respect go to my parents, whom I can always feel at my side.

Liverpool, 8th April 2019

ABSTRACT

Long-term virological and clinical outcomes of treating Human Immunodeficiency Virus infection in an urban setting in Ghana

Giovanni Villa

The World Health Organisation recommends virological monitoring to guide the management of HIV treatment. Implementation is proving challenging, not least because of overburden health services. Work presented in this thesis was conducted in the HIV-positive cohort of Kumasi, Ghana, a typical programmatic setting that offers antiretroviral therapy (ART) free of charge but has yet to implement virological monitoring. Patients typically remain on first-line NNRTI-based ART until clinical events mandate a change.

Novel molecular platforms can now facilitate adoption of virological monitoring at point of care. In this research project we investigated rates of resuppression with a point-of-care (POC) assay in a prospective cohort: patients with a detectable viral load received immediate adherence review and counselling, and were invited to attend a second visit after 8 weeks, where POC viral monitoring was repeated and patients' treatment optimised based on the viral load findings. Plasma samples with detectable viraemia >200 copies/mL at the first visit were tested for drug resistance by population sequencing. This large study had several important findings. At the initial screening, almost half of the patients had a detectable viral load and one in five above the WHO threshold used to define virological failure (1000 copies/mL). Despite detailed adherence counselling, resuppression was achieved by only one in five subjects after 8 weeks, and was virtually never achieved in those with viral load >1000 copies/ml due to the presence of drug resistance. A small subgroup of patients switched treatment between the two study visits, with reassuring virological declines. They study demonstrated that waiting for the outcomes of an adherence interventions for patients with a detectable viral load >1000 copies/mL might be counterproductive, as they are not likely to resuppress due to the presence extensive drug resistance.

NRTI and NNRTI drug resistance is a growing problem in the Kumasi cohort. In this research project I obtained a detailed characterisation of the emerging drug resistance patterns using deep sequencing in a cohort of HIV/HBV co-infected patients that changed their regimen from zidovudine or stavudine to tenofovir in the absence of virological monitoring. Complex patterns of mutations were found in the reverse transcriptase gene of patients that had a detectable viral load (typically >1000 copies/ml) at the time of treatment change. Coexistence of multiple discriminatory mutations (i.e. K65R, K70E/T, L74V/I, Y115F, M184V) was observed following prolonged treatment with tenofovir, lamivudine and efavirenz. This unusual resistance profile was predicted to confer extensive NRTI resistance. Worryingly, it was accompanied by high viral loads, indicating no loss of fitness for the resistant variants and risk of clinical disease progression and onward transmission.

Along with drug resistance, adherence to ART is the second major determinant of virological suppression. While viral load monitoring and drug resistance testing might not be routine available in sub-Saharan Africa, adherence measures that are simple to adopt may help targeting viral load testing to those at the greatest risk of virological failure. In this research study I used a visual analogue scale and a multi-item questionnaire to measure self-reported adherence, and showed that these measures were predictive of virological outcomes. A self-reported history of previous treatment interruption (for ≥3 days), usually reflecting unavailability of the drug dispensary, doubled the risk of a detectable viral load. In addition, I developed a combined continuous adherence score that increased the discriminatory ability of the single measures, which could be implemented in routine HIV clinics to select patients that could benefit from viral load testing.

These data clearly indicate that improving HIV control must be the priority for the Kumasi cohort. The scale-up of ART in the region has led to a massive improvement in life-expectancy and reduction in HIV-related morbidity and mortality, and with current recommendation of treating all patients the number of individuals on ART is destined to increase. A further concern for the patients on long-term ART is related to the lack of routine screening for drug toxicity, highly prevalent co-infection with the hepatitis B virus, and non-communicable diseases. In this research study I explored the prevalence of tubular proteinuria in a HIV/HBV co-infected cohort from Kumasi after a median 4-year exposure to tenofovir, an antiretroviral agent with known potential nephrotoxicity. I found that 16% of patients had tubular proteinuria at the time of the assessment and 22% of patients had a rapid eGFR decline. Concomitant use of lopinavir/ritonavir significantly increased the risk of eGFR decline in this cohort, identifying a group that would benefit from routine renal monitoring. Improved detection and management of often undiagnosed hypertension and diabetes are also required to improve the renal health of patients in Kumasi, as hypertension was independently associated with greater odds of tubulopathy.

In addition to the evaluation of the renal health, in this research project I performed a comprehensive evaluation of the metabolic status and the liver health of patients attending the HIV outpatient clinic in Kumasi. A diagnosis of metabolic syndrome was made in almost one in every four subjects, reflecting high rates of central obesity (60%), and high prevalence of hypertension (42%), dyslipidaemias (34% of hypercholesterolemia, 21% of hypertriglyceridemia) and glucose dysregulation (5% with impaired regulation and 5% with overt diabetes). Using a Fibroscan to measure liver steatosis (as controlled attenuation parameter, CAP) and fibrosis (as liver stiffness), steatosis was detected in one in every five subjects and was associated with metabolic syndrome. Fibrosis was present in 17% of the cohort and was more common in HBV co-infected subjects.

All these projects were linked by a strong gender theme. Women in our study were more represented, in line with the overall Kumasi cohort and other cohorts from sub-Saharan Africa. In this study, they showed overall better adherence and virological outcomes, albeit poorer socioeconomic and mental health measures. In addition, they were more likely to develop tubular proteinuria and were less commonly affected by liver fibrosis.

To conclude, optimised HIV care in this programmatic setting requires an integrated approach that incorporates virological monitoring and detection and management of comorbidities in routine practice. Further research is needed to understand differences

in treatment outcomes between women and men and drives of poorer adherence and worse liver health in men, and higher prevalence of kidney injury in women.

TABLE OF CONTENTS

Declaration of authorship II
Acknowledgements III
Abstract V
List of tables XIII
List of figures XVI
List of abbreviations XVII
1 First chapter – Introduction 1
1.1 Classification and Origin 1
1.2 Viral Structure 3
1.3 Viral Replication 4
1.4 Transmission and Prevention Strategies 8
1.5 Pathogenesis 11
1.6 Antiretroviral Therapy and Drug Resistance 15
1.6.1 NRTIs 17
1.6.2 NNRTIs 19
1.6.3 PIs 19
1.6.4 INSTIs 20
1.6.5 Entry inhibitors 20
1.6.6 Interactions between mutational pathways 21
1.7 Treatment Guidelines 22
1.8 Virological Monitoring and Genotypic Testing 23
1.9 Acquired drug-resistance, transmitted drug-resistance and pre-treatment drug resistance 25
1.10 The Challenges of Adherence to ART 27
1.11 The HIV Epidemics in Ghana 30
1.11.1 HIV infection in Ghanaian key populations 31
1.11.2 HIV treatment guidelines in Ghana 32
1.11.3 Antiretroviral drugs management within the Ghanaian healthcare system 33
1.11.4 Drugs stock-outs in Ghana 33
1.11.5 Pre-treatment HIV drug resistance in Ghana 34
1.11.6 Prevalence of infective comorbidities in the HIV population in Ghana 34
1.12 Aim And Objectives 36

1.12.1 Aim of the research project....................36
1.12.2 Objectives....................36
1.13 Thematic Framework and Thesis Structure....................38
2 Second chapter - Materials and methods....................40
2.1 Study Cohorts....................40
2.1.1 The OPTIMISE Cohort....................40
2.1.2 The HEPIK Cohort....................41
2.2 Setting....................43
2.3 Ethics....................45
2.3.1 Abnormal laboratory findings and diagnosis of comorbidities....................45
2.3.2 Abnormal findings at the clinical assessments and their management 46
2.3.3 Dissemination of the findings to the scientific community and locally....................46
2.3.4 Ethical Considerations on Study Design and Sample Size....................47
2.4 Data Collection....................51
2.4.1 Adherence and well-being questionnaires....................51
2.4.2 Clinical data and Fibroscan readings....................55
2.4.3 Laboratory Data....................57
2.5 HIV Drug Resistance Testing....................62
2.5.1 Sanger Sequencing....................62
2.5.2 Next-generation Sequencing....................65
2.6 Data Storage and Statistical Analysis....................67
2.7 Personal Contribution in The Research Projects....................68
2.7.1 Personal Contribution in the Research Project Presented in Chapter 3....................68
2.7.2 Personal Contribution in the Research Project Presented in Chapter 4....................68
2.7.3 Personal Contribution in the Research Project Presented in Chapter 5....................69
2.7.4 Personal Contribution in the Research Project Presented in Chapter 6....................69
2.7.5 Personal Contribution in the Research Project Presented in Chapter 7....................70
3 Third Chapter – Point-of-care viral load testing to guide immediate adherence support and subsequent resuppression rates in a programmatic HIV setting in sub-Saharan Africa....................71
3.1 Introduction....................71

3.2 Methods 73
3.2.1 Setting and population 73
3.2.2 Patients' flow 73
3.2.3 Adherence measures 75
3.2.4 Viral load testing 76
3.2.5 Resistance testing 76
3.2.6 Analysis 77
3.3 Results 78
3.3.1 Study population at T0 78
3.3.2 Predictors of a suppressed viral load at T0 81
3.3.3 Viral load resuppression at T1 84
3.3.4 Adherence measures 89
3.3.5 Resistance 89
3.4 Discussion 103
4 Fourth Chapter - Drug resistance outcomes of long-term antiretroviral therapy (ART) with tenofovir disoproxil fumarate in the absence of virological monitoring ... .107
4.1 Introduction 107
4.2 Patients and methods 109
4.2.1 Study population 109
4.2.2 Retrospective viral load and resistance testing 111
4.2.3 Statistical analysis 112
4.3 Results 114
4.3.1 Treatment status at T1 114
4.3.2 Viral load and drug-resistance associated mutations at T0 and T1 ...116
4.3.3 Evolution of viral load and drug resistance 119
4.3.4 Discussion 127
5 Fifth Chapter - Self-reported adherence to antiretroviral therapy in relation to viral load and drug resistance outcomes in a programmatic HIV care setting in sub-Saharan Africa 131
5.1 Introduction 131
5.2 Methods 133
5.2.1 Study population 133
5.2.2 Adherence 133
5.2.3 Socio-economic status 136
5.2.4 Physical and psychological wellbeing 136

5.2.5 Laboratory testing ... 137
5.2.6 Statistical analysis ... 137
5.3 Results ... 139
5.3.1 Study population ... 139
5.3.2 Socio-economic status and physical and psychological wellbeing ... 142
5.3.3 Adherence ... 145
5.3.4 Predictors of optimal adherence ... 146
5.3.5 Adherence as a predictor of outcomes ... 149
5.3.6 Characteristics of the cohort by gender ... 152
5.4 Discussion ... 156
6 Sixth Chapter - Renal health after long-term exposure to tenofovir disoproxil fumarate (TDF) in HIV/HBV positive adults in Ghana ... 161
6.1 Introduction ... 161
6.2 Methods ... 163
6.2.1 Setting ... 163
6.2.2 Study population ... 163
6.2.3 Laboratory tests ... 164
6.2.4 Analysis ... 165
6.3 Results ... 166
6.3.1 Characteristics of the study population ... 166
6.3.2 Markers of renal health ... 169
6.3.3 Relationship between TuPr, eGFR, and urinary dipstick results ... 177
6.4 Discussion ... 180
7 Seventh Chapter - Liver steatosis and fibrosis in HIV-positive adults with and without hepatitis B virus co-infection accessing programmatic care in sub-Saharan Africa ... 184
7.1 Introduction ... 184
7.2 Methods ... 186
7.2.1 Study population ... 186
7.2.2 Laboratory investigations ... 186
7.2.3 Definitions and grading ... 187
7.2.4 Statistical analysis ... 188
7.3 Results ... 189
7.3.1 Study population ... 189
7.3.2 HIV status ... 193
7.3.3 Viral hepatitis co-infection ... 193

7.3.4 Metabolic syndrome ... 193
7.3.5 Liver steatosis and fibrosis status ... 195
7.3.6 Factors associated with liver fibrosis ... 199
7.4 Discussion ... 204
8 Eighth Chapter – General discussion ... 208
8.1 Recommendations ... 215
8.2 Future directions ... 216
References ... 217
Appendix 1: Non-commercial material Transfer Agreement ... 245
Appendix 2: HEPIK-2 Study Questionnaire ... 248
Appendix 3: OPTIMISE Study Questionnaire 1 ... 254
Appendix 4: OPTIMISE Study Questionnaire 2 ... 257
Appendix 5: OPTIMISE Study Questionnaire 3 ... 259

LIST OF TABLES

Table 2-1. **RT-PCR steps** 63

Table 2-2. **Nested-PCR steps**. 63

Table 2-3. **Cycle sequencing PCR steps** 64

Table 2-4. **RT-PCR steps for next-generation sequencing** 66

Table 3-1 **Adherence score (0-3 points)** 76

Table 3-2 **Baseline characteristics of the study population according to the viral load** 79

Table 3-3 **Univariable and multivariable logistic regression analysis of predictors of suppressed T0 viral load (<40 copies/ml) in the OPTIMISE cohort** 82

Table 3-4 **Univariable and multivariable logistic regression analysis of predictors of suppressed T0 viral load (<40 copies/ml) in the OPTIMISE cohort** 83

Table 3-5 **Baseline characteristics of patients with detectable T0 viral load according to the T1 viral load** 85

Table 3-6 **Univariable and multivariable logistic regression model of predictors of resuppression at T1 (n=32/150, 21.3%)** 88

Table 3-7 **Prevalence of resistance associated mutations detected in subjects with T0 viral load >200 copies/ml (n=87) stratified by T0 viral load < or ≥1000 copies/ml** 90

Table 3-8 **Resistance associated mutations (RAMs) in the OPTIMISE cohort. Subjects with HIV RNA > 5 log copies/ml at T0** 93

Table 3-9 **Resistance associated mutations (RAMs) in the OPTIMISE cohort. Subjects with HIV RNA 4-5 log copies/ml at T0** 95

Table 3-10 **Resistance associated mutations (RAMs) in the OPTIMISE cohort. Subjects with HIV RNA 3-4 log copies/ml at T0** 97

Table 3-11 **Resistance associated mutations (RAMs) in the OPTIMISE cohort. Subjects with HIV RNA 400-1000 copies/ml at T0** 99

Table 3-12 **Resistance associated mutations (RAMs) in the OPTIMISE cohort. Subjects with HIV RNA 200-400 copies/ml at T0** 101

Table 4-1 **Resistance-associated mutations included in the genotypic susceptibility score** 112

Table 4-2 **Characteristics of the study population at the time of switching from zidovudine or stavudine to tenofovir disoproxil fumarate (time zero, T0) and after a median of four years (T1) (n=87)** 115
Table 4-3 **Viral load after a median of 8.1 years of antiretroviral therapy (ART) according to reported treatment interruptions and adherence**† 117
Table 4-4 **Univariate logistic regression analysis of factors associated with a detectable plasma HIV-1 RNA (>40 copies/mL) after a median of 8.1 years of antiretroviral therapy (T1, n=19)**† 118
Table 4-5 **Univariate and multivariable logistic regression analysis of factors associated with the combined outcome of either showing a detectable viral load at T1 or having introduced lopinavir/ritonavir between T0 and T1 (n=21)** 118
Table 4-6 **Intention-to-treat univariate and multivariable logistic regression analysis: all patients who had died or were lost to follow-up after T0 were included in the outcome measure** 119
Table 4-7 **Patients on efavirenz or nevirapine showing a detectable viral load at T0 and a suppressed viral load at T1**† 121
Table 4-8 **Patients on efavirenz or nevirapine showing a suppressed viral load at T0 and a detectable viral load at T1**† 122
Table 4-9 **Patients on efavirenz or nevirapine showing a detectable viral load at both T0 and T1**† 124
Table 4-10 **Patients that introduced lopinavir/ritonavir between T0 and T1**† . 126
Table 5-1 **Demographic and clinical characteristic of the study population at study entry, stratified by the adherence grade** 140
Table 5-2 **Socio-economic status and physical and psychological wellbeing of the study population at study entry, stratified by the adherence grade** 143
Table 5-3 **Numbers and proportions reporting levels within EQ-5D dimensions** 144
Table 5-4 **Logistic regression analysis of predictors of optimal adherence** 147
Table 5-5 **Logistic regression analysis of predictors of optimal adherence (n=19) for participants in a partnership (n=54)** 148
Table 5-6 **Sensitivity and specificity of the 3-grade adherence scale and the continuous adherence score** 152
Table 5-7 **Spearman correlation of CD4 cell counts and viral load with the continuous adherence scale** 152

Table 5-8 **Demographics, clinical characteristics, socio-economic status and physical psychological wellbeing of the study population stratified by gender (n=106)** 153
Table 6-1 **Laboratory reference ranges** 164
Table 6-2 **Characteristics of HIV/HBV positive patients with long-term TDF exposure according to the detection of tubular proteinuria (TuPr; n=101 evaluated) and rapidly declining estimated glomerular filtration rate (RD-eGFR; n=97 evaluated)[a]** 167
Table 6-3 **Relationship between markers of renal health in HIV/HBV positive patients with long-term TDF exposure** 170
Table 6-4 **HIV/HBV positive patients with significant tubular proteinuria (sTuPr)[a]** 171
Table 6-5 **Logistic regression analysis of factors associated with TuPr and RD-eGFR in HIV/HBV positive patients with long-term TDF exposure[a]** 173
Table 6-6 **Additional multivariable logistic regression analyses of factors associated with TuPr in HIV/HBV positive patients with long-term TDF exposure** 174
Table 6-7 **Linear regression analysis of factors associated with changes in eGFR in HIV/HBV positive patients with long-term TDF exposure** 176
Table 6-8 **Urinary dipstick in relation to a diagnosis of TuPr, RD-eGFR, hypertension, diabetes, and positive urinary Schistosoma antigen** 179
Table 7-1 **Participants' characteristics according to HBsAg status** 190
Table 7-2 **Comorbidities and their grading of severity in the study population** 194
Table 7-3 **Univariate and multivariable linear regression analysis of factors associated with hepatic steatosis** 197
Table 7-4 **Univariable and multivariable linear regression analysis of predictors of hepatic steatosis grade** 200
Table 7-5 **Univariable and multivariable linear regression analysis of factors associated with hepatic fibrosis** 202

LIST OF FIGURES

Figure 1-1. **The HIV life cycle and targets of antiretroviral therapy** 4
Figure 1-2 **Synthesis of the integration-competent HIV-1 DNA from the viral RNA template** 6
Figure 1-3. **HIV prevention tools** 10
Figure 1-4. **Natural course of untreated and treated HIV infection** 13
Figure 1-5. **Potency and barrier to resistance of main ARVs** 17
Figure 1-6 **HIV prevalence according to WHO region** 30
Figure 1-7 Thematic Framework of the dissertation 39
Figure 2-1 **Study cohorts** 40
Figure 2-2 **Map of Ghana and of the Ashanti region.** Adapted from Osei FB et al.[139] 43
Figure 2-3 **Visual Analogue Scale** 52
Figure 3-1 **OPTIMISE cohort at T0 and at T1. Patients flow-chart** 74
Figure 3-2 **Viral load at T1 among study participants with detectable viral load at T0 (n=150)** 87
Figure 3-3 **Prevalence of at least one resistance associated mutation per drug class according to T0 viral load** 91
Figure 3-4 **Genotypic susceptibility score according to T0 viral load: box and whisker plots** 91
Figure 4-1 **Disposition of the cohort** 110
Figure 4-2 **Proportion of subjects with suppressed (<40 copies/ml) or detectable plasma HIV-1** 117
Figure 5-1 **Calculation of the continuous adherence score** 134
Figure 5-2 **Calculation of the 3-level adherence grade** 135
Figure 5-3 **Treatment interruptions since the start of treatment and in the previous three months** 145
Figure 5-4 **ROC analyses** 151
Figure 6-1 **ROC analysis on the performance of dipstick proteinuria (a), dipstick glycosuria (b) and dipstick haematuria (c) as predictors of TuPr** 178
Figure 7-1 **Prevalence of liver steatosis and fibrosis after exclusion of patient with excessive alcohol consumption** 196

LIST OF ABBREVIATIONS

3TC	Lamivudine
ABC	Abacavir
ADR	Acquired drug resistance
AIDS	Acquired immunodeficiency syndrome
ALT	Alanine aminotransferase
ART	Antiretroviral treatment
ARV	Antiretroviral
AST	Aspartate aminotransferase
ATV/r	Ritonavir-boosted atazanavir
AZT	Zidovudine
BIC	Bictegravir
BMI	Body mass index
BP	Blood pressure
c	cobicistat
CAP	Controlled attenuation parameter
CCR5	CC-chemokine receptor 5
cDNA	Copy deoxyribonucleic acid
CI	Confidence interval
CHB	Chronic hepatitis B
CKD	Chronic kidney disease
CKD-EPI	Chronic kidney disease epidemiology collaboration-derived equation
CRF	Circulating recombinant form
CXCR4	CXC-chemokine receptor 4
D4T	Stavudine
DDC	Zalcitabine
DDI	Didanosine
DNA	Deoxyribonucleic acid
DRC	Democratic Republic of Congo
DRV/r	Ritonavir-boosted darunavir
DSS	Depression severity score
DTG	Dolutegravir

eGFR	Estimated glomerular filtration rate
EIA	Enzyme immune assay
ELISA	Enzyme-linked immunosorbent assay
ETR	Etravirine
EVG	Elvitegravir
FACS	Fluorescence-activated cell sorter
FTC	Emtricitabine
FSW	Female sex worker
GAD	Generalised anxiety disorder
GFR	Glomerular filtration rate
GGT	Gamma-glutamyltransferase
gp41	Glycoprotein 41
gp121	Glycoprotein 121
gp160	Glycoprotein 160
HAND	HIV-associated neurocognitive disorder
HbA1c	Glycated haemoglobin
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HDL	High density lipoprotein
HDV	Hepatitis delta virus
HEPIK	Hepatitis B infection in Kumasi
HPLC	High performance liquid chromatography
HIV	Human immunodeficiency virus
HIV-1	Human immunodeficiency virus type 1
HIV-2	Human immunodeficiency virus type 2
HIVAN	HIV-associated nephropathy
HRQoL	Health-related quality of life
IDU	Injecting drug user
IN	Integrase
INSTI	Integrase strand inhibitor
IQR	Inter-quartile range
KATH	Komfo Anokye Teaching Hospital
KNUST	Kwame Nkrumah University of Science and Technology
KP	Key populations

LDL	Low-density lipoprotein
LLQ	Lower limit of quantification
LPV/r	Ritonavir-boosted lopinavir
LTR	Long terminal repeat
MA	Matrix
MCV	Maraviroc
MSAS	Memorial symptoms assessment scale
MSM	Men who have sex with men
mRNA	Messenger ribonucleic acid
NAFLD	Non-alcoholic fatty liver disease
NC	Nucleocapsid
NCD	Non-communicable disease
NGS	Next-generation sequencing
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NRTI	Nucleoside reverse-transcriptase inhibitor
NVP	Nevirapine
OI	Opportunistic Infection
OR	Odds ratio
ORF	Open reading frame
PBMC	Peripheral blood mononuclear cell
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PDR	Pre-treatment HIV-1 drug resistance
PMTCT	Prevention of mother-to-child transmission
POC	Point-of-care
PHQ-9	Patient Health Questionnaire-9
r	ritonavir
RAL	Raltegravir
RAM	Resistance associated mutation
RCT	Randomised controlled trial
RD-eGFR	Rapid eGFR decline
RNA	Ribonucleic acid
RT	Reverse transcriptase
SIV	Simian immunodeficiency virus
SSA	Sub-Saharan Africa

STI	Sexually transmitted infection
sTuPr	Significant tubular proteinuria
T20	Enfuvirtide
TAF	Tenofovir alafenamide fumarate
TB	Tuberculosis
TC	Total cholesterol
TDF	Tenofovir disoproxil fumarate
TDR	Transmitted drug resistance
TE	Transient elastography
TuPr	Tubular proteinuria
uACR	Urinary albumin-to-creatinine ratio
uPCR	Urinary protein-to-creatinine ratio
uAPR	Urinary albumin-to-protein ratio
UK	United Kingdom
URF	Unique recombinant form
VAS	Visual analogue scale
Vpr	Viral protein
WHO	World Health Organisation

1 First chapter – Introduction

1.1 Classification and Origin

The human immunodeficiency virus (HIV) belongs to the Retroviridae family, subfamily Orthoretrovirinae, genus *Lentivirus*. Two types of HIV are responsible for the pathogenesis of the human acquired immunodeficiency syndrome (AIDS): *Human immunodeficiency virus type 1* (HIV-1) and *Human immunodeficiency virus type 2* (HIV-2). Despite being both aetiological agents of AIDS, HIV-2 is confined to West Central Africa, is a much less pathogenic virus and is less easily transmissible than HIV-1.[1] Both HIV-1 and HIV-2 have a zoonotic origin: they originated from West Central African chimpanzees and gorillas (HIV-1), and sooty mangabeys (HIV-2), and closely resemble Simian immunodeficiency viruses (SIVs) found in these animals.[2]

HIV-1 is further classified into four genetically distinct groups, which represent four separate crossing of the species barrier: M and N, whose simian precursors infected chimpanzees in Southern Cameroon, and O and P, which originated from gorillas in the same region.[2] HIV-1 group M had a subsequent global distribution, whereas groups N, O and P remain confined to West Central Africa. Zoonotic transmission is likely to have occurred via blood contact during hunting and butchering of these primates. Data from a 1960 biopsy and a 1959 plasma sample document the circulation of HIV-1 M strains two decades before their first recognition in the United States, and molecular analysis demonstrated that the M group diverged in the human population at the beginning of the 20th century.[2] Phylogeographic analyses located the origin of the HIV-1 group M pandemic in Kinshasa, Democratic Republic of Congo (DRC).[3] From here, subsequent expansion in the region was facilitated by urbanisation, expanding transport networks, increase access to commercial sex, and use of unsterilized injections at sexually transmitted disease clinics.[3]

HIV-2 has nine distinct groups (A-I) which are mainly confined to Western Africa and countries with close links to the region. Dual infection with both HIV-1 and HIV-

2 has been documented in areas where both viruses circulate, but no recombination has been observed between the two.[2]

This introduction will focus on HIV-1 to reflect the theme of the research work. No analysis was conducted on HIV-2 in this project.

Evolution of HIV-1 group M strains within the human population led to the emergence of nine distinct genotypes (A-D, F-H, J, K), of different sub-subtypes (A1-A4, F1, F2) and numerous circulating recombinant forms (CRFs) and unique recombinant forms (URFs). CRFs and URFs are the product of recombination of different subtypes.[4] Where co-circulation of different virus types or strains occurs, co-infection of the same individual can lead to the emergence of mosaic strains within the same subject.[5]

The highest HIV-1 genetic diversity is observed in Central Africa;[3] outside this region, subtypes have a very specific geographic distribution; for example, subtype B is predominant in North America, Western Europe and Australia; subtype C in Eastern and Southern Africa, and India; CRF02_AG in Western Africa.[6] However, the epidemiology of HIV subtypes is constantly evolving with new strains emerging following movement of populations and evolving transmission networks.[7,8]

1.2 VIRAL STRUCTURE

The HIV-1 particle measures 100 nm in diameter.[9] The outer membrane of HIV-1, called the envelope, originates from the membrane of the infected cell and inherits its lipid bilayer structure. It contains several host membrane proteins, along with approximately 10 viral trimeric non-covalently linked heterodimers, which consist of the surface glycoprotein 120 (gp120), and the transmembrane glycoprotein 41 (gp41).[10,11] The envelope encloses a conical capsid core made of the viral capsid (CA) protein. The viral capsid houses the viral genome, which is composed of 2 identical molecules of single-stranded ribonucleic acid (RNA), and the viral enzymes reverse transcriptase (RT) and integrase (IN). HIV-1 genome contains nine open reading frames (ORFs) ending in a 3' and a 5' long terminal repeat (LTR).

The viral genome encodes for three polyprotein precursors: Gag, GagPol and Env. *Gag* encodes for matrix and structural proteins of the capsid; *Pol* for the viral enzymes RT, protease and IN; and *Env* for gp160, which is the precursor of gp120 and gp41. Six additional ORFs encode for accessory and regulatory proteins tat, rev, vif, vpr, nef and vpu.[9]

1.3 VIRAL REPLICATION

The HIV life cycle is illustrated in Figure 1-1.

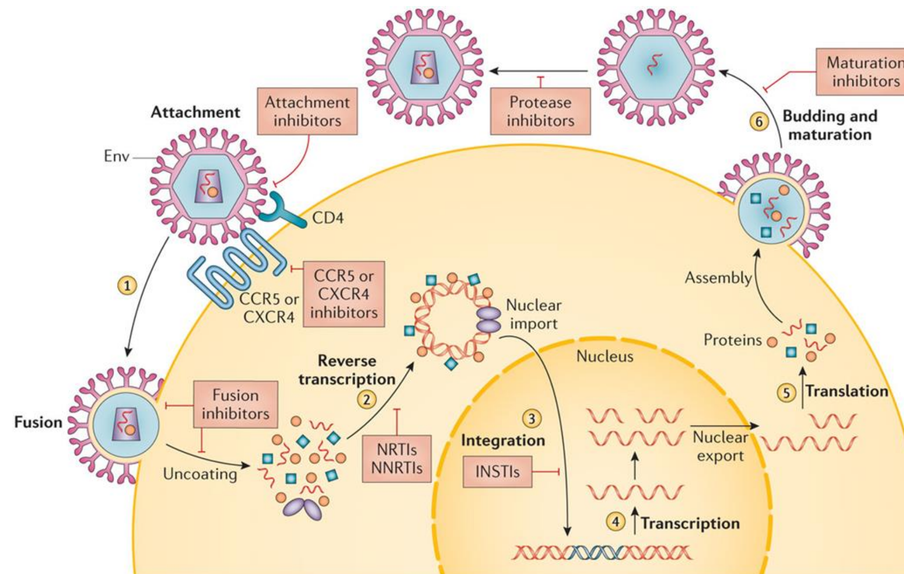


Figure 1-1. **The HIV life cycle and targets of antiretroviral therapy**

Adapted from Deeks et al.[12] The figure illustrated the various steps in the viral replication and the site of action of the different classes of antiretroviral agents. NRTI=nucleoside reverse transcriptase inhibitor; NNRTI=non-nucleoside reverse transcriptase inhibitor; INSTIs=integrase strand transfer inhibitors.

Viral envelope proteins mediate the interaction between the virus and the host cell, which ultimately leads to the fusion of the envelope with the cell membrane and the release of the viral core into the cytoplasm of the host cell. The first step is binding of gp120 to CD4, its receptor on the host cell membrane. CD4 is expressed by macrophages and $CD4^+$ T-cell lymphocytes; other cells, such as monocytes, dendritic cells, $CD8^+$ T lymphocytes, endothelial cells, haematopoietic stem cells, and astrocytes, may be susceptible to HIV infection, but do not sustain efficient viral replication.[13] Binding of gp120 to CD4 leads to a conformational change in gp120, exposing the binding site for a second receptor on the membrane host cell, which is predominantly the CC-chemokine receptor 5 (CCR5) or CXC-chemokine receptor 4 (CXCR4).[11,13] HIV-1 variants use one or the other co-receptor and are accordingly classified as R5 or X4 tropic strains, respectively; some strains may use both co-receptors and are termed R5/X4 dually tropic viruses. CCR5 is expressed mainly on

macrophages and a subset of memory T lymphocytes, whereas CXCR4 is more widely expressed, including in naïve T lymphocytes.[12] R5 viruses are primarily responsible for establishing new infections. Binding of the co-receptor leads to consecutive steps that encompass insertion of the gp41 fusion peptide into the cell membrane and further rearrangements and conformational changes in gp41 that ultimately lead to the fusion of the virus envelope with the host cell membrane and release of the viral capsid into the host cell cytoplasm.

After entry, the viral capsid undergoes dissolution, followed by release of the viral RNA strands and reverse transcription. Within the cell, the virus must evade the cellular restriction factors such as the apolipoprotein B mRNA-editing catalytic polypeptide-like 3 (APOBEC3) proteins, which are capable of mutating viral genome. The viral protein Vif is responsible for neutralising the activity of APOBEC3.[12] The HIV-1 RT enzyme is composed of two subunits, p51 and p66, which contain two active sites: the N-terminal RNA- and deoxyribonucleic acid (DNA)-dependent DNA polymerase and the C-terminal RNase H. The RT enzyme converts single-stranded RNA into double-stranded DNA. The reverse transcription and DNA polymerisation step is crucial for the establishment of the genetic diversity that characterises HIV-1 infection, including emergence of mutations and recombination events.[14] RT is an error prone enzyme: it produces errors at a rate of 3×10^{-5} per base pair per replication cycle. Due to lack of proofreading capability, mis-incorporations become point mutations in progeny virus, allowing emergence in an infected host of a variety of related but diverse virus strains that are termed the quasispecies. In addition, during polymerisation, RT switches between viral templates, creating further opportunities for errors and recombination. Some of the genetic variants that naturally emerge during replication in a host may be severely incapacitated and either fail to replicate or replicate at a low level due to loss of fitness. The quasispecies is rapidly responsive to selective pressures (e.g., immune responses, drug pressure) that favour the emergence and expansion of escape variants.[15] *Env* is able to tolerate the highest prevalence of mutations; within the same individual, the *Env* sequence varies by 0.6-1% per year and, between different subjects infected by different subtypes of HIV-1, this variability can reach 35%.[12]

Initially, cellular tRNA hybridizes to the genome-encoding binding site of the viral genome and initiates negative-strand DNA synthesis, which is directed towards the 5’

terminus of the molecule. This created a RNA/DNA hybrid; the RNA molecule is degraded by the RNase H domain of the viral RT enzyme, and the newly formed negative-strand copy of DNA forms the template for the complementary positive-strand (Figure 1-2).[16]

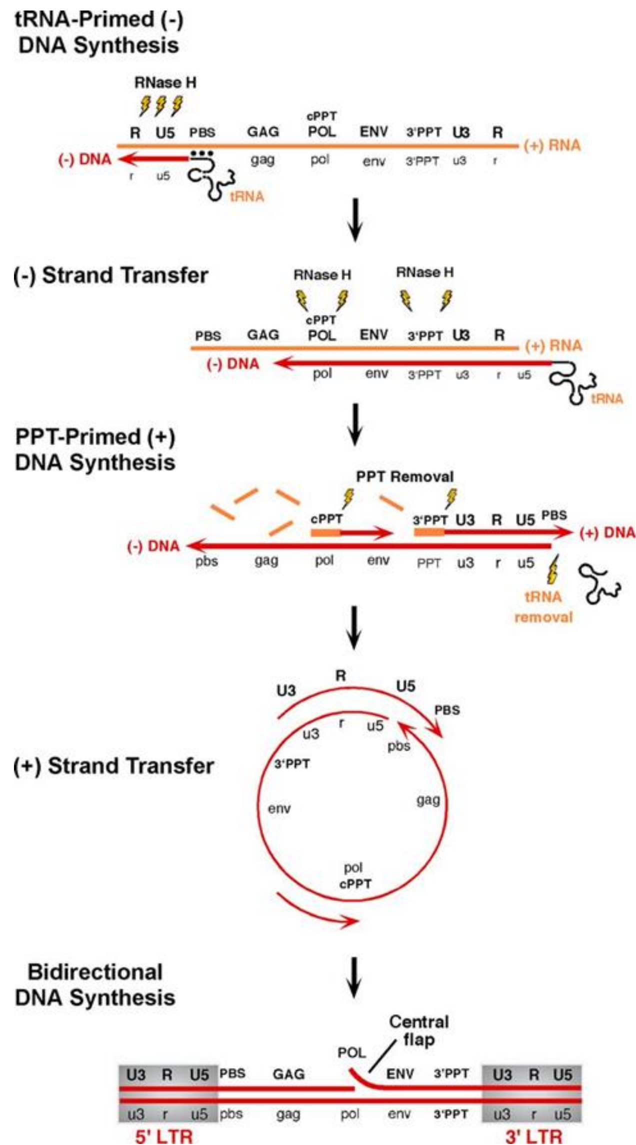


Figure 1-2 **Synthesis of the integration-competent HIV-1 DNA from the viral RNA template**

Adapted from Stuart F. J. et al.[16]

The double stranded DNA subsequently binds to host and viral proteins to constitute the pre-integration complex. This complex enters into the host cell nucleus and the linear viral DNA can either integrate into the host genome or form episomal circular

DNA termed LTR circles.[17] Integration in the host genome is a process mediated by IN; the integrated viral DNA is termed *provirus*, and represents the template for virus replication and a stable reservoir.

Replication of the provirus requires the machinery of activated cells and expression of promoting factors, such as NF-κB, which mediate transcription of provirus into pregenomic RNA and messenger ribonucleic acid (mRNA).[9] These are subsequently transported into the cytoplasm, where viral proteins are translated at two sites. Synthesis of gp160 occurs in the rough endoplasmic reticulum, followed by transport to the host cell membrane via the Golgi apparatus. Free polyribosomes of the cytosol mediate the production of Gag and GagPol: unspliced RNA forms the template for the production of the Gag protein and, via a frame-shift, the polyprotein precursor GagPol. Subsequent events include assembly of viral components and budding of the newly formed virion from the cell membrane. Maturation of the budding virion requires cleavage of the Gag precursor by the viral protease and the acquisition of the conical shape of the capsid that characterises the mature HIV-1.[18]

1.4 TRANSMISSION AND PREVENTION STRATEGIES

HIV infection is transmitted through blood exchange from person to person (parenteral exposure), via sexual contact (sexual exposure) or vertically from an infected mother to her child *in utero*, at delivery or through breastfeeding.[9] The risk of HIV transmission varies significantly. The highest risk is with blood transfusions from an HIV-positive donor to an HIV-negative recipient (>90%), and with mother-to-child transmission (~23%). Risk is lower for parenteral exposure, including sharing drug injection paraphernalia contaminated by infected blood (0.63%) and percutaneous needle stick injuries (0.23%). For sexual exposure, the highest risk is found in receptive anal intercourse (1.4% per act), followed by insertive anal intercourse (0.11% per act), receptive penile-vaginal intercourse (0.08%) and insertive penile-vaginal intercourse (0.04%). The risk is negligible for oral sex.[19] The risk of both transmission and acquisition for sexual exposure is enhanced by the concomitant presence of sexually-transmitted infections (STIs), such as genital ulcers in the HIV-negative partner,[20] which increase the concentration of target cells, and by a high plasmatic HIV-1 RNA, the *viral load*, in the HIV-positive partner,[20] which usually characterises HIV-1 acute infection or advanced stages of the disease.[21,22]

Given these routes of transmission, certain individuals are at higher risk of HIV infection: these include children born to infected mothers, men who have sex with men (MSM), injecting drugs users (IDUs), prisoners and people in other closed settings, sex workers and transgender people.[12]

Antiretroviral treatment (ART) initiation and suppression of HIV-1 viral load during pregnancy; scheduled caesarean section for women with viral load >1000 copies/ml at delivery, infant ART prophylaxis and avoidance of breastfeeding can prevent vertical transmission.[23] Expanded access and uptake of ART have dramatically reduced vertical transmission globally. Currently, it is estimated that 80% of HIV-positive pregnant women receive ART for the prevention of mother-to-child transmission (PMTCT). This has to date resulted in 14.8 million averted transmissions to newborn children worldwide.[24] Along with global access to ART, strategies to achieve universal PMTCT must include implementation and routine HIV

screening in antenatal clinics, effective engagement with ART services, and optimal adherence to ART.[25]

Treatment as prevention (TasP) is an effective strategy for preventing sexual transmission among HIV serodiscordant heterosexual and homosexual couples, and there is evidence to indicate that the risk of sexual transmission from a HIV-positive subject is negligible if the subject is taking effective ART and has a suppressed viral load.[26-28]

In HIV-negative subjects at risk of sexual exposure, use of antiretrovirals (ARVs) as pre-exposure prophylaxis (PrEP) can also prevent HIV infection. This strategy was first proved effective in simian models and subsequently confirmed by several studies that documented the efficacy of the combination of two nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) comprising tenofovir disoproxil fumarate (TDF) with emtricitabine (FTC).[29-32] It should be noted however that PrEp showed poor efficacy in women in some African studies, thought to reflect mainly poor adherence.[33] Currently, use of PrEP is recommended for high risk groups (risk greater than 3 per 100 person-year in the absence of PrEP) including MSM and transgender women who report condomless anal sex and serodiscordant couples when the HIV-positive partner has undiagnosed or untreated infection.[34,35] Further use is recommended on a case-by-case basis.[35] Chemsex, or the sexualised use of illicit substances in the practice of sex (e.g., cocaine, ecstasy, mephedrone, GHB/GBL, methamphetamine, ketamine), has been linked to increased risk of HIV acquisition due to engagement in riskier practices.[36]

Post-exposure prophylaxis (PEP) and post-exposure prophylaxis after sexual exposures (PEPSE) is the administration of ARVs as soon as possible (no later than 72 hours) after exposure. Evidence in support of this approach comes from animal models.[37,38] PEP applications include exposure in the occupational health setting, e.g., via needlestick or mucosal exposure, and in children born to infected mothers. PEPSE is currently recommended when the risk of acquisition is higher than 0.1% and consists in the administration of three ARVs for four weeks. Current drugs of choice for PEP/PEPSE are TDF/FTC in association with the integrase strand inhibitor (INSTI) raltegravir (RAL),[39] or dolutegravir (DTG).[40]

Condoms are barrier contraceptives that are effective in preventing acquisition of HIV and STIs.[41] The efficacy of male condoms has been summarised in a meta-analysis of cohorts including HIV serodiscordant couples engaging in sexual intercourse and where condom use was reported either as "always" or "never". This analysis demonstrated a 80% reduction in HIV incidence in people reporting consistent use of condoms.[42]

Finally, male circumcision has been demonstrated to be an effective tool in HIV prevention by reducing HIV acquisition by 51% in heterosexual men in rural settings in Uganda.[43,44] It has been suggested that the foreskin plays a role in HIV acquisition due to its large surface area which is rich in target cells and the presence of microabrasions from sexual activity.[12] The evidence of the efficacy of male circumcision in MSM is controversial due to lack of randomised-controlled trials;[45] this strategy may be protective for those that practise insertive anal intercourse.[46]

Prevention tools against HIV transmission and acquisition are illustrated in Figure 2. There is currently no vaccine available for HIV prevention, although research continues.

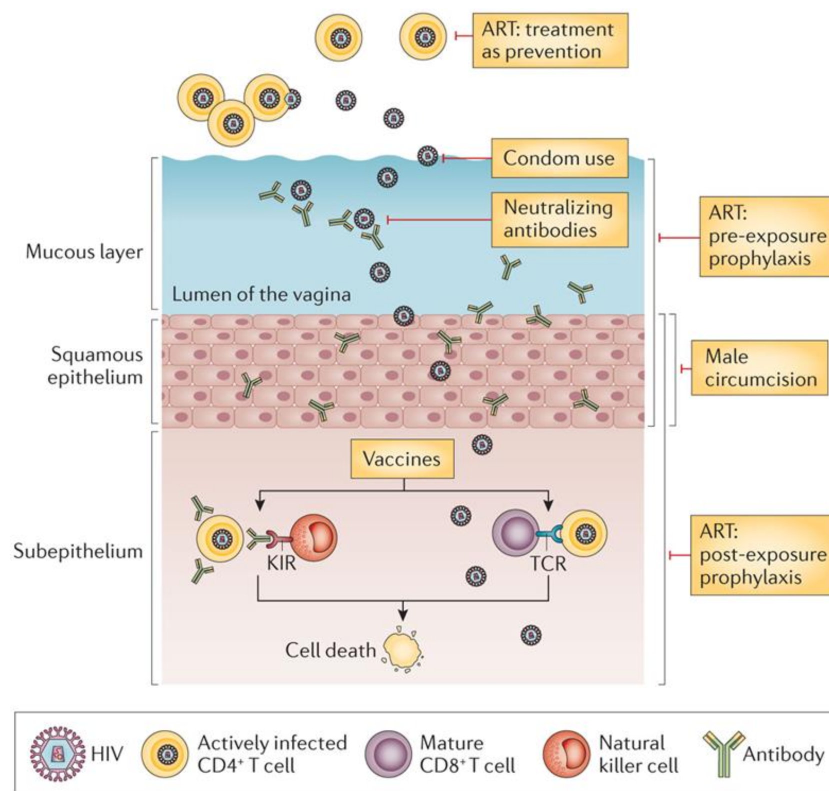


Figure 1-3. **HIV prevention tools**

Adapted from Deeks et al.[12] KIR= killer-cell immunoglobulin-receptor; TCR=T cell receptor.

1.5 Pathogenesis

The natural course of untreated HIV-1 infection is characterised by three consecutive phases: primary infection, asymptomatic phase, and AIDS phase (Figure 1-4).[9] Primary infection can be subdivided into six different stages based on unique patterns of laboratory assay reactivity during these early events (Fiebig stages).[47] The first stage is called eclipse and no plasmatic marker of the virus, including viral RNA, is detectable in plasma. It usually lasts a mean of 10 days from the infection and represents initial replication of the virus at the inoculation site and its spread to the satellite lymphoid organs. As viral sequences of the virus at the earliest stages of the infection are very homogeneous, only a single virion or a very small number of closely related ones, usually CCR5-tropic, are transmitted and are responsible for the onset of the infection in the newly infected individual.[48] In Fiebig stage I the viral RNA becomes detectable in plasma. Subsequent stages II-VI are characterised by the following consecutive events: detection of the p24 antigen (stage II); seroconversion with appearance of anti-HIV antibodies detectable by immune assays (stage III); indeterminate Western Blot (stage IV); positivity of Western blot with negative p31 antigen (stage V); and positivity of Western blot with positive p31 antigen (stage IV).[47] The viral load peaks at the third-forth week from inoculation, usually during Fiebig stage II, and starts declining during Fiebig stage V, plateauing at Fiebig stage VI.[28] High viral load and a high risk of transmission characterise the acute phase.[49] It is at this early time of the infection that the virus disseminates and seeds widely establishing the viral reservoir in lymphoid tissues.[50] Acute infection is also characterised by an intense immune-inflammatory response, with high levels of chemokines and cytokines. This phase can be accompanied by the onset of clinically overt symptoms, such as cutaneous rash, diarrhoea, and an influenza-like syndrome.[28] The marked rise in plasmatic viral load that is observed during acute infection is typically accompanied by a sharp decline in CD4 cell counts. Coinciding with the development of host immunity, the viral load starts to decline to reach its plateaux (the viral load "set-point") in Fiebig stage VI. The set point is nearly stable during the asymptomatic phase of the infection, showing a small gradual increase over time, and the level is highly predictive of the rate of disease progression. Along with the decline in viral load there is an initial partial restoration in CD4 cell counts, followed by a

progressive decline during the asymptomatic phase culminating in the AIDS phase. The duration of the asymptomatic phase varies and depends greatly on the set point of the viral load, being shorter for higher levels.[9] The host immune response is not capable of controlling viral replication completely, as the virus constantly develops mechanisms to elude it. Chronic antigenic stimulation progressively drives immune exhaustion and immune dysfunction. The AIDS stage is characterised by marked rebound in viral load, CD4 count decline to less than 200 cells/mm^3, and development of opportunistic infections and malignancies. HIV-1 causes CD4 cell death to an extent by a direct cytopathic effect, and mainly via indirect mechanisms, which include enhanced inflammatory responses, accelerated thymic loss, fibrosis of the lymphoid tissue and impairment of haematopoiesis.[12] Apoptosis of infected CD4 cells is an important contributing factor to the CD4 count decline and it is mediated by the cellular enzyme *caspase-3*. *Caspase-3* mediates cellular apoptosis in T cells that are permissive to HIV infection. A subgroup of T cells are not permissive and do not support HIV replication. In this group cell death occurs via the enzyme *caspase-1*, which elicits pyroptosis. Pyroptosis is a highly pro-inflammatory form of apoptosis, in which there is the release of all the cytoplasmic contents from the cells, which in turn triggers pyroptosis in other cells sustaining a deadly vicious cycle.[51]

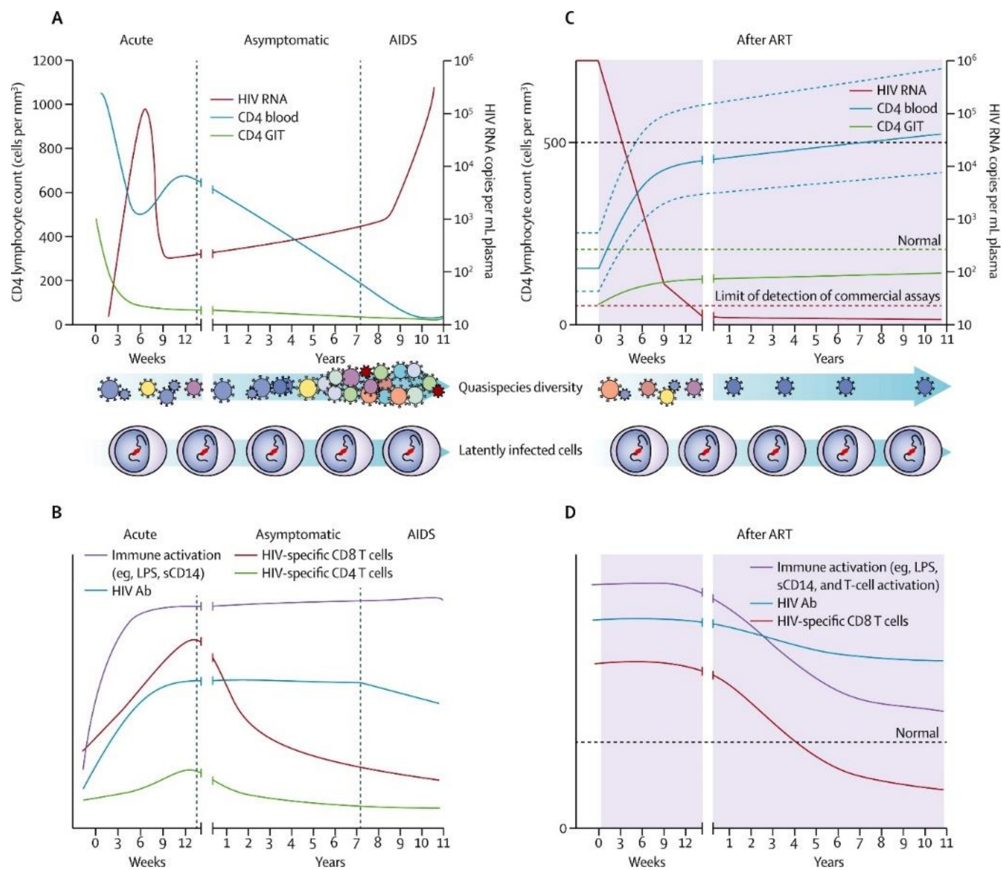


Figure 1-4. **Natural course of untreated and treated HIV infection**

Adapted from Marteens et al.[52] The figure depicts the natural course of the infection in untreated subjects (diagrams A and B) and in subjects on ART (diagrams C and D). GIT=gastrointestinal tract; LPS=lipopolysaccharides.

The pathogenesis of HIV-related disease depends on two distinct but interrelated mechanisms. Along with immunosuppression with development of opportunistic infections and AIDS-related malignancies, HIV promotes a constant state of immune-activation in the host, along with activation of the endothelium and of the coagulation system. This begins early, and it is characterised by increased proinflammatory mediators, low CD4/CD8 ratio, and exhaustion and senescence of T cells and monocytes (Figure 1-4).[53] The inflammatory state is believed to be responsible for the premature ageing and multi-organ disease observed in patients with HIV infection. This is at the basis of premature onset of cardiovascular disease, neurocognitive decline, liver disease, non-AIDS related cancers, and overall increase in mortality.[52] In addition, HIV infection profoundly alters the architecture of lymphoid tissues: changes in the intestinal lymphoid tissues observed in HIV positive subjects lead to a marked increase in microbial translocation due to the disruption of the physiological

gut barrier, increase plasmatic circulation of lipopolysaccharides, a component of the bacterial cell wall, which in turns enhances persistent immune activation.[54]

Direct pathogenesis of HIV is observed in other sites beyond the immune system. In the central nervous system and in the kidneys, the virus can infect via a CD4-independent mechanism, astrocytes and renal epithelial cells, respectively. These mechanisms are at the basis of the onset of HIV-associated neurocognitive disorder (HAND) and HIV-associated nephropathy (HIVAN).[52]

1.6 ANTIRETROVIRAL THERAPY AND DRUG RESISTANCE

In the absence of ART, HIV infection ultimately leads to the death of the infected individual within two years after the onset of AIDS, which occurs a median of ten years after infection.[55] The development of the first ARVs in the late 1980s, and the subsequent introduction of triple therapy in 1996, has set the scene for one of the most dramatic successes of modern medicine. From almost certain death, thanks to these agents, which are capable of suppressing viral replication via the blockage of crucial steps in the viral life cycle, life-expectancy of HIV-positive individuals has significantly increased, albeit still gaps exist with HIV-negative subjects in middle and low-income countries.[56]

After treatment initiation, restoration of $CD4^+$ cells counts occur in two phases: in the initial phase there is redistribution of activated memory T cells, reduced apoptosis and improvement in thymopoiesis; a second phase begins 4-6 weeks later, and it is characterised by a shift in the T-cell receptor repertoire from Th2 to Th1.[57]

In sub-Saharan Africa, 20%-25% of HIV-positive individuals present for care with advanced immunodepression, characterised by a depleted $CD4^+$ cells count <100 cells/µL.[58] This exposes them to a higher risk of opportunistic infections (OI) and to a weaker virological response once ART is initiated. In addition, in a subgroup of patients with advanced immunodepression, a paradoxical worsening of the clinical condition can be observed after treatment initiation, due to the restoration of the immune response to the active OI. This is called immune reconstitution inflammatory syndrome (IRIS), and its onset and severity might depend on the antigen load of the opportunistic pathogen, on the specific nature of the immune response, and on the genetic characteristics and HLA haplotypes of the host. IRIS can complicate ART initiation; morbidity and mortality caused by IRIS can be significant, and prompt management with delay ART start, corticosteroids and eventually ART discontinuation must be considered in specific cases.[57]

As of today, ART is not able to eradicate HIV infection and HIV-positive individuals must remain on continuous and uninterrupted treatment for life. Upon discontinuation of suppressive ART, viral replication almost inevitably resumes from the viral reservoir, which is unaffected by current treatment strategies. The reservoir is

established during the acute phase and is highly stable.[59,60] It consists of integrated HIV DNA within the host genome,[61] predominantly within memory $CD4^+$ T-cells. These cells are characterised by a long life in a quiescent state and can undergo both homeostatic and antigen-drive proliferation, resulting in expansion of the reservoir without virus production .[61] In addition, it is thought that persistent HIV replication with virus production may occur in anatomical sites with suboptimal drug penetration or activity, such as highly fibrotic lymphoid tissue[62] and the central nervous system.[63,64]

An additional challenge is the ability of the virus to develop resistance. As discussed, viral replication is an error prone process which is capable of generating several mutations in the viral genome. This process is caused by the high rate of transcription errors generated by RT, its lack of proofreading ability and the high rate of recombination events between different RNA molecules.[15] Drug-resistant variants carrying one or sometimes two resistance-associated mutations (RAMs) in the targets of therapy arise spontaneously during replication. In the absence of selective drug pressure, resistant variants tend to occur at low frequency within the viral quasispecies reflecting low fitness relative to wild-type virus.[65] If virus replication continues under drug pressure however, for example as a result of poor adherence, resistant variants acquire a selective advantage, expand becoming dominant within the quasispecies, and can be detected by sequencing the viral genome. The number of RAMs that HIV requires to become resistant to a certain ARV depends on the drug. The barrier to resistance of a certain ARV depends on both the number of RAMs required to confer resistance and the effect of each RAM on viral fitness.[66] The viral genome is highly plastic. With continued virus replication under drug pressure, the viral genome continues to evolve and may acquire additional RAMs that increase resistance and cross-resistance, and additional mutations that restore viral fitness and are termed compensatory mutations. RAMs can thus be classified into primary, if they directly reduce drug-susceptibility, and accessory, if they increase viral fitness. Potency of an ARV is a measure of its ability to suppress virus replication and reduce the viral load. Potency and barrier to resistance are the intrinsic shields that an ARV possesses against the development of drug resistance and virological failure (Figure 1-5). There is no cross-resistance between drug classes, while cross-resistance within the same class is highly common.[66]

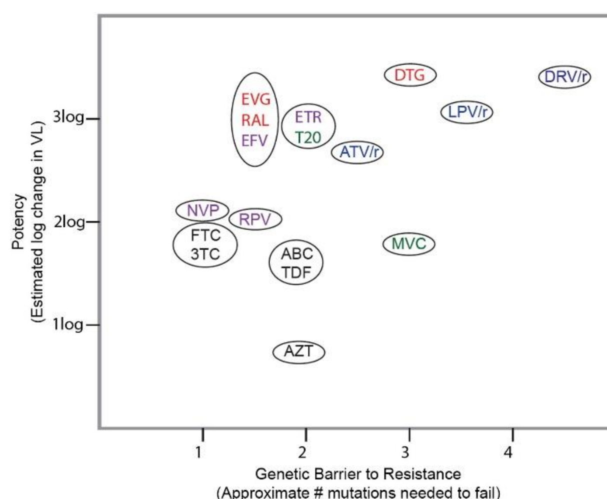


Figure 1-5. **Potency and barrier to resistance of main ARVs**

Adapted from Clutter DS et al.[66] NVP=nevirapine; FTC=emtricitabine; 3TC lamivudine; EVG=elvitegravir; RAL=raltegravir; EFV=efavirenz; RPV=rilpivirine; ETR=etravirine; T20=enfuvirtide; ABC=abacavir; TDF=tenofovir disoproxil fumarate; AZT=zidovudine; ATV/r=atazanavir/ritonavir; DTG=dolutegravir; MCV=maraviroc; LPV/r=lopinavir/ritonavir; DRV/r=darunavir/ritonavir

1.6.1 NRTIs

The first ARV introduced in clinical practice was zidovudine (AZT), a thymidine analogue that belongs to the NRTI class. AZT, after undergoing intracellular phosphorylation to AZT triphosphate, binds to the active site of RT, and is incorporated into the elongating DNA chain causing its termination. After the introduction of AZT, several other NRTIs were developed comprising the nucleoside analogues stavudine (d4T), didanosine (ddI), zalcitabine (ddC), lamivudine (3TC), FTC, and abacavir (ABC), and the nucleotide analogues TDF and more recently tenofovir alafenamide (TAF).[9] Due to their toxicity profile, d4T, ddC, and ddI are no longer recommended.[67] Tenofovir (TFV, as either TDF or TAF) or ABC in combination with FTC or 3TC are the preferred NRTI backbones for currently recommended ART regimens.[68,69]

Prior to 1995-1996, attempts at treating HIV with either one or two NRTIs alone failed to maintain suppression of HIV replication due to the emergence of drug resistance. There are two distinct genetic mechanisms of NRTI resistance: discriminatory RAMs enable RT to distinguish between the NRTI and natural nucleotides, thereby reducing

binding of the NRTI, whereas primer unblocking RAMs facilitate the excision of the incorporated NRTI from the elongating DNA chain, usually using ATP as the acceptor. Thymidine analogue mutations (TAMs) are selected by the thymidine analogues AZT and d4T. Emergence of TAMs during treatment with AZT or d4T occurs in a step-wise fashion, with gradually increasing levels of resistance and cross-resistance. Two distinct patterns are recognised: type 1 TAMs include M41L, L210W and T215Y, while type 2 TAMs include D67N, K70R, T215F and K219Q/E.[70] Overall the TAM 1 profile is associated with greater levels of NRTI resistance than the TAM 2 profile. The T215Y mutation is a primer unblocking RAM which has a significant impact on NRTI resistance. Although selected by AZT and d4T, TAMs cause cross-resistance ddI, ddC, TFV and ABC, and have less significant effects on 3TC and FTC. [66] By affecting the active site of a key viral enzyme, TAMs tend to markedly reduce viral fitness. As a result NRTIs such as TFV and AZT can retain significant antiviral activity despite the presence of TAMs.[71]

Resistance to the cytosine analogues 3TC and FTC is mainly mediated by the discriminatory mutations M184I and M184V. During treatment with 3TC or FTC, M184I may emerge first and is then followed by the emergence of M184V as the fitter variant. Nonetheless M184V retains a significant effect on viral fitness. These mutations cause high-level resistance to both 3TC and FTC, and also reduce susceptibility to ABC. However, they can increase susceptibility to TDF and AZT by countering the effect of primer unblocking mutations.

Other common discriminatory mutations are K65R, K70E/G/Q, L74V/I, and Y115F. K65R is selected primarily by TVF and ABC and to a lesser extent d4T, and confers resistance to these NRTIs as well as 3TC and FTC. K70/E/G/Q can be selected by both ABC and TDF, and confer resistance to these drugs, and potentially reduce susceptibility of 3TC and FTC.[72] L74V/I are selected primarily by ABC and ddI, and confer resistance to ABC and 3TC and FTC. Y115F is selected primarily by ABC and confers resistance to ABC; in combination with K65R or Q151M, it reduces susceptibility to both TDF and ABC. Thus, this group of discriminatory RAMs tend to cause substantial NRTI cross resistance. However, the mutations either do not reduce or in fact increase susceptibility to AZT.

Certain mutations are able to cause multi-NRTI resistance. This is the case of the Q151M complex: Q151M can occur with two or more of the accessory mutations A62V, V75I, F77L, and F116Y. Alone, Q151M causes high-level resistance to AZT, ddI, d4T, and low-level resistance to 3TC, FTC and TDF. Resistance to these last three drugs increases in the presence of the accessory mutations.[73]

1.6.2 NNRTIs

The non-nucleoside reverse transcriptase inhibitors followed the NRTIs in the drug-development timeline. Nevirapine (NVP), efavirenz (EFV), etravirine (ETR), rilpivirine (RPV) and more recently doravirine belong to this class. NNRTIs cause RT inhibition with a different mechanism from the NRTIs. They do not require intracellular phosphorylation and act by binding to a hydrophobic pocket of the enzyme. Binding causes an allosterical effect that modifies the shape of the active site reducing its enzymatic function.[9] The most common NNRTI-related RAMs are L100I, K101E/P, K103N/S, V106A/M, Y181C/I/V, Y188C/H/L, G190A/S/E, and M230L.[74] NNRTIs are characterised by a low barrier to resistance, as a single RAM is often sufficient to cause high-level resistance (e.g., K103N with EFV or NVP). Moreover, there is extensive cross-resistance within the class. NNRTI RAMs reduce binding of the NNRTI. Owing to the indirect mode of action of the NNRTIs, RAMs cause reduced susceptibility without a significant impact on viral fitness.

1.6.3 PIs

An additional class of ARVs targets the enzyme protease, which mediates the maturation of the newly formed virion. They are called protease inhibitors (PIs), and were available for the treatment of HIV since the mid-1990s. They are currently used in association with the enzymatic boosters ritonavir (r) or cobicistat (c) to improve their plasmatic concentration and their pharmacokinetic profile. This class is characterised by the highest genetic barrier to resistance, as multiple RAMs are required for the virus to become resistant to PIs, and for this reason molecules of this

group are recommended as part of second-line regimens for patients failing treatment.[67] Lopinavir (LPV), darunavir (DRV), atazanavir (AVT) are the most widely used PIs; major RAMs in this class affect the substrate cleft of the enzyme and include V32I, G48V/M, I50V/L, V82A/T/L/F/S/C/M, and I84V/A/C. Reduced susceptibility can be caused by mutations of the enzyme core, as the case of L33F, and the enzyme flap, as the case of M46I/L and I54V/M/L/T/S/A.[66]

1.6.4 INSTIs

INSTIs block the activity of the viral integrase enzyme by binding to its active site. Raltegravir (RAL), elvitegravir (EVG), dolutegravir (DTG) and bictegravir (BIC) are INSTIs available for use in clinical practice. EVG is used with the booster cobicistat. RAL and EVG are characterised by a relatively low barrier to resistance, whereas DTG and BIC retain variable degree of activity against mutated viruses. RAL and EVG cannot be used sequentially, whereas there is evidence from clinical trials that INSTI-experienced patients with RAMs can respond to high doses of DTG in combination with other active drugs.[75-77] INSTIs share common mutational pathways, and the combination Q148H/R/K ± G140S/A confers the highest level of resistance and cross-resistance.[78] This is mediated by the high resistance effects of mutations at position Q148 combined with the compensatory effects of mutations at codon G140 which restore viral fitness. There is evidence that the occurrence of Q148H/R/K + G140S/A is less frequent in non-B subtypes.[79] Interestingly, in clade B sequences only one nucleotide is required to replace glycine with serine at codon 140, whereas two nucleotides are required for non-subtype B viruses, which consequently have a higher genetic barrier to resistance to this drug. As fitness cannot as easily be restored, viruses harbouring mutations at codon 148 are observed less frequently in non-B subtypes as well.

1.6.5 Entry inhibitors

The CCR5-antagonist maraviroc (MVC) and the fusion inhibitor enfuvirtide (T20) block virus entry by two distinct mechanisms. MVC blocks the interaction between the V3 region of gp120 and the co-receptor CCR5 and is only active against R5 virus. It is the first and to date only example of an antiretroviral agent that targets a host protein to exert antiviral activity. Resistance to MVC can occur either by emergence of viral populations that use alternative co-receptors for entry, or less commonly through emergence of mutations in R5 strains to allow use of the occupied co-receptor. T20 is administered subcutaneously. It binds to gp41 blocking fusion of the viral envelope with the cell membrane. Resistance can develop relatively easy through the emergence of mutations in gp41. These compounds currently have limited application in clinical practice.

1.6.6 Interactions between mutational pathways

Some mutations rarely occur together due to a marked increase in fitness cost for the virus (e.g., K65R with L74V).[80] In contrast, others are frequently seen in combination as they are capable of restoring the replicative ability of the mutated virus. This is the case of K65R with L74I or L74I with K103N, combinations that are found to be advantageous for viral replication.[81,82] A further example is provided by the association of M184I with E138K: both mutations reduce the replication capacity of the virus if in isolation, while when present in combination they can restore a fitness level comparable to the wild-type virus. This association confers resistance to rilpivirine and etravirine.[83]

1.7 TREATMENT GUIDELINES

It was thanks to the combination of 2NRTIs plus either a NNRTI or a PI that, in 1995-1996, it was possible to suppress viral replication effectively in an infected individual, and profoundly change the natural course of the infection.[84] Currently, both the World Health Organisation (WHO)[85] and guidelines from high income settings[68,69] recommend starting ART soon after diagnosis regardless of the CD4 cell count, based on data from a large clinical trial showing a higher all-cause mortality in the arm that deferred the start of ART based on CD4 count thresholds.[86] Most guidelines from high-income settings currently recommend treatment initiation with 2NRTIs + 1INSTI;[68,69] some guidelines still include RPV or ATV/r or DRV/r in first line agents.[69] The WHO recommends treatment initiation with 2NRTIs + DTG in programmatic settings in low and middle income countries regardless of the CD4 cell count. A DTG-containing regimen is currently recommended for second-line treatment for those patients failing a non-DTG based first-line regimen.[40] [87]

1.8 VIROLOGICAL MONITORING AND GENOTYPIC TESTING

The target of ART is achieving and maintaining the plasma viral load below the limit of detection of available assays. The plasmatic viral load represents the direct measure of the virological effectiveness of ART and provides the best surrogate marker for clinical effectiveness.[88] If the virus does not harbour RAMs and with optimal ART adherence, the vast majority of patients can achieve and maintain an undetectable viral load. In order to monitor treatment outcomes, viral load is superior to clinical assessment or CD4 cell counts. Care can be optimised by the information provided by the viral load, and resources invested for those with detectable viraemia, such as reinforced adherence support, closer virological monitoring, more frequent visits and prompt change of ART regimen. Viral-load-informed differentiated care is likely to be a cost-effective solution globally and especially in resource-limited settings, where universal virological monitoring is not yet fully implemented despite WHO guidance.[89] Current WHO guidelines recommend virological monitoring as the preferred approach to diagnose and confirm treatment failure. After ART initiation, a viral load test should be performed after 6 and 12 months, and every 12 months thereafter; virological failure is defined as two consecutive measures >1000 copies/mL in a 3-month interval, with an adherence support after the first measure.[90]

Virological monitoring can limit the emergence of drug resistance, by guiding early interventions to manage viraemia. Monitoring of the viral load has been established since the late 1990s in resource-rich settings and alongside improved drug potency and tolerability is believed to be a key determinant of the success of ART in countries such as the United Kingdom, where over 90% of patients receiving ART show a suppressed viral load.[91] In addition, genotypic resistance testing is routinely adopted in high-income settings at the time of HIV diagnosis or before treatment initiation to detect transmitted RAMs and optimise treatment selection.[92] However, its implementation in low resource settings to guide the choice of first-line or second-line regimens at the individual patient level is not feasible. Rather, programmatic care is complemented and informed by surveillance programme that monitor rates of drug resistance at the population levels.[93] Currently, in low income countries, a switch to second line ART regimens remains largely guided by the CD4 cell count trend and

clinical outcome, an approach that can result in a delayed switch and accumulation of drug resistance.[94,95]

Point-of-care (POC) viral load testing offers the advantage to potentially expedite the detection of viraemia, decentralise testing and increase rates of resuppression by improving retention in care. Use of POC technologies is currently expanding is low and middle income countries; however, in decentralised and remote conditions, POC technologies must function under extreme environmental conditions, inconsistent power supply, and must be easy to use by unskilled personnel.[96] Dried blood spots specimens can be used for virological monitoring and to detect treatment failure by using a virological cut-off of 1000 copies/mL under those circumstances, in remote areas with poor access to laboratory facilities. Plasma samples remain however to be preferred.[90,97]

Lack of testing capacity for monitoring the viral load should not represent a barrier to treatment initiation. The World Health Organisation recommends prioritising viral load testing for pregnant and breastfeeding women especially around the time of delivery; for infants and children; and for adolescents.[67]

1.9 ACQUIRED DRUG-RESISTANCE, TRANSMITTED DRUG-RESISTANCE AND PRE-TREATMENT DRUG RESISTANCE

Acquired drug resistance (ADR) is defined as the development of RAMs in an individual on ART, whereas transmitted drug resistance (TDR) is the presence of RAMs in ART-naïve individuals and it occurs when uninfected individuals become infected by strains harbouring RAMs. Pre-treatment drug resistance (PDR) is defined as the presence of RAMs in ART naïve individuals initiating treatment or in subjects with prior exposure to antiretrovirals either as part of prevention strategies, as for PMTCT, or for treatment, who are initiating or re-initiating first-line ART. PDR can be either ADR or TDR, or both.[98]

Prevalence of PDR is rapidly growing in sub-Saharan Africa, in contrast with high-income countries, where prevalence figures are stable around 10%.[99,100] PDR is associated with an increased risk of virological failure, poor immunological outcomes and future ADR once ART is initiated.[101] If prevalence of PDR were to reach 10% in sub-Saharan Africa, modelling indicates that it would result in 890,000 deaths due to AIDS and 4,500,000 deaths in the region between 2016 and 2030.[102] In pooled analyses of studies from sub-Saharan Africa, 65% and 62% of treated patients had a suppressed viral load by intention to treat analysis after 24 months of first-line NNRTI-based and second-line PI/r-based ART, respectively.[71,103] Reported rates of virological suppression differ by region, and tend to be higher in participants of randomised clinical trials than in those of observational cohorts.[71] Among individuals with treatment failure, 70%-90% harbour RAMs, with most resistance to NNRTIs.[72]

A prevalence of 7.2% was estimated in 2016 for NNRTI-related PDR in Western and Central Africa and an annual increase in the odds of PDR of 17%; prevalence estimates for Southern Africa and Eastern Africa are higher, with figures of 11% and 10%, respectively. [99] Prevalence of NRTI-related PDR appeared to be overall stable and <5% in Western and Central Africa; while PI-associated PDR was very rare (<1%).[99] Most common RAMs associated with NNRTI-related PDR were K103N, Y181C and G190A, while most common RAMs associated with NRTI-related PDR were M184V, and AZT-associated TAMs D67N and M41L.[99] TFV-associated RAMs K65R and L74I/V were relatively uncommon (<3%). Prevalence data on INSTI-

related PDR confirm that it occurs rarely in sub-Saharan Africa, and INSTI-associated RAMs detected mostly at low frequency <20%.[104]

In 2017, the WHO recommended a regimen not including a NNRTI in subjects at high risk of NNRTI-related PDR and urged countries with a prevalence of NNRTI-PDR >10% to consider alternative first-line regimens that do not include NNRTI.[105] Current recommendations have removed NNRTI from first-line regimens in favour of DTG, to overcome the issue of NNRTI-related PDR resistance in the region with a novel, more potent and robust agent.[40]

1.10 THE CHALLENGES OF ADHERENCE TO ART

In order to avoid the development of drug resistance and maximise treatment efficacy, optimal adherence to ART is essential. The level of adherence required to achieve improved immune faction and virological suppression varies and depends on the ART regimens and prior duration of virological suppression.[106] Adherence levels higher than 90-95% are believed to be needed to sustain viral suppression, allow immunological reconstitution and prevent hospitalisation.[107] Adherence to treatment is one of the strongest determinants of patient's survival and improvement of CD4 count over time.[108,109] Factors that influence adherence to ART in HIV-positive individuals are multiple and multifaceted. Begley et al. describe them, as intrapersonal, interpersonal and extra-personal.[110] Intrapersonal elements include the patient's cognitive and psychological processes, whereas interpersonal factors encompass relationships with others, social support, living conditions and interactions with the health-care providers. Extra-personal variables encompass all the elements that are not directly linked to the individual and its interrelations with others, such as medication side-effects, comorbidities, alcohol and drug use, lifestyle and socio-economic factors.[110]

In their review, Bolsewicz et al describe factors associated with suboptimal ART adherence in HIV-positive subjects in resource-rich countries, including poor health and well-being, emergence of drug-resistance, treatment failure, faster progression to AIDS and death.[111] Among intrapersonal barriers to ART adherence, the Authors highlight how low perception of the need for treatment and behavioural adjustments that ART adherence requires can cause deferral of ART initiation. Furthermore, mental health issues (i.e. depression, low mood), coping skills and poor information about HIV treatment and care can act as additional obstacles. In this scenario, ART represents a constant reminder of the disease and its internalised stigma, which can contribute to poor adherence.[111] People living with HIV fear the unwanted disclosure of their status and the subsequent discrimination if they are seen taking the medications, and can decide not to take them if travelling or in social contexts.[111] Health care providers play a fundamental role in the decision of the patient of starting, changing or stopping the treatment. Moreover, social relations and social support are among the interpersonal factors that strongly influence adherence, as maintaining a

meaningful role within the family and the community represents a fundamental target for patients.[111] According to the Authors, additional extra-personal barriers to ART adherence include medication side effects, comorbidities, use of recreational drugs and socio-demographics characteristics. The latter group includes age, gender, employment, housing conditions, and ethnicity. Moreover, barriers to accessibility to ART can include costs of ART if not provided free of charge, distance travelled to health services and economic hardship.[111]

The work of Mills et al. provides additional insights from sub-Saharan Africa, where optimal adherence may encounter additional obstacles.[112] Despite high rates of optimal treatment adherence, significant structural barriers, such as poor social support, inadequate transportation and food insecurity represent additional challenges. In addition, an overburdened health system, which does not include adequate facilities that address mental health and HIV counselling, and political and structural barriers, such as health illiteracy, gender inequalities and local believes on HIV contribute further to sub-optimal adherence to ART.[113] Finally, disclosure of HIV is recognised as an important step towards health-seeking behaviours and functional social support, ultimately facilitating treatment adherence; however, it is still very difficult in these contexts for fear of stigmatisation, discrimination or violence.[114,115]

Several interventions can be used to improve adherence to ART, which range from education and counselling; information and communication technology-enhanced solutions (i.e. the use of use of mobile phone to communicate with the patient on ART; electronic dosing monitoring; and electronic pharmacy refills tracking systems); healthcare delivery restructuring; economic incentives; and social protection interventions.[116] Adherence counselling are central in ART prescription and delivery, and included in WHO guidelines upon recognition of viraemia.[67] However, they are not easily standardised, their quality highly dependent on the training of the counsellor, and difficult to implement in busy HIV clinics in sub-Saharan Africa.[116]

Long waiting times at the clinics, fragile supply chain leading to stock-outs, and long journeys to attend the health care provider represent additional challenges to optimal adherence to ART.[116] In low-resource settings, the health care delivery remains inadequate to provide long-term care, due to the high patient load that over burden the system and the limited resources.

1.11 THE HIV EPIDEMICS IN GHANA

With a median estimate of 4% in HIV prevalence in the entire WHO African region, Africa bears the highest burden of disease. Figure 5 illustrates the global HIV prevalence by WHO region.

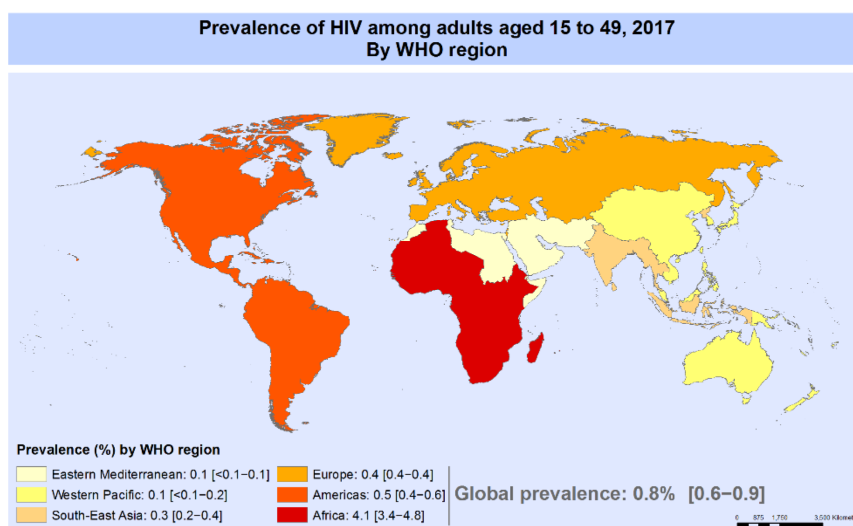


Figure 1-6 **HIV prevalence according to WHO region**

Adapted from https://www.who.int/gho/hiv/en/.

From the year 2010, there has been a decrease in the HIV incidence and HIV-related mortality in the sub-Saharan region, with marked differences between Eastern and Southern Africa, which have had historically the highest rates of infection and also experienced the greatest reductions (-30% in HIV incidence and -42% in AIDS-related mortality), and Western and Central Africa, where these declines have been smaller (-8% in HIV incidence and -24% in AIDS –related mortality). The latest estimates place the number of individuals living with HIV at 19.6 million in Eastern and Southern Africa and 6.1 million individuals in Western and Central Africa.[24]

Despite the great achievements of the scale-up of ART, a number of challenges persist in treating HIV in Africa: ART coverage remains suboptimal due to insufficient availability and poor affordability, HIV incidence remains high and there is very limited access to virological monitoring and to second and third lines.[53] As the

infection takes hold in young adults at the peak of their economic productivity, this altered the economies of the countries that bear the highest prevalence.

Ghana is a lower middle-income country located in Western Africa and is the country where the entire research presented in this thesis was conducted. HIV prevalence in adult Ghanaians is 1.7% (1.4-2.0),[24] greater than the 1% threshold set by the WHO to define a generalised epidemic.[67] The total number of people living with HIV is estimated to be 310,000 (260,000-370,000), with an incidence of 19,000 (15,000-24,000) new infections per year (84% adults, 16% children) (HIV adult incidence in 2017: 0.7%) and 16,000 (12,000 – 19,000) estimated AIDS-related deaths per year. ART coverage for is 40% (33-47).[24] HIV prevalence in the Ashanti region is currently estimated at 1.5%, where 18.5% of the total HIV population of the country live.[117] In terms ART coverage, it is estimated that there is a 60% of HIV-positive individuals not on treatment.[118]

The following figures come from the latest report of the Ghana AIDS Commission. Despite an almost universal awareness of the epidemic in the population, knowledge about HIV prevention and transmissibility is still below 30% in young men and women aged 15-24. This goes along with a high prevalence of high-risk behaviours, namely having more than one sexual partner (e.g. 53.8% in men aged 25-49 years), young sexual debut (before 15 years of age) and a low adherence to condom use among people reporting high sexual risk behaviours (<20%).[117]

1.11.1 HIV infection in Ghanaian key populations

One of the recognised determinants of HIV spread in Ghana is marginalisation of key populations (KPs), such as female sex workers (FSWs), men who have sex with men (MSM), and injecting drug users (IDUs). Fears of discrimination, stigma, social hostility and criminalisation of their activities prevent these groups to access HIV prevention services.[117] Data on KPs and on their linkage to care and treatment are lacking;[119] it is believed that KPs are disproportionally affected by the epidemics in the country. Women are the most affected by the epidemic; men with multiple sex partners or clients of FSWs represent the bridge of the infection to the female population. It is estimated that there are about 65,000 FSWs and about 55,000 MSM

in Ghana.[119] Prevalence of HIV was 6.9% in FSWs in 2015;[119] and 18.1% in MSMs in 2017.[119] FSW and their clients, and MSM, account for 28% of all new infections in the country.[119] Interventions targeting these key groups have decreased the HIV incidence in FSWs; currently, the main drive of HIV spread is casual heterosexual sex. [117]

1.11.2 HIV treatment guidelines in Ghana

Prior to September 2016, patients were eligible for treatment if their CD4 count was below 350 cells/mm^3 or had symptomatic HIV infection in WHO clinical stage 3 or 4. Recommended first-line was AZT+3TC+NVP, with EFV as an alternative drug to NVP in case of liver disease, and TDF as an alternative to AZT in case of anemia.[120]

From September 2016, according to national guidelines for the treatment of the HIV infection, Ghanaian HIV-positive subjects are eligible to receive ART regardless of WHO clinical stage or CD4 cell count.[118] Two pre-treatment adherence counselling sessions are required prior to the initiation of ART to ensure patient's motivation. Recommended laboratory testing include viral load (with cost currently to be covered by the patient) and CD4 cell count at ART initiation (with intermittent availability free of charge due to stock-outs of reagents),[121] after six months, and then yearly thereafter. Viral load testing is available in 9/10 geographical regions of the country, but coverage remains low (10%-14%).[119]

Current recommended first-line regimens are TDF+3TC (or FTC) +EFV; alternative options are TDF+3TC (or FTC)+NVP or AZT+3TC (or FTC)+NVP (or EFV). First choice for second line ART is AZT+3TC (or FTC) +LPV/r (or ATV/r) (if TDF was used in the first-line regimen) or TDF+3TC (or FTC)+LPV/r (or ATV/r) (if AZT was used in the first-line regimen). All HIV-positive individuals should receive a test for hepatitis B surface antigen (HBsAg) among baseline tests; in case of a positive result, a second test should be repeated after 6 months to confirm chronic hepatitis B (CHB). This recommendation is not routinely implemented however. Thus, whereas first-line and second-line ART for HIV/HBV co-infected individuals should include or retain TDF+3TC (or FTC) as part of the regimen, ART continues to remain largely HBV-blind.[118]

1.11.3 Antiretroviral drugs management within the Ghanaian healthcare system

Provision and funding for ART in Ghana started in 2003 through Global Fund grants that covered ART, $CD4^{+}$ cells counts and viral loads. Up to 2010, donor funding accounted for 75% of the HIV/AIDS spending of the country. From 2011, the Ghanaian government committed to providing 150 million Ghana cedis (approximately US$98.8 million in June 2011) to continue the country HIV-related programmes and to guarantee continuous drug provision. The necessary provisions to ensure a steady drug supply were not made by the country; the Global Fund grants were consequently extended to 2014 to cover the initial cohort. However, with the changing treatment guidelines, the eligible population more than doubled. These needs surpassed the grants supplied by the Global Fund and the government provisions, and emergency shipments of drugs were partially covered by the United States government and other donors, providing treatment for 64% of the eligible population.[121]

The United States President's Emergency Plan for AIDS Relief (PEPFAR) is currently supporting Ghana in expanding treating programs to cover all eligible individuals. From an initial support that was limited to technical assistance, PEPFAR supplemented its funds to Ghana in 2016-2017 due to the increased needs caused by the change in the guidelines that made all HIV-positive individuals eligible for treatment.

1.11.4 Drugs stock-outs in Ghana

Qualitative research has highlighted the presence of drug stock-outs in Ghana as a continuous problem, especially after the expiration of the original Global Fund in 2011; between 10% and 30% of patients experienced a treatment interruption due to drugs stock-outs.[122,123] Drugs stock-outs inevitably increase the number of patients' accesses to the healthcare centres due to the partial filling of the prescriptions: this causes an increment in the healthcare costs, and a greater economic burden on the

patients as well, who have to sustain multiple journeys to collect their medications. Procurement of the drugs is from abroad, as the only supplier in the country has not met WHO standards yet. Provision from suppliers that are external to the country faces the problems of transportation and long stay at the ports, which causes additional delays.[121]

In a qualitative study conducted at the Komfo Anokye Teaching Hospital (KATH), in Kumasi, which explored reasons for treatment discontinuations, 28% of the participants reported a history of ART discontinuation due to drugs stock-outs. About a third of patients reported that they interrupted treatment during the stock-out; in addition, others reported coping strategies like reducing the frequency of how they take their medication (i.e. from twice to once daily) and reducing the number of pills. For most patients it was too expensive to buy medicines from the private sector or take days off to travel to other hospitals.[124]

1.11.5 Pre-treatment HIV drug resistance in Ghana

An early study from 2009 investigated the prevalence of PDR in pregnant ART-naïve women in Ghana and documented a low prevalence <5%;[125] another study conducted in the same year included newly diagnosed subjects and did not document any TDR prior treatment initiation in this group.[126] However, more recent studies place the prevalence of TDR in ART-naïve Ghanaian pregnant women between 5%-15%.[127,128] Use of ART for PMTCT has been available in Ghana since 2003; more worrisome figures come from studies that reported data on women with previous exposure to ART for PMTCT who reinitiated treatment for their own health: they were more likely to harbour RAMs, with figures as high as 32%.[127] In line with data from the entire region reported earlier, most common RAMs included K103N, M230L and L100I affecting NNRTIs and M184v and the TAMs T215Y and M41L affecting susceptibility to 3TC and AZT, respectively.[127]

1.11.6 Prevalence of infective comorbidities in the HIV population in Ghana

Infectious comorbidities that were investigated in this research project include hepatitis B, hepatitis C, hepatitis D and schistosomiasis.

A systematic review and meta-analysis of the data presented in the literature pooled the prevalence of HBV/HIV coinfection in Ghana at 13.6%, ranging from 2.4% to 41.7%;[129] subsequent prevalence studies from Ghanaian HIV-positive cohorts reported a prevalence of the coinfection from 6.1% to 12.5%.[130,131] Data from the HIV cohort at KATH place this figure at 16.7%.[132] Screening for HBV co-infection is of paramount importance prior ART initiation, where inclusion of tenofovir or retention of tenofovir in a changing regimen is not necessarily part of routine practice, and to guide the evaluation of liver health. Yet, it is not uncommon for HIV/HBV co-infected patients to be receiving ART regimens with 3TC as the sole anti-HBV active agent.[133] In areas with high HBV prevalence such as Ghana, initiation of first-line ART or switch to second-line ART must not be HBV "blind", however HBV screening is not part of routine care, meaning that tenofovir is not always used in HBsAg-positive subjects.

A systematic review and meta-analysis on the prevalence of HCV/HIV co-infection in Ghana pooled the figure at 2.8%; prevalence reported from two studies conducted in the HIV-positive population at KATH varied from 1.0% to 5.5%.[131,134]

A systematic review and meta-analysis on the prevalence of HDV infection in sub-Saharan Africa documented that, in West Africa, general prevalence of HDV co-infection in the HBsAg-positive population is estimated at 7.3%, with great variation according to geographical region; seroprevalence of hepatitis D virus does not differ according to HIV prevalence, after adjustment for African region.[135] In Ghana, it is estimated at 2.3%.[135]

A systematic review and geostatistical analysis on the prevalence of schistosomiasis in Ghana in school-aged children estimated it at 23.3%, of which 22.3% caused by *Schistosoma haematobium* and 1.3% by *Schistosoma mansoni*.[136]

1.12 AIM AND OBJECTIVES

1.12.1 Aim of the research project

The aim of this research project was to investigate current clinical needs of a mature cohort of HIV-positive individuals on long-term ART in an urban setting in Ghana in order to find implementable strategies to optimise their health care.

1.12.2 Objectives

1.12.2.1 Objective 1

To explore rates of resuppression after virological monitoring at point of care followed by an immediate adherence intervention.

1.12.2.2 Objective 2

To determine viral load and drug resistance outcomes of patients on first-line NNRTI-based ART that underwent a changed in the NRTI-backbone from AZT or d4T to TDF in the absence of virological monitoring.

1.12.2.3 Objective 3

To explore how self-reported adherence to ART measured by a visual analogue scale, multi-item questionnaires and composite scores correlates with viral load and drug resistance.

1.12.2.4 Objective 4

To investigate prevalence and determinants of tubular proteinuria and rapid decline of estimated glomerular filtration rate (eGFR) in HIV/HBV co-infected patients after long-term exposure to TDF.

1.12.2.5 Objective 5

To assess the liver health of the HIV cohort attending for outpatient care by measuring prevalence of viral hepatitis coinfections and prevalence and determinants of liver steatosis and fibrosis.

1.13 THEMATIC FRAMEWORK AND THESIS STRUCTURE

Figure 1.7 summarises the thematic framework of this research dissertation. Each research question and related study objective, as stated in sub-chapter 1.12, are addressed in the result chapters 3 to 7. The result chapters are structured in paper format.

Chapter 1 provides an introductory background for the reader. Chapter 2 reports overall materials and methods used for this work; more specific references are made to the result chapters, which have their own methodology section. Chapter 8 provides a general discussion on the findings.

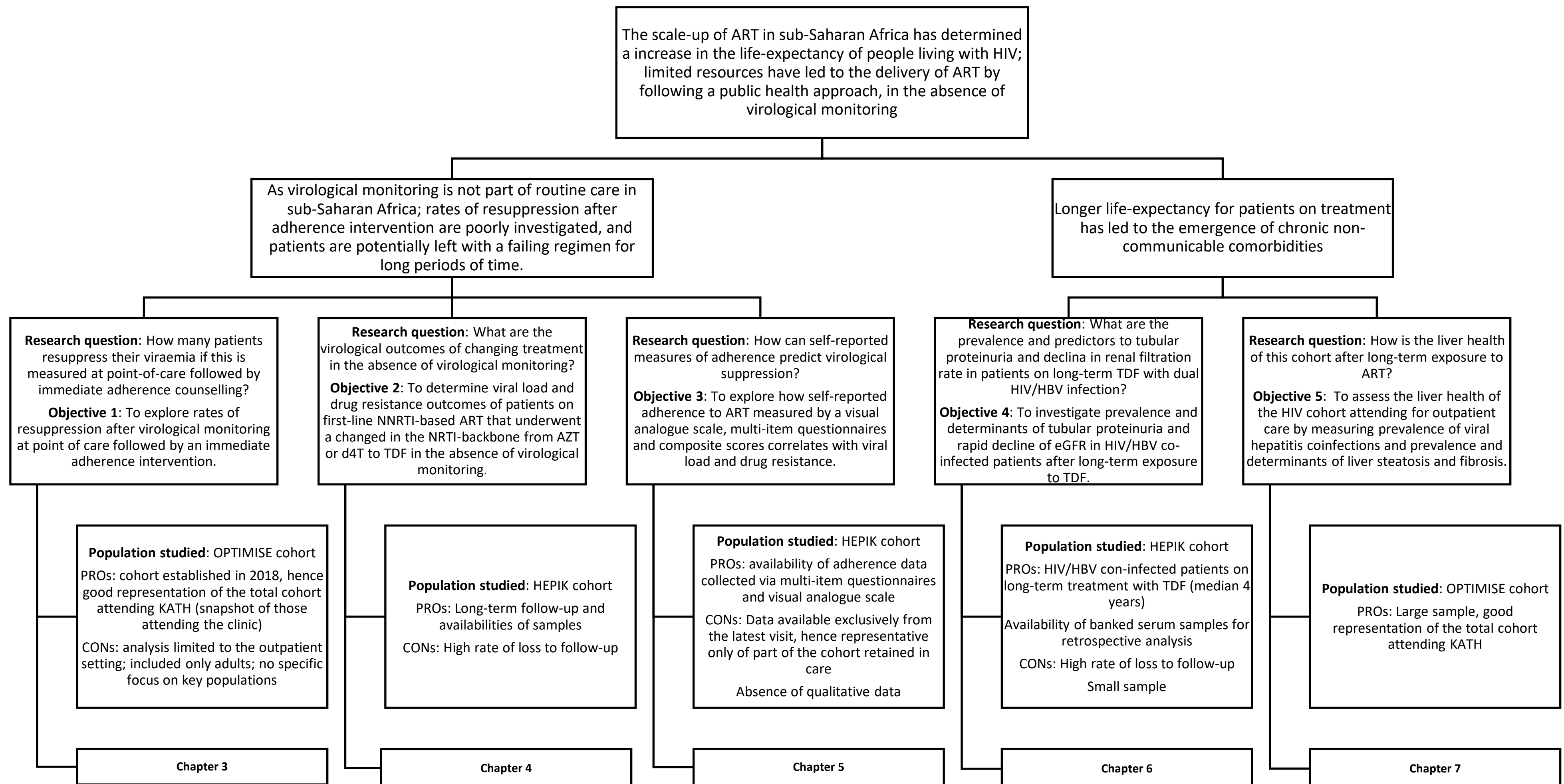


Figure 1-7 Thematic Framework of the dissertation

2 SECOND CHAPTER - MATERIALS AND METHODS

2.1 STUDY COHORTS

The Venn diagram in figure 2.1 illustrates the study cohorts.

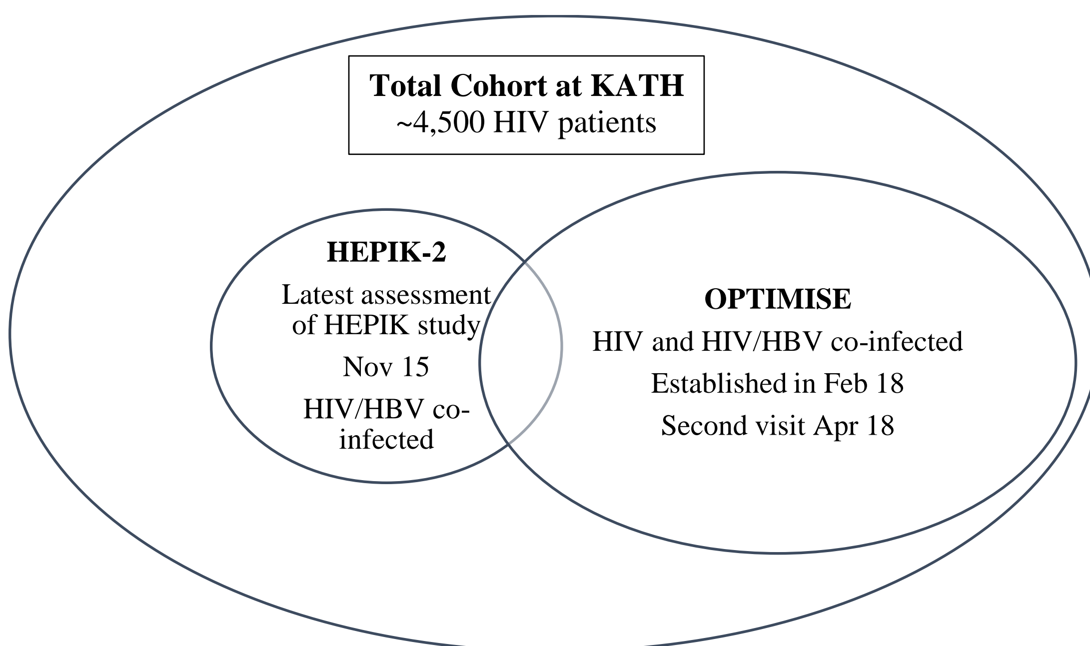


Figure 2-1 **Study cohorts**

2.1.1 The OPTIMISE Cohort

The OPTIMISE Study is a prospective cohort study that recruited HIV-infected adults (≥18 years old) receiving out-patient care at KATH. The overall aim of the study was to optimise their chronic care, including: (i) improving ART outcomes by identifying patients that should be fast-tracked for POC viral load testing and immediate adherence support upon detection of viraemia; (ii) improving the recognition of liver disease, by assessing patients for HBV status and hepatic fibrosis and steatosis; (iii) improving the diagnosis of unrecognised non-communicable diseases, with a focus on hypertension, diabetes, raised blood lipids, and chronic kidney disease. The cohort

was established in February 2018. Participants were adults patients aged more than 18 years and were enrolled from the KATH outpatient HIV clinics. The study did not include in-patients, pregnant women, children, adolescents and patients on treatment for tuberculosis. Data on other key populations, such as MSM, IDUs, FSWs, were not collected.

2.1.2 The HEPIK Cohort

The Hepatitis B in Kumasi (HEPIK) study is a prospective cohort study of HIV/HBV co-infected individuals receiving care at KATH. Initially, consecutive HIV-positive patients with unknown HBsAg status were screened in 2007 for HBsAg as part of a study whose principal aim was to determine the HBsAg seroprevalence in HIV-positive individuals in this setting.[132] HIV/HBV co-infected subjects were recruited to the HEPIK study from 2010, when participants introduced TDF in their regimen following WHO 2013 guidelines, which included phasing out of d4T and inclusion of TDF in all ART regimens for HIV/HBV co-infected individuals.[137] The HEPIK study consisted in a cross-sectional analysis of TDF-naïve, 3TC-experienced subjects, and a prospective analysis of 3TC-experienced subjects who were assessed before and after (median 8 months) the introduction of TDF as part of ART.[138] This study presented the first analysis of liver fibrosis by transient elastography (TE) and associated markers of liver disease and virological status among HIV/HBV co-infected subjects with long-term 3TC exposure in sub-Saharan Africa, and was the first to analyse prospectively the effect of introducing TDF in such populations.[138] This evaluation was conducted in July 2011 and July 2012. The main findings were that after nearly four years of 3TC-containing ART, over half of patients had persistent HBV replication, one third had HBV DNA levels >2000 IU/mL, nearly a third had HBV drug-resistance, and one in eight had TE measurements consistent with cirrhosis. HBV responses to the introduction of TDF, while continuing 3TC, were highly encouraging, with marked reductions in HBV DNA levels and reducing TE measurements in those with higher baseline measurements. After just 8 months of TDF, the proportion of subjects with predicted cirrhosis by TE fell from 7% to 4%.[138] The last study visit was conducted in November 2015 (HEPIK-2 study), with the aim of determining the degree of long-term change in liver elasticity by TE after a median

of 4 years since the introduction of TDF as part of ART and relative to the assessment made in the same patients in 2011-2012.

2.2 SETTING

KATH is a 1200-bed facility and the main hospital of the metropolis of Kumasi, the second municipality in Ghana and the main city of the Ashanti Region (Figure 2.2). Kumasi is inhabited by ~1.5 million people.

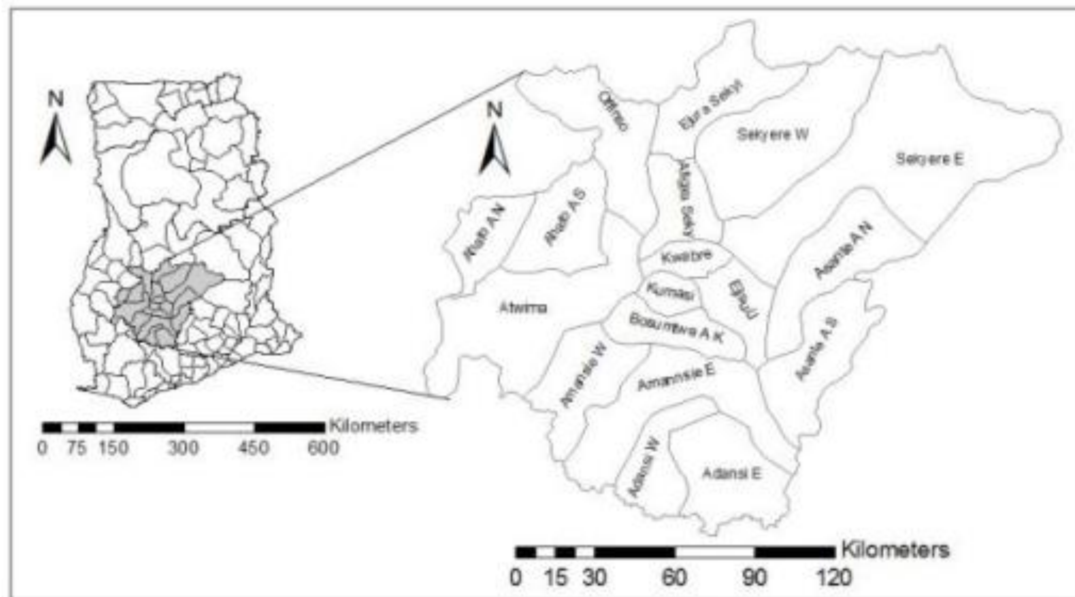


Figure 2-2 **Map of Ghana and of the Ashanti region.** Adapted from Osei FB et al.[139]

KATH in located in the main urban area of Kumasi, where it offers HIV outpatient and inpatient care. HIV outpatient clinics are hold twice weekly: patients, in the absence of clinical symptoms, are evaluated by a nurse and prescriptions of ART are delivered by the hospital pharmacy, where antiretrovirals are stored and dispensed every three months. ART is supplied free of charge, but patients pay every additional test. Patients are evaluated by a physician if they report physical complaints. Patients normally collect their treatment every three to four months. KATH has yet to adopt routine virological monitoring. CD4 cells counts are available, but CD4 count monitoring is not part of routine management of the patients.

As of May 2019, a precise figure of all patients attending the HIV Clinic at KATH is unknown. Once interrogated, health care personnel at KATH estimate it to be between

4,000 and 5,000 individuals. In 2019, during the first five months, 262 new HIV cases were registered, of whom 176/262 (67.2%) were females.

2.3 ETHICS

The ethical approval for the HEPIK Study was granted in September 2010 by the Committee of Human Research, Publication and Ethics, Kwame Nkrumah University of Science and Technology (protocol number CHRPE/143/10) and obtained renewal in October 2015 (protocol number CHRPE/AP/347/15) for the projects presented in this dissertation. The original approval in 2010 was sponsored by the Royal Society, and was conferred before studies conducted in foreign countries with approval from the local ethics committee required concomitant approval from Institutions in the United Kingdom.

The ethical approval for the OPTIMISE Study was granted in January 2018 by the Committee of Human Research, Publication and Ethics, Kwame Nkrumah University of Science and Technology (protocol number CHRPE/AP/017/18) as an amendment and extension of the approval granted for the HEPIK Study. Transfer agreement of the samples collected in Kumasi was obtained from the University of Liverpool (Appendix 1).

2.3.1 Abnormal laboratory findings and diagnosis of comorbidities

Results from biochemistry testing, urinary dipsticks, CD4 counts, HIV viral load and screening of hepatitis coinfection and Schistosomiasis were made available to the HIV clinic for inclusion in the medical files of the patients. Abnormal biochemistry results that were deemed to require an immediate intervention, CD4 counts <200 cells/mm^3, HIV viral loads >1000 copies/mL and diagnosis of coinfection were flagged and discuss with the medical personnel.

Upon diagnosis of hepatitis B co-infection via a positive surface antigen (HBsAg), antiretroviral treatment was reviewed to ensure inclusion of TDF; patients diagnosed with Schistosomiasis were treated with appropriate therapy (praziquantel) and retested 4-8 weeks later to confirm a negative result. HCV co-infection and HBV/HDV co-infection were recorded in the clinical notes for closer monitoring of their liver health, as currently treatment is not provided at KATH.

2.3.2 Abnormal findings at the clinical assessments and their management

Blood pressure readings suggestive of a diagnosis of hypertension were repeated and, if confirmed abnormal, reported to the patient and to the medical staff for eventual inclusion or optimisation of anti-hypertensive treatment and adequate follow-up.

Abnormal findings obtained from the Fibroscan suggestive of advanced fibrosis/cirrhosis and advanced steatosis were reported to both the patient and the medical personnel for appropriate management.

2.3.3 Dissemination of the findings to the scientific community and locally

Preliminary results from Chapter 3 were presented at the Conference on Retroviruses and Opportunistic Infections in Seattle, Washington, in March 2019 and a manuscript has been submitted to the *Lancet HIV*. The findings reported in Chapter 4 were published in the *Journal of Antimicrobial Chemotherapy*.[140] Preliminary results from Chapter 5 were presented as a poster presentation at the Glasgow HIV conference, Glasgow, United Kingdom, in October 2016.[141] Preliminary results from Chapter 6 were initially presented as a poster presentation at the Glasgow HIV conference, Glasgow, United Kingdom, in October 2016,[141] and subsequently published in the *Journal of Infection*.[142] Preliminary results from Chapter 7 were presented at the European Association for the Study of the Liver conference in Vienna, Austria, in April 2019. Co-authorship of these works was shared between collaborators in the United Kingdom and in Ghana.

Given the alarming findings on virological control in the OPTIMISE cohort, a seminar was organised at the Kwame Nkrumah University of Science and Technology in May 2019, where all the local medical and scientific community were invited to attend and participate and results presented and discussed.

2.3.4 Ethical Considerations on Study Design and Sample Size

2.3.4.1 Objective 1

In order to respond to objective 1 (i.e. *To explore rates of resuppression after virological monitoring at point of care followed by an immediate adherence intervention*) I chose the OPTIMISE cohort, as OPTIMISE was designed to define strategies to improve ART outcomes. Rate of resuppression were measured at the second study visit conducted after 8 weeks from enrolment and included only participants with quantifiable viraemia (≥40 copies/mL) that received an immediate adherence intervention (see Methods Chapter 3).

OPTIMISE was designed as a prospective cohort rather than a randomised controlled trial for a number of considerations, including: 1) we wanted to obtain estimates of the prevalence of certain conditions in this setting (i.e. virological failure, hepatic steatosis and fibrosis, co-infection with HBV); 2) availability of resources and time were limited if a randomised-controlled trial (RCT) were to be designed for the parts that included an intervention, such as the one presented here; 3) RCT often provides overly optimistic scenarios due to the number of criteria used for inclusions, albeit our cohort was not entirely representative as well as it did not include children and adolescents, pregnant women, in-patients, and other key populations (e.g. MSMs, FSWs). Therefore, the research project conducted for objective 1 lacked a controlled arm(s), which could have included the standard of care at KATH (i.e. lack of virological monitoring) and/or virological monitoring via a laboratory based facility.

Inclusion into the study was offered to all patients attending the clinic based on their number of arrival until we reached our capacity limit. We used a convenient sample: despite enrolling patients in a consecutive fashion to try to minimise selection biases, during the busiest days, when capacity was reached, we excluded patients that arrived at the clinic late, who might have represented a subgroup with specific characteristics (i.e. younger patients, employed, with poorer adherence). This occurred only during one of the days of enrolment; all efforts were made to try to enrol everybody to minimise this potential bias, and I am confident that the OPTIMISE cohort has a fair representation of the HIV-positive adult population receiving care at KATH.

The cohort included 340 patients, 333 on ART (see Results Chapter 3), 164/333 (49.2%) with detectable viral load ≥40 copies/mL. Based on an estimate of lack of suppression of 40%[103] and a total cohort of ~4,500 individuals attending for HIV care at KATH, a sample of 341 individuals would have been necessary in order to have a precision of 5% and a confidence of 95% to estimate a prevalence of lack of suppression in the Kumasi cohort, which is a very close number to the one we were able to recruit. In order to have an estimate of the rate of resuppression of the 164 subjects, with a precision of 5% and a confidence interval of 95% we would have needed to recall for the second visit at least 116 subjects, assuming an unknown prevalence of resuppression. We decided to recall all participants, not to create further selection biases based potentially on the viral load (i.e. those with higher viraemia would have had to be prioritised due to their higher risk of failure), albeit that meant greater costs and distress for the patients. In addition, a greater number of participants allowed for better outcomes of secondary endpoints (i.e. predictors of resuppression) and eventual loss to follow-up. Finally, our assessment had no potential of causing any harm to the patients and was deemed beneficial for their overall care. We covered the patients' travel costs, we offered refreshments and, ultimately, provided additional care that would probably not have received otherwise (i.e. treatment switches based on virological failure).

2.3.4.2 Objective 2

In order to address objective 2 (i.e. *To determine viral load and drug resistance outcomes of patients on first-line NNRTI-based ART that underwent a changed in the NRTI-backbone from AZT or d4T to TDF in the absence of virological monitoring*) I chose to investigate the HEPIK cohort. As explained, the HEPIK cohort had a long follow-up of patients on TDF that switched treatment in the absence of virological monitoring. A rich dataset and an extensive repository of banked plasma samples were available and that allowed me to explore the emergence of drug resistance over time. The sample size of the original cohort was estimated based on a different objective, i.e. the evolution of liver fibrosis after the introduction on TDF in the drug regimen.[138] At the last visit, 87 patients of the HEPIK cohort were eligible for the analysis (see Results Chapter 4). The cohort experienced >20% loss to follow-up over time, which

is in line with data from African cohorts, but consists in a severe limitation, as patients with poor virological control might have died or be too sick to attend the study visit.

In order to investigate the prevalence of patients with detectable viral in this cohort, with a precision of 5%, a confidence interval of 95%, and an unknown prevalence of patients with detectable viral load, I would have needed to test at least 72 subjects. Testing the entire cohort did not increase costs significantly, avoided the introduction of selection biases and allowed a better power for the statistical analysis.

2.3.4.3 Objective 3

In order to address objective 3 (i.e. *To explore how self-reported adherence to ART measured by visual analogue scale, multi-item questionnaires and composite scores correlates with viral load and drug resistance*) I chose to use the data collected at the last study visit of the HEPIK cohort, where adherence measures were taken for all participants via a visual analogue scale and a multi-item questionnaire (see Methods Chapter 5). This represents an exploratory analysis for a secondary outcome, and a formal sample size calculation was not performed.

2.3.4.4 Objective 4

In order to respond to objective 4 (i.e. *To investigate prevalence and determinants of tubular proteinuria and rapid decline of estimated glomerular filtration rate (eGFR) in HIV/HBV co-infected patients after long-term exposure to TDF*) I chose to use data from the HEPIK cohort. Participants of the HEPIK cohort introduced TDF in their regimen by following WHO 2013 guidelines and, at the time of the last assessment in November 2015, had a median of 4 years of exposure to the drug (see Methods Chapter 6). Over the years, the cohort has experienced a 26% of loss to follow-up: this high rate consists of a severe limitation for the generalisability of the findings, and results have to be interpreted with caution (see Discussion Chapter 6).

The main outcome measure of the study is the prevalence of tubular proteinuria among HIV/HBV co-infected patients attending the last visit. Tubular proteinuria is a marker of TDF-mediated nephrotoxicity. The cohort was powered to estimate a different primary outcome, hence no formal sample size calculation was performed.

TDF is the drug of choice for the treatment of HIV/HBV co-infected patients, for its dual activity against both HIV and HBV replication and is currently recommended for all co-infected patients.[67] Therefore, in order to study TDF nephrotoxicity, a different study design, such as a RCT with a control arm of co-infected patients not on TDF would have not been ethically acceptable, and HIV-monoinfected patients not on TDF do not represent an ideal comparator as HBV-infection might exert a role in kidney function.

2.3.4.5 Objective 5

In order to address objective 5 (i.e. *To assess the liver health by measuring prevalence of viral hepatitis coinfections and prevalence and determinants of liver steatosis and fibrosis*) I chose the OPTIMISE cohort. The OPTIMISE cohort was powered to address a different primary objective (see 2.4.1). I used cross-sectional data from the enrolment visit that was conducted in February 2018. As explained in 2.4.1, I believe that the OPTIMISE cohort represents a fair snapshot of the population attending KATH for HIV care, hence prevalence of non-communicable diseases reflects the one of the total population.

In terms of sample size calculation, the main outcome measure was the prevalence of steatosis in the cohort; out of 340 enrolled participants, 329 had a valid Fibroscan reading (see Results Chapter 7). With a precision of 5% and a confidence interval of 95%, the minimum sample would have been of 178 subjects. Offering the test to all participants allowed for an increase in our power to perform secondary analyses, such as the ones on predictors of hepatic steatosis and fibrosis. The Fibroscan test is a non-invasive procedure and does not cause any harm to the patients. On the contrary, it provides useful information that can be used to guide treatment choices and life-style counselling for the patients.

2.4 DATA COLLECTION

2.4.1 Adherence and well-being questionnaires

HEPIK-2 study (November 2015)

A detailed description of the self-reported adherence measures used in the HEPIK-2 study is reported in the methodology section of Chapter 5. Data on self-reported adherence is also reported in the methodology sections of Chapter 4 and 6. After consenting, all participants answered a structured questionnaire administered by trained local interpreters due to literacy barriers (Appendix 2). The interpreters translated the questionnaires from English to Akan by the translators at the time of the interview on patient request; the questionnaire was filled in by the interpreter. A pilot was conducted prior the study visit to assess the duration of the interview and acceptability of the patients, and the questionnaire was considered implementable.

All interviews were conducted at KATH in a quiet and separate area from the HIV Clinic. The interviewers were instructed to use neutral and non-judgemental tones. Information was collected on demographics (gender, age, ethnicity), family composition (whether participant had a partner; degrees of HIV disclosure; duration of partnership, partner HIV serostatus, number of children), means of travel to the HIV clinic and duration of the journey, and markers of socio-economic status (employment, level of skilled training, education, housing and self-assessment of financial hardship). In addition, participants were asked whether they were present or past alcohol consumers. Heavy consumption was inferred when patients reported drinking at least three times a week or stopping drinking for excessive consumption.

Adherence was assessed with four different questions: i) number of times from the start of treatment individuals had an interruption of at least three consecutive days (0, 1, 2, ≥3); ii) number of ART doses missed in the previous week (0, 1, 2-3, >3); iii) number of interruptions of at least three days in the previous three months (0, 1, 2-3, >3); and iv) ordinal visual analogue scale (VAS) assessing ART adherence in the previous three months (from 0%=complete non-adherence to 100%=perfect adherence).[143] The VAS used in the questionnaire is reported in Figure 2.2.

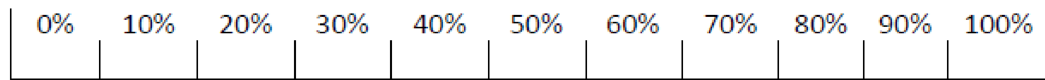


Figure 2-3 **Visual Analogue Scale**

The interpreter explained to the patient the meaning of the VAS and asked the patient to sign with an "X" their self-reported adherence in increments of 10%. It was explained to the patients that 0% meant that they had been taking no medications at all and that 100% meant they had been perfectly adherent and had never missed a single dose in the previous three months. They could choose any space in between, including the extremes, in 10% increments. If they put the "X" on the line between the values, a mid-value was chosen (i.e. 85% if the "X" was on the line between 80% and 90%).

This adherence questionnaire was adapted from the Antiretrovirals, Sexual Transmission Risk and Attitudes (ASTRA) study,[144,145] which has been validated in the United Kingdom. The questionnaire had not been previously validated in Ghana.

The multiple-item questionnaires used different recall times (i.e. previous week, previous three months, ever since ART was started) in order to have the most comprehensive assessment (i.e. patients might have had poor adherence in the previous week because of concomitant event, but overall optimal one in the longer term, and vice versa). In addition, the use of a visual mean as the VAS might be preferable and more intuitive for some patients than questions on number of missed doses. In Chapter 5, I explored the use of these tools when combined in a unique scale and score (see Methods Chapter 5).

Each question relating to psychosocial parameters was assessed on an ordinal three-point scale, with scores of 2, 1 and 0 attributed to each response option. Depression was assessed through an adaptation of the validated Patient Health Questionnaire-9 (PHQ-9).[146] Four of the original 9 questions were separated into their components for ease of understanding, and then combined back to the 9-domain format. A total score was then calculated, and an adaptation of the original Depression Severity Score

(DSS) was attributed: 0 (no symptoms), 1-3 (minimal), 4-6 (mild), 7-9 (moderate), 10-12 (moderate/severe), 13-18 (severe). Each threshold was assigned proportionally to the original PHQ-9 questionnaire cut-offs. Generalised Anxiety Disorder (GAD) was assessed similarly using 6 questions from the validated GAD-7.[147] Degrees of anxiety were defined by re-scaling the original categorisations: none (0-3), mild (4-6), moderate (7-9), and severe (10-12). Physical distress symptoms were assessed using the Memorial Symptoms Assessment Scale (MSAS) questionnaire,[148] and categorised as recommended: minimal (0-2), low (3-11), moderate (12-23) and high (24-64). Health-related quality of life (HRQoL) was calculated using EQ-5D-3L questionnaire,[149] attributing the utility sets from Zimbabwe as a proxy of Ghana, for which no utility set was available.[150] Scores ranged from 0 (death) to 1 (highest quality of life). All these questionnaires had not been validated in this cohort nor in other Ghanaian settings.

OPTIMISE Study

All participants answered a structured questionnaire administered by trained local interpreters due to literacy barriers, by following the same approach that was conducted for the HEPIK-2 Study. Interviews were conducted in a separate and quiet area. The questionnaire collected information on adherence, and socio-economic and life-style parameters: having enough food to be able to eat regular meals, alcohol consumption (i.e., if drinking alcohol never, occasionally [once a week or less frequently], or regularly), and use of traditional or herbal remedies.

Adherence was assessed with 3 structured questionnaires administered at different times (Appendix 3-5) (see Methods Chapter 3). T0 questionnaire 1, which was administered at recruitment, asked participants about any previous treatment interruption, defined as discontinuation of all antiretroviral drugs for at least three consecutive days since first starting ART, and how the patient would describe adherence in the previous three months on a VAS ranging from 0% (complete non-adherence) to 100% (complete adherence) in 10% increments. T0 adherence questionnaire 2, which was administered at adherence review, asked about the number of doses missed in the previous week and in the previous month (none; 1; 2 to 3; more than 3). The T1 questionnaire, which was administered at follow-up, assessed

adherence with a VAS and number of missed doses in the previous week and the previous month (none; 1; 2 to 3; more than 3).

Adherence measures were combined in scoring tools, which allowed stratification of adherence into different grades. Details on the scoring system are reported in Chapter 3 and Chapter 5.

2.4.2 Clinical data and Fibroscan readings

HEPIK-2 (November 2015) and OPTIMISE study (February 2018)

Clinical history on ART, co-morbidities and co-medications was collected by interviewing study participants and by retrieving relevant information from the patients' medical records. Interviews were conducted by local nurses to overcome the language barrier. Data was collected on pre-designed case report forms and carried out at each visit.

Blood pressure was measured in all participants with a manual sphygmomanometer; all abnormal readings were repeated after letting the patient rest for 20 minutes. Anthropometric measures, including height, weight, (and waist circumference for the OPTIMISE study), were collected for all patients.

All patients, who had consumed the last meal at least 2.5 hours prior the medical assessment, underwent measurement of liver transient elastography (TE) and, for the OPTIMISE study, of controlled attenuation parameter (CAP), by using portable equipment (Fibroscan, Ecosens, France). All female participants were tested for pregnancy prior the Fibroscan test (SureScreen Pregnancy Test, Medisave, Weymouth, United Kingdom).

Liver biopsy represents the gold-standard for the diagnosis of liver fibrosis and cirrhosis. However, it is an invasive procedure with the risk of potential complications, sampling can be inadequate and there can be interobserver variability.[151] Measures of TE with Fibroscan correlate with the degree of fibrosis, and are not invasive.[152] Sensitivity and specificity are estimated to be 70%-79% and 78%-84% for ≥F2 stage and 83%-87% and 89%-91% for cirrhosis.[152,153] Valid liver stiffness measurements should be ≥10, with a success rate (successful readings/total number of attempts) ≥60% and a IQR/median ratio <0.30. Under these conditions, using a cut-off of 8 kPa, the negative predictive value of valid evaluation is superior to invalid ones for the detection of significant (84% vs 71%) and advanced fibrosis (100% vs 93%).[154]

Several conditions can lead to an overestimation of liver fibrosis by TE, and these include: raised transaminases;[155,156] extrahepatic cholestasis;[157] heart failure with subsequent hepatic congestion;[158] non-fasting status;[159] and severe steatosis.[160]

Additional factors can influence TE results, such as operator's experience,[161] and interobserver variability.[161,162]

2.4.3 Laboratory Data

2.4.3.1 Full blood count and CD4 cell count

HEPIK-2 Study (November 2015) and OPTIMISE Study (February 2018)

After consenting, blood was collected on-site in a dedicated phlebotomy room by following standard phlebotomy procedures. Aliquots of whole blood destined to full blood count and CD4 cell count were collected in two distinct 4 ml K_2EDTA tubes (BD Vacutainer, Wokingham, United Kingdom) and processed on site at the KATH Haematology laboratory. Full blood count was determined by using laser-based flow cytometry (CELL-DYN 3700 System, Abbott Diagnostics, Abbott Park, United States) by following manufacturer's instructions. CD4 cell count was measured by immune-labelling and fluorescence-activated cell sorter (FACS) analysis by using BD FACSCount automated reader (Becton Dickinson, San Jose, United States) by following manufacturer's instructions.

2.4.3.2 Biochemistry tests

HEPIK-2 Study (November 2015)

Whole blood was collected in one 4 ml silica tube (BD Vacutainer, Wokingham, United Kingdom) and centrifuged at 4500g per 10 minutes to separate serum. Biochemistry testing was conducted on freshly separated serum at the Malaria Laboratory at KATH. Aliquots of serum were stored at -80 ºC for subsequent shipment to the United Kingdom in dry ice. Testing included determination of serum concentration of i) alanine aminotransferase (ALT), ii) aspartate aminotransferase (AST), iii) gamma-glutamyltransferase (GGT), iv) total bilirubin and v) urea. Analyses were conducted with the Selectra ProS instrument (EliTech Group, Puteaux, France), according to manufacturer's instructions. Creatinine concentrations were measured at the Biochemistry Department of the Royal Liverpool and Broadgreen University Hospital. For this analysis, serum was thawed from frozen aliquots that were shipped in dry ice from Kumasi to the United Kingdom and stored at -80ºC in the Institute of Infection and Global Health of the University of Liverpool, United

Kingdom. Serum creatinine was quantified by using the Roche/Hitachi cobas c systems (Cobas, Roche Diagnostics, Indianapolis, United States) according to manufacturer's instructions. Whole blood, collected in sodium fluoride, was thawed from frozen aliquots and glycated haemoglobin concentrations measured at the same laboratory by using ion exchange high performance liquid chromatography (HPLC).

Urine was collected on site in standard containers. Aliquots of urine from the last study visit were frozen at -80ºC and subsequently shipped in dry ice to the United Kingdom, where they were testes at the Biochemistry Department of the Royal Liverpool and Broadgreen University Hospital for urinary albumin-to-creatinine ratio (uACR) and urinary protein-to-creatinine ratio (uPCR); quantification of albuminuria and proteinuria was obtained by using the Roche/Hitachi cobas c systems (Cobas, Roche Diagnostics, Indianapolis, United States) according to manufacturer's instructions. Urine samples were also tested on site with urinary dipsticks (Medi-Test Combi 8, Medisave, Weymouth, United Kingdom), which comprised a qualitative estimation of the presence and the degree of haematuria, glycosuria and proteinuria.

OPTIMISE Study (February 2018)

Whole blood was collected in a 10 ml silica tube (BD Vacutainer, Wokingham, United Kingdom) and centrifuged at 4500g per 10 minutes to separate serum. Serum was frozen at -80ºC and subsequently shipped in dry ice to the United Kingdom, where it was tested at the Biochemistry Department of the Royal and Broadgreen University Hospital. ALT, AST, creatinine, total cholesterol (TC), low-density lipoproteins (LDL), high-density lipoproteins (HDL), and triglycerides were measured by using the Roche/Hitachi cobas c systems (Cobas, Roche Diagnostics, Indianapolis, United States) according to manufacturer's instructions. HbA1c was measured by using ion exchange HPLC or by boronate affinity with fluorescence detection when the first method yielded invalid results.

2.4.3.3 HIV viral load quantification

HEPIK Study

Thawed aliquots of plasma were used to extract and quantify HIV-1 RNA by using the standard protocol of the Abbott m2000sp/m2000rt HIV-1 assay (Abbott, Maidenhead, United Kingdom). This analysis was conducted at the Institute of Infection and Global Health, University of Liverpool. Lower limit of quantification (LLQ) of the assay is 40 copies/ml. Briefly, 1ml of plasma per sample was placed into Sarstedt tubes (Sarstedt AG & Co., Nümbrecht, Germany) and centrifuged at room temperature for 5 minutes to allow cellular debris to pellet at the bottom of the tube. Tubes containing the samples were placed on the sample rack along negative, low positive and high positive controls as per Abbott instructions. Samples and controls were loaded into the m2000sp platform for the subsequent automated steps of RNA extraction. Eluted RNA was dispensed by the automated platform into a 96-well optimal plate. After addition of the mastermix solution containing primers, probe and polymerase enzyme, the Abbott 96-well optimal plate was manually sealed and transferred to the Abbott m2000rt for the real-time polymerase chain reaction. The Abbott ROW Software (Abbott) was used to analyse the results.

OPTIMISE Study

HIV-1 viral load was quantified on-site at KATH. Blood was collected from patients by standard phlebotomy procedures into EDTA tubes. Plasma was separated immediately after collection by centrifuging the tubes for 10 minutes at 4500g and aliquoted into 1.8 ml Eppendorfs; 1 ml of plasma was transferred with a pipette into a cartridge of the Cepheid Xpert HIV-1 Viral Load (Cepheid, Sunnyvale, United States). The Cepheid Xpert HIV-1 Viral Load is validated for the HIV-1 RNA quantification via RT-PCR from HIV-1 Group M (subtypes A, B, C, D, F, G, H, J, K, CRF01_AE, CRF02_AG, and CRF03_AB), Group N and Group O. In the Cepheid platform, specimen preparation, nucleic acid extraction and amplification are automated and integrated; PCR reagents and processes are hosted in self-contained, single-use disposable cartridges, hence cross-contamination between specimens is minimized. The platform consisted of an instrument that could run 8 tests at a time,

independently, a laptop with preloaded software for running tests and viewing the results. Turn-around-time for the results was 90 minutes and quantification range 40 to 10^7 copies/ml; 22 copies/ml was the lower limit of detection (LLD).[163] Sensitivity and specificity for virological failure defined at 1000 copies/ml are 94.1% and 98.5%, respectively.[164] The platform was installed in a laboratory adjacent to the clinic site.

2.4.3.4 Hepatitis B screening and viral quantification

All participants of the HEPIK-2 cohort were known HBV/HIV co-infected subjects, hence screening with HBsAg was not repeated. HBV DNA was quantified by using the standard protocol of the Abbott m2000sp/m2000rt HBV assay (Abbott, Maidenhead, United Kingdom). Lower limit of quantification (LLQ) of the assay is 15 IU/ml. HBV DNA quantification was conducted at the Institute of Infection and Global Health of the University of Liverpool, United Kingdom.

All participants of the OPTIMISE cohort were screening for HBsAg status by using Architect (Abbot Diagnostics, Sligo, Ireland), which has both sensitivity and specificity >99% for HBsAg detectability.[165] Testing was conducted on frozen aliquots of serum at the Berkshire and Surrey Pathology Services, Frimley Health NHS Foundation Trust, United Kingdom. HBV DNA was quantified on plasma from HBsAg-positive samples at the Virology Department of the Royal and Broadgreen University Hospital by using the Cepheid Xpert HBV Viral Load (Cepheid, Sunnyvale, United States). The Cepheid Xpert HBV Viral Load can quantify HBV DNA for genotypes A to H; LLD and LLQ of the platform are, for plasma, 3.2 IU/ml and 10 IU/ml, respectively.[166] Turnaround time for the results is <60 minutes.

2.4.3.5 Hepatitis C screening and viral quantification

All participants of the OPTIMISE cohort were screened for active HCV infection. Pools of 10 plasma samples were prepared using 100 µl per samples and screened for HCV RNA with Xpert HCV Viral Load (Cepheid, Sunnyvale, United States), followed by testing of individual samples of HCV RNA positive pools. Testing was

conducted at the Virology Department of the Royal and Broadgreen University Hospital. The Cepheid Xpert HCV Viral Load is capable of quantifying HCV RNA from genotypes 1 to 6; lower limit of detection (LLD) and LLQ of the platform are, for plasma, 4 IU/ml and 10 IU/ml, respectively. Turnaround time for the results is 105 minutes.[167]

2.4.3.6 Hepatitis delta screening and viral quantification

Serum from HBsAg-positive subjects was screened for total HDV (Hepatitis delta virus) antibodies by enzyme immune assay (EIA) (LaunchDiagnostics Limited, Longfield, UK). Reported diagnostic sensitivity and specificity of the assay are >98%.[168] HDV RNA was detected in plasma at the accredited diagnostic laboratory Micropathology (Coventy, United Kingdom) using a real-time assay that targets the ribozyme region.

2.4.3.7 Schistosomiasis screening

Participants of the HEPIK-2 study were screened for *Schistosoma spp.* co-infection: urinary samples were tested for the presence of the circulating catholid antigen (CCA) of the parasite via urine-CCA rapid test (RapidMedical Diagnostics, Pretoria, South Africa). A positive urine-CCA test indicates active infection: sensitivity for *S. mansoni* infection varies from 70% to 100% and it depends on the parasite load; specificity in non-endemic setting is 95%.[169] Sensitivity for *S. japonicum* and *S. haematobium* infection is lower. Patients with a positive result were recalled and treated with praziquantel.

2.5 HIV DRUG RESISTANCE TESTING

2.5.1 Sanger Sequencing

HIV Sanger sequencing was conducted at the Institute of Infection and Global Health, University of Liverpool, United Kingdom.

RNA extraction

Plasma samples were thawed and RNA extracted manually from 140 µl of sample by using the QIAamp Viral RNA mini kit (QIAGEN, Manchester, United Kingdom). Manufacturer's instructions were followed. Briefly, after the lysing stages, the sample solution was loaded onto the QIAmp Mini spin column to allow binding of the RNA to the silica-based membrane via two brief centrifugations. Carrier RNA was used to enhance binding of RNA to the membrane and reduce the chance of RNA degradation. Two subsequent consecutive washing steps allowed removal of contaminants. The RNA was subsequently eluted in 60 µl of the manufacturer's RNase-free buffer (AVE buffer).

Reverse Transcription – PCR (RT-PCR)

The entire protease region (amino acids 1-99, HXB2 position 2253-2549) and two-thirds of the RT region (1-335, HXB2 position 2550-3554) were amplified from the RNA extracts by RT-PCR and nested-PCR to generate a 1.8 kb amplicon. The RT-PCR step was performed by using Invitrogen Superscript III One-Step RT-PCR system with Platinum Taq High Fidelity DNA polymerase (ThermoFisher Scientific, Life Technologies Corporation, Carlsband, United States). The primers used for this step were RES1 (GAAGAAATGATGACAGCATGTCAGGG) and RES2 (TAATTTATCTACTTGTTCATTTCCTCCAAT). A master mix solution was prepared using, per sample, 25 µl of 2x reaction mix, 1 µl of RES1 (from 100 µM concentration), 1 µl of RES2 (from 100 µM concentration), 1 µl of enzyme mix and 12 µl of H20; 40 µl of master mix were added to 10 µl of RNA extract.

The RT-PCR protocol followed the thermal cycler steps summarised in table 2-1.

Table 2-1. **RT-PCR steps**

Step	Temperature (°C)	Time	Cycles
cDNA synthesis	55	30 minutes	1
Denaturation	94	2 minutes	1
Denature	94	15 secs	40
Anneal	52	30 secs	
Extend	68	2 minutes	
Final extension	68	5 minutes	1
Hold	4	Hold (<24 hours)	1

Nested-PCR

Products of the RT-PCR underwent a nested-PCR step by using Invitrogen Platinum PCR SuperMix high fidelity (ThermoFisher Scientific, Life Technologies Corporation, Carlsband, United States). The primers used for this step were RES3 (ATGGYTCTTGATAAATTTGATATGTCC) and RES4 (AGACAGGCTAATTTTTTAGGGA). A master mix solution was prepared using, per sample, 45 µl of Platinum PCR SuperMix high fidelity, 1 µl of RES3 (from 100 µM concentration), 1 µl of RES4 (from 100 µM concentration), and 0.5 µl of H20; 47.5 µl of master mix were added to 2.5 µl of the RT-PCR product.

The nested-PCR protocol followed the thermal cycler steps summarised in table 2-2.

Table 2-2. **Nested-PCR steps**.

Step	Temperature (°C)	Time	Cycles
Denaturation	94	2 minutes	1
Denature	94	30 secs	35
Anneal	52	30 secs	
Extend	68	2 minutes	
Hold	4	Hold (<24 hours)	1

The nested-PCR products were subsequently visualised by gel electrophoresis.

Purification

PCR products were subsequently purified by using the QIAquick PCR purification kit (QIAGEN, Manchester, United Kingdom) and concentration of the purified PCR products estimated via NanoDrop 1000 Spectrophotometer. They were subsequently diluted in order to obtain 40 ng of DNA per well of the sequencing plate used in the following steps.

Cycle sequencing

The following primers were used for the sequencing steps: RES3, SEQ1 (GAGCCAACAGCCCCACC), SEQ2 (CAATGGCCATTGACAGAAG), SEQ3 (GGATCACCAGCAATATTCCA), SEQ5 (TGGGCCATCCATTCC TGGCTT), SEQ6 (CATCCCTGTGGAAGCACATT), NE135(CCTACTAACTTCTGTATGTCATTGACAGTCCAGCT), RES4. In the PCR optical plate, eight wells were required per sample. A master mix of the following solution was created and 12 µl added in each well: 2 µl of BigDye Terminator v3.1 Ready Reaction Mix, 1 µl of primer, 9 µl of BigDye Terminator 1X Sequencing buffer; 8 µl of diluted purified product was subsequently added into each well. Once ready, the solution underwent the PCR steps summarised in Table 2-3.

Table 2-3. **Cycle sequencing PCR steps**

Temperature (°C)	**Time**	**Cycles**
96	10 seconds	25
50	5 seconds	
60	4 minutes	
4	Hold	< 24 hours

Purification

PCR products underwent two distinct steps of purification. In the first, a solution of sodium acetate 3.0 M pH 5.5 (2 µl per well) and 100% ethanol (50 µl per well) was added in each well. The plate was subsequently centrifuged at 2000g for 20 minutes. In the second step, after removal of the previous solution, 150 µl of 70% ethanol were added in each well and the plate was centrifuged at 2000g for 5 minutes. Finally, after

removal of the second solution, 10 µl of Hi-Di Formamide were added in each well and left at room temperature for 30 minutes before loading of the plate into the sequencer.

The plate was loaded into the AB 3730 genetic analyser; results were subsequently analysed with Seqscape. All sequences were checked against the reference sequence by looking at the corresponding chromatograms. The FSTA files were then uploaded into the Stanford HIV database for the interpretation.

2.5.2 Next-generation Sequencing

The steps described from extraction to purification of the amplicon were conducted at the Institute of Infection and Global Health, University of Liverpool, United Kingdom. Sequencing and bioinformatics analysis were conducted at Public Health of England, Colindale, United Kingdom.

RNA extraction

RNA was extracted manually by using the QIAamp Viral RNA mini kit (QIAGEN, Manchester, United Kingdom). Manufacturer's instructions were followed as described previously. The total input was targeted to be ≥10000 virions. For samples with viral load >35000 copies/ml, 280 µl of samples were used, whereas for samples with viral load between 18000-35000 copies/ml, 560 µl of samples were used with an increased number of subsequent centrifugation steps. Samples with viral load <18000 copies/ml underwent ultracentrifugation prior RNA extraction: samples were centrifuged at 35000g for 20 minutes at 4 °C with the Optima XPN (Beckman Coulter, ThermoFisher Scientific, Loughborough, United Kingdom). After ultracentrifugation, the supernatant was discarded and the pellet reconstituted with 280 µl of phosphate buffered Saline. This solution underwent the steps of RNA extraction described above.

RT-PCR

The steps described previously for Sanger sequencing were followed. The primers used were AZT3.1 (CCAGGAATGGATGGCCCAA) and RES3. The master mix used for the reaction was created by adding, per reaction well: 25 µl of 2x reaction mix, 1.25 µl of AZT3.1, 1.25 µl of RES3, 1 µl of reaction enzyme (Superscript III RT/Platinum one step *taq* hi fi enzyme), and 6.5 µl of water. Reactions were conducted in triplicates by adding to 15 µl of RNA extract to the master mix.

The RT-PCR followed the steps described in table 2-4.

Table 2-4. **RT-PCR steps for next-generation sequencing**

Step	**Temperature (ºC)**	**Time**	**Cycles**
cDNA synthesis	54	30 minutes	1
Denaturation	94	2 minutes	1
Denature	94	15 seconds	30
Anneal	56	30 seconds	
Extend	68	90 seconds	
Final extension	68	5 minutes	1
Hold	4	Hold (<24 hours)	1

Purification

After visualization of the PCR products with gel electrophoresis, the PCR products of the triplicate wells were combined and purified by using Ampure XP (Beckman Coulter, ThermoFisher Scientific, Loughborough, United Kingdom), which is a paramagnetic bead based system used to remove contaminants. Manufacturer's instructions were followed.

Sequencing and Bioinformatic Analysis

Purified amplicons were sent to Public Health of England (Colindale, United Kingdom), for the further steps of sequencing and bioinformatics analysis. Nextera XT DNA Sample Prep Kit (Illumina) was used for DNA library preparation from 1 µl of PCR product as per the kit protocol. NGS was performed using the MiSeq Reagent Kit version 2 (Illumina).

2.6 DATA STORAGE AND STATISTICAL ANALYSIS

All patients' data were anonymised for the research purposes. Anonymization was guaranteed by creating a unique alpha-numeric code per participant. All consent forms, questionnaires and case record forms are kept in dedicated folders in a locked cabinet at the study site. Anonymised data were entered into a pre-designed Excel database (HEPIK-2 Study) and in an EPI-Info database (OPTIMISE Study). The data spreadsheets were subsequently imported into the STATA software version 14 (StataCorp LLC, College Station, United States) for the statistical analysis.

Data were summarised in frequencies and medians with interquartile ranges (IQRs) if categorical or continuous, respectively. Categorical variables were compared with Fisher's exact test or X^2, continuous variable were compared with Mann-Whitney U tests. Factors associated with the outcome measure were explored with regression models, either logistic, if the outcome was a dichotomous variable, or linear, if continuous. Assumptions of linear regression models were checked (i.e. the normal distribution of the outcome measure and that of the residuals), by plotting the distribution of the outcome and by plotting the residuals vs the fitted values. Details on multivariable adjustment are reported in each chapter. Receiver operating characteristic (ROC) curve analysis was used to test discriminatory abilities of tests and scores.

2.7 PERSONAL CONTRIBUTION IN THE RESEARCH PROJECTS

2.7.1 Personal Contribution in the Research Project Presented in Chapter 3

Prior the data collection and the fieldworks, I designed the three questionnaires administered to the patients at the different time points and the database where the data were subsequently stored. I liaised with the local collaborators in Kumasi to ensure that the team was prepared to deliver the questionnaires and a sufficient number of phlebotomists and interpreters was employed. I helped with organising the accommodation and the transport of the research team from Liverpool to Kumasi.

I took part of both study visits in February 2018 and April/May 2018. I was personally involved in the enrolment of the patients of the OPTIMISE cohort, in the registration of the patients in the study and I was responsible for the patients' flow among the different stations, including interviews and phlebotomy. I helped with the viral load testing on site and with the adherence reviews, aided by a local nurse, as described. I transferred all data that were collected in the questionnaires in the electronic database.

In Liverpool, I personally performed the sequencing of the samples that had a viral load >200 copies/mL, as described, and interpreted the results. Finally, I performed all the statistical analysis, including the descriptive summary and the regression models.

2.7.2 Personal Contribution in the Research Project Presented in Chapter 4

For the research project presented in Chapter 4, I was involved in the fieldwork in Ghana that took place in November 2015, for which I designed the case record form and I helped with the processing of the blood samples.

In Liverpool, I performed the viral load quantification of the samples collected in Kumasi in November 2015, the Sanger sequencing and prepared the amplicons that were subsequently used for the deep sequencing. I interpreted the results of the

sequencing. Finally, I performed all the statistical analysis, including the descriptive summary and the regression models.

2.7.3 Personal Contribution in the Research Project Presented in Chapter 5

For the research project presented in Chapter 5, I was involved in the fieldwork in Ghana that took place in November 2015, for which I helped with the processing of the blood samples. In Kumasi, I transferred all the data from the questionnaires into a pre-designed database to allow subsequent analysis.

In Liverpool, I performed the viral load quantification of the samples collected in Kumasi and the Sanger sequencing and prepared the amplicons that were subsequently used for the deep sequencing. I interpreted the results of the sequencing. Finally, I performed all the statistical analysis, including the descriptive summary and the regression models, and developed the different adherence scores.

2.7.4 Personal Contribution in the Research Project Presented in Chapter 6

For the research project presented in Chapter 6, I was involved in the fieldwork in Ghana that took place in November 2015, for which I designed the case report form and I helped with the processing of the blood samples. In addition, I performed all the urinary dipsticks and tested all urinary samples for schistosomiasis. In Kumasi, I transferred all the data from the questionnaires and case record forms into a pre-designed database to allow the subsequent analysis.

In Liverpool, I performed the viral load quantification of the samples collected in Kumasi and the Sanger sequencing and prepared the amplicons that were subsequently used for the deep sequencing. I interpreted all the results of the sequencing. Finally, I performed all the statistical analysis, including the descriptive summary, the regression models, and the evaluation of the urinary dipstick as a predictor of renal disease.

2.7.5 Personal Contribution in the Research Project Presented in Chapter 7

Prior the data collection and the fieldworks, I designed the questionnaire administered to the patients, the case record form and the database where the data were subsequently stored. I liaised with the local collaborators in Kumasi to ensure that the team was prepared to deliver the questionnaires and a sufficient number of phlebotomists and interpreters was employed. I helped with organising the accommodation and the transport of the research team from Liverpool to Kumasi.

I took part of the study visits in February 2018. I was personally involved in the enrolment of the patients of the OPTIMISE cohort, in the registration of the patients in the study and I was responsible for the patients' flow among the different stations, including interviews, phlebotomy, and the clinical area. I helped with the viral load testing on site and with the Fibroscan assessment of the participants. I transferred all data that were collected both in the questionnaires and in the case record forms in the electronic database. I organised the shipment of the frozen samples from Kumasi to the United Kingdom.

Finally, I performed all the statistical analysis, including the descriptive summary and the regression models.

3 Third Chapter – Point-of-care viral load testing to guide immediate adherence support and subsequent resuppression rates in a programmatic HIV setting in sub-Saharan Africa

3.1 Introduction

Plasma HIV-1 RNA levels provide a direct measure of the efficacy of antiretroviral therapy (ART), predicting immunological and clinical outcomes[170] and the risk of onward transmission.[27] Modelling indicates that differentiating care based on the viral load is cost-effective for low-income settings,[89] whereby virologically suppressed patients attend clinic visits less frequently and more resources are focused on patients with viraemia. The approach is endorsed by the World Health Organisation (WHO), whose guidelines recommend virological monitoring for all treated patients.[67] Viraemic patients should in the first instance receive counselling to enhance adherence,[171] followed by a repeat viral load measurement taken three months later.[67] There is limited evidence indicating that resuppression is common after interventions to re-enforce adherence, especially in patients with a low viral load.[172-174] A change of the treatment regimen is recommended with a confirmed viral load >1000 copies/ml.[67]

Access to ART has been expanding in sub-Saharan Africa. Of the 25.7 million people estimated to be living with HIV, 15.4 million (60%) were receiving treatment in 2018,[24] aiming for 90% by 2020.[175] Viral load monitoring of patients receiving ART ought to follow the same trajectory; however, routine access remains limited due to overburdened healthcare provision, financial constraints, poor training, and weak transport and laboratory systems.[176] Implementation of POC viral load-informed differentiated care in sub-Saharan Africa may benefit from testing solutions that obviate the need for multiple clinic visits, which in turn is likely to improve retention into care and clinical outcomes.[177]

The Cepheid Xpert HIV-1 viral load assay was the first molecular system to be approved by the WHO for implementation at point of care in resource-limited settings.[178] The Xpert platform is used widely for the diagnosis of tuberculosis across sub-Saharan Africa.[179] Its modular, cartridge-based system is easy to use by non-specialised personnel and offers a low risk of contamination, a fast turn-around time for results, and no requirement for sample batching,[180] features that make the platform suitable for same-day testing at point of care. In three comparative studies in Botswana, Malawi and South Africa, the Xpert HIV-1 viral load assay showed a high level of agreement with standard laboratory-based testing.[181-183]

The aim of this study was to measure rates of resuppression after virological monitoring at point of care followed by an immediate adherence intervention. Given that the centre is yet to adopt routine virological monitoring, the study was conducted observationally rather than randomise patients to the point of care intervention versus standard of care. The assay results were used to fast track viraemic patients to adherence counselling, and the rates of resuppression after the intervention were measured 8 weeks later by repeat POC viral load testing.

3.2 METHODS

3.2.1 Setting and population

The study took place at the HIV clinic of the Komfo Anokye Teaching Hospital (KATH), a 1200-bed facility in the city of Kumasi and the second-largest hospital in Ghana, serving a population of around 10 million people in the Ashanti Region. At the time of the study, all HIV-infected individuals had universal access to treatment in the country. Approval was granted by the Ethics Committee of the Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. Eligible participants were HIV-positive adults (≥18 years) attending for routine HIV care and on ART for at least 3 months. In February 2018 (time point zero, T0), consecutive patients attending 4 out-patient clinics over two weeks were offered participation in the study and all accepted.

3.2.2 Patients' flow

The patients' flow is shown in Figure 3.1.

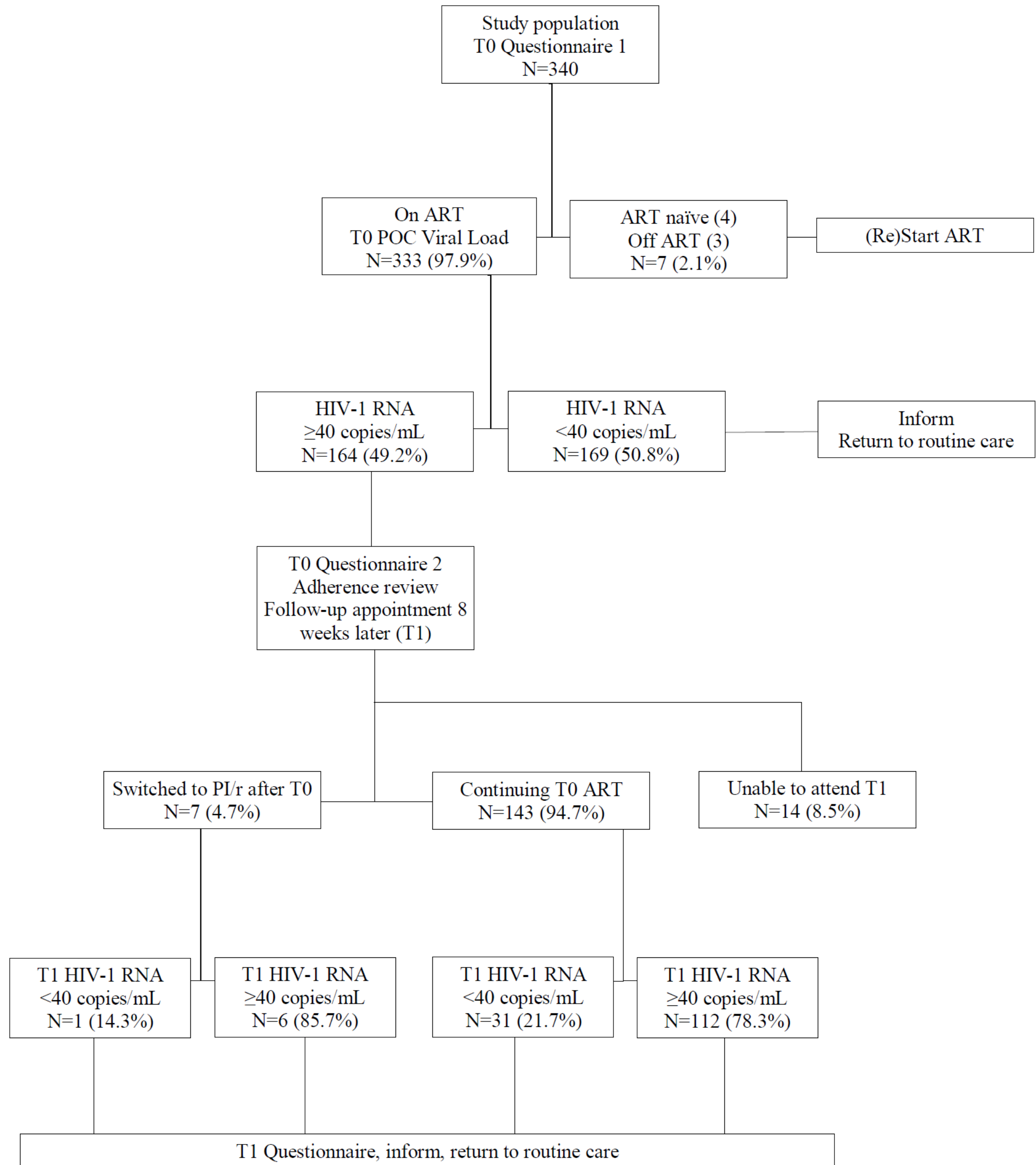


Figure 3-1 **OPTIMISE cohort at T0 and at T1. Patients flow-chart**

At T0 participants underwent blood sampling and were then invited to complete structured questionnaires, which were administered by trained local interpreters to overcome literacy barriers. The questionnaires collected information on adherence (T0 adherence questionnaire 1, see below) and socio-economic and life-style parameters: having enough food to be able to eat regular meals, alcohol consumption (i.e., if drinking alcohol never, occasionally [once a week or less frequently], or regularly), and use of traditional or herbal remedies. After completing the questionnaires, ART-naïve patients and subjects that reported having discontinued ART were directed to initiating or reinitiating treatment. Participants taking ART were invited to wait for the viral load test results. Patients with undetectable (<40 copies/ml) viral load were informed and returned to routine care. Patients with detectable viral load were fast-tracked to an in-depth adherence review with a local trained nurse, which was informed by the viral load result and used a structured questionnaire (T0 adherence questionnaire 2, see below). Potential reasons for poor adherence were addressed, including problems with tolerability, and strategies to improve adherence were suggested, including the use of memory aids (e.g., setting an alarm on a mobile phone). Following adherence review, patients were invited to attend a follow-up visit 8 weeks later (May 2018; T1), when the viral load test was repeated and adherence was re-assessed using the T1 adherence questionnaire (see below). CD4 cell counts and full blood counts were measured at the KATH diagnostic laboratory at T0. All patients with CD4 cell count <200 cells/mm^3 were referred to the HIV Clinic for appropriate ART management. Available clinical data were collected from the medical records.

3.2.3 Adherence measures

Adherence was assessed with 3 structured questionnaires administered at different times of the patients' flow (Figure 3-1). T0 questionnaire 1, which was administered at recruitment, asked participants about any previous treatment interruption, defined as discontinuation of all antiretroviral drugs for ≥3 consecutive days since first starting ART, and how the patient would describe adherence in the previous three months on an ordinal visual analogue scale (VAS) ranging from 0% (complete non-adherence) to 100% (complete adherence) in 10% increments. T0 adherence

questionnaire 2, which was administered at adherence review, asked about the number of doses missed in the previous week and in the previous month (none; 1; 2 to 3; more than 3). The T1 questionnaire, which was administered at follow-up, collected VAS and number of missed doses in the previous week and the previous month (none; 1; 2 to 3; more than 3). Results of T0 questionnaire 2 and the T1 questionnaire were used to calculate a 4-point adherence score (Table 3-1), where patients with best adherence scored 3 points and patients with worse adherence 0 points.

Table 3-1 **Adherence score (0-3 points)**

Reported doses missed previous month	Reported doses missed previous week	Adherence score
0	0	3
1	0	2
1	1	2
2-3	0	1
2-3	1	1
2-3	2-3	1
>3	0	1
>3	1	1
>3	2-3	0
>3	>3	0

3.2.4 Viral load testing

In a room adjacent to the clinical area, plasma was separated from whole blood in EDTA immediately after collection by centrifugation at 4,500g for 10 minutes. HIV-1 RNA was quantified with the Xpert HIV-1 viral load assay (Cepheid, Sunnyvale, US). The assay employs real-time PCR to quantify HIV-1 Group M, N and O; the quantification range is 40 to 10^7 copies/ml.[163] Specimen preparation, nucleic acid extraction and amplification are integrated in self-contained, single-use disposable cartridges which once loaded on the Xpert system provide a result within 90 minutes. See Chapter 2 for details.

3.2.5 Resistance testing

In patients with T0 viral load >200 copies/ml, plasma samples underwent testing for the presence of resistance-associated mutations (RAMs) in reverse transcriptase (RT,

amino acids 14–345) and protease (amino acids 1–99) by Sanger sequencing, as previously described in Chapter 2.[184] Major RAMs and genotypic susceptibility scores (GSS) were determined using the Stanford HIV Drug Resistance database (v8.7); each drug in the regimen was assigned a score of 0 for high-level resistance, 0.25 for intermediate resistance, 0.5 for low level resistance and 1 for potential low-level resistance or full predicted susceptibility. A GSS of 3 was assigned to those with viral load <200 copies/ml. Five samples failed to yield an amplicon; GSS of 3 was assigned to them, as all had a viral load between 200-400 copies/ml and no RAMs were detected in all other samples within this category (i.e. all had a GSS=3).

3.2.6 Analysis

Fisher's, Chi-squared, or Mann Whitney U tests were used as appropriate to compare the characteristics of study participants according to the T0 viral load, and those with detectable viral load at T0 according to whether their T1 viral load was detectable or undetectable. Predictors of a suppressed T0 viral load in the entire population, and predictors of resuppression at T1 in the population with detectable T0 viral load were identified in multivariable logistic regression models, where all variables with $p<0.1$ were included for adjustment. The T0 CD4 cell count was not included in the multivariable models as part of the causal pathway of the outcomes. The association between viral load and CD4 cell counts was investigated by univariate linear regression analysis. T0 and T1 adherence scores were compared with Wilcoxon signed rank sum test. Correlation between T0 viral load and GSS were assessed with Spearman correlation. Statistical analyses were performed with STATA software, version 14 (StataCorp Inc, College Station, Texas, USA).

3.3 RESULTS

3.3.1 Study population at T0

Among 340 consecutive HIV-positive subjects invited to participate in February 2018, 340 (100%) agreed and were enrolled in the study (Figure 3-1). Of these, 4/340 (1.2%) were ART-naïve and 3/340 (0.9%) had discontinued ART for >3 months; these subjects were referred for prompt initiation or re-initiation of treatment. The characteristics of the 333 patients who were on ART at T0 are shown in Table 3-2. The cohort included a majority of women (246/333, 73.9%), had a long-standing HIV diagnosis (median 9.5 years), was long established on ART (median 8.9 years), and showed overall good immune status (median CD4 count 626 cells/mm^3). Most (297/333, 89.2%) were receiving an NNRTI-based regimen (predominantly efavirenz) whereas 36/333 (10.8%) were on a ritonavir-boosted protease inhibitor (predominantly lopinavir/ritonavir), usually combined with either tenofovir disoproxil fumarate/lamivudine (187/333, 56.2%) or zidovudine/lamivudine (141/333, 42.3%). Overall, virological control was poor, with just over half (169/333, 50.8%) showing a viral load <40 copies/ml. The median viral load among those with quantifiable levels was 423 copies/ml (IQR 92-23,400); there were 71/333 (21.3%) patients with viral load >1000 copies/ml (Table 3-2). Each 1 log_{10} copies/ml increase in viral load was associated with a 131 cells/mm^3 lower CD4 cell count (95% CI -151 to -111 cells/mm^3, $p<0.01$).

Table 3-2 **Baseline characteristics of the study population according to the viral load**

Characteristics		Total	HIV-1 RNA (copies/ml)		p
			<40	≥40	
Total number (%)		333 (100)	169 (100)	164 (100)	
Female gender, n (%)		246 (73.9)	135 (79.9)	111 (67.7)	0.01
Age, median years (IQR)		48 (42-54)	49 (42-55)	47 (41-52)	0.09
Time since HIV diagnosis, median years (IQR)		9.5 (6.3-12.0)	10.1 (6.6-12.4)	9.1 (6.1-11.7)	0.10
Enough food, n (%)	Every day/most days	267 (80.2)	131 (77.5)	136 (82.9)	0.10
	Sometime/never	61 (18.3)	37 (21.9)	24 (14.6)	
	No data	5 (1.5)	1 (0.6)	4 (2.4)	
Alcohol consumption, n (%)	Never	317 (95.2)	164 (97.0)	153 (93.3)	0.14
	Occasionally	13 (3.9)	5 (3.0)	8 (4.9)	
	Regularly	3 (0.9)	0 (0)	3 (1.8)	
Use of traditional or herbal remedies, n (%)		11 (3.3)	4 (2.4)	7 (4.3)	0.37
Duration of ART, median years (IQR)		8.9 (5.7-11.3)	9.5 (5.9-11.3)	8.7 (5.4-11.2)	0.26
ART regimen NNRTI-based, n (%)		297 (89.2)	155 (91.7)	142 (86.6)	0.13
ART regimen PI/r-based, n (%)		36 (10.8)	14 (8.3)	22 (13.4)	-
NRTI backbone TDF/3TC, n (%)		187 (56.2)	92 (54.4)	95 (57.9)	0.52
NRTI backbone AZT/3TC, n (%)		141 (42.3)	76 (45.0)	65 (39.6)	0.32
NNRTI-based regimens, n (%)	TDF 3TC EFV	155 (46.6)	77 (45.6)	78 (47.6)	
	TDF 3TC NVP	12 (3.6)	7 (4.1)	5 (3.1)	
	AZT 3TC EFV	60 (18.0)	26 (15.4)	34 (20.7)	
	AZT 3TC NVP	70 (21.0)	45 (26.6)	25 (15.2)	
PI/r-based regimens, n (%)	TDF 3TC LPV/r	18 (5.4)	7 (4.1)	11 (6.7)	
	TDF 3TC ATV/r	2 (0.6)	1 (0.6)	1 (0.6)	
	AZT 3TC LPV/r	10 (3.0)	5 (3.0)	5 (3.1)	
	AZT 3TC ATV/r	1 (0.3)	0 (0)	1 (0.6)	
	ABC 3TC ATV/r	1 (0.3)	0 (0)	1 (0.6)	
	Other	4 (1.2)	1 (0.6)	3 (1.8)	

History of treatment interruptions, n (%)	None	250 (75.1)	147 (87.0)	103 (62.8)	<0.01
	1	42 (12.6)	8 (4.7)	34 (20.7)	
	≥2	41 (12.3)	14 (8.3)	27 (16.5)	
VAS, n (%)	100%	258 (77.5)	146 (86.4)	112 (68.3)	<0.01
	90-100%	37 (11.1)	13 (7.7)	24 (14.6)	
	80-90%	23 (6.9)	7 (4.1)	16 (9.8)	
	<80%	15 (4.5)	3 (1.8)	12 (7.3)	
Nadir CD4 cell count, median cells/mm^3 (IQR)		177 (76-286)	195 (104-308)	162 (60-253)	0.07
CD4 cell count, median cells/mm^3 (IQR)		626 (373-840)	757 (575-970)	447 (235-700)	<0.01
CD4 cell count, cells/mm^3, n (%)	<200	34 (10.2)	6 (3.6)	28 (17.1)	<0.01
	200-500	84 (25.3)	22 (13.1)	62 (37.8)	
	>500	214 (64.5)	140 (83.3)	74 (45.1)	
Detectable viral load, copies/ml	40-199	63 (18.9)	-	63 (38.4)	
	200-399	17 (5.1)	-	17 (10.4)	
	400-999	13 (3.9)	-	13 (7.9)	
	1000-9999	17 (5.1)	-	17 (10.4)	
	10000-99999	29 (8.7)	-	29 (17.7)	
	≥100000	25 (7.5)	-	25 (15.2)	

IQR=interquartile-range; ART=antiretroviral treatment; NRTI=nucleoside reverse transcriptase inhibitor; NNRTI=non-nucleoside reverse transcriptase inhibitor; PI/r=ritonavir-boosted protease inhibitor; TDF=tenofovir disoproxil fumarate; 3TC=lamivudine; AZT=zidovudine; EFV=efavirenz; NVP=nevirapine; LPV/r=ritonavir-boosted lopinavir; ATV/r=ritonavir-boosted atazanavir; ABC=abacavir; VAS=visual analogue scale

3.3.2 Predictors of a suppressed viral load at T0

In univariable analysis, variables found to be significantly associated with a suppressed viral load comprised female gender, having enough food at least sometime, reporting no history of treatment interruption, showing higher VAS scores, and higher nadir and current CD4 cell counts (Table 3-3). After adjustment, reporting no history of treatment interruption remained significantly associated with virological suppression. In a separate model excluding a history of treatment interruption, VAS (adjusted OR 1.55 per each 10% increment in the VAS; 95% CI 1.11-2.15, p=0.01) and having enough food at least sometimes (adjusted OR 0.21; 95% CI 0.04-1.03, p=0.05) independently predicted a suppressed viral load (Table 3-4).

Table 3-3 **Univariable and multivariable logistic regression analysis of predictors of suppressed T0 viral load (<40 copies/ml) in the OPTIMISE cohort**

Variable		Univariate analysis			Multivariable analysis		
		OR	95% CI	p	OR	95% CI	p
Gender	female vs male	1.90	1.15-3.12	0.01	1.64	0.84-3.21	0.15
Age	per 5-year older	1.13	0.99-1.29	0.06	1.14	0.96-1.36	0.14
Enough food	never vs at least sometime	0.30	0.09-0.95	0.04	0.26	0.05-1.31	0.10
Alcohol consumption[a]	yes vs no	0.19	0.02-1.64	0.13			
Use of traditional or herbal remedies	yes vs no	0.54	0.16-1.89	0.34			
Third agent	PI/r vs NNRTI	0.58	0.29-1.18	0.14			
Backbone	TDF/3TC vs AZT/3TC	0.83	0.53-1.28	0.40			
Duration of ART	per 1 year longer	1.03	0.98-1.09	0.29			
Treatment interruptions, number	≥1 vs never	0.25	0.15-0.44	<0.01	0.31	0.15-0.65	<0.01
VAS	per 10% score higher	1.69	1.26-2.26	<0.01	1.22	0.85-1.76	0.29
Time since HIV diagnosis	per year longer	1.05	0.99-1.11	0.12			
Nadir CD4 cell count	per 100 cells/mm^3 higher	1.14	1.00-1.31	0.06	1.05	0.96-1.14	0.27
CD4 cell count	per 100 cells/mm^3 higher	1.38	1.27-1.51	<0.01	NI		

[a]Occasional or regular. In model b, nadir CD4 count was excluded from the analysisOR=odds ratio; CI=confidence interval; ART=antiretroviral treatment; VAS=visual analogue scale; PI/r=ritonavir-boosted protease inhibitor; NNRTI=non-nucleoside reverse transcriptase inhibitor; TDF=tenofovir disoproxil fumarate; 3TC=lamivudine; AZT=zidovudine

Table 3-4 **Univariable and multivariable logistic regression analysis of predictors of suppressed T0 viral load (<40 copies/ml) in the OPTIMISE cohort**

Variable		Univariable analysis			Multivariable analysis		
		OR	95% CI	p	OR	95% CI	p
Gender	female vs male	1.90	1.15-3.12	0.01	1.69	0.88-3.25	0.12
Age	per 5-year older	1.13	0.99-1.29	0.06	1.16	0.97-1.37	0.10
Enough food	never vs at least sometimes	0.30	0.09-0.95	0.04	0.21	0.04-1.03	0.05
Alcohol consumption[a]	yes vs no	0.19	0.02-1.64	0.13			
Use of traditional or herbal remedies	yes vs no	0.54	0.16-1.89	0.34			
Third agent	PI/r vs NNRTI	0.58	0.29-1.18	0.14			
Backbone	TDF/3TC vs AZT/3TC	0.83	0.53-1.28	0.40			
Duration of ART	per 1 year longer	1.03	0.98-1.09	0.29			
Treatment interruptions, number	≥1 vs never	0.25	0.15-0.44	<0.01	NI		
VAS	per 10% score higher	1.69	1.26-2.26	<0.01	1.55	1.11-2.15	0.01
Time since HIV diagnosis	per year longer	1.05	0.99-1.11	0.12			
Nadir CD4 cell count	per 100 cells/mm^3 higher	1.14	1.00-1.31	0.06	1.04	0.96-1.14	0.31
CD4 cell count	per 100 cells/mm^3 higher	1.38	1.27-1.51	<0.01	NI		

[a]Occasional or regular. OR=odds ratio; CI=confidence interval; ART=antiretroviral treatment; VAS=visual analogue scale; PI/r=ritonavir-boosted protease inhibitor; NNRTI=non-nucleoside reverse transcriptase inhibitor; TDF=tenofovir disoproxil fumarate; 3TC=lamivudine; AZT=zidovudine

3.3.3 Viral load resuppression at T1

Of 164 participants with detectable T0 viral load, 150/164 (91.5%) returned at T1. The rate of viral load resuppression between T0 and T1 was 32/150 (21.3%). The characteristics of T1 attendees according to their resuppression status are summarised in Table 3-5 and in Figure 3-2. There were 7/150 (4·7%) patients who changed from NNRTI-based to PI/r-based ART after T0 owing to low CD4 cell counts; in this subset the viral load changed from 5·3 (IQR 4·9-5·8) to 2·2 (IQR 1·6-2·5) (p=0·018); 1/7 (14·3%) achieved suppression. Among subjects with T0 viral load 40-199 and 200-999 copies/mL, 21/58 (36·2%) and 10/27 (37·0%) respectively achieved suppression at T1. Only 1 subject among the 65 (1·5%) with T0 viral load ≥1000 copies/mL achieved suppression at T1; this subject was in the subset that changed to PI/r-based ART after T0. Predictors of resuppression were explored by logistics regression analysis. In univariable analysis, factors associated with lack of resuppression comprised a lower T0 CD4 cell count, a higher T0 viral load, a T0 viral load ≥1000 copies/ml, lower T1 VAS and higher GSS. After adjustment, the only independent predictor of lack of resuppression was a T0 viral load ≥1000 copies/ml (Table 3-6).

Table 3-5 **Baseline characteristics of patients with detectable T0 viral load according to the T1 viral load**

Demographics data		Total	T1 HIV-1 RNA (copies/ml)		p
			<40	≥40	
Total number (%)		150 (100)	32 (100)	118 (100)	
Female gender, n (%)		102 (68.0)	22 (68.7)	80 (67.8)	0.92
Age, median years (IQR)		47 (41-52)	48 (41-54)	47 (42-52)	0.72
Enough food to eat regular meals, n (%)	Every day/most days	123 (84.3)	99 (86.8)	24 (75.0)	0.10
	Sometimes/never	23 (15.8)	15 (13.2)	8 (25.0)	
	no data	4 (2.7)	0 (0)	4 (3.4)	
Alcohol consumption, n (%)	Never	140 (93.3)	31 (96.9)	109 (92.4)	0.33
	Occasionally	7 (4.7)	0 (0)	7 (5.9)	
	Regularly	3 (2.0)	1 (3.1)	2 (1.7)	
Use of traditional or herbal remedies, n (%)		7 (4.7)	1 (3.1)	6 (5.1)	1.0
Duration of ART, median years (IQR)		8.8 (5.4-11.2)	8.8 (3.6-10.6)	8.8 (5.4-11.3)	0.87
T0 ART regimen NNRTI-based, n (%)		129 (86.0)	26 (81.3)	103 (87.3)	0.40
T0 ART regimen PI/r-based, n (%)		21 (14.0)	6 (18.8)	15 (12.7)	
T0 NRTI backbone TDF/3TC, n (%)		87 (58.0)	15 (46.9)	72 (61.0)	0.16
T0 NRTI backbone AZT/3TC, n (%)		59 (39.3)	16 (50.0)	43 (36.4)	0.22
T0 NNRTI-based regimens, n (%)	TDF 3TC EFV	71 (47.3)	12 (37.5)	59 (50.0)	-
	TDF 3TC NVP	5 (3.3)	1 (3.1)	4 (3.4)	
	AZT 3TC EFV	31 (20.7)	8 (25.0)	23 (19.5)	
	AZT 3TC NVP	22 (14.7)	5 (15.6)	17 (14.4)	
T0 PI/r-based, n (%)	TDF 3TC LPV/r	10 (6.7)	1 (3.1)	9 (7.6)	-
	TDF 3TC ATV/r	1 (0.7)	1 (3.1)	0 (0)	
	AZT 3TC LPV/r	5 (3.3)	3 (9.4)	2 (1.7)	
	AZT 3TC ATV/r	1 (0.7)	0 (0)	1 (0.9)	
	ABC 3TC ATV/r	1 (0.7)	0 (0)	1 (0.9)	
	Other	3 (2.0)	0 (0)	3 (2.5)	
ART switch between T0 and T1, n (%)	yes	7 (4.7)	1 (3.1)	6 (5.1)	1.0
	no	143 (95.3)	31 (96.9)	112 (94.9)	
T0 Treatment interruptions, n (%)	none	92 (61.3)	17 (53.1)	75 (63.6)	0.28
	at least once	58 (38.7)	15 (46.9)	43 (36.4)	
T0 VAS score, n (%)	100 %	103 (68.7)	25 (78.1)	78 (66.1)	0.17
	90 %	21 (14.0)	1 (3.1)	20 (17.0)	

	80 %	14 (9.3)	4 (12.5)	10 (8.5)	
	<80 %	12 (8.0)	2 (6.3)	10 (8.5)	
T1 VAS score, n (%)	100 %	68 (45.3)	17 (53.1)	51 (43.2)	0.33
	90 %	48 (32.0)	12 (37.5)	36 (30.5)	
	80 %	12 (8.0)	1 (3.1)	11 (9.3)	
	<80 %	21 (14.0)	2 (6.3)	19 (16.1)	
	no data	1 (0.7)	0 (0)	1 (0.9)	
T0 adherence score, median (IQR)		1.0 (0.5-3.0)	2.0 (1.0-3.0)	1.0 (0.0-3.0)	0.03
T1 adherence score, median (IQR)		3.0 (1.0-3.0)	3.0 (3.0-3.0)	3.0 (1.0-4.0)	0.01
Change adherence score, median (IQR)		0.0 (0.0-2.0)	0.0 (0.0-2.0)	0.5 (0.0-2.0)	0.70
Nadir CD4 cell count, median cells/mm^3 (IQR)		160 (62-251)	144 (67-344)	164 (55-250)	0.84
CD4 cell count, median cells/mm^3 (IQR)		457 (235-703)	658 (481-770)	390 (220-641)	<0.01
CD4 cell count, cells/mm^3 (IQR)	<200	25 (16.7)	1 (3.1)	24 (20.3)	<0.01
	200-500	56 (37.3)	8 (25.0)	48 (40.7)	
	>500	69 (46.0)	23 (71.9)	46 (39.0)	
T0 HIV viral load, median log_{10} copies/ml (IQR)		2.63 (1.96-4.37)	2.00 (1.85-2.48)	3.37 (2.09-4.69)	<0.01
T0 HIV viral load, copies/ml, n (%)	40 – 199	58 (38.7)	21 (65.6)	37 (31.4)	
	200 – 399	15 (10.0)	6 (18.8)	9 (7.6)	
	400 – 999	12 (8.0)	4 (12.5)	8 (6.8)	
	1,000 – 9,999	17 (11.3)	0 (0)	17 (14.4)	
	10,000 – 99,999	25 (16.7)	0 (0)	25 (21.2)	
	≥ 100,000	23 (15.3)	1 (3.1)	22 (18.6)	

IQR=interquartile-range; ART=antiretroviral treatment; NRTI=nucleoside reverse transcriptase inhibitor; NNRTI=non-nucleoside reverse transcriptase inhibitor; PI/r=ritonavir-boosted protease inhibitor; TDF=tenofovir disoproxil fumarate; 3TC=lamivudine; AZT=zidovudine; EFV=efavirenz; NVP=nevirapine; LPV/r=ritonavir-boosted lopinavir; ATV/r=ritonavir-boosted atazanavir; ABC=abacavir; VAS=visual analogue scale.

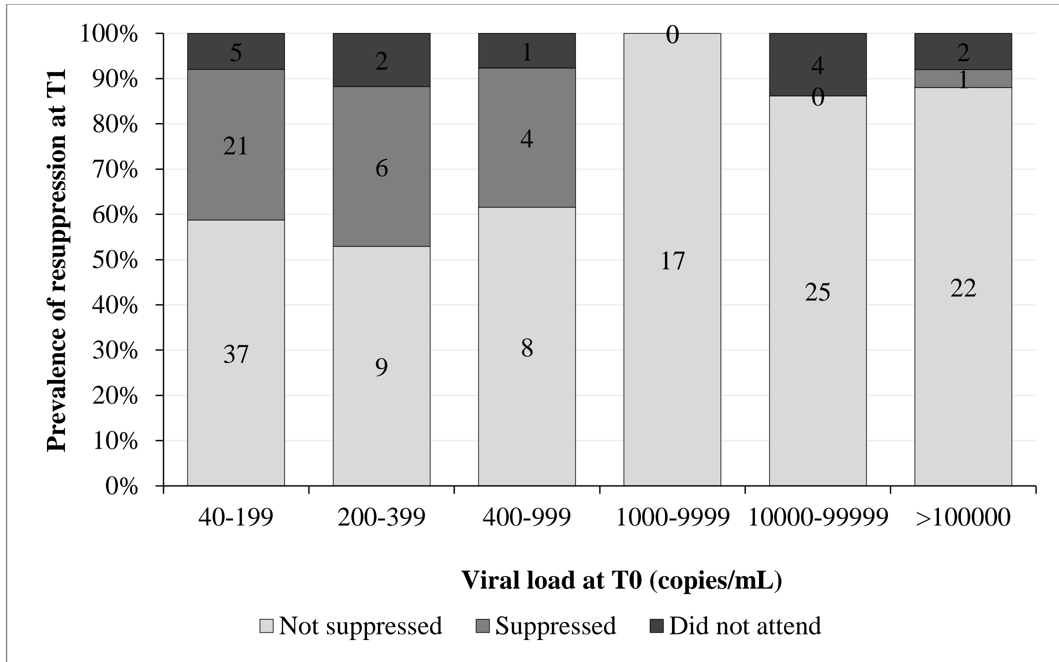


Figure 3-2 **Viral load at T1 among study participants with detectable viral load at T0 (n=150)**

Table 3-6 **Univariable and multivariable logistic regression model of predictors of resuppression at T1 (n=32/150, 21.3%)**

Variable		Univariable analysis			Multivariable analysis								
					Model 1			Model 2			Model 3		
		OR	95% CI	p	OR	95% CI	p	OR	95% CI	p	OR	95% CI	p
Gender	Female vs male	1.05	0.45-2.42	0.92									
Age	per 5-year older	1.02	0.80-1.29	0.89									
Enough food	never vs at least sometimes	1.89	0.53-6.74	0.33									
Alcohol consumption[a]	yes vs no	0.39	0.05-3.20	0.38									
Use of herbal remedies	yes vs no	0.60	0.07-5.19	0.64									
Third agent	PI/r vs NNRTI	1.58	0.56-4.48	0.39									
Duration of ART	per 1 year longer	0.99	0.89-1.09	0.83									
Treatment interruptions, number	≥1 vs never	1.54	0.70-3.39	0.28									
T1 VAS	per 10% score higher	1.50	1.00-2.26	0.05	1.31	0.77-2.23	0.32	1.38	0.84-2.27	0.21	1.32	0.78-2.23	0.31
Change in adherence score	per unit higher	0.98	0.73-1.32	0.89									
Time since HIV diagnosis	per year longer	1.01	0.91-1.12	0.81									
T0 CD4 cell count	Per 100-cells higher	1.25	1.09-1.44	<0.01	NI			NI			NI		
Nadir CD4 cell count	Per 100-cells higher	0.96	0.82-1.13	0.61									
T0 viral load	Per log_{10} copies/ml higher	0.33	0.19-0.58	<0.01	0.46	0.21-1.00	0.05	NI			0.36	0.20-0.63	<0.01
T0 viral load, copies/ml	40-199	1			NI			NI			NI		
	200 – 399	1.17	0.37-3.76	0.79									
	400 – 999	0.88	0.24-3.28	0.85									
	≥1,000	0.03	0.00-0.21	<0.01									
T0 GSS score	per 0.5 unit higher	1.80	1.27-2.56	<0.01	1.21	0.77-1.90	0.41	1.73	1.22-2.45	<0.01			

Due to the collinearity between T0 viral load and GSS score, their effect in the multivariable adjustment was explored separately in models 2 and 3. [a]Occasional or regular. OR=odds ratio; CI=confidence interval; ART=antiretroviral treatment; VAS=visual analogue scale; PI/r=ritonavir-boosted protease inhibitor; NNRTI=non-nucleoside reverse transcriptase inhibitor; TDF=tenofovir disoproxil fumarate; 3TC=lamivudine; AZT=zidovudine

3.3.4 Adherence measures

At recruitment, 295/333 (88.6%) patients reported a VAS score ≥90% (Questionnaire 1). The median adherence score for individuals with quantifiable viral load at T0 (computed from Questionnaire 2) was 1.0 (IQR 1.0-3.0), at T1, the adherence score (computed from Questionnaire 3) had a median of 3.0 (IQR 1.0-3.0), higher than the one measured at T0 (p<0.01).

3.3.5 Resistance

Resistance testing was performed in 87/92 (91.6%) patients with T0 viral load >200 copies/ml (Table 3-7). No amplicon was obtained from 5/92 (5.4%) subjects, all with viral load <400 copies/ml. Prevalence of at least 1 NRTI RAM, 1 NNRI RAM and 1 PI RAM was 55/87 (63.2%), 60/87 (69.0%) and 4/87 (4.6%) respectively. The proportion of patients with at least 1 RAM increased by T0 viral load stratum (Figure 3-3) whereas the GSS scores decreased in parallel (Figure 3-4); there was a strong correlation between viral load and GSS (Spearman's rho=-0.66, p<0.01).

Table 3-7 **Prevalence of resistance associated mutations detected in subjects with T0 viral load >200 copies/ml (n=87) stratified by T0 viral load < or ≥1000 copies/ml**

			HIV-1 RNA (copies/ml)			
			<1000		**>1000**	
			n=22 (100)		**n=65 (100)**	
NRTI RAMs			**n**	**%**	**n**	**%**
Discriminatory		M184V/I	3	13.6	48	73.8
		K65R	-	-	12	18.5
		K70E/G/N	-	-	7	10.8
		L74I/V	1	4.5	5	7.7
		Y115F	-	-	3	4.6
TAMs	type 1	M41L	2	9.1	12	18.5
		L210W	1	4.5	6	9.2
		T215Y	2	9.1	9	13.8
	type 2	D67N/G	1	4.5	7	10.8
		K70R	-	-	8	12.3
		T215F	-	-	4	6.2
		K219Q/E/R	1	4.5	14	21.5
	revertants	T215D/I/V	-	-	3	4.6
	accessory	E44D	1	4.5	2	3.1
Miscellaneous		T69G	-	-	1	1.5

	HIV-1 RNA (copies/ml)			
	<1000		**>1000**	
	n=22 (100)		**n=65 (100)**	
NNRTI RAMs	**n**	**%**	**n**	**%**
A98G	-	-	10	15.4
L100I	-	-	1	1.5
K101E/H/P	-	-	9	13.8
K103N/S	3	13.6	43	66.2
V108I	1	4.5	10	15.4
E138G/Q	-	-	5	7.7
G190A/S	-	-	8	12.3
Y181C	-	-	10	15.4
Y188L	-	-	3	4.6
H221Y	-	-	4	6.2
P225H	-	-	16	24.6
F227L	-	-	3	4.6
M230L	-	-	5	7.7
L234I	-	-	3	4.6
K238T	1	4.5	6	9.2
Y318F	-	-	1	1.5

	HIV-1 RNA (copies/ml)			
	<1000		**>1000**	
	n=22 (100)		**n=65 (100)**	
PI RAMs	**n**	**%**	**n**	**%**
L33F	-	-	1	1.5
M46I	1	4.5	1	1.5
L76V	1	4.5	-	-
V82A	-	-	1	1.5
I84V	1	4.5	-	-

NRTI=nucleoside reverse transcriptase inhibitors; NNRTI=non-nucleoside reverse transcriptase inhibitors; PI=protease inhibitors; RAMs=resistance-associated mutations; TAMs=thymidine analogue mutations

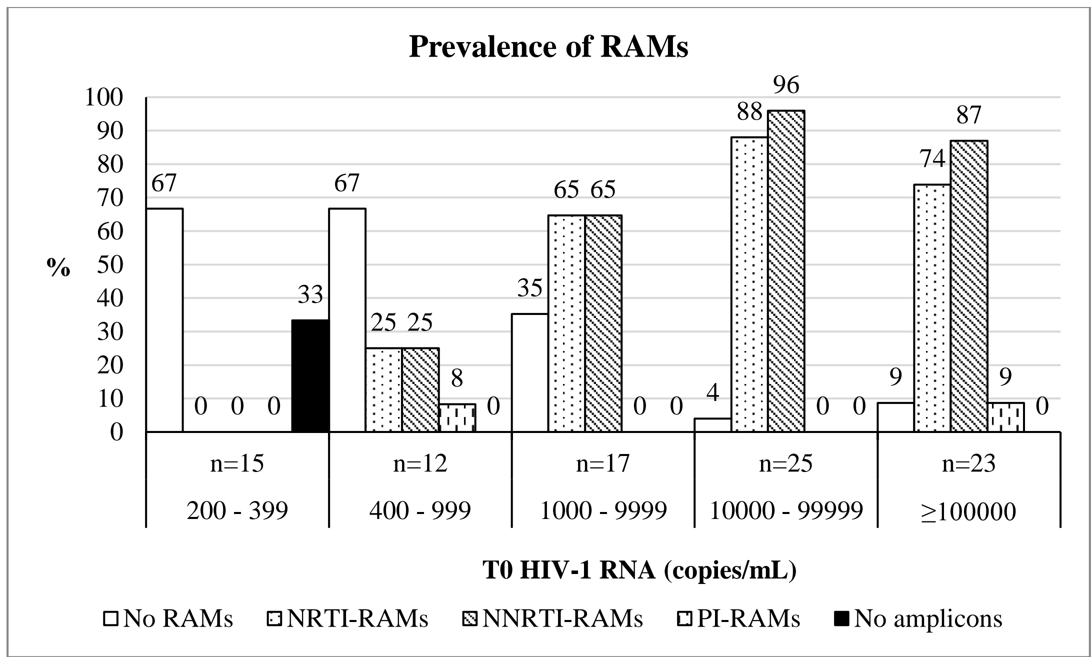


Figure 3-3 **Prevalence of at least one resistance associated mutation per drug class according to T0 viral load**

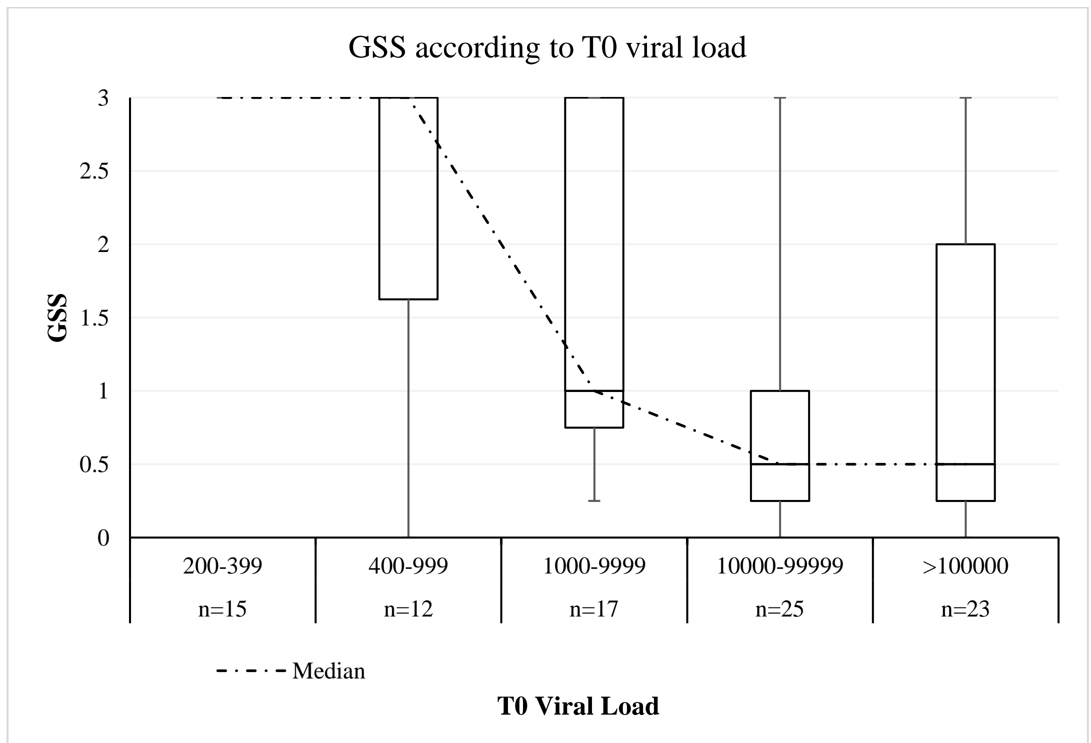


Figure 3-4 **Genotypic susceptibility score according to T0 viral load: box and whisker plots**

Patients with T0 viral load >1000 copies/ml were 65/87 (74.7%), 17/87 (19.5%) with viral load between 1000-9999 copies/ml, 25/87 (28.7%) between 10000-99999 copies/ml, and 23/87 (26.4%) ≥100000 copies/ml. They showed complex resistance patterns. The most prevalent RAM was M184V/I, which was detected in 48/65 (73.8%) subjects. TDF-associated discriminatory RAMs at codon 65 (K65R), 70 (K70E/G/N), 74 (L74I/V), and 115 (Y115F) were detected in 12/65 (18.5%), 7/65 (10.8%), 5/65 (7.7%), and 3/65 (4.6%) subjects, respectively. Thymidine-analogue mutations (TAMs) were common and detected in 14/65 (21.5%) participants: type 1 (M41L, L210W, T215Y), type 2 (D67N, K70R, T215D, K219E/Q) and mixed patterns were detected in 9/65 (13.9%), 9/65 (13.9%), and 5/65 (7.7%) individuals, respectively. NNRTI-associated RAMs were very frequent: at least 1 was detected in 55/65 subjects (84.6%); out of the patients on second-line treatment, 2/65 (3.1%) harboured PI-associated RAMs conferring intermediate to high resistance to LPV/r. No RAM was detected in patients with T0 viral load in the range 200-400 copies/ml (n=15). Four subjects had RAMs in the 400-1000 group (n=12): detected RAMs were M184V in 3/12 (25%) individuals, type 1 TAMs in 1/12 (8.3%) individual, TAMs mixed pattern in 1/12 (8.3%), and NNRTI and PI related RAMs in 3/12 (25.0%) and 1/12 (8.3%) subject, respectively. CRF02_AG was the most prevalent subtype (68/92, 73.9%), followed by CRF06_cpx (10/92, 10.9%), A (5/92, 5.4%), CRF09_cpx (4/92, 4.3%), G (2/92, 2.2%), and B (1/92, 1.1%). Patient-level data are reported in Supplementary Tables 4-8 to 4-12.

Table 3-8 **Resistance associated mutations (RAMs) in the OPTIMISE cohort. Subjects with HIV RNA > 5 log copies/ml at T0**

ID	SUBTYPE	YEARS ON ART	CD4 cells /µl	T0 ART	T0 HIV RNA log copies/ ml	T1 ART	T1 HIV RNA log copies/ ml	NRTI RAMS	NNRTI RAMS	PI RAMS	T0 GSS	T1 GS S
OPT/270	CRF02_AG	11.5	326	AZT 3TC EFV	6.37	AZT 3TC EFV	6.98	M41ML, D67N, K70R, L74LI, M184V, L210LW, T215DFVY, K219Q	A98G, K103N, P225H, K238T	NONE	0	0
OPT/107	CRF02_AG	5.2	222	AZT 3TC EFV	6.09	AZT 3TC EFV	5.34	M184MV	K103N, E138EG, P225H, K238T	NONE	1	1
OPT/261	CRF02_AG	3.1	247	AZT 3TC EFV	6.02	AZT 3TC EFV	2.21	NONE	K103N	NONE	2	2
OPT/023	CRF02_AG	12.7	20	TDF 3TC EFV	6.01	TDF 3TC EFV	5.97	K65R, M184V, K219KE	L100I, K103N	NONE	0.25	0.25
OPT/149	CRF02_AG	1.51	45	TDF 3TC EFV	5.98	TDF 3TC EFV	6.20	M41L, D67DN, K70KR, V75VM, M184V, T215F, K219Q	A98G, K103N, P225H, K238T	NONE	0.25	0.25
OPT/009	CRF02_AG	11.3	201	TDF 3TC EFV	5.90	TDF 3TC LPV/r	2.54	NONE	NONE	NONE	3	3
OPT/033	CRF02_AG	9.6	9	AZT 3TC EFV	5.76	TDF 3TC LPV/r	2.38	NONE	K103N, E138EG, P225H,K238T	NONE	2	3
OPT/208	CRF02_AG	8.9	185	AZT 3TC EFV	5.72	AZT 3TC EFV	4.35	M184V	K103N, E138A, V179E, P225H	NONE	1	1
OPT/255	CRF02_AG	5.4	204	AZT 3TC EFV	5.69	AZT 3TC EFV	5.40	M184V	K103N, V108I, P225H	NONE	1	1
OPT/329	CRF02_AG	11.8	42	TDF 3TC EFV	5.64	TDF 3TC EFV	5.48	K70N, L74I, M184V	K103N, V179E, P225H	NONE	0.5	0.5
OPT/234	CRF02_AG	9.2	115	AZT 3TC EFV	5.61	AZT 3TC EFV	2.31	NONE	K103N	NONE	2	2
OPT/007	CRF02_AG	9.4	7	TDF 3TC EFV	5.39	TDF 3TC LPV/r	1.63	A62V, K65R, V75I, Y115F, M184V	K101E, V179T, Y181C, G190S	NONE	0	1

OPT/111	A	Na	216	TDF 3TC EFV	5.37	TDF 3TC NVP	6.06	K65R, M184V, K219E	A98AG, V108I, Y181C	NONE	0.25	0.25
OPT/128	A	5.9	192	TDF 3TC EFV	5.37	TDF 3TC EFV	4.59	K65R, M184I	K103N, P225H	NONE	0.25	0.25
OPT/285	CRF06_cpx	10.8	56	TDF 3TC EFV	5.32	TDF 3TC LPV/r	2.08	M41L, L74I, M184V, L210W, T215Y, K219Q	A98G, K103N, V108VI, V179E, K238T	L33F	0.25	1.25
OPT/277	CRF02_AG	4.8	176	TDF 3TC LPV/r	5.29	TDF 3TC LPV/r	2.19	NONE	NONE	NONE	3	3
OPT/043	CRF02_AG	11.4	6	AZT 3TC LPV/r	5.28	ABC 3TC ATV/r TDF	2.90	D67G, K70E, T215Y	E138A	M46I, V82A	1.5	1.75
OPT/104	CRF02_AG	9.6	327	AZT 3TC NVP	5.19	AZT 3TC NVP	5.12	M184V	A98AG, K101KE, Y181C, H221HY	NONE	1	1
OPT/175	CRF02_AG	6.9	191	AZT 3TC EFV	5.19	TDF 3TC EFV	2.11	NONE	K103N	NONE	2	2
OPT/302	CRF02_AG	13.6	422	AZT 3TC EFV	5.16	AZT 3TC EFV	5.33	K70KR, M184V	K103N, P225H	NONE	1	1
OPT/242	CRF02_AG	1.5	89	TDF 3TC EFV	5.11	TDF 3TC EFV	5.23	K65R, M184V	K103N, V179E	NONE	0.25	0.25
OPT/216	A	6.6	220	AZT 3TC EFV	5.07	AZT 3TC EFV	4.87	M41L, E44ED, V75I, M184V, L210W, T215Y	K103N, V108I, Y318F	NONE	0	0
OPT/142	CRF09_cpx	6.8	7	TDF 3TC NVP	5.03	ABC 3TC ATV/r	1.30	K65R, V75M, M184V, K219E	K103N, Y181C	NONE	0.25	1

ART=antiretroviral treatment; NRTI=nucleoside reverse transcriptase inhibitors; RAMs=resistance-associated mutations; NNRTI=non-nucleoside reverse transcriptase inhibitors; PI=protease inhibitors; GSS=genotypic susceptibility score; AZT=zidovudine; 3TC=lamivudine; EFV=efavirenz; TDF=tenofovir disoproxil fumarate; LPV/r=ritonavir-boosted lopinavir; ABC=abacavir; ATV/r=ritonavir-boosted atazanavir; NVP=nevirapine

Table 3-9 **Resistance associated mutations (RAMs) in the OPTIMISE cohort. Subjects with HIV RNA 4-5 log copies/ml at T0**

ID	SUBTYPE	YEARS ON ART	CD4 cells /µl	T0 ART	T0 HIV RNA log copies/ml	T1 ART	T1 HIV RNA log copies/ml	NRTI RAMS	NNRTI RAMS	PI RAMS	T0 GSS	T1 GSS
OPT/064	CRF02_AG	8.9	383	TDF 3TC EFV	4.92	TDF 3TC EFV	2.63	NONE	P225HY, F227L	NONE	2	2
OPT/311	CRF06_cpx	7.5	484	AZT 3TC EFV	4.92	AZT 3TC EFV	4.77	M41L, E44ED, M184V, L210W, T215Y	K101H, K103N, G190A	NONE	0	0
OPT/077	CRF02_AG	9.7	154	TDF 3TC EFV	4.91	TDF 3TC EFV	5.03	M41ML, K65KR, K70KE, M184V, K219KE	K103N, V179VE, P225H, F227FL, M230ML	NONE	0	0
OPT/110	CRF02_AG	8.8	88	TDF 3TC EFV	4.88	TDF 3TC LPV/r	2.54	L74I, M184V	A98G, K103N, P225H	NONE	1	2
OPT/137	CRF02_AG	10.6	234	AZT 3TC EFV	4.87	AZT 3TC EFV	4.62	M184V	K103N, V108I, P225PH	NONE	1	1
OPT/138	CRF02_AG	7.2	288	TDF 3TC EFV	4.80	TDF 3TC EFV	5.59	K65R, M184V	K103KNRS, V108VI, Y181C, G190A	NONE	0.25	0.25
OPT/124	CRF02_AG	7.6	25	AZT 3TC NVP	4.79	AZT 3TC NVP	4.82	M41L, V75M, M184V, L210W, T215Y	A98G, K101H, G190A	NONE	0	0
OPT/276	CRF02_AG	7.5	264	AZT 3TC LPV/r	4.69	AZT 3TC LPV/r	2.75	NONE	NONE	NONE	3	3
OPT/278	CRF02_AG	5.7	307	TDF 3TC EFV	4.58	TDF 3TC EFV	2.93	NONE	K103N	NONE	2	2
OPT/074	CRF02_AG	10	155	TDF 3TC EFV	4.55	TDF 3TC EFV	4.69	K70E, M184V	K103N, V106I, H221Y, M230L, L234I	NONE	0.5	0.5
OPT/244	CRF02_AG	12.7	220	TDF 3TC EFV	4.53	TDF 3TC EFV	4.85	M41L, M184V, T215Y	K103N, V179E	NONE	0.5	0.5
OPT/002	CRF02_AG	6.1	44	TDF 3TC EFV	4.50	TDF 3TC EFV	4.46	K65R, L74I, Y115F, M184V	K103N, V179E, Y181C, P225PH	NONE	0	0
OPT/155	CRF02_AG	13.7	371	AZT 3TC NVP	4.49	AZT 3TC NVP	5.11	M41ML, M184V, T215Y	K103S, E138Q, G190A	NONE	0.25	0.25

OPT/044	CRF02_AG	11.4	176	AZT 3TC EFV	4.45	AZT 3TC EFV	4.64	M184V	K103N, H221Y, M230L, L234I	NONE	1	1
OPT/148	CRF02_AG	9.2	127	AZT 3TC EFV	4.37	AZT 3TC EFV	5.36	K70R, M184V, T215F, K219KQ	K103N	NONE	0	0
OPT/037	CRF06_cpx	6.7	104	TDF 3TC EFV	4.35	TDF 3TC EFV	3.93	M41L, V75M, M184V	K101E, G190S	NONE	1	1
OPT/201	CRF02_AG	5.1	271	AZT 3TC NVP	4.19	TDF 3TC NVP	4.41	K70N, M184V, K219R	K103N, F227L	NONE	1	0.5
OPT/280	CRF02_AG	3.9	325	TDF 3TC EFV	4.16	TDF 3TC EFV	3.54	K65R, M184I, K219E	K103N, M230L	NONE	0.25	0.25
OPT/084	CRF02_AG	9.2	89	AZT 3TC EFV	4.15	AZT 3TC EFV	4.20	D67DN, K70R, M184V, K219KQ	K103N, P225H	NONE	0.25	0.25
OPT/239	CRF09_cpx	6.3	279	TDF 3TC EFV	4.14	TDF 3TC EFV	4.21	K70EG, M184V	K101P, V179D, Y188L	NONE	0.5	0.5
OPT/072	CRF02_AG	6.6	330	TDF 3TC EFV	4.14	TDF 3TC EFV	4.32	K70G, Y115F, M184V	A98G, E138G, V179E, Y188L, H221Y	NONE	0.25	0.25
OPT/310	CRF02_AG	9.3	234	AZT 3TC NVP	4.13	AZT 3TC NVP	3.99	M184V	K103N, K238KT	NONE	1	1
OPT/225	CRF02_AG	2.8	195	TDF 3TC NVP	4.08	TDF 3TC NVP	3.65	K65R, M184I	K101E, Y181C	NONE	0.25	0.25
OPT/094	CRF02_AG	13.1	447	AZT 3TC EFV	4.08	AZT 3TC EFV	4.62	M184V	K103N, V108VI	NONE	1	1
OPT/170	CRF02_AG	13.4	463	AZT 3TC NVP	4.06	AZT 3TC NVP	3.77	M184V	A98AG, K101H, Y181C	NONE	1	1

ART=antiretroviral treatment; NRTI=nucleoside reverse transcriptase inhibitors; RAMs=resistance-associated mutations; NNRTI=non-nucleoside reverse transcriptase inhibitors; PI=protease inhibitors; GSS=genotypic susceptibility score; AZT=zidovudine; 3TC=lamivudine; EFV=efavirenz; TDF=tenofovir disoproxil fumarate; LPV/r=ritonavir-boosted lopinavir; ABC=abacavir; ATV/r=ritonavir-boosted atazanavir; NVP=nevirapine

Table 3-10 **Resistance associated mutations (RAMs) in the OPTIMISE cohort. Subjects with HIV RNA 3-4 log copies/ml at T0**

ID	SUBTYPE	YEARS ON ART	CD4 cells /µl	T0 ART	T0 HIV RNA log copies/ ml	T1 ART	T1 HIV RNA log copies/ ml	NRTI RAMS	NNRTI RAMS	PI RAMS	T0 GSS	T1 GSS
OPT/078	CRF02_AG	6.9	504	TDF 3TC NVP	3.92	TDF 3TC NVP	4.15	D67N, K70R, M184V, K219E	Y188L	NONE	0.5	0.5
OPT/031	CRF02_AG	8.9	527	AZT 3TC EFV	3.90	AZT 3TC EFV	3.81	M184V, T215Y	A98G, K103N	NONE	0.25	0.25
OPT/222	A	3.7	229	AZT 3TC ATV/r	3.86	AZT 3TC ATV/r	3.48	M41L, M184I	K103N, P225H	NONE	2	2
OPT/167	CRF06_cpx	9.5	290	AZT 3TC NVP	3.80	AZT 3TC NVP	3.64	M184V	K103N, E138Q	NONE	1	1
OPT/045	CRF02_AG	6.9	471	AZT 3TC NVP	3.74	AZT 3TC EFV	2.97	M184V	K103N, V108I	NONE	1	1
OPT/154	CRF02_AG	10.7	315	TDF 3TC NVP	3.70	TDF 3TC NVP	4.08	M184MV	K103KN, Y181YC	NONE	1	1
OPT/153	CRF02_AG	0.5	535	TDF 3TC EFV	3.68	TDF 3TC EFV	2.18	NONE	NONE	NONE	3	3
OPT/299	CRF02_AG	11.9	625	TDF 3TC EFV	3.68	TDF 3TC LPV/r	2.16	D67Deletion, T69G, K70R, M184V, T215I, K219E	K103N, V108I, M230L, L234I	NONE	0.25	1.25
OPT/251	CRF02_AG	0.2	202	TDF 3TC EFV	3.54	TDF 3TC EFV	1.97	NONE	NONE	NONE	3	3
OPT/325	CRF02_AG	6.8	895	TDF 3TC EFV	3.47	TDF 3TC EFV	3.07	NONE	NONE	NONE	3	3
OPT/191	CRF02_AG	6.4	235	AZT 3TC NVP	3.46	AZT 3TC NVP	3.12	M184V	Y181C	NONE	1	1
OPT/294	CRF02_AG	0.3	519	TDF 3TC EFV	3.45	TDF 3TC EFV	2.69	NONE	NONE	NONE	3	3
OPT/304	CRF06_cpx	13.6	830	AZT 3TC NVP	3.28	AZT 3TC NVP	2.37	NONE	NONE	NONE	3	3

OPT/015	CRF02_AG	11	708	TDF 3TC EFV	3.22	TDF 3TC EFV	2.89	K65R	K103N, V108I, Y188N	NONE	0.25	0.25
OPT/257	CRF02_AG	14	859	AZT 3TC NVP	3.20	AZT 3TC NVP	3.31	M184V	K103S, G190A	NONE	1	1
OPT/041	CRF06_cpx	11.4	708	TDF 3TC LPV/r	3.03	TDF 3TC LPV/r	2.21	M41L, D67N, K70R, M184V, L210W, T215F, K219E	K101E, V106I, G190A	NONE	1	1
OPT/189	CRF02_AG	11.2	629	AZT 3TC NVP	3.03	AZT 3TC NVP	2.35	NONE	E138A	NONE	3	3

ART=antiretroviral treatment; NRTI=nucleoside reverse transcriptase inhibitors; RAMs=resistance-associated mutations; NNRTI=non-nucleoside reverse transcriptase inhibitors; PI=protease inhibitors; GSS=genotypic susceptibility score; AZT=zidovudine; 3TC=lamivudine; EFV=efavirenz; TDF=tenofovir disoproxil fumarate; LPV/r=ritonavir-boosted lopinavir; ABC=abacavir; ATV/r=ritonavir-boosted atazanavir; NVP=nevirapine

Table 3-11 **Resistance associated mutations (RAMs) in the OPTIMISE cohort. Subjects with HIV RNA 400-1000 copies/ml at T0**

ID	SUBTYPE	YEARS ON ART	CD4 cells/ µl	T0 ART	T0 HIV RNA log copies/ ml	T1 ART	T1 HIV RNA log copies/ ml	NRTI RAMS	NNRTI RAMS	PI RAMS	T0 GSS	T1 GSS
OPT/248	G	0,3	235	TDF 3TC EFV	2,96	TDF 3TC EFV	1,92	NONE	NONE	NONE	3	3
OPT/035	CRF02_AG	9,4	473	AZT 3TC LPV/r	2,95	AZT 3TC LPV/r	1,3	M41L, M184V, T215Y	E138A	NONE	1,25	1,25
OPT/156	CRF02_AG	1,7	416	ABC 3TC ATV/r	2,82	ABC 3TC ATV/r	3,19	L74V, M184V	K103N	NONE	1	1
OPT/166	CRF02_AG	8,9	720	AZT 3TC NVP	2,78	AZT 3TC NVP	2,24	NONE	NONE	NONE	3	3
OPT/163	CRF02_AG	13,2	1319	AZT 3TC NVP	2,75	AZT 3TC NVP	1,3	NONE	NONE	NONE	3	3
OPT/082	CRF02_AG	10,5	718	TDF 3TC EFV	2,73	TDF 3TC EFV	2,6	NONE	NONE	NONE	3	3
OPT/279	CRF06_cpx	1,1	1022	TDF 3TC EFV	2,66	TDF 3TC EFV	3,11	NONE	NONE	NONE	3	3
OPT/024	A	1,4	695	TDF 3TC EFV	2,65	TDF 3TC EFV	1,3	NONE	NONE	NONE	3	3
OPT/054	CRF09_cpx	na	1208	TDF ABC LPV/r	2,65	TDF ABC LPV/r	2,66	NONE	NONE	NONE	3	3
OPT/288	CRF02_AG	13,1	163	TDF 3TC LPV/r	2,63	TDF 3TC LPV/r	4,38	M41L, E44D, D67N, V75M, M184 VL210W, T215Y, K219E	K103N, V108I, K238T	M46I, L76V, I84V	0	0
OPT/109	CRF09_cpx	na	489	AZT 3TC LPV/r	2,62	AZT 3TC LPV/r	1,3	NONE	NONE	NONE	3	3
OPT/309	CRF02_AG	6,1	945	TDF 3TC EFV	2,61	TDF 3TC EFV	2,89	NONE	K103N	NONE	2	2

ART=antiretroviral treatment; NRTI=nucleoside reverse transcriptase inhibitors; RAMs=resistance-associated mutations; NNRTI=non-nucleoside reverse transcriptase inhibitors; PI=protease inhibitors; GSS=genotypic susceptibility score; AZT=zidovudine; 3TC=lamivudine; EFV=efavirenz; TDF=tenofovir disoproxil fumarate; LPV/r=ritonavir-boosted lopinavir; ABC=abacavir; ATV/r=ritonavir-boosted atazanavir; NVP=nevirapine

Table 3-12 **Resistance associated mutations (RAMs) in the OPTIMISE cohort. Subjects with HIV RNA 200-400 copies/ml at T0**

ID	SUBTYPE	YEARS ON ART	CD4 cells /µl	T0 ART	T0 HIV RNA log copies/ ml	T1 ART	T1 HIV RNA log copies/ ml	NRTI RAMS	NNRTI RAMS	PI RAMS	T0 GSS	T1 GSS
OPT/131	CRF02_AG	9,3	617	TDF 3TC ATV/r	2,59	TDF 3TC ATV/r	1,30	NONE	NONE	NONE	3	3
OPT/230	no amplicon	1,7	720	TDF 3TC EFV	2,55	TDF 3TC EFV	0,70	no amplicon	no amplicon	no amplicon	.	.
OPT/164	no amplicon	11,8	709	TDF 3TC EFV	2,54	TDF 3TC EFV	2,45	no amplicon	no amplicon	no amplicon	.	.
OPT/287	B	8,7	604	AZT 3TC EFV	2,53	AZT 3TC EFV	1,30	NONE	NONE	NONE	3	3
OPT/173	CRF02_AG	3,9	349	TDF 3TC EFV	2,52	TDF 3TC EFV	2,63	NONE	NONE	NONE	3	3
OPT/169	CRF02_AG	12,4	822	AZT 3TC NVP	2,43	AZT 3TC NVP	1,30	NONE	NONE	NONE	3	3
OPT/076	CRF06_cpx	8,8	587	AZT 3TC EFV	2,43	AZT 3TC EFV	1,30	NONE	NONE	NONE	3	3
OPT/250	CRF02_AG	2,4	429	TDF 3TC EFV	2,43	TDF 3TC EFV	2,03	NONE	NONE	NONE	3	3
OPT/292	no amplicon	8,7	375	AZT 3TC NVP	2,37	AZT 3TC NVP	2,68	no amplicon	no amplicon	no amplicon	.	.
OPT/008	no amplicon	11,1	799	TDF 3TC LPV/r	2,36	TDF 3TC LPV/r	2,07	no amplicon	no amplicon	no amplicon	.	.
OPT/316	no amplicon	7,0	897	TDF 3TC EFV	2,34	TDF 3TC EFV	2,47	no amplicon	no amplicon	no amplicon	.	.
OPT/051	G	5,0	593	TDF 3TC LPV/r	2,34	TDF 3TC LPV/r	2,30	NONE	NONE	NONE	3	3
OPT/176	CRF02_AG	11,8	912	TDF 3TC EFV	2,33	TDF 3TC EFV	1,90	NONE	NONE	NONE	3	3

OPT/289	CRF06_cpx	5,4	293	AZT 3TC NVP	2,32	AZT 3TC NVP	2,15	NONE	V179E	NONE	3	3
OPT/101	CRF02_AG	0,1	212	TDF 3TC EFV	2,32	TDF 3TC EFV	1,30	NONE	NONE	NONE	3	3

ART=antiretroviral treatment; NRTI=nucleoside reverse transcriptase inhibitors; RAMs=resistance-associated mutations; NNRTI=non-nucleoside reverse transcriptase inhibitors; PI=protease inhibitors; GSS=genotypic susceptibility score; AZT=zidovudine; 3TC=lamivudine; EFV=efavirenz; TDF=tenofovir disoproxil fumarate; LPV/r=ritonavir-boosted lopinavir; ABC=abacavir; ATV/r=ritonavir-boosted atazanavir; NVP=nevirapine

3.4 DISCUSSION

In this prospective study of a mature HIV cohort accessing treatment in a real-life setting in Ghana, POC viral load testing was technically feasible and effectively informed immediate adherence counselling for patients with viraemia. After long-term, mainly NNRTI-based ART, nearly half of the patients had a detectable viral load, and 1 in 5 had a viral load ≥1000 copies/mL, the WHO recommended threshold for defining virological failure.[67] Eight weeks later, there was high retention into follow-up and evidence of improved adherence. Resuppression rates were poor however, and none of the patients with a viral load ≥1000 copies/mL achieved resuppression while continuing NNRTI-based ART. Co-existence of high viral loads and complex drug resistance patterns was common in this group. It can be concluded that waiting to confirm a viral load ≥1000 copies/mL – as currently recommended[67] - was unnecessary in NNRTI-treated patients with viral load ≥1000 copies/mL. Delaying a switch to second-line ART may in fact be counterproductive in terms of risk of disease progression and transmission of drug-resistant strains. Encouragingly, good viral load responses were seen in the few patients who switched to second-line PI/r-based ART while in the study.

In pooled analyses of studies from sub-Saharan Africa, 65% and 62% of patients had a suppressed viral load (by intention to treat) after 24 months of first-line NNRTI-based ART and second-line PI/r-based ART, respectively.[71,103] Rates of virological suppression differ by region, and tend to be higher in randomised clinical trials than in observational cohorts.[71] Long-term data are scarce. In this study, after a median of 8·9 years of predominantly NNRTI-based ART, 49·2% had detectable viraemia and 21·3% had a viral load ≥1000 copies/mL. Similar alarming data were reported from Togo: after a median of 6 years of predominantly NNRTI-based ART, nearly 60% of patients had viraemia, and 40% had a viral load ≥1000 copies/mL.[185] New strategies are needed to improve management of HIV-positive cohorts in West Africa, where care continues to be delivered largely in the absence of virological monitoring.

This is the first study to report on the use of Xpert to measure the viral load at point of care. A previous multicentre study in rural Zimbabwe used Xpert for on-site testing of a selected population, but patients did not wait for the results.[186] Adherence

counselling was planned for subjects with a viral load ≥1000 copies/mL; however, 1-3 weeks after the initial viral load test, about half (53/96, 55%) of those with viral load ≥1000 copies/mL were lost to follow-up. In our study, all patients waited for their results, and all patients with any level of viraemia received adherence counselling. Furthermore, perhaps aided by the first adherence counselling and knowledge that results would be available on the same day, attendance at the follow-up visit was 91·5% among viraemic patients.

POC viral load testing was technically successful, and knowledge of the result often unmasked problems with adherence that had not emerged at the first interview a few hours earlier. However, implementation on a larger, routine scale requires a number of operational solutions. Firstly, back-up batteries of sufficient potency are required to ensure continuous supply of electricity and avoid assay failure. Second, the size of the Xpert unit dictates the number of tests that can be run within a typical clinic day. Where larger or multiple units are not available, strategies must be defined to stratify patients according to their risk of viraemia. In our study, both a history of treatment interruption and the VAS score were associated with the viral load. Other studies from Mozambique,[187] Nigeria, Uganda, Kenya and Tanzania[188] similarly indicated that measured or self-reported adherence independently predicted a viral load ≥1000 copies/mL. Thus, simple measures of adherence could be used to fast track patients to POC vs. deferred viral load testing, a hypothesis that needs investigating in controlled studies. Additional factors could be used. For example, age below 50 years more than doubled the odds of a viral load ≥1000 copies/mL in our study, along with the use of AZT/3TC rather than TDF/3TC, whereas males and the most indigent were at an increased risk of low-level viraemia.

This is also the first study to investigate post-counselling resuppression by POC testing. Eight weeks after viral load-informed adherence counselling, only 1 in 5 subjects with a detectable viral load achieved suppression, and the proportion fell to 1 in 65 for those with ≥1000 copies/mL. The only patient to show resuppression after a viral load ≥1000 copies/mL had switched from NNRTI-based to PI/r-based ART between viral load measurements. A meta-analysis of 5 studies conducted between 2004 and 2013 explored rates of resuppression after adherence counselling in high and middle-low income countries, including Mali, Burkina Faso, Swaziland, and South Africa.[189] A pooled estimate of 70% was derived from a total population of 406

patients that differed in terms of treatment history and definition of viraemia and resuppression. Resuppression rates following adherence counselling in patients that showed a viral load ≥1000 copies/mL while receiving first-line NNRTI-based ART were also reported from a trial in Uganda (resuppression rate 19/70, 27%),[173] a multicentre study from Burkina Faso, Cameroon, and Senegal (81/584, 14%),[190] and a prospective study from rural Lesotho (39/110, 35%).[191] One important limitation of these studies was that the viral load was deferred and quantified at a central laboratory and rates of loss to follow-up ranged up to 33%.

A study from Uganda reported that adherence measured by electronic pill count predicted the likelihood of resuppression for patients with viral load 500-1000 copies/mL, but not for those with viral load ≥1000 copies/mL.[174] This is in line with our findings. The viral load predicted the likelihood of resuppression after adherence counselling independently of self-reported adherence. Although the effect moved along a continuum, none of the patients with viral load ≥1000 copies/mL resuppressed while remaining on an NNRTI-based regimen, whereas a substantial proportion of those with a lower viral load did. This observation identifies a group that would benefit the most from adherence counselling to prevent viral load increases and accumulation of resistance.

In previous studies, prevalence of RAMs in NNRTI-treated patients with viral load ≥1000 copies/mL was 89% (54/61) after a median of 3 years in Mozambique,[187] 99% (440/446) after 4 years in Burkina Faso, Senegal and Cameroon,[190] 92% (77/84) after 5 years in Mali,[192] and 99% (163/164) after 6 years in Togo.[185] After a median of 8·9 years of predominantly NNRTI-based ART in this study, 55/65 (85%) patients with viral load ≥1000 copies/mL had ≥1 RAM, usually including multiple NRTI and NNRTI RAMs. We noted a relatively high prevalence of tenofovir RAMs in this group, with 21/65 (32%) patients showing K65R, K70E/G/N, L74I/V, or Y115F. As previously observed by us[140] and others,[190] the highly mutated virus strains did not show evidence of impaired fitness given the high viral loads and low CD4 cell counts. Tenofovir remains a key component of first- and second-line ART in sub-Saharan Africa, including forthcoming regimens with dolutegravir. It will be important to monitor the impact of tenofovir RAMs in treated populations, and assess risk of transmission and impact on the efficacy of pre-exposure prophylaxis.

There are limitations to this study. As the cohort had no routine access to viral load testing, no randomised comparison with standard of care was possible. Direct measures such as pill-counts could have enhanced the evaluation of adherence, although there is evidence that self-reported measures may perform better than pill-counts in these settings.[193] Detection of RAMs by population sequencing might have failed to detect low-frequency variants. However, we previously documented that in a population long established on NNRTI-based ART deep sequencing affords a rather modest increase in yield.[140] Finally, longer follow-up is required to determine the outcomes of viraemic patients with viral load <1000 copies/mL that achieved resuppression while continuing NNRTI-based ART. Meanwhile, we can conclude that POC viral load testing is technically feasible and highly informative, and that in a mature HIV cohort long established NNRTI-based ART, a viral load ≥1000 copies/mL predicts lack of resuppression if therapy is continued unchanged, independently of adherence levels. Controlled studies are required to determine the optimal screening strategy for assigning patients to POC versus deferred viral load testing.

.

Box of recommendations

In HIV-positive cohorts from sub-Saharan Africa on long-term NNRTI-based ART, in the absence of virological monitoring

1. **Prioritise patients with a history of treatment interruption for viral load measurement;**
2. **Switch immediately those on first-line and a HIV-1 viral load ≥1000 copies/mL to a second-line regimen;**
3. **Retest patients with viraemia 40-999 copies/mL after providing an adherence intervention;**
4. **Monitor 3rd 90-target by using POC technologies.**

4 FOURTH CHAPTER - DRUG RESISTANCE OUTCOMES OF LONG-TERM ANTIRETROVIRAL THERAPY (ART) WITH TENOFOVIR DISOPROXIL FUMARATE IN THE ABSENCE OF VIROLOGICAL MONITORING

4.1 INTRODUCTION

Access to ART has been increasing in sub-Saharan Africa (SSA), where an estimated 25.5 million people live with HIV of whom 36% (Western and Central Africa) to 61% (Eastern and Southern Africa) were receiving treatment in 2016.[194] WHO recommends a public health approach to managing HIV in SSA, based upon rapid treatment initiation regardless of CD4 cell counts, and use of standardised regimens for first-line and second-line therapy.[67] Recommended first-line regimens comprise of two NRTIs with either an NNRTI, principally efavirenz, or more recently with the integrase inhibitor dolutegravir.[40,67]

Treatment programmes for SSA initially employed zidovudine or stavudine, each typically combined with lamivudine, as first-line NRTIs. In 2009, WHO recommended phasing out stavudine in favour of less toxic NRTIs, including tenofovir disoproxil fumarate, (henceforth referred to as tenofovir).[195] Current WHO guidelines place tenofovir, in combination with lamivudine or emtricitabine, as the preferred NRTI backbone for the treatment of HIV infection in SSA, including the treatment of highly prevalent co-infection with the hepatitis B virus (HBV).[196] Use of tenofovir as part of ART has been increasing as a result.[197] In 2013, WHO also recommended that plasma viral load monitoring should be adopted in SSA to guide treatment changes, replacing reliance on CD4 cell counts and clinical indicators of treatment failure.[67] However, implementation of viral load monitoring varies across the region, and even in settings with access to testing, delays in identifying treatment failure are commonly reported.[71,198-200] HIV-positive individuals in SSA also face additional challenges: inconsistent drug supplies due to stock-out can lead to

unintended ART interruptions, and travel-related and other costs of accessing care pose an obstacle to retention into regular follow-up.[177,201] In a meta-analysis of 163 studies, the observed rates of virological suppression were 89% after 48 months of predominantly NNRTI-based first-line ART in SSA, declining to 62% in the intention-to-treat analysis that excluded those who had died, were lost to follow-up, or had interrupted ART.[103]

The aim of this study was to determine the viral load and drug resistance outcomes of first-line ART in a typical HIV programmatic setting in SSA, where changes in the preferred NRTI backbone, introduced to reflect updated guidelines, occurred without virological monitoring. Using stored samples from a separate prospective study,[138] viral load and drug resistance-associated mutations (RAMs) were determined retrospectively to reflect four years of follow-up, and the findings related to the self-reported history of treatment interruptions and adherence.

4.2 Patients and methods

4.2.1 Study population

The study investigated HIV-1/HBV positive adults receiving care at the Komfo Anokye Teaching Hospital, a 1200-bed facility in the city of Kumasi and the second-largest hospital in Ghana, serving a population of 4 million people in the Ashanti Region. Recruitment into a prospective observational cohort occurred in 2010-2012,[138] and the last observation took place in November 2015. Given the observational nature of the study, management between study visits was at the discretion of the treating clinician and reflective of routine care; testing for viral load and drug resistance was not routinely available. Subjects eligible for this analysis were those that at study entry (time zero, T0) replaced zidovudine or stavudine with tenofovir while continuing lamivudine and the NNRTI (efavirenz or nevirapine), and remained in care at the last study visit in November 2015 (T1). The disposition of all subjects is shown in Figure 4-1.

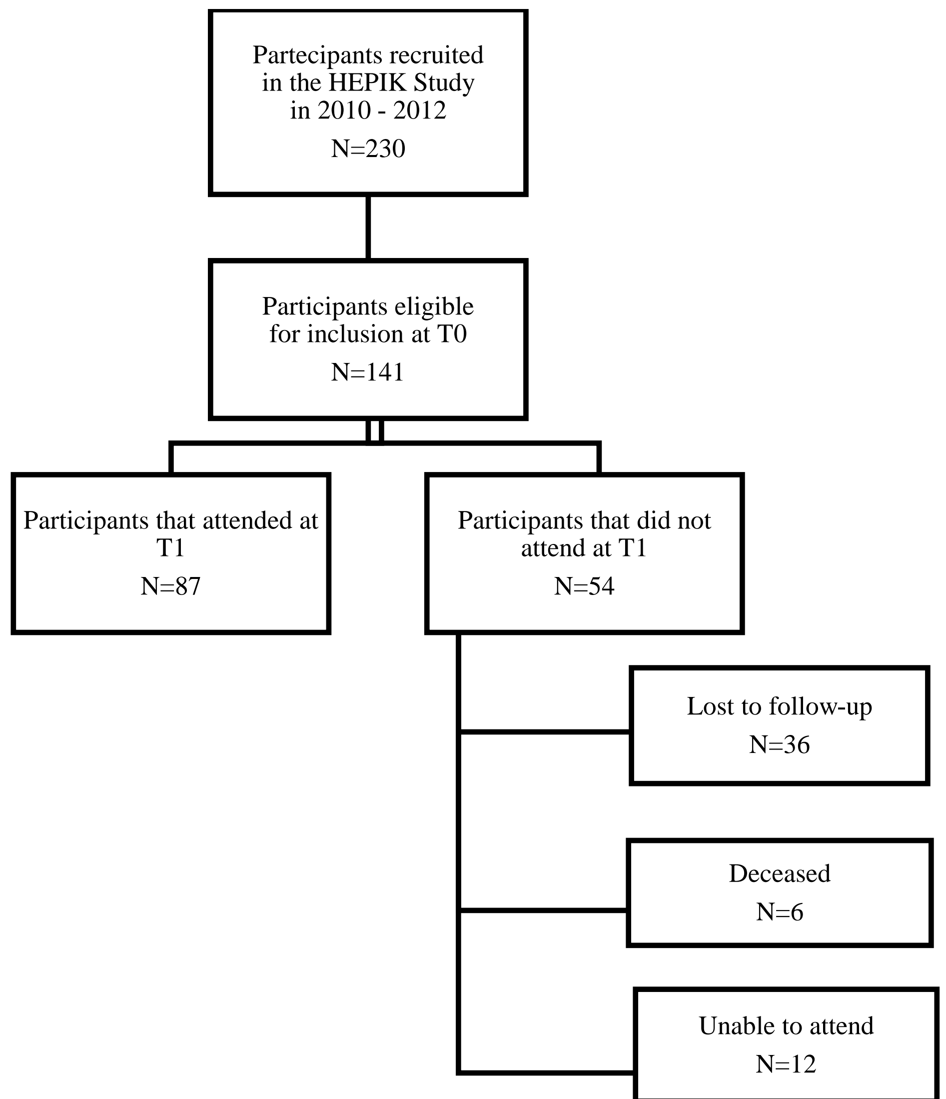


Figure 4-1 **Disposition of the cohort**

Loss to follow-up was documented through at least three calls made to the patient's and next of kin's telephone number over three months.

At study visits, patients underwent clinical examination and blood sampling, and available clinical and laboratory data were collected from the medical records. Plasma samples were stored at -80ºC at T0, T1 and at least one additional study visit between T0 and T1. At T1, participants were invited to respond to a questionnaire about the number of times they had interrupted ART for ≥3 consecutive days since first starting treatment and in the previous three months. Adherence to ART in the previous three months was also determined at T1 using a visual analogue scale, which scored adherence from zero (complete non-adherence) to 100% (complete adherence) in 10% increments;[202,203] optimal adherence was defined as a score ≥90%. Ethical approval was granted by the Kwame Nkrumah University of Science and Technology, Ghana (Ref: CHRPE/AP/347/15) and all participants gave written informed consent. The study was conducted in accordance with the Declaration of Helsinki and national and institutional standards.

4.2.2 Retrospective viral load and resistance testing

Plasma was separated from whole venous blood in EDTA within one hour of collection by centrifugation at 4,500g for 10 minutes and stored at -80ºC. Samples were shipped frozen to the United Kingdom (UK) for retrospective testing. Plasma HIV-1 RNA was quantified by the RealTime HIV-1 assay (Abbott Diagnostics, Maidenhead, UK) with a lower limit of quantification of 40 copies/ml. Samples with detectable HIV-1 RNA underwent testing for the presence of RAMs in reverse transcriptase (RT, amino acid 14-345) and protease (PR, amino acids 1-99) by Sanger sequencing, as described.[184] Genotypic susceptibility scores (GSS) were determined using the Stanford HIV Drug Resistance algorithm (v8.4): each drug in the regimen was assigned a score of 0 for high-level resistance, 0.25 for intermediate resistance, 0.5 for low-level resistance, and 1 for potential low-level resistance or full predicted susceptibility. Patients that did not yield an amplicon for sequencing (all with viral load <200 copies/ml) were assigned a GSS of 3. Samples also underwent deep sequencing similarly to how has been described previously.[204,205] Briefly, a 1000bp RT amplicon was generated, purified with the Agencourt Ampure XP system (Beckman Coulter, High Wycombe, UK), and quantified with the Qubit dsDNA High

Sensitivity Assay Kit using the Qubit 3.0 fluorometer (Invitrogen, Loughborough, UK). A DNA library was prepared with the Nextera XT DNA Sample Prep Kit (Illumina, San Diego, CA, USA), followed by sequencing with the MiSeq Reagent Kit v2. Consensus sequences and frequencies of reads were produced as previously described; reads were analysed applying a 1% interpretative cut-off.[204,206] RAMs considered major in the resistance analysis are reported in Table 4-1.

Table 4-1 **Resistance-associated mutations included in the genotypic susceptibility score**

NRTIs	Discriminatory K65R/N/E K70E/G/Q/T/N/S L74V/I Y115F M184V/I	TAMs M41L D67N/G/E K70R L210W T215Y/F T215 revertants K219Q/E/N/R	MDR T69ins Q151M/L [with or without A62V V75I F77L F116Y]	Miscellaneous T69D/N/G V75T/M/A/S
NNRTI	Non-polymorphic and minimally polymorphic A98G L100I/V K101E/H/P/Q/N/A/T K103N/S/H V106A/M V108I I132L/M E138K/Q/G/R V179L/F Y181C/I/V/F/S/G Y188L/C/H/F G190A/S/E/Q/C/T/V H221Y P225H F227L/C M230L/I Y232H L234I P236L K238T/N Y318F			Combinations K103R + V179D/E V106I + V179D
Protease inhibitors	Major D30N V32I L33F M46I/L/V I47A/V G48V/M/A/S/T/Q/L I50V/L I54V/A/S/T/L/M L76V V82A/T/S/F/L/M/C I84V/A/C N88D/S/T/G L90M			

TAMs=thymidine analogue mutations; MDR=multidrug resistance

4.2.3 Statistical analysis

Characteristics of participants at T0 versus T1 were compared by Wilcoxon matched-pairs/paired t-test or Fisher's exact test. The prevalence of reported treatment interruptions and suboptimal adherence according to viral load status at T1 were compared by chi-squared test. Factors associated with a detectable viral load at T1 were explored by univariable logistic regression analysis. Variables included in the univariable analysis comprised gender, age, viral load, CD4 cell count and presence of RAMs at T0; and reported treatment interruptions and adherence at T1. A separate model analysed factors associated with the combined outcome of showing a detectable viral load at T0 or having introduced lopinavir/ritonavir between T0 and T1. A

sensitivity analysis explored factors associated with a detectable viral load at T1 by an intention-to-treat approach, including all subjects that started tenofovir at T0 regardless of whether they remained in follow-up at T1 (missing=failure). The relationship between viral load and CD4 cell count at T1 was determined by univariable linear regression analysis. Analyses were performed with STATA version 14 (StataCorp, College Station, Texas, USA).

4.3 RESULTS

4.3.1 Treatment status at T1

The study population comprised 87 subjects that after receiving zidovudine or stavudine plus lamivudine and an NNRTI for a median of 4.2 years (IQR 2.5-5.4) replaced zidovudine or stavudine with tenofovir while continuing lamivudine and the NNRTI, in the absence of viral load testing (Table 4-2).

Table 4-2 **Characteristics of the study population at the time of switching from zidovudine or stavudine to tenofovir disoproxil fumarate (time zero, T0) and after a median of four years (T1) (n=87)**

Characteristic		T0	T1
Gender, female, n (%)		57 (65.5)	57 (65.5)
Age, median years (IQR)		40 (34-44)	44 (39-48)
BMI, median kg/m^2 (IQR)		24.0 (21.0-26.3)	23.2 (20.3-27.1)
Time from HIV diagnosis, median years (IQR)		4.5 (3.2-6.3)	8.6 (7.2-10.3)
CD4 count at HIV diagnosis, median cells/mm^3 (IQR)		185 (87-333)	185 (87-333)
CD4 cell count, median cells/mm^3 (IQR)		580 (360-742)	558 (346-711)
Antiretroviral agent, n (%)	Efavirenz	49 (56.3)	77 (88.5)
	Nevirapine	38 (43.7)	2 (2.3)
	Lopinavir/ritonavir	0 (0)	5 (5.7)
	Stavudine + lamivudine	13 (14.9)	0 (0)
	Zidovudine + lamivudine	74 (85.1)	2 (2.3)
	Tenofovir + lamivudine	0 (0)	82 (94.3)
	None	0 (0)	3 (3.4)
Total ART duration, median years (IQR)		4.2 (2.5-5.4)	8.1 (6.5-9.2)
Total tenofovir duration, median years (IQR)		0 (0)	4.0 (3.8-4.1)
HIV-1 RNA copies/ml, n (%)	<40	68 (78.2)	68 (78.2)
	40-399	9 (10.3)	5 (5.7)
	1,000-9,999	4 (4.6)	1 (1.1)
	>10,000	6 (6.9)	13 (14.9)
RAMs, n (%)	Any	8 (9.2)	11 (12.6)
	NNRTI only	1 (1.1)	2 (2.3)
	NRTI + NNRTI	7 (8.0)	9 (10.3)
	Protease inhibitor	0 (0)	0 (0)
	None	7 (8.0)	5 (5.7)
	No amplicon	4 (4.6)[†]	2 (2.3)[‡]
Treatment interruption[§], n (%)	None	-	59 (67.8)
	1-2	-	19 (21.8)
	≥3	-	9 (10.3)
Adherence[¶], n (%)	100%	-	54 (62.1)
	90%	-	21 (24.1)
	70-80%	-	9 (10.3)
	Off ART	-	3 (3.4)

[†]Four samples with viral load 40-60 copies/ml and [‡]two samples with viral 40-200 copies/ml did not yield an amplicon for sequencing in repeated attempts; [§]Defined as interrupting ART for ≥3 consecutive days since first starting treatment; [¶]Measured by visual analogue scale. BMI=body mass index; RAMs=resistance-associated mutations.

After a median of 4.0 years (IQR 3.8-4.1), 82/87 (94.3%) subjects continued on tenofovir plus lamivudine and 79/87 (90.8%) remained on a NNRTI, with greater efavirenz use in preference of nevirapine. A small number (5/87, 5.7%) had introduced ritonavir-boosted lopinavir. The remaining 3/87 (3.4%) subjects were no longer on ART, having interrupted treatment three months, two years, and three years prior to T1, respectively. In the questionnaires, 28/87 (32.6%) respondents reported that they had interrupted treatment for ≥3 consecutive days since first starting ART, although most (25/28) had subsequently resumed treatment. Overall 9/87 (10.3%) subjects reported ≥3 interruptions and 16/87 (18.4%) reported an interruption within the previous three months. Reasons given for interrupting ART were primarily temporary closure of the HIV dispensary and less commonly use of herbal remedies or misunderstanding instructions. By visual scale, 12/87 (13.8%) respondents reported adherence <90% in the previous three months.

4.3.2 Viral load and drug-resistance associated mutations at T0 and T1

Retrospectively, across the whole population, 19/87 (21.8%) subjects at T0 and 19/87 (21.8%) subjects at T1 had a viral load >40 copies/ml (Table 4-2). Proportions with viral load >10,000 copies/ml increased at T1 compared with T0 (14.9% versus 6.9%, p=0.14), whereas median CD4 cell counts did not change significantly between time points (558 versus 580 cells/mm^3; p=0.47). Reporting treatment interruptions and adherence levels <90% was significantly more prevalent among subjects with a detectable viral load at T1 than in subjects with suppressed viral load (Figure 4-2) (Table 4-3).

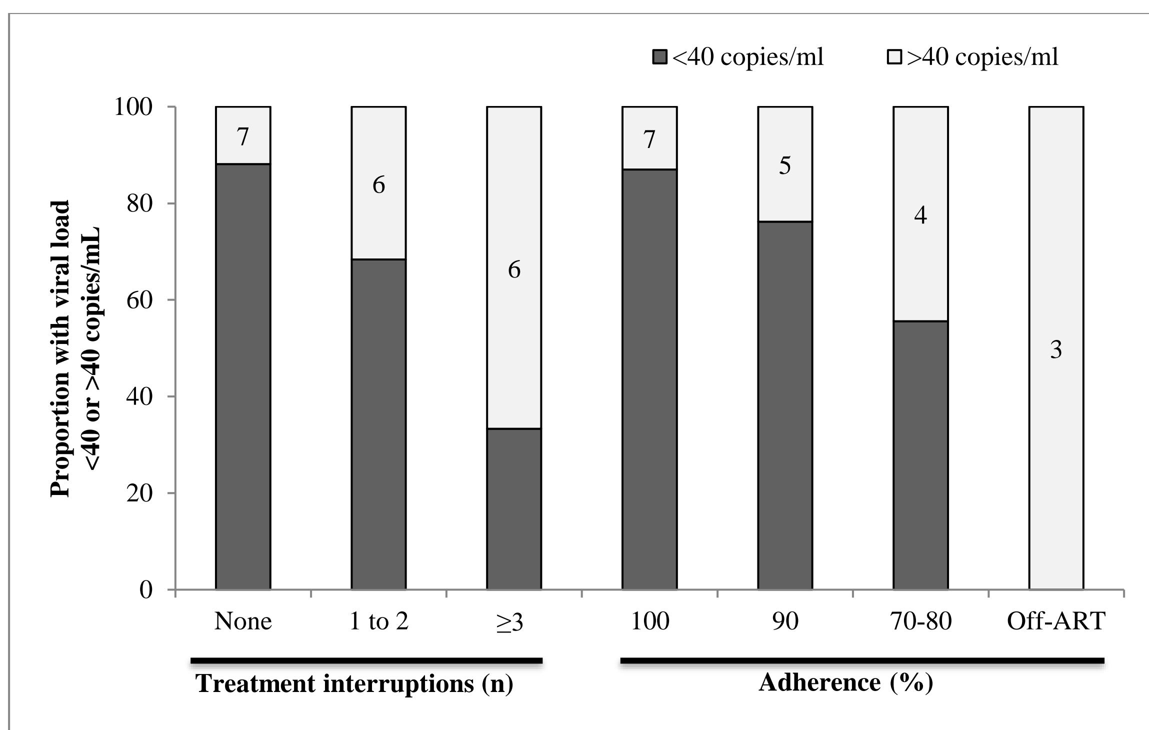


Figure 4-2 **Proportion of subjects with suppressed (<40 copies/ml) or detectable plasma HIV-1**

RNA after a median of 8.1 years of antiretroviral therapy (ART) according to reported treatment interruptions and adherence. The number of subjects with detectable viral load is indicated in each column. A treatment interruption was defined as interrupting ART for ≥3 consecutive days since first starting treatment. Adherence was measured through a visual analogue scale.

Table 4-3 **Viral load after a median of 8.1 years of antiretroviral therapy (ART) according to reported treatment interruptions and adherence**†

		Total population	HIV-1 RNA (copies/ml)		P
			<40	>40	
		n=87	n=68	n=19	
Interruption since first starting ART, n (%)	None	59 (67.8)	52 (76.15	7 (36.8)	<0.001
	1-2	19 (21.8)	13 (19.1)	6 (31.6)	
	≥3	9 (10.3)	3 (4.4)	6 (31.6)	
Interruption in previous three months, n (%)	No	71 (81.6)	61 (89.7)	10 (52.6)	0.001
	Yes	16 (18.4)	7 (10.3)	9 (47.4)	
Adherence, n (%)	100%	54 (62.1)	47 (69.2)	7 (36.8)	<0.001
	90%	21 (24.1)	16 (23.5)	5 (26.3)	
	70-80%	9 (10.3)	5 (7.4)	4 (21.1)	
	Off-ART	3 (3.5)	0 (0)	3 (15.8)	

†Treatment interruption was defined as interrupting ART for ≥3 consecutive days since first starting treatment; adherence was measured by visual analogue scale.

At T0, 8/87 (9.2%) subjects had ≥1 major NNRTI RAM and 7/87 (8.0%) had ≥1 major NRTI RAM (Table 4-2); most subjects (82/87; 94.3%) showed full predicted susceptibility to tenofovir. The GSS of the tenofovir-containing regimen started at T0 was median 3 and ranged from 0.5 to 3. By logistic regression analysis, showing a

detectable viral load and RAMs at T0 and reporting treatment interruptions and suboptimal adherence at T1 were each predictive of a detectable viral load at T1 (Table 4-4).

Table 4-4 **Univariate logistic regression analysis of factors associated with a detectable plasma HIV-1 RNA (>40 copies/mL) after a median of 8.1 years of antiretroviral therapy (T1, n=19)†**

Variable	OR	95% CI	P-value
Gender (female versus male)	0.38	0.13-1.06	0.07
Age (per 5-year increment)	1.01	0.74-1.38	0.96
T0 CD4 count (per 50 cells lower)	1.10	1.00-1.22	0.06
T1 CD4 count (per 50 cells lower)	1.51	1.24-1.84	<0.01
T0 HIV-1 RNA (per 1 log_{10} copies/mL higher)	1.97	1.15-3.35	0.01
T0 NNRTI RAMs (yes versus no)	15.2	2.76-84.0	<0.01
Treatment interruption (per each interruption)‡	2.32	1.41-3.82	<0.01
Adherence (per 10% lower)§	2.10	1.19-3.70	0.01

†T0 (time zero) variables were measured at the introduction of tenofovir and after a median of 4.2 years of antiretroviral therapy (ART); T1 variables were measured a median of 4.0 years later; ‡Defined as interrupting ART for ≥3 consecutive days since first starting treatment; §Measured by visual analogue scale. OR=odds ratio; RAMs=resistance-associated mutations.

Results were confirmed in two separate models considering a) the combined outcome of a detectable viral load at T1 or having introduced lopinavir/ritonavir between T0 and T1, and b) adopting an intention-to-treat approach that included patients who had died or were lost to follow-up after T0 (Table 4-5 and Table 4-6).

Table 4-5 **Univariate and multivariable logistic regression analysis of factors associated with the combined outcome of either showing a detectable viral load at T1 or having introduced lopinavir/ritonavir between T0 and T1 (n=21)**

Variable	Univariable		
	OR	95% CI	p
Gender (female vs. male)	0.48	0.18-1.31	0.15
Age (per 5-year increment)	1.04	0.77-1.40	0.81
T0 CD4 count (per 50 cells lower)	1.15	1.03-1.27	0.01
T1 CD4 count (per 50 cells lower)	1.62	1.29-2.02	<0.01
T0 HIV-1 RNA (per 1 log_{10} copies/ml higher)	2.12	1.22-3.69	0.01
T0 NNRTI-RAMs (yes versus no)	32.5	3.70-286	<0.01
Adherence (per 10% lower)	2.00	1.15-3.48	0.01
Treatment interruption (per each interruption)	2.22	1.36-3.63	<0.01

OR=odd ratio; CI=confidence interval; ART=antiretroviral treatment; GSS=genotypic susceptibility score; PI=protease inhibitor; NNRTI=non-nucleoside reverse transcriptase inhibitor; HIV=human immunodeficiency virus; RNA=ribonucleic acid.

Table 4-6 **Intention-to-treat univariate and multivariable logistic regression analysis: all patients who had died or were lost to follow-up after T0 were included in the outcome measure**

Variable	Univariable		
	OR	95% CI	p
Gender (female vs. male)	0.60	0.30-1.20	0.15
Age (per 5-year increment)	1.01	0.74-1.38	0.96
T0 CD4 count (per 50 cells lower)	1.06	1.00-1.13	0.06
T1 CD4 count (per 50 cells lower)	1.61	1.28-2.03	<0.01
T0 HIV-1 RNA (per 1 log_{10} copies/ml higher)	1.66	1.08-2.55	0.02
T0 NNRTI-RAMs (yes versus no)	8.53	1.87-38.9	0.01
Adherence (per 10% lower)	2.09	1.18-3.71	0.01
Treatment interruption (per each interruption)	2.32	1.41-3.82	<0.01

OR=odd ratio; CI=confidence interval; ART=antiretroviral treatment; GSS=genotypic susceptibility score; PI=protease inhibitor; NNRTI=non-nucleoside reverse transcriptase inhibitor; HIV=human immunodeficiency virus; RNA=ribonucleic acid.

At T1, by linear regression analysis, CD4 cell counts were 135 cells/mm^3 lower for each log_{10} increase in viral load (95% CI 93-176; p<0.01).

4.3.3 Evolution of viral load and drug resistance

Four patterns were identified among subjects receiving an NNRTI throughout follow-up: a) 58/82 (70.7%) subjects had a suppressed viral load at both T0 and T1; b) 8/82 (9.8%) subjects with detectable viral load at T0 achieved viral load suppression at T1; c) 8/82 (9.8%) subjects with supressed viral load at T0 experienced viral load rebound at T1; d) 8/82 (9.8%) subjects had a detectable viral load at both time points. Patient-level data are presented in Tables 4-7 to 4-10, which also include the three subjects that had discontinued ART at T1. Within viral load group b (T0 detectable/T1 suppressed; Table 4-7), most patients had viral load <200 copies/ml at T0 and all had a suppressed viral load at the next study visit after T0 and prior to T1. In this group, one patient on tenofovir, lamivudine and efavirenz showed the major NNRTI RAM K103N at T0; the T0 viral load was 101 copies/ml and the patient reported no treatment interruptions and 100% adherence. Within viral load group c (T0 suppressed/T1 detectable, Table 4-8), 5/8 patients showed emergence of major RAMs at viral load rebound: all had the lamivudine mutation M184V and ≥2 NNRTI RAMs and three subjects had thymidine analogue mutations (TAMs). In addition, three subjects on tenofovir, lamivudine and efavirenz showed ≥1 discriminatory NRTI RAM (RT codons 65, 70, 74, 115). Within viral load group d (T0 detectable/T1

detectable; Table 4-9), 4/8 subjects showed emergence of ≥1 discriminatory NRTI RAM (RT codons 65, 70, 74, 115), always alongside M184V and with or without TAMs. In this group, between T0 and T1 the number of NNRTI RAMs increased from a median of 0 (range 0-3) to a median of 3 (range 0-4), the median viral load increased from 2.6 (IQR 1.7-3.7) to 4.3 (IQR 4.1-5.1) log_{10} copies/ml (p=0.02), whereas the median CD4 count declined from 544 (IQR 368-590) to 215 (IQR 167-278) (p=0.08). The profile of the five subjects on lopinavir/ritonavir is shown in Table 4-10. The patients had introduced lopinavir/ritonavir a median of 3.7 (IQR 1.1-3.9) years prior to T1 as a result of a decline in CD4 cell counts. At T1, three subjects showed a detectable viral load, all at levels <200 copies/ml; one subject on tenofovir plus lamivudine showed discriminatory NRTI RAMs (RT codons 65, 70).

Overall, considering the entire population at risk, 8/87 (9.2%) subjects on tenofovir developed ≥1 discriminatory NRTI RAM over a median of 4.0 years of exposure. Discriminatory NRTI RAMs usually occurred at high frequency in each patient's sample and were therefore detected by both Sanger and deep sequencing. Low-frequency (1-5%) variants detected only by deep sequencing comprised K70E (n=1), L74I (n=2), L74V (n=1), and Y115F (n=1). Between T0 and T1, the number of NRTI and NNRTI RAMs increased by 5 and 6 per year, respectively. At T1, prevalence of predicted intermediate or high-level resistance to lamivudine or emtricitabine, abacavir, tenofovir, and zidovudine was 12/87 (13.8%), 10/87 (11.5%), 4/87 (4.6%), and 4/87 (4.6%) respectively. Tenofovir and zidovudine resistance did not usually overlap.

Table 4-7 **Patients on efavirenz or nevirapine showing a detectable viral load at T0 and a suppressed viral load at T1**[†]

ID Subtype	T	Regimen	Yrs of ART	Yrs of TDF	VL	CD4 count	NRTI RAMs	NNRTI RAMs	GSS
029	T0	D4T/3TC/NVP	0.5	0	5.5	278	None	None	3
CRF02		TDF/3TC/EFV	1.9	1.4	UD	507	-	-	3
	T1	TDF/3TC/EFV	4.5	4.0	UD	711	-	-	3
050	T0	ZDV/3TC/NVP	1.1	0	5.1	243	None	None	3
CRF02		TDF/3TC/EFV	2.5	1.3	UD	214	-	-	3
	T1	TDF/3TC/EFV	5.0	3.9	UD	293	-	-	3
086	T0	ZDV/3TC/EFV	3.3	0	1.8	689	None	None	3
CRF02		TDF/3TC/EFV	3.8	0.5	UD	849	-	-	3
	T1	TDF/3TC/EFV	7.4	4.1	UD	655	-	-	3
130	T0	D4T/3TC/EFV	4.0	0	2.3	225	None	None	3
CRF02		TDF/3TC/EFV	4.9	0.9	UD	504	-	-	3
	T1	TDF/3TC/EFV	8.2	4.2	UD	443	-	-	3
147	T0	ZDV/3TC/EFV	4.3	0	3.2	173	None	None	3
CRF02		TDF/3TC/EFV	4.7	0.5	UD	ND	-	-	3
	T1	TDF/3TC/EFV	7.7	3.4	UD	265	-	-	3
216	T0	ZDV/3TC/EFV	2.5	0	2.0	703	None	K103N (96)	2
A1		TDF/3TC/EFV	3.8	1.3	UD	494	-	-	2
	T1	TDF/3TC/EFV	6.4	3.9	UD	514	-	-	2
003	T0	ZDV/3TC/EFV	5.0	0	1.7	580	No amplicon	No amplicon	3
		TDF/3TC/EFV	5.8	0.8	UD	588	-	-	3
	T1	TDF/3TC/EFV	9.1	4.1	UD	528	-	-	3
115	T0	ZDV/3TC/EFV	1.8	0	1.8	159	No amplicon	No amplicon	3
		TDF/3TC/EFV	2.9	1.1	UD	259	-	-	3
	T1	TDF/3TC/EFV	5.2	3.4	UD	345	-	-	3

[†]RAMs were detected by both Sanger sequencing and deep sequencing. The frequency of each RAM in the deep sequencing reads is reported in brackets. T=time point; Yrs=years; VL=viral load; RAMs=resistance-associated mutations; GSS=genotypic susceptibility score; UD=undetectable (<40 copies/ml); D4T=stavudine; 3TC=lamivudine; NVP=nevirapine; TDF=tenofovir disoproxil fumarate; EFV=efavirenz; ZDV=zidovudine.

Table 4-8 **Patients on efavirenz or nevirapine showing a suppressed viral load at T0 and a detectable viral load at T1[†]**

ID Subtype	T	Regimen	Yrs of ART	Yrs of TDF	VL	CD4 count	NRTI RAMs	NNRTI RAMs	GSS
146	T0	ZDV/3TC/EFV	4.3	0	UD	790	-	-	3
CRF02		TDF/3TC/EFV	4.9	0.6	UD	743	-	-	3
	T1	TDF/3TC/EFV	7.8	3.5	5.1	337	None	None	3
188	T0	ZDV/3TC/EFV	5.0	0	UD	452	-	-	3
CRF02		TDF/3TC/EFV	5.7	0.7	UD	732	-	-	3
	T1	TDF/3TC/EFV	9.0	4.0	5.3	172	None	None	3
218	T0	ZDV/3TC/NVP	1.0	0	UD	214	-	-	3
		TDF/3TC/EFV	2.7	1.7	UD	ND	-	-	3
	T1	TDF/3TC/EFV	4.8	3.8	2.1	939	No amplicon	No amplicon	3
010	T0	ZDV/3TC/NVP	2.8	0	UD	758	-	-	3
CRF02		TDF/3TC/EFV	3.3	0.5	UD	823	-	-	3
	T1	ZDV/3TC/NVP	6.6	1.4	4.7	346	M184V (15)	K103N (100) P225Y (8) F227L (92)	1
030	T0	ZDV/3TC/NVP	1.3	0	UD	256	-	-	3
CRF02		TDF/3TC/EFV	1.8	0.4	2.0	279	None	None	3
		TDF/3TC/EFV	2.5	1.1	2.1	294	None	None	3
	T1	TDF/3TC/EFV	5.3	4.0	5.7	8	L74I (5) M184I (14)	K101E (30) K103N(100) Y181C (19) G190A (20)	1
018	T0	D4T/3TC/EFV	3.7	0	UD	672	-	-	3
CRF02		TDF/3TC/EFV	4.1	0.5	UD	273	-	-	3
	T1	TDF/3TC/EFV	7.8	4.1	4.7	14	K65R (97) D67N (81) K70T (20) Y115F (99) M184V (100) K219E (86)	K103N (99) V108I (99) Y181C (100)	0
099	T0	ZDV/3TC/EFV	6.7	0	UD	593	-	-	3
CRF06		TDF/3TC/EFV	8.2	1.4	UD	457	-	-	3
	T1	ZDV/3TC/EFV	10.9	1.4	4.7	347	D67N (100) T69D (99) K70R (100) M184V (100) T215V (100) K219Q (100)	A98G (100) K103N (100) V108I (2) E138G (66)	0
258[‡]	T0	ZDV/3TC/EFV	5.2	0	UD	565	-	-	3
CRF02		TDF/3TC/EFV	5.8	0.6	UD	269	-	-	3
		TDF/3TC/EFV	6.5	1.3	5.3	238	K70E (1) M184V (3) T215F (2)	L100I (5) K103N (96)	0.5

[†]RAMs were detected by Sanger sequencing and deep sequencing. The frequency of each RAM in the deep sequencing reads is reported in brackets; RAMs detected only by deep sequencing are underlined; [‡]Subject 258 interrupted ART two years prior to T1; the T1 viral load and CD4 counts were 5.1 log_{10} copies/ml and 54 cells/mm^3, respectively. T=time point; Yrs=years; VL=viral load; RAMs=resistance-associated mutations; GSS=genotypic susceptibility score; UD=undetectable (<40 copies/ml); ZDV=zidovudine; 3TC=lamivudine; EFV=efavirenz; TDF=tenofovir disoproxil fumarate; NVP=nevirapine; D4T=stavudine.

Table 4-9 **Patients on efavirenz or nevirapine showing a detectable viral load at both T0 and T1**[†]

ID Subtype	T	Regimen	Yrs of ART	Yrs of TDF	VL	CD4 count	NRTI RAMs	NNRTI RAMs	GSS
061	T0	ZDV/3TC/NVP	1.0	0	1.8	126	None	None	3
CRF02		TDF/3TC/EFV	2.0	1.0	UD	536	-	-	3
	T1	TDF/3TC/EFV	5.0	4.0	1.8	306	None	None	3
134	T0	ZDV/3TC/EFV	2.5	0	1.7	284	No amplicon	No amplicon	3
CRF02		TDF/3TC/EFV	4.3	1.9	UD	465	-	-	3
	T1	TDF/3TC/EFV	6.6	4.1	4.2	435	None	K101E (26) K101N (4) K103N (39)	2
048	T0	ZDV/3TC/EFV	3.0	0	1.6	590	None	None	3
CRF02		TDF/3TC/EFV	3.8	0.8	UD	515	-	-	3
	T1	TDF/3TC/EFV	6.8	3.8	5.5	176	None	K103N (90)	2
004 CRF06	T0	ZDV/3TC/NVP	4.3	0	3.5	547	D67N (99) K70R (99) M184V (100) T215I (5) T215V (66) K219Q (99)	A98G (92) K101E (99) G190A (99)	0.5
		TDF/3TC/NVP	5.1	0.8	3.7	327	D67N (99) K70R (100) M184V (100) K219Q (100)	A98G (100) K101E (99) K103N (1) V108I (52) G190A (97) P225H (63)	0.5
	T1	TDF/3TC/EFV	7.9	3.6	4.2	192	D67N (99) T69N (68) K70R (99) L74I (89) M184V (100) T215V (99) K219Q (100)	A98G (100) K101E (73) K103N (26) V108I (98) G190A (100) P225H (99)	0.5
040	T0	D4T/3TC/NVP	1.8	0	4.1	541	M184V (100)	V106A (100)	1
CRF02		TDF/3TC/EFV	2.4	0.6	4.0	511	K65R (80) K70E (18) L74V (1) M184V (100)	K103N (85) V106A (100) G190A (11)	0
		TDF/3TC/EFV	2.9	0.9	5.1	386	K65R (99) Y115F (46) M184V (100)	K103N (99) V106A (100)	0
	T1	TDF/3TC/EFV	6.0	4.1	5.6	139	K65R (100) K70T (100) L74I (2) Y115F (100) M184V (100)	K103N (100) V106A (100) F227L (100)	0
101	T0	ZDV/3TC/NVP	0.9	0	3.6	396	M184V (100)	K103N (100)	1
CRF02		TDF/3TC/NVP	1.5	0.8	2.0	344	No amplicon	No amplicon	1
	T1	TDF/3TC/EFV	4.8	3.9	4.5	269	L74I (14) M184V (100)	A98G (2) K103N (100) P225H (100) Y318F (1)	1
150[‡]	T0	ZDV/3TC/EFV	7.4	0	1.7	1009	No amplicon	No amplicon	3
		TDF/3TC/EFV	7.9	0.4	UD	870	-	-	3
113[§]	T0	ZDV/3TC/EFV	3.0	0	4.1	591	M184V (99)	L100I (88) K103N (99) Y188L (8)	1
CRF02		TDF/3TC/EFV	3.8	0.9	4.4	150	K70R (62) Y115F (1) M184V (100)	L100I (97) K103N (99) V108I (3) Y188L (1)	1
		TDF/3TC/EFV	4.3	1.4	5.2	147	K70E (31) K70R (8) M184V (100) T215F (74) K219E (3) K219Q (2)	L100I (96) K103N (99) V108I (1) Y188L (2)	0.25

†RAMs were detected by Sanger sequencing and deep sequencing. The frequency of each RAM in the deep sequencing reads is reported in brackets; RAMs detected only by deep sequencing are underlined; ‡Subject 150 interrupted all ART three years prior to T1; the T1 viral load and CD4 counts were 3.9 $\log_{10}$ copies/ml and 238 cells/mm^3 respectively; §Subject 113 interrupted all ART three months prior to T1; the T1 viral load and CD4 counts were 5.0 $\log_{10}$ copies/ml and 40 cells/mm^3 respectively. T=time point; Yrs=years; VL= viral load; RAMs=resistance-associated mutations; GSS=genotypic susceptibility score; UD=undetectable (<40 copies/ml); ZDV=zidovudine; 3TC=lamivudine; NVP=nevirapine; TDF=tenofovir disoproxil fumarate; EFV=efavirenz; D4T=stavudine.

Table 4-10 **Patients that introduced lopinavir/ritonavir between T0 and T1**†

ID Subtype	T	Regimen	Yrs of ART	Yrs of TDF	VL	CD4 count	NRTI RAMs	NNRTI RAMs	GSS
020	T0	ZDV/3TC/NVP	3.9	0	3.5	161	K70R (2) M184V (100)	K101E (99) G190A (100)	1
CRF02		TDF/3TC LPV/r	4.8	0.8	3.1	61	No amplicon	No amplicon	2
	T1	TDF/3TC LPV/r	8.1	4.2	UD	270	-	-	2
127	T0	ZDV/3TC/NVP	3.8	0	UD	175	-	-	3
		TDF/3TC LPV/r	5.2	1.3	UD	215	-	-	3
	T1	TDF/3TC LPV/r	8.5	4.7	2.0	391	No amplicon	No amplicon	3
082	T0	D4T/3TC/NVP	2.4	0	4.6	306	D67N (2) M184V (100) T215Y (99)	Y181C (99)	1
CRF06		TDF/3TC EFV	4.4	2.0	2.9	36	M184V (72) T215Y (74)	K101E (16) K101Q (9) K103N (7) V108I (58) Y181C (74) G190A (58)	1
	T1	TDF/3TC ZDV LPV/r	6.5	4.1	1.8	287	M184V T215Y	V108I Y181C G190A	2.25
186 CRF02	T0	ZDV/3TC/EFV	4.4	0	4.8	109	M184V (100)	K103N (22) V106A (80) V108I (81) M230L (78)	1
		TDF/3TC/EFV	5.0	0.6	4.2	231	M184V (100)	V108I (100) H221Y (56) M230L (100)	1
		TDF/3TC/EFV	5.5	1.2	4.6	64	K65R (88) <u>T215F (5)</u> M184V (100)	V108I (99) <u>H221Y (12)</u> M230L (99)	0
	T1	TDF/3TC ZDV LPV/r	8.3	3.9	2.1	337	K65R K70T M184V	V108I M230L	1.5
016	T0	D4T/3TC/EFV	4.2	0	UD	177	-	-	3
		TDF/3TC LPV/r	5.3	1.1	UD	288	-	-	3
	T1	TDF/3TC LPV/r	7.9	3.7	UD	463	-	-	3

†At T0 and intermediate time points RAMs were detected by Sanger sequencing and deep sequencing. The frequency of each RAM in the deep sequencing reads is reported in brackets; RAMs detected only by deep sequencing are underlined. At T1 RAMs were detected by Sanger sequencing alone; protease sequences were also obtained at T1 and showed no major RAMs. T=time point; Yrs=years; VL= viral load; RAMs=resistance-associated mutations; GSS=genotypic susceptibility score; UD=undetectable (<40 copies/ml); ZDV=zidovudine; 3TC=lamivudine; NVP=nevirapine; TDF=tenofovir disoproxil fumarate; LPV/r=lopinavir/ritonavir; D4T=stavudine; EFV=efavirenz.

4.3.4 Discussion

This study investigated the long-term viral load and drug resistance outcomes of subjects accessing first-line NNRTI-based ART in a programmatic setting in SSA where implementation of virological monitoring has yet to take place. Focusing on subjects that remained in care, the study found that a median of four years after first introducing tenofovir in place of zidovudine or stavudine, most patients were still receiving tenofovir, lamivudine and efavirenz and only a minority (5.7%) had started second-line ART with a boosted protease inhibitor as a result of immunological failure. While most patients maintained or achieved viral load suppression during follow-up, having a detectable viral load with evidence of NNRTI resistance at the time of introducing tenofovir was predictive of a lack of viral load suppression after four years. Notably, prior to introducing tenofovir, patients had received a thymidine analogue (zidovudine or stavudine) with lamivudine for a median of 4.2 years, but the prevalence of TAMs was limited and most patients retained full predicted susceptibility to tenofovir. Patients who subsequently experienced viraemia while on tenofovir, lamivudine, and efavirenz acquired discriminatory NRTI RAMs, including well recognised tenofovir RAMs (K65R, K70E/T) as well as RAMs not typically associated with tenofovir (L74I/V, Y115F), alongside M184V and with or without TAMs. The complex mutation patterns have uncertain effects on continued tenofovir susceptibility. Importantly, there was no suggestion of impaired viral fitness based on viral load and CD4 cell counts.

The observed high prevalence and progressive accumulation of NNRTI RAMs among patients experiencing viraemia on NNRTI-based ART is in line with other studies from SSA.[95,190,207-214] We observed interesting patterns of NRTI resistance associated with tenofovir, lamivudine and efavirenz exposure in this cohort comprising predominantly CRF02 and CRF06 strains. Rhee *et al.* recently compared RT sequences from subjects with virological failure on a first-line tenofovir-containing regimens to sequences from ART-naïve patients and patients on thymidine analogues.[215] Overall 12 mutations – A62V, K65R/N, S68G/N/D, K70E/Q/T, L74I, V75L, and Y115F – were statistically associated with tenofovir exposure. It should be noted however that only some of these (e.g., K65R and K70E) are recognised as predicting reduced tenofovir susceptibility in commonly used resistance interpretation

algorithms. Our prospectively collected, quantitative resistance data provide strength to the statistical association reported by Rhee *et al.* L74I was common in our cohort. Whereas most RAMs occurred at high frequency, L74I also occurred at a low frequency, below the detection limit of Sanger sequencing. We observed co-occurrence of multiple discriminatory mutations at codons 65, 70, 74 and 115, including co-occurrence of K65R with L74I or K70T. L74V is known to rarely coexist with K65R due to a marked fitness effect.[80] In contrast, the combination of K65R with L74I increases reverse transcriptase processivity and viral replication is preserved.[81] It has also been proposed that L74I restores the fitness of variants with the NNRTI RAM K103N.[82] Taken together, the data indicate that selective pressure by tenofovir, lamivudine and efavirenz drove viral genetic evolution towards high drug resistance and preserved viral fitness. Further studies are needed to determine the impact of K70T, L74I/V, and Y115F, and the combination of multiple discriminatory RAMs on phenotypic susceptibility and clinical responses to tenofovir. We had insufficient samples to perform phenotypic resistance testing in this cohort. Growing rates of NNRTI resistance in SSA are of concern, and it expected that patients will likely benefit from the planned introduction of the fixed dose combination of tenofovir, lamivudine, and dolutegravir.[216,217] However, efficacy in patients harbouring multiple discriminatory mutations affecting tenofovir and in the context of the high diversity of viral strains circulating in SSA remains to be determined. Implementation should be accompanied by enhanced efforts to establish virological monitoring and by public health programmes to survey efficacy.

The observed rate of virological suppression was 78% after a median of 4.2 years of NNRTI-based first-line ART, and in line with published data from SSA.[103] It is encouraging that the observed suppression rate was maintained during a further four years of follow-up. Previous systematic analyses have shown that taking an intention-to-treat approach leads to lower suppression rates in sub-Saharan African populations due to mortality and loss to follow-up and this was also true of our cohort.[103] We have previously reported on the large variations in the rates of switching to second-line ART in SSA, with higher rates reported in populations undergoing virological monitoring than in those without routine access to viral load testing, as also reflected in this study.[71] Emphasis has been placed on providing adherence support prior to changing ART for patients experiencing viraemia in SSA, given that re-suppression

is frequently observed. In our cohort, it was common for viraemic patients to gain suppression while remaining on first-line ART. However, this was generally only true of patients that showed a viral load <200 copies/ml or had a higher viral load but no detectable resistance. Thus, the impact of adherence support is likely to be limited with regimens that pose a low barrier to resistance, and once NNRTI RAMs have emerged if patients receive efavirenz (or other NNRTIs). In our study, virological outcomes were also significantly affected by a history of treatment interruptions. One third of patients reported that they had interrupted ART for ≥3 continuous days at least once since first starting treatment, in most cases due to the unavailability of the ART dispensary. A previous qualitative study from the same centre in Kumasi reported that three quarters of patients on ART had experienced drug stock-outs and treatment interruptions lasting for an average of 30 days.[218] While the previous study did not measure viral load outcomes, we found that each reported episode of treatment interruption more than doubled the risk of viral load detectability at follow-up. Thus, in addition to general measures to support adherence, structural barriers to treatment provision must be removed to optimise outcomes and reduce loss to follow-up and mortality in SSA.[113,219] A reduction of clinical visits and ART pick-ups, improving linkage between communities and clinics, community dispensing of ART, and immediate start of ART at diagnosis are proposed as viable options.[177,220-222] Providers and patients should also be alerted to the risk of NNRTI resistance associated with abrupt ART interruptions, due to the long half-life of efavirenz and nevirapine.[223]

A number of considerations apply to this study. We used viral load detectability (>40 copies/ml) as an end-point, rather than apply a viral load cut-off to the definition of virological failure.[67] We based this approach on our previous observation that in Western cohorts low-level viraemia is predictive of higher viral load rebound;[184] a similar observation has been recently made for SSA.[224] However, although we attempted resistance testing at all detectable viral loads, both sequencing success and detection of resistance were higher at viral load >200 copies/ml. A further point relates to the clinical significance of the observed NRTI resistance patterns. Recent studies in SSA have indicated that genotypic resistance testing might not accurately predict NRTI activity during protease inhibitor-based second-line ART.[225] Interestingly, detection of NRTI resistance, most commonly M184V and TAMs, was found to predict significantly higher (rather than lower) odds of virological suppression on

second-line ART.[71] One proposed explanation is that patients who develop resistance at failure of first-line ART may have higher levels of adherence (hence higher drug selective pressure) than subjects who fail without resistance. Furthermore, it is well established that NRTIs such as tenofovir and zidovudine retain significant residual antiviral activity in the presence of TAMs, and that this is enhanced by the concomitant presence of M184V and continuation of lamivudine.[226] There is currently scarce evidence that similar principles apply to populations with multiple discriminatory NRTI RAMs, and the benefit of continuing tenofovir and lamivudine in such populations remains to be demonstrated. In addition the high viral loads associated with the observed mutation profiles raise concerns about clinical progression, while potential onward transmission of tenofovir RAMs may impact both treatment and pre-exposure prophylaxis programmes. In this scenario, it has been argued that the most cost-effective strategy to prevent transmission of resistance lies in a prompt switch to second line ART.[227] Further studies are needed to optimise the adoption of viral load monitoring, and strategies for use of second-line ART in the region.

Box of recommendations

In HIV-positive cohorts from sub-Saharan Africa on long-term ART:

1. **Do not change a single component of the ART regimen without performing a viral load measurement.**

5 FIFTH CHAPTER - SELF-REPORTED ADHERENCE TO ANTIRETROVIRAL THERAPY IN RELATION TO VIRAL LOAD AND DRUG RESISTANCE OUTCOMES IN A PROGRAMMATIC HIV CARE SETTING IN SUB-SAHARAN AFRICA

5.1 INTRODUCTION

Access to antiretroviral therapy (ART) has been expanding across sub-Saharan Africa (SSA), with coverage increasing from 22.1% in 2010 to 59.5% in 2017.[24] The World Health Organization (WHO) recommends a public health approach to treating HIV infection in the region, based upon standardized ART regimens for first-line and second-line therapy.[67] Optimal adherence to ART is required to maintain virological suppression and prevent emergence of drug resistance, comprising of both consistent and persistent dose taking, i.e., taking doses as prescribed and avoiding treatment interruptions.[228] Multiple factors are proposed to influence adherence to ART and these may act at the level of the individual (e.g., socio-economic status, mental health, lifestyle), the community (e.g., social support, stigma, disclosure), and the system (e.g., infrastructure, standards of service provision).[145,229-232]

Adherence to ART can be estimated by different measures such as electronic monitoring, pill counts, and pharmacy refill records, but these can be expensive and labor intensive. Among methods for collecting self-reported adherence, visual analogue scales (VAS) and multi-item questionnaires (MIQ) are practical and inexpensive, and may be more easily implemented in resource-constrained settings.[203,233-235] Self-reported adherence scales have been validated against adherence measured by electronic monitoring.[236] Studies have compared measured and self-reported adherence in relation to viral load in HIV-positive cohorts in SSA.[203,233-235,237,238] Results have not been entirely consistent: some studies indicated

that measured and self-reported adherence were equally predictive of viral load,[203,235] whereas others indicated a better performance of direct measures.[233,238]

Implementation of virological monitoring faces practical challenges in sub-Saharan Africa and simple adherence measures may help stratify patients based on their risk of failure. This is the first report on the correlation between self-reported adherence to ART and viral load and drug resistance in HIV-positive patients in Ghana. The aim of the study was to explore how VAS and answers gathered through an MIQ correlated with viral load and drug resistance in patients established on long-term ART, with the ultimate goal of guiding implementation of virological monitoring into routine care. Results were analyzed in the context of self-reported indicators of socio-economic status and of physical and psychological well-being.

5.2 METHODS

5.2.1 Study population

The study investigated 106 consecutive adults (≥18 years) accessing the HIV out-patient clinic of the Komfo Anokye Teaching Hospital (KATH), which is located in the Ashanti Region of Ghana. HIV care in Ghana is government funded. Ethical approval was granted by the Kwame Nkrumah University of Science and Technology; informed consent was obtained from all individual participants included in the study. Clinic attendees were invited to complete an MIQ (Appendix 2), which was administered by trained local health care assistants in the local language to overcome literacy barriers. The questionnaire collected data on the following domains: demographic characteristics (gender, age); adherence to ART; socio-economic status; physical and psychological well-being; and regular alcohol consumption, defined as drinking alcohol at least three times a week or stopping drinking for excessive consumption. Medical records were reviewed to collect CD4 cell counts at HIV diagnosis and ART history.

5.2.2 Adherence

The MIQ asked: a) the number of times the patient had interrupted ART for ≥3 consecutive days since first starting treatment; b) the number of times the patient interrupted ART for ≥3 consecutive days in the three months prior to the study visit; and c) the number of treatment doses missed in the week prior to attending clinic.[145] Participants were also asked to indicate adherence in the previous three months through an ordinal VAS that quantified adherence in 10% increments from 0% (complete non-adherence) to 100% (complete adherence).[143] The answers were compiled into a continuous adherence score, as detailed in Figure 5-1. Adherence was also graded into three categories: optimal, intermediate, and incomplete, as detailed in Figure 5-2.

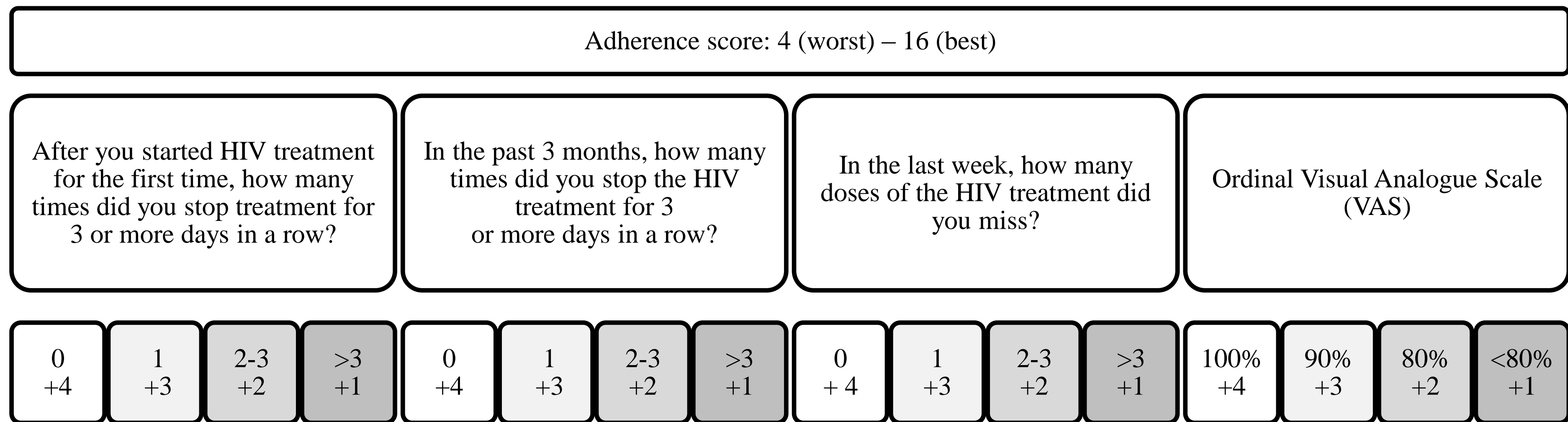
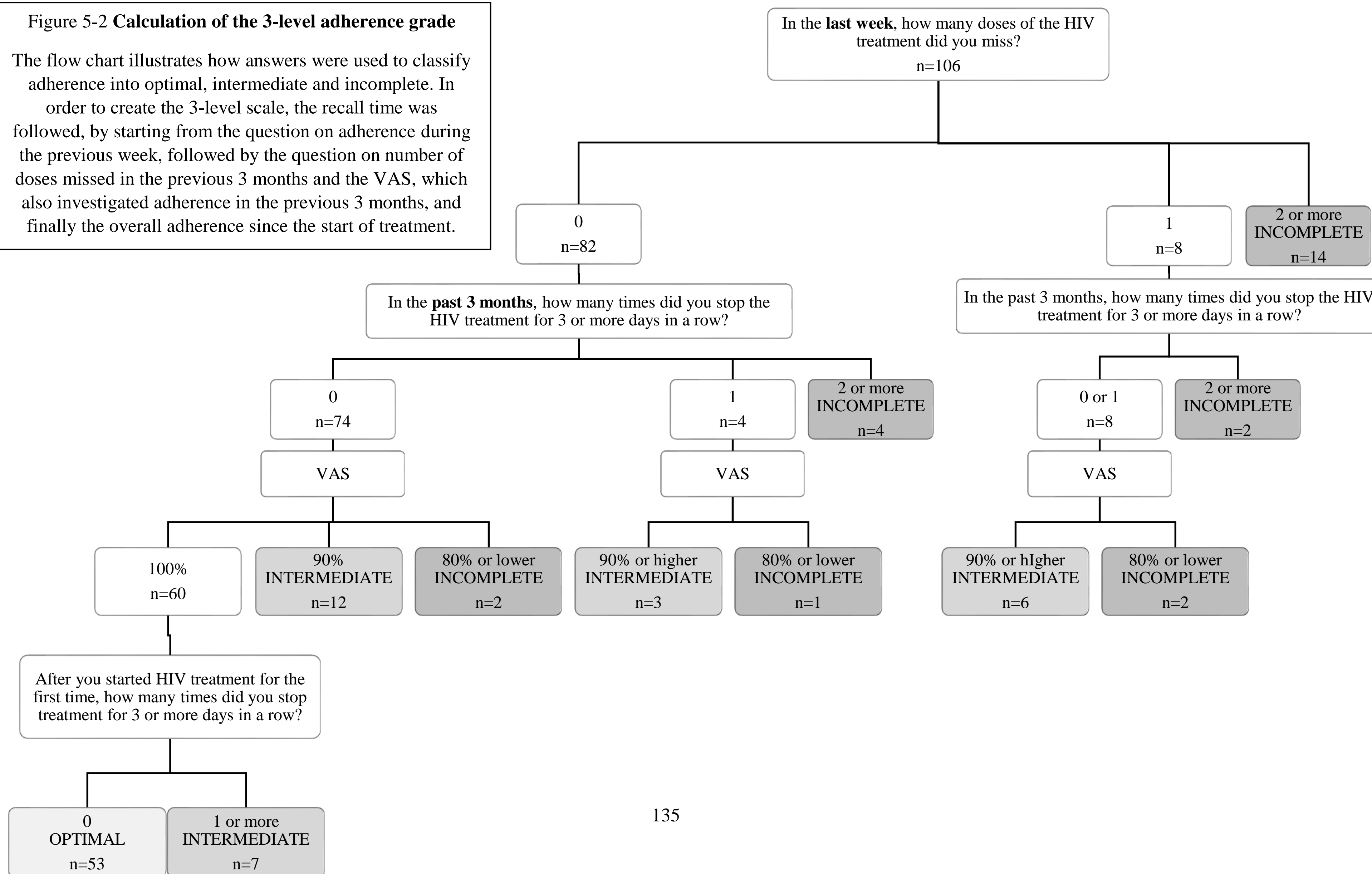


Figure 5-1 **Calculation of the continuous adherence score**

Figure 5-2 **Calculation of the 3-level adherence grade**

The flow chart illustrates how answers were used to classify adherence into optimal, intermediate and incomplete. In order to create the 3-level scale, the recall time was followed, by starting from the question on adherence during the previous week, followed by the question on number of doses missed in the previous 3 months and the VAS, which also investigated adherence in the previous 3 months, and finally the overall adherence since the start of treatment.



5.2.3 Socio-economic status

Participants answered questions about a) relationship status, whereby being married or co-habiting with a partner was defined as being in a partnership; b) HIV serostatus of the partner (if applicable); c) HIV disclosure to the partner (if applicable), and/or to at least one person other than a partner; d) number of children in the household; e) length of journey to KATH; f) whether in regular paid employment; g) education level, categorized as primary/below primary or secondary/above secondary; h) financial hardship, defined as lacking sufficient money to meet basic needs (food, clothing) and categorized as always/most of the time or rarely/never.

5.2.4 Physical and psychological wellbeing

Participants responded to 44 questions describing symptoms, mood, and emotion, each to be self-scored as 0, 1, or 2, according to the intensity or frequency (Appendix 2). Depression was assessed through 15 questions; each item reflected a well-defined mental mood or health status and represented a simplification of the Patient Health Questionnaire (PHQ)-9. The 15 questions were converted into a 9-domain format to mirror the original PHQ-9: symptoms of depression were classed as none (0), minimal (1-3), mild (4-6), moderate (7-9), moderate/severe (10-12), and severe (13-18).[146] Six additional questions consisted of an adaptation of the generalized anxiety disorder (GAD)-7 item scale: symptoms of anxiety were classed as none (0-3), mild (4-6), moderate (7-9), and severe (10-12).[147] Psychological and physical distress symptoms were assessed using the Total Memorial Symptoms Assessment Scale (MSAS)[148] to generate a total symptoms distress score, which was classed as minimal (0-2), low (3-11), moderate (12-23), and high (24-64). Health-related quality of life (HRQoL) was calculated using EQ-5D-3L (19), applying the utility sets from Zimbabwe,[150] which ranged from 0 (death) to 10 (perfect health).

5.2.5 Laboratory testing

CD4 cell counts were measured at the KATH diagnostic laboratory (reference range 410-1,590 cells/mm^3). Plasma was separated from whole venous blood in EDTA within one hour of collection by centrifugation at 4,500g for 10 minutes and stored immediately at -80°C. Samples were shipped frozen to the United Kingdome (UK) for HIV-1 RNA quantification by the RealTime HIV-1 assay (Abbott Diagnostics, Maidenhead, UK), as previously described.[140,184,204] Samples with detectable HIV-1 RNA (>40 copies/ml) underwent testing for the presence of resistance-associated mutations (RAMs) in reverse transcriptase (RT, amino acid 14-345) and protease (PR, amino acids 1-99) by Sanger sequencing and deep sequencing on the Illumina MiSeq system (Saffron Walden, UK), as described. [140,184,204] Genotypic susceptibility scores (GSS) were determined using the Stanford HIV Drug Resistance algorithm (v8.5): each drug in the regimen was assigned a score of 0 for high-level resistance, 0.25 for intermediate resistance, 0.5 for low-level resistance, and 1 for potential low-level resistance or full predicted susceptibility. Patients that did not yield an amplicon for sequencing (all with viral load <200 copies/ml) were assigned a GSS of 3. Resistance testing was not performed in patients who had discontinued ART for ≥3 months, given the absence of drug pressure.

5.2.6 Statistical analysis

Receiver operating characteristic (ROC) analysis was used to assess the performance of the number of treatment interruptions, VAS, continuous adherence score, and 3-grade adherence scale in predicting a detectable viral load. The analyses applied three viral load cut-offs: >40, >200, and >1000 copies/ml. Areas under the ROC curves (AUROC) were compared between adherence measures. The sensitivity and specificity of each point of the continuous score and of the 3-grade scale in predicting a patient with detectable viral load was calculated for each of the three viral load cut-offs. Factors associated with optimal adherence were analyzed by univariable logistic regression analyses. Variables showing an association with $p<0.2$ were included in a multivariable model: the main multivariable model included gender, being in a

partnership, number of children in the household, being in regular paid employment, length of journey to KATH, total symptoms distress score, and HRQoL. A separate model was restricted to participants in a partnership and included partner HIV serostatus and disclosure to the partner in place of being in a partnership; the model adjusted for gender, length of journey to KATH and HRQoL, and applied a stepwise approach with $p<0.1$ for model entry and exit to account for the more limited number of events. The ART regimen (categorized as first-line NNRTI-based and second-line protease inhibitor [PI]-based), and the GSS of the regimen were not included as predictors of optimal adherence in the multivariable models because they lie on the causal pathway to the outcome measure, as lower adherence can lead to increased likelihood of receipt of second-line ART and drug resistance, which in turn may lead to virological non-suppression. Correlation between continuous adherence score, CD4 cell counts, and viral load was assessed by Spearman's correlation coefficient. Analyses were performed with STATA software, version 14 (StataCorp Inc, College Station, Texas, USA).

5.3 RESULTS

5.3.1 Study population

Among consecutive ART-experienced patients invited to participate, 106/106 (100%) agreed and completed a study questionnaire (Table 5-1). Participants had started ART a median (IQR) of 7.8 (5.6-8.2) years earlier and showed a median (IQR) CD4 count of 559 (346-711) cells/mm^3. Most (91/106; 90.1%) were on a first-line ART regimen with two NRTIs (predominantly tenofovir disoproxil fumarate [TDF] and lamivudine [3TC]) and one NNRTI (predominantly efavirenz [EFV]); 10/106 (9.4%) were on a second-line ART regimen with two NRTIs and ritonavir-boosted lopinavir (LPV/r); the remaining 5/106 (4.7%) were NNRTI-experienced but had discontinued ART a median of 2.3 years (range 0.3-4.6) prior to the study visit. Plasma HIV-1 RNA was detected in 26/106 (24.5%) participants, at median levels of 4.6 $\log_{10}$ copies/ml (IQR 2.6-5.1). Among the 101/106 (95.3%) subjects on ART, 21/101 (20.8%) showed a detectable viral load, and most (19/101, 18.8%) had a level >1000 copies/ml; resistance testing in the treated population detected a high prevalence of NNRTI RAMs (15/21, 71.4%), usually co-existing with NRTI RAMs (12/21, 57.1%). PI RAMs were uncommon: one subject on LPV/r (1/21, 4.8%) showed I54V. The GSS of the ART regimen was <3 in 18/101 (17.8%) subjects, including 5/101 (5.0%) subjects with a GSS of 0.

Table 5-1 **Demographic and clinical characteristic of the study population at study entry, stratified by the adherence grade**

		Total	Adherence grade[a]			p
			Incomplete	Intermediate	Optimal	
Total, n		106	25	28	53	-
Gender, female n (%)		68 (64.2)	10 (40.0)	15 (53.6)	43 (81.1)	<0.01
Age, median years (IQR)		44 (39-48)	45 (42-48)	43 (38-47)	44 (39-50)	0.85
CD4 count at HIV diagnosis, median cells/mm^3 (IQR)		199 (90-334)	208 (87-278)	206 (89-284)	172 (93-358)	0.72
CD4 count at study visit, median cells/mm^3 (IQR)		559 (346-711)	391 (269-573)	519 (346-671)	612 (481-787)	<0.01
Duration of ART, median years (IQR)		7.8 (5.6-9.2)	7.4 (5.2-8.8)	8.0 (6.2-10.2)	7.9 (6.1-9.2)	0.44
Calendar year of starting ART, n (%)	2004-2009	79 (74.5)	17 (68.0)	22 (78.6)	40 (75.5)	0.70
	2010-2014	27 (25.5)	8 (32.0)	6 (21.4)	13 (24.5)	
ART class experienced, n (%)	NRTI	106 (100)	25 (100)	28 (100)	53 (100)	-
	NNRTI	96 (90.6)	21 (84.0)	25 (89.3)	50 (94.3)	
	Protease inhibitor	10 (9.4)	4 (16.0)	3 (10.7)	3 (5.7)	
ART regimen at study visit, n (%)	Tenofovir/Lamivudine	100 (99.0)	19 (95.0)	28 (100)	53 (100)	-
	Zidovudine/Lamivudine	1 (1.0)	1 (5.0)	0 (0)	0 (0)	
	Efavirenz or Nevirapine[b]	91 (85.9)	21 (84.0)	25 (89.3)	50 (94.3)	0.14
	Lopinavir/ritonavir	10 (9.4)	4 (16.0)	3 (10.7)	3 (5.7)	
	None[c]	5 (4.7)	5 (20.0)	0 (0)	0 (0)	
HIV-1 RNA copies/ml, n (%)	≤40	80 (75.5)	10 (40.0)	22 (78.6)	48 (90.6)	<0.01
	41-400	6 (5.7)	3 (12.0)	1 (3.6)	2 (3.8)	
	401-1000	1 (0.9)	0 (0)	0 (0)	1 (1.9)	
	>1000	19 (17.9)	12 (48.0)	5 (17.9)	2 (3.8)	
RAMs, n (%)	NNRTI only	3 (2.8)	3 (12.0)	0 (0)	0 (0)	-
	NRTI+NNRTI	12 (11.3)	5 (20.0)	4 (14.3)	3 (5.7)	
	NRTI+PI	1 (0.9)	0 (0)	0 (0)	1 (1.9)	
	None	3 (2.8)	1 (4.0)	1 (3.6)	1 (1.9)	
	No amplicon	2 (1.9)	1 (4.0)	1 (3.6)	0 (0)	
	Off ART, not tested[d]	5 (4.7)	5 (20.0)	0 (0)	0 (0)	
GSS of ART regimen, n (%)	3	83 (78.3)	12 (48.0)	23 (82.1)	48 (90.6)	<0.01
	2	9 (8.5)	5 (20.0)	1 (3.6)	3 (5.7)	
	1	4 (3.8)	2 (8.0)	2 (7.1)	0 (0)	
	0	5 (4.7)	1 (4.0)	2 (7.1)	2 (3.8)	
	Off ART, not tested[d]	5 (4.7)	5 (20.0)	0 (0)	0 (0)	

[a]The adherence grade comprised the following composite self-reported measures of adherence: i) number of treatment interruptions (≥3 consecutive days) since first starting treatment; ii) number of treatment interruptions (≥3 consecutive days) in the three months prior to the study visit; (iii) number of individual treatment doses missed in the week prior to the study visit; and iv) percentage adherence in the three months prior to the study visit recorded on an ordinal visual scale. The five individuals who had discontinued ART for ≥3 months at the time of the study visit were classified as having incomplete adherence. [b]Overall, 87/91 subjects were on efavirenz and 4/91 on nevirapine. [c]All had discontinued treatment while on first-line NNRTI-based ART. [d]Resistance testing was not performed in the five patients that had discontinued ART. IQR=interquartile range; ART=antiretroviral therapy; NRTI= nucleoside reverse transcriptase inhibitor; NNRTI=non-nucleoside reverse transcriptase inhibitor; RAMs=resistance-associated mutations; GSS=genotypic susceptibility score.

5.3.2 Socio-economic status and physical and psychological wellbeing

Just over half (54/106, 50.9%) of the participants reported being in a partnership (Table 5-2), and 36/106 (34%) reported a relationship with a HIV-positive partner; half of those participants who were not in a relationship reported being a widow (26/52, 50.0%). Most participants had disclosed their HIV status to either a partner (45/54 of those in a partnership, 83.3%) or at least one other person (75/106, 70.8%). Economic status was overall poor, with 40/106 (37.7%) participants reporting no regular paid employment, and 88/106 (83.0%) reporting that they always or mostly lacked sufficient money to cover basic needs. Depression and anxiety scores were moderate/severe in 27/106 (25.5%) and 10/106 (9.4%) subjects, respectively. The total symptoms distress score, reflecting distress caused by physical or psychological symptoms, was severe in 10/106 (9.4%) participants. The most commonly reported physical symptoms were tiredness (65/106, 61.3%), headache (60/106, 56.6%), pain (62/106, 58.5%), and muscle aches (60/106, 56.6%). In terms of HRQoL, most patients reported full health (56/106, 52.8%); for scores <10, most difficulties were observed in the "pain/discomfort" (34/106, 32.1%) and "depression/anxiety" (33/106, 31.1%) domains (Table 5-3).

Table 5-2 **Socio-economic status and physical and psychological wellbeing of the study population at study entry, stratified by the adherence grade**

		Total	Adherence grade			p
			Incomplete	Intermediate	Optimal	
Total n		106	25	28	53	-
Partnership status, n (%)	Single	13 (12.3)	1 (4.0)	4 (14.3)	8 (15.1)	
	Separated/divorced	13 (12.3)	3 (12.0)	1 (3.6)	9 (17.0)	
	Widow	26 (24.5)	5 (20.0)	4 (14.3)	17 (32.1)	
	In partnership	54 (50.9)	16 (64.0)	19 (67.9)	19 (35.9)	0.01
HIV-positive partner, n (%)		36 (34.0)	8 (32.0)	14 (50.0)	14 (26.4)	0.15
Disclosed to partner, n (%)		45 (42.5)	9 (36.0)	18 (64.3)	18 (34.0)	<0.01
Disclosure to others, n (%)		75 (70.8)	14 (56.0)	21 (75.0)	40 (75.5)	0.11
Children in the household, median number (IQR)		2 (1-3)	2 (2-3)	2 (1-3)	2 (1-3)	0.41
Age of youngest child, median years (IQR)		11 (6-19)	11 (7-18)	8 (6-11)	14 (6-20)	0.58
Education level, n (%)	Not stated/none/primary	52 (49.1)	14 (56.0)	12 (42.9)	26 (49.1)	0.75
	Secondary/post-secondary	54 (50.9)	11 (44.0)	16 (57.1)	27 (50.9)	
In regular paid employment, n (%) (n=95)		55 (51.9)	14 (56.0)	18 (64.3)	23 (43.4)	0.27
Financial hardship, n (%)	Always/mostly	18 (17.0)	3 (12.0)	7 (25.0)	8 (15.1)	0.94
Length of journey to KATH, median minutes (IQR)		60 (60-120)	55 (25-75)	60 (55-120)	90 (60-150)	<0.01
Regular alcohol consumption, n (%)		5 (4.7)	3 (12.0)	0 (0)	2 (3.8)	0.27
Depression score[a], median (IQR)		4 (2-7)	4 (1-5)	4 (3-7)	5 (3-7)	0.23
Anxiety score[b], median (IQR)		2 (1-4)	1 (1-4)	2 (1-5)	3 (1-4)	0.18
Total Symptoms Distress score[c], median (IQR)		13 (9-17)	12 (7-17)	13 (6-16)	14 (10-18)	0.12
HRQoL[d], median utility values (IQR)		10 (7.9-10)	10 (8.4-10)	10 (8.4-10)	0.9 (0.0-10)	0.06

[a]Adapted from the PHQ-9 score, with scores classed as 0=none; 1-3=minimal; 4-6= mild; 7-9=moderate; 10-12=moderate/severe; and 13-18=severe symptoms of depression. [b]Adapted from the GAD-7 score, with scores classed as 0-3=none; 4-6= mild; 7-9=moderate; and 10-12=severe symptoms of anxiety. [c]Adapted from the MSAS score, with scores classed as 0-2=minimal; 3-11=low; 12-23= moderate; and 24-64= high distress. [d]HRQoL adapted from the EQ-5D-3L questions with value sets from Zimbabwe, and ranging from 0 (death) to 10 (perfect health). IQR=interquartile range; KATH=Komfo Anokye Teaching Hospital; HRQoL=Health-Related Quality of Life; PHQ-9=Patient Health Questionnaire-9; GAD-7=Generalised Anxiety Disorder-7; MSAS=Memorial Symptoms Assessment Scale.

Table 5-3 **Numbers and proportions reporting levels within EQ-5D dimensions**

		Mobility	Self-care	Usual activities	Pain/discomfort	Anxiety/depression
Level, n (%)	1	91 (85.8)	103 (97.2)	97 (91.5)	72 (67.9)	73 (68.9)
	2	15 (14.2)	2 (1.9)	6 (5.7)	29 (27.4)	29 (27.4)
	3	0 (0)	1 (0.9)	3 (2.8)	5 (4.7)	4 (3.8)
	Total	106 (100)	106 (100)	106 (100)	106 (100)	106 (100)
Number reporting some problems		15 (14.2)	3 (2.8)	9 (8.5)	34 (32.1)	33 (31.1)

5.3.3 Adherence

By the 3-grade scale, adherence was graded as optimal, intermediate, and incomplete in 53/106 (50.0%), 28/106 (26.4%), and 25/106 (23.6%) subjects, respectively (Tables 3-1 and 3-2). A total of 35/106 subjects (33.0%) reported ≥1 treatment interruption since first starting ART, including 15/106 (14.9%) who had interrupted ART in the previous three months. Of these 35 subjects, 30 had resumed ART, whereas five remained off ART at the study visit. Among the 101 participants who were on ART, 15 (14.9%) reported missing ≥1 treatment dose in the previous week; the reasons described comprised: running out of tablets (7/15, 46.7%), forgetfulness (4/15, 26.7%), believing that treatment was making them ill (3/15, 20.0%), feeling depressed (1/15, 6.7%), having sleepless nights due to nightmares (1/15, 6.7%), using traditional remedies instead of ART (1/15, 6.7%), having had an accident (1/15, 6.7%), and being away from home (1/15, 6.7%). VAS was graded 100% for 63/106 (59.4%) subjects, 90% for 25/106 (23.6%), 80% for 8/106 (7.6%) and <80% for 10/106 (9.4%). Subjects reporting 1, 2 or ≥3 treatment interruptions a) since the start of treatment and b) during the previous three months were a) 19/106 (17.9%), 5/106 (4.7%), 11/106 (10.4%) and b) 9/106 (8.5%), 5/106 (4.7%), 8/106 (7.6%), respectively (Figure 3-3).

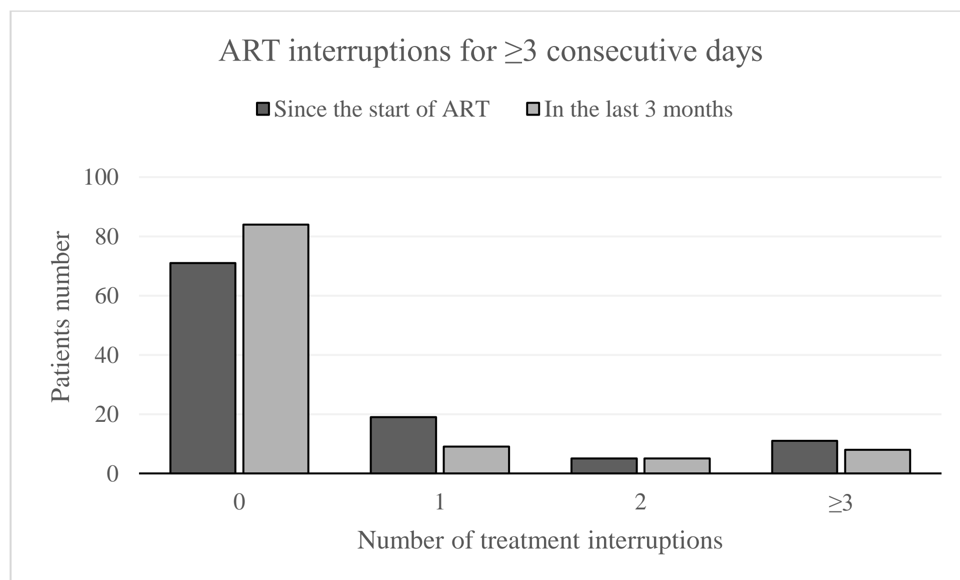


Figure 5-3 **Treatment interruptions since the start of treatment and in the previous three months**

5.3.4 Predictors of optimal adherence

In the univariable analysis (Table 3-4), optimal adherence as defined by the 3-level scale, was more likely among women, those not in a partnership, those describing a longer journey to KATH, and those reporting a higher total symptoms distress score. After adjustment, women, those not in a partnership, and those with a longer journey to KATH retained a higher odd of reporting optimal adherence. A separate model restricted to subjects in a partnership (Table 5-5) confirmed that women were more likely to report optimal adherence than men after adjustment for length of journey to KATH and HRQoL, with an adjusted odds ratio (OR) of 4.11 (95% confidence interval [CI] 1.61-10.5; $p<0.01$). Partner HIV status and HIV disclosure did not show an effect.

Table 5-4 **Logistic regression analysis of predictors of optimal adherence**

		Univariable analysis			Multivariable analysis[a]		
		OR	95% CI	p	OR	95% CI	p
Gender	female vs. male	4.82	2.00-11.5	<0.01	3.56	1.22-10.3	0.02
Age	per 5-year older	1.15	0.89-1.47	0.28			
Time on ART	per year longer	1.02	0.87-1.19	0.84			
ART regimen	PI vs. NNRTI	0.35	0.09-1.45	0.15			
GSS	per point higher	1.78	0.98-3.22	0.06			
CD4 count at diagnosis	per 50 cells higher	1.05	0.95-1.16	0.33			
In partnership	yes vs. no	0.29	0.13-0.64	0.02	0.38	0.15-0.99	0.05
Number of children in the household	per each one higher	1.16	0.95-1.42	0.14	1.27	0.99-1.64	0.06
In regular paid employment	yes vs. no	0.50	0.23-1.09	0.08	1.01	0.40-2.56	0.99
Education level	none/not stated/primary	1					
	secondary/post-secondary	1.00	0.47-2.14	1.00			
Financial hardship	yes vs. no	0.76	0.28-2.12	0.61			
Length of journey to KATH	per 30 minutes longer	1.39	1.11-1.74	<0.01	1.39	1.09-1.77	0.01
HIV disclosure[b]	yes vs. no	1.49	0.64-3.51	0.36			
Regular alcohol consumption	yes vs. no	0.65	0.10-4.08	0.65			
Depression	per 1 unit higher	1.06	0.94-1.19	0.37			
Anxiety	per 1 unit higher	1.05	0.91-1.22	0.50			
Total symptom distress score	per 4 units higher	1.24	1.00-1.52	0.04	1.00	0.76-1.31	0.99
HRQoL	per 1 utility value increase	0.79	0.61-1.04	0.09	0.82	0.60-1.12	0.21

[a]Factors included in the multivariable model comprised gender, being in partnership, number of children per household, employment status, length of journey to KATH, total symptoms distress, HRQoL. [b]HIV disclosure to someone other than a partner. ART=antiretroviral treatment; PI=protease inhibitor; NNRTI=non-nucleoside reverse transcriptase inhibitors; GSS=genotypic susceptibility score; KATH=Komfo Anokye Teaching Hospital; HRQoL=Health-related quality of life.

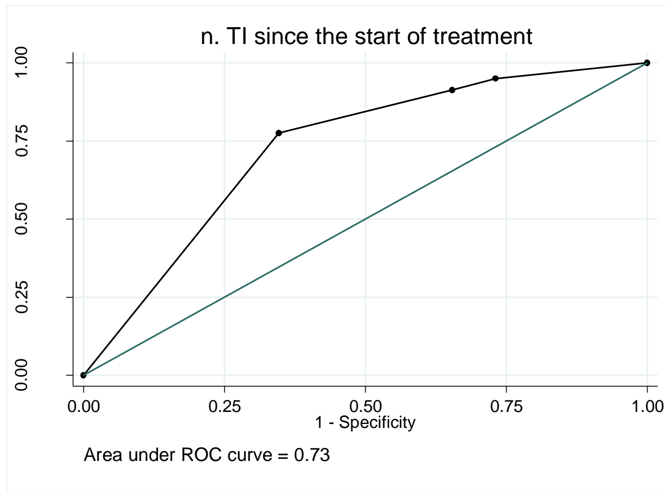
Table 5-5 **Logistic regression analysis of predictors of optimal adherence (n=19) for participants in a partnership (n=54)**

		Univariable analysis			Multivariable analysis[a]		
		OR	95% CI	p	OR	95% CI	p
Gender	female vs. male	4.82	2.00-11.5	<0.01	4.11	1.61-10.5	<0.01
Age	per 5-year older	1.15	0.89-1.47	0.28			
Time on ART	per year longer	1.02	0.87-1.19	0.84			
ART regimen	PI vs. NNRTI	0.35	0.09-1.45	0.15			
GSS	per point higher	1.78	0.98-3.22	0.06			
CD4 count at diagnosis	per 50 cells higher	1.05	0.95-1.16	0.33			
Partner HIV serostatus	negative/unknown	1					
	positive	1.65	0.48-5.66	0.42			
Number of children per household	per one higher	1.16	0.95-1.42	0.14			
In regular paid employment	yes vs. no	0.50	0.23-1.09	0.08			
Education level	none/not stated/primary	1					
	secondary/post-secondary	1.00	0.47-2.14	1.00			
Financial hardship	yes vs. no	0.76	0.28-2.12	0.61			
Length of journey to KATH	per 30-minute longer	1.39	1.11-1.74	<0.01	1.39	1.10-1.75	0.01
HIV disclosure[b]	yes vs. no	1.49	0.64-3.51	0.36			
HIV disclosure to the partner	yes vs. no	5.33	0.61-46.4	0.13			
Regular alcohol consumption	yes vs. no	0.65	0.10-4.08	0.65			
Depression	per 1 unit higher	1.06	0.94-1.19	0.37			
Anxiety	per 1 unit higher	1.05	0.91-1.22	0.50			
Total symptoms distress	per 4 units higher	1.24	1.00-1.52	0.04			
HRQoL	per 1-utility value increase	0.79	0.61-1.04	0.09	0.78	0.59-1.04	0.09

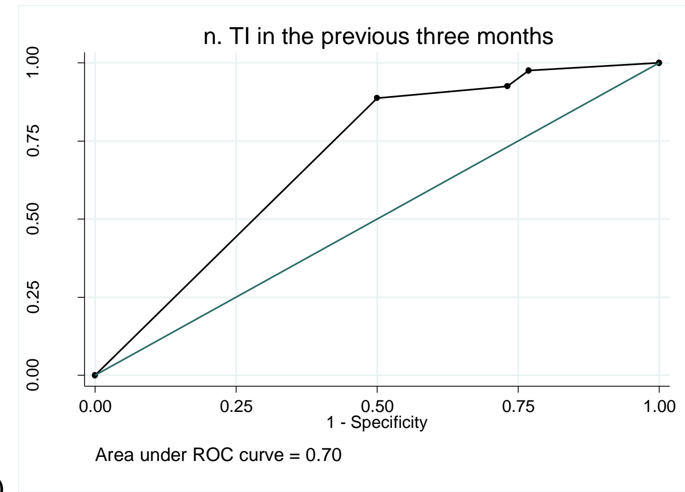
[a]Stepwise selection with p<0.10 for entry and exit from the multivariable model, with final adjustment for gender, length of journey to KATH and health-related quality of life.
[b]HIV disclosure to someone other than a partner. ART=antiretroviral treatment; PI=protease inhibitor; NNRTI=non-nucleoside reverse transcriptase inhibitor; GSS=genotypic susceptibility score; KATH=Komfo Anokye Teaching Hospital. HRQoL=Health-related quality of life.

5.3.5 Adherence as a predictor of outcomes

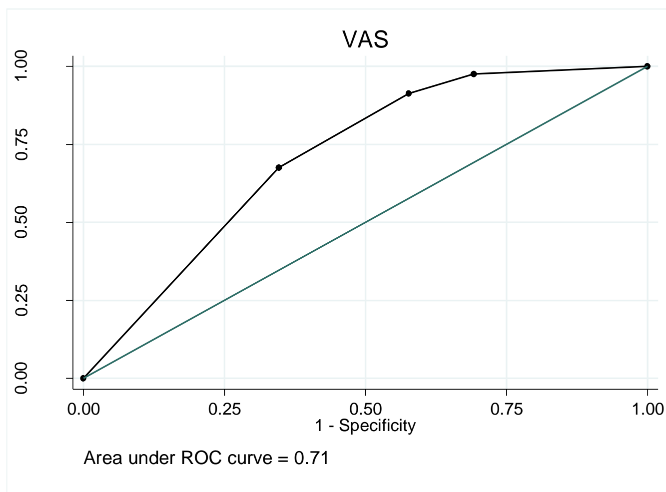
Subjects with optimal adherence showed higher rates of virological suppression, less drug resistance with a higher GSS score, and higher CD4 cell counts (Table 5-1). In ROC analyses, the number of treatment interruptions, the VAS, the 3-grade scale, and the continuous score all had acceptable discriminatory ability (area under the ROC curve [AUROC] ≥0.7) for predicting a detectable viral load (Figure 5-4). After comparison of the AUROC curves with each other, the 3-grade scale and continuous score outperformed the other measures (p=0.01). The 3-grade scale to define optimal adherence showed 60% sensitivity and 81% specificity for predicting a detectable viral load using a >40 copies/ml threshold (Table 5-6); specificity increased to 85% and 90% while sensitivity declined to 58% and 59% when applying higher viral load thresholds of >200 copies/ml and >1,000 copies/ml, respectively. Across all viral load thresholds, a continuous score ≥15/16 showed 72-75% sensitivity and 69-74% specificity for predicting viraemia.



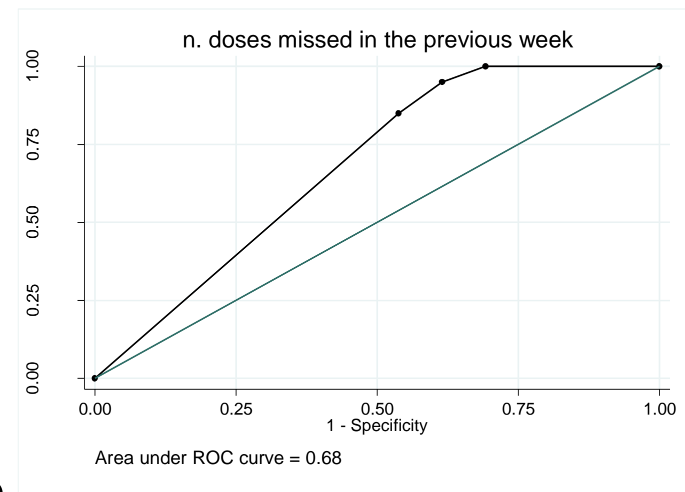
a)



b)



c)



d)

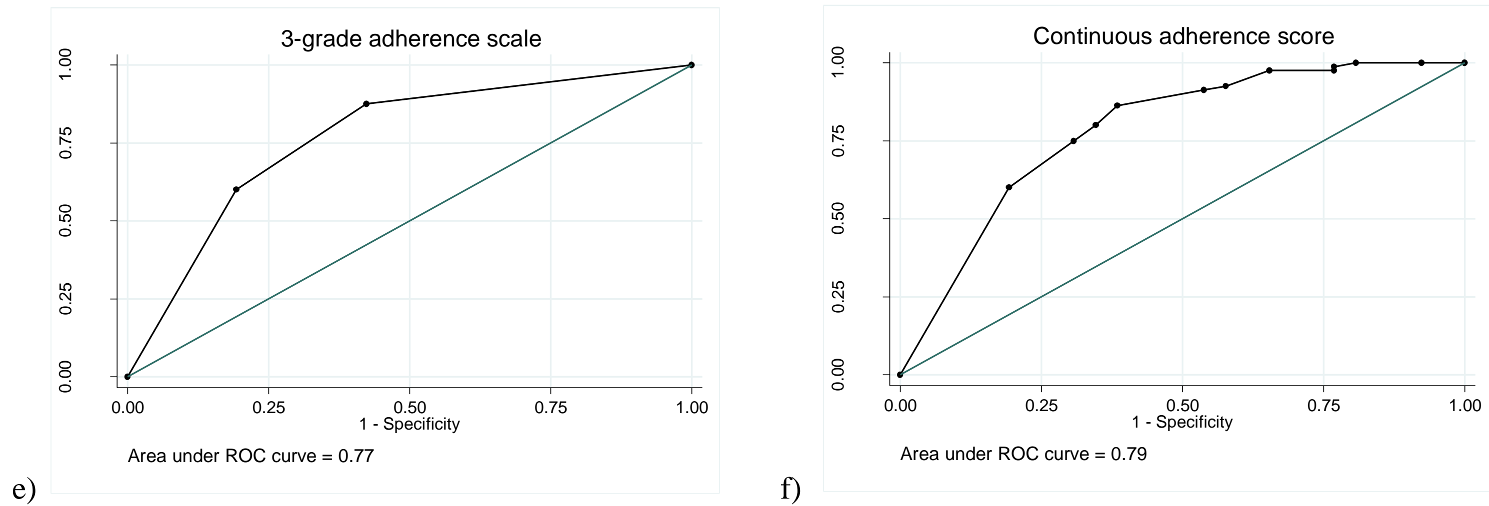


Figure 5-4 **ROC analyses**

Performance of the a) number of treatment interruptions since the start of HIV treatment, b) number of treatment interruptions in the previous three months, c) visual analogue scale, d) number of missed doses in the previous week, e) 3-grade adherence scale and f) continuous adherence score in predicting detectable viral load (>40 copies/ml) (n=106). TI=treatment interruptions.

Table 5-6 **Sensitivity and specificity of the 3-grade adherence scale and the continuous adherence score**

		Viral load (copies/ml)		
		>40	>200	>1000
Optimal adherence in the 3-grade scale (n=53) [1]	Sensitivity	60%	58%	59%
	Specificity	81%	85%	90%
≥15 points in the continuous score (n=68)[2]	Sensitivity	75%	72%	72%
	Specificity	69%	72%	74%

[1]Optimal adherence in the 3-grade scale represents an ideal scenario of no missed doses and no history of treatment interruptions. [2]A score of ≥15/16 was chosen to indicate an ideal level of adherence (>90%).

Correlations between the continuous score, viral load, and CD4 counts are reported in Table 3-7.

Table 5-7 **Spearman correlation of CD4 cell counts and viral load with the continuous adherence scale**

		Adherence score*	
		rho	p
CD4 count	per 50-cell increment	0.35	<0.01
Viral load	per $\log_{10}$ increment	-0.47	<0.01

*min score 4 (participants off treatment), max score 16 (participants with optimal adherence)

5.3.6 Characteristics of the cohort by gender

As gender was significantly associated with adherence, the baseline characteristics of men and women were compared (Table 3-8).

Table 5-8 **Demographics, clinical characteristics, socio-economic status and physical psychological wellbeing of the study population stratified by gender (n=106)**

		Total	Men	Women	p
Total, n		106	38	68	
Age, median years (IQR)		44 (39-48)	47 (43-48)	43 (38-49)	0.03
CD4 count at HIV diagnosis, median cells/mm^3 (IQR) (n=104)		199 (90-334)	180 (93-243)	214 (87-419)	0.13
CD4 count at study visit, median cells/mm^3 (IQR)		559 (346-711)	419 (265-575)	613 (449-793)	<0.01
Duration of ART, median years (IQR)		7.8 (5.6-9.2)	8.2 (6.6-9.9)	7.8 (5.2-9.1)	0.21
Calendar year of starting ART, n. (%)	2004-2009	79 (74.5)	30 (79.0)	49 (72.1)	0.49
	2010-2014	27 (25.5)	8 (21.0)	19 (27.9)	
ART class experienced, n (%)	NRTI	106 (100)	38 (100)	68 (100)	-
	NNRTI	91 (90.1)	29 (85.3)	62 (92.5)	
	Protease inhibitor	10 (9.4)	5 (13.2)	5 (7.4)	
ART regimen at study visit, n. (%)	Tenofovir/Lamivudine	100 (99.0)	34 (89.5)	66 (97.0)	-
	Zidovudine/Lamivudine	1 (1.0)	0 (0)	1 (1.5)	
	Efavirenz or Nevirapine[a]	91 (90.1)	29 (85.3)	62 (92.5)	0.06
	Lopinavir/ritonavir	10 (9.4)	5 (13.2)	5 (7.4)	
	None[b]	5 (4.7)	4 (10.5)	1 (1.5)	
HIV RNA, copies/mm^3, n. (%)	≤40	80 (75.5)	24 (63.2)	56 (82.4)	0.03
	41-400	6 (5.7)	2 (5.3)	4 (5.9)	
	401-1000	1 (0.9)	0 (0)	1 (1.5)	
	>1000	19 (17.9)	12 (31.6)	7 (10.3)	
RAMs, n (%)	NNRTI only	3 (2.8)	1 (2.6)	2 (2.9)	
	NRTI+NNRTI	12 (11.3)	7 (18.4)	5 (7.4)	
	NRTI+PI	1 (0.9)	0 (0)	1 (1.5)	
	None	3 (2.8)	2 (5.3)	1 (1.5)	
	No amplicon	2 (1.9)	0 (0)	2 (2.9)	
	Off ART, not tested[c]	5 (4.7)	4 (10.5)	1 (1.5)	
GSS, n. (%) (n=101)	3	83 (82.2)	26 (76.5)	57 (85.1)	0.18
	2	9 (8.9)	3 (8.8)	6 (9.0)	
	1	4 (4.0)	1 (2.9)	3 (4.5)	
	0	5 (5.0)	4 (11.8)	1 (1.5)	
Partnership status, n (%)	Single	13 (12.3)	3 (7.9)	10 (14.7)	<0.01
	Separated/divorced	13 (12.3)	5 (13.2)	8 (11.8)	
	Widow	26 (24.5)	2 (5.3)	24 (35.3)	

	In partnership	54 (50.9)	28 (73.7)	26 (38.2)	
HIV-positive partner, n (%)		36 (66.7)	21 (75.0)	15 (57.7)	0.25
Disclosed to partner, n (%)		45 (42.5)	22 (57.9)	23 (33.8)	0.47
Disclosed to others, n (%)		75 (70.8)	22 (57.9)	53 (77.9)	0.04
Children in the household, median number (IQR)		2 (1-3)	2 (2-3)	2 (1-3)	0.73
Age of youngest child, median years (IQR)		11.0 (6.0-19.0)	11.0 (7.0-13.0)	13.0 (6.0-20.0)	0.38
Education level, n. (%)	None/not stated/primary	52 (49.1)	15 (39.5)	37 (54.4)	0.14
	Secondary/post-secondary	54 (50.9)	23 (60.5)	31 (45.6)	
In regular paid employment, n (%) (n=95)		55 (51.9)	27 (71.1)	28 (41.2)	<0.01
Financial hardship, n (%)	Always/mostly	18 (17.0)	9 (23.7)	9 (13.2)	0.19
Length journey to KATH, median minutes (IQR)		60 (60-120)	60 (45-90)	90 (60-120)	0.04
Regular alcohol consumption, n (%)		5 (4.7)	4 (10.5)	1 (1.5)	0.04
Depression score[d], median (IQR)		4 (2-7)	3 (1-4)	5 (3-7)	<0.01
Anxiety score[e], median (IQR)		2 (1-4)	1 (0-3)	3 (1-5)	<0.01
Total Symptoms Distress (PSS) score[f], median (IQR)		13 (9-17)	8 (4-13)	15 (11-18)	<0.01
HRQoL[g], median utility values (IQR)		10 (7.9-10)	10 (8.3-10)	9.3 (7.9-10)	0.30

[a]Overall, 87/91 subjects were on efavirenz and 4/91 on nevirapine. [b]All had discontinued treatment while on first-line NNRTI-based ART. [c]Resistance testing was not performed in the five patients that had discontinued ART. [d]Adapted from the PHQ-9 score, with scores classed as 0=none; 1-3=minimal; 4-6= mild; 7-9=moderate; 10-12=moderate/severe; and 13-18=severe symptoms of depression. [e]Adapted from the GAD-7 score, with scores classed as 0-3=none; 4-6= mild; 7-9=moderate; and 10-12=severe symptoms of anxiety. [f]Adapted from the MSAS score, with scores classed as 0-2=minimal; 3-11=low; 12-23= moderate; and 24-64= high distress. [g]HRQoL adapted from the EQ-5D-3L questions with value sets from Zimbabwe, and ranging from 0 (death) to 10 (perfect health). IQR=interquartile range; ART=antiretroviral treatment; NRTI= nucleoside reverse transcriptase inhibitor; NRTI=nucleoside reverse transcriptase inhibitor; NNRTI=non-nucleoside reverse transcriptase inhibitor; PI=protease inhibitor; GSS=genotypic susceptibility score; KATH=Komfo Anokye teaching hospital; HRQoL=Health-Related Quality of Life; PHQ-9=Patient Health Questionnaire-9; GAD-7=Generalised Anxiety Disorder-7; MSAS=Memorial Symptoms Assessment Scale.

Women were younger by a median of 4 years (p=0.03), had a higher rate of virological suppression (56/68, 82.4% vs. 24/38, 63.2%; p=0.03), higher CD4 cell counts (median 613 vs. 419 cells/mm^3; p<0.01), disclosed their HIV status to circles outside the partnership more often than men (53/68, 77.9% vs. 22/38, 57.9%; p=0.04), and reported greater disadvantage in all socio-economic parameters evaluated, above all being in regular paid employment (28/68, 46.7% in women vs. 27/38 in men, 77.1%; p<0.01). Women reported regular alcohol consumption less frequently (1/68, 1.5% vs 4/28, 10.5%; p=0.04). Women were also less likely to be in a partnership than men (26/68, 38.2% vs 28/38, 73.7%, p<0.01); more than a third of women were widows (24/68, 35.3%). Self-reported wellbeing measures were worse for women, including higher scores for depression (23/68, 33.8% vs 4/38, 10.5%; p<0.01), anxiety (9/68, 13.2% vs 1/38, 2.6%; p<0.01) and total distress symptoms (7/68, 10.3% vs 3/38, 7.9%; p<0.01).

5.4 Discussion

This study was the first to investigate the relationship between self-reported adherence and virological status among HIV-positive adults attending for routine care in Ghana. Despite reporting greater socio-economic disadvantage and worse physical and psychological wellbeing, women had a >3-fold greater likelihood of reporting optimal adherence than men. In this mature programmatic HIV care setting where virological monitoring has yet to be implemented routinely, self-reported adherence was predictive of virological outcomes, and two adherence grading systems were developed that showed good specificity and sensitivity for predicting viremia across a range of clinically recommended viral load thresholds.

Optimal adherence as defined by the 3-level scale was reported by half of the participants in this study, and by 63% of women and 26% of men. These rates were lower than pooled estimates described both globally (62-63%)[239,240] and for SSA (67-77%).[112,240] A previous study from Ghana reported overall adherence rates of 62%, without investigating associated virological measures;[241] 79% of participants were females and 81% has started ART within the previous 1 to 4 years, which may explain the higher rates of optimal adherence relative to our study where the proportion of women was lower (64.2%) and participants had received ART for longer (median 7.8 years).

Our data are consistent with previous studies indicating that men in SSA tend to have worse adherence to ART than women,[242] and this is in contrast with data from Europe and North America.[232,243,244] Gender differences in behavioral predictors of health are well recognized.[245] Proposed modulating factors comprise side-effects of treatment, mental health, and multiple psycho-social and socio-economic characteristics, including education level, perceived efficacy of treatment, social support, standard of care, and HIV disclosure.[231,232,244,246,247] Depressive symptoms are frequent in HIV-positive individuals,[248] and can affect treatment outcomes regardless of gender.[231,232] In SSA, pooled prevalence estimates of depression in HIV-positive adults range from 9% to 32%, and women and those with poor socio-economic status are disproportionately affected.[249] In our cohort, one in four patients reported symptoms consistent with moderate to severe depression, and this was more common in women

than in men. Women were also at a greater socio-economic disadvantage than men. As most participants were receiving efavirenz, the relationship between plasma exposure to efavirenz, gender, and mental health should be explored in this setting.

The previous study from Ghana detected an association between adherence and other ailments, side effects of treatment, self-perceived wellness, family support, and regular follow-up.[241] Women in our cohort described more severe symptoms, which is in line with previous observations.[250] Additional predictors of adherence in our cohort included longer journeys to the clinic, which likely reflects higher motivation in the most adherent patients, and not being in a partnership, which is more complex to explain. For individuals in a partnership, there was a trend between HIV disclosure to the partner and optimal adherence, which may provide a partial insight into the observation. As the majority of patients not in a partnership had been widowed, it could also be speculated that having lost their partner might have strengthen resolution to seek and maintain treatment.

In a meta-analysis of 43 studies including 14 studies from SSA, adherence was predictive of virological outcomes across various types of adherence measures, ART regimens, study populations, and reporting methods.[239] The cut-off to define optimal adherence ranged from ≥80% to 100% in published studies, and may also depend on the overall "forgiveness" of the regimen, such as that resulting from the long half-life of NNRTIs.[239,251] Some studies highlighted that patients who achieve perfect or nearly perfect adherence do not necessarily have better virological outcomes than patients who maintain good enough (≥80-90%) adherence. [239,251] Conversely, it has been pointed out that optimal adherence does not necessarily translate into improved virological outcomes in resource-limited countries.[239] Poor availability of virological monitoring and prior accumulation of drug resistance could prevent virological suppression despite improved adherence.[140] In addition, treatment interruptions can occur as a consequence of drugs stock-outs.[218] We previously observed that each reported treatment interruption in the Kumasi cohort was associated with a two-fold increase in the risk of viremia.[140] Abrupt ART interruptions can lead to the development of NNRTI resistance, due to the long half-life of efavirenz and nevirapine and their low genetic barrier to resistance.[252]

In our study, optimal adherence was defined as the absence of any missed dose in a recall period ranging from the previous week to the previous three months, along with no history of treatment interruptions, hence reflecting an ideal scenario of 100% in dose-taking execution without disruption of drug refill or supply. Optimal adherence was associated with lower rates of viraemia and drug resistance, and with higher CD4 cell counts. In a setting where most patients were on NNRTI-based ART, we found that 79% of subjects with intermediate adherence achieved viral suppression compared with only 40% of those with incomplete adherence. The rates increased to 91% in those with optimal adherence. In addition, NNRTI RAMs were more likely to be isolated from patients with incomplete or intermediate adherence (32% and 14%, respectively), than from those with optimal adherence (6%).

There are limitations to this study. It was a cross-sectional analysis of a prospective cohort, and only participants retained in care for quite a long time took part, creating a bias in favor of those who had better adherence, were healthier, and were able to afford travel to KATH. Measures indicative of depression and anxiety were self-reported and the relative high rates of depression and anxiety suggested by the answers warrant a more formal investigation. Furthermore, a health care assistant administered the questionnaire to overcome literacy barriers, and social desirability bias in the responses cannot be excluded. It has been argued that direct adherence measures, above all medication event monitoring system, have a better discriminatory ability in detecting virological failure than self-reported adherence.[239] However, they are hard to implement in routine care where resources are limited. Measuring adherence by VAS was previously shown to be predictive of virological suppression and CD4 cell counts in African cohorts receiving first-line ART, either as a single measure,[253] or combined with other measures such as pill counts.[254] A study from Cameroon describing a cohort on NNRTI-based ART found that self-reported adherence (defined by the number of missed doses) and a history of treatment interruptions (defined as a discontinuation of at least two consecutive days), were independent predictors of virological failure.[255] In a cohort study from South Africa, adherence self-reported by a MIQ was the only independent predictor of virological failure during first-line ART.[256] However, self-reported measures of adherence have not always been successful in predicting virological outcomes in Africa.[257] In our study, a composite measure of self-reported adherence, comprising multiple recall times (i.e., from

immediately prior to the study visit to the whole life span on ART), different measures (i.e. number of missed doses, VAS), and inclusion of historical treatment discontinuations, achieved a good performance, as shown by the greater AUROC curves of the two proposed combined scores over single measures. Our continuous adherence score showed better sensitivity (75% vs 60%) and worse specificity (69% vs 81%) than the 3-grade adherence scale in discriminating patients with detectable (>40 copies/ml) viral load, hence proving to be a better measure for screening. The specificity of both composite measures increased when higher thresholds were applied, reaching values of 74% and 90% for the continuous score and the 3-grade adherence scale, respectively, for a cut-off of 1000 copies/ml. Importantly, the findings apply to a population that had not been monitored virologically and were predominantly on first-line NNRTI-based ART. Further studies are needed to define the performance of the composite adherence measures in other settings, in cohorts receiving other regimens, and in the presence of regular virological monitoring.

Religion and beliefs as potential determinants of adherence and treatment interruptions have not been formally investigated in our work, along with the role of stigma, and this represents a limitation for the analysis of determinants of adherence. In Ghanaian tradition, health advice was provided by a variety of traditional healers, including herbalists, cult healers, fetish priests, and church leaders,[258] and illness is usually believed to be the result of natural or spiritual agents.[259] HIV infection and AIDS, along with venereal diseases, belong to the second group, and often believed to be caused by witchcraft.[260] Despite the discouragement and disapproval of Christian priests on the beliefs and practise of witchcraft, Christian fundamentalism represented an obstacle itself to the discussion of HIV prevention practices.[261] HIV is believed to be caused by a 'basabasa' (i.e. improper) life-style, implying promiscuity, prostitution, and extramarital relationships.[260] It has been reported that there is belief that God can cure the infection, and some leaders in spiritual or charismatic churches have the power to do so.[260] There is growing popularity in Ghana in new churches that involve the use of 'healing' and 'salvation',[262] and patients might interrupt their medications because of the instructions of a spiritual leader. Stigma of the disease represents a barrier to treatment initiation and retention, along with financial difficulties, and health system challenges, particularly in key populations.[263] Discrimination is worse in key populations than that experienced by those that are HIV-positive alone, and key

populations, particularly MSM and FSWs, might additionally fear criminalization in the African settins. Stigma can isolate HIV-positive individuals from their social context and reduces their motivation to take ART; fear of the consequences of disclosure further impacts adherence and engagement in care.[264]

Self-reported measures of adherence are inexpensive tools that clinicians in SSA can implement in routine care, including the selection of patients that require fast-tracking for virological monitoring where testing programs are starting. We recommend the implementation of composite scores to improve the performance of self-reported adherence in predicting virological failure.

Box of recommendations

In HIV-positive cohorts from sub-Saharan Africa on long-term ART:

1. **Use self-reported measures of adherence to fast-track patients at higher risk of failure for viral load testing;**
2. **Prefer composite tools to include different recall times; our continuous score, at a cut-off of 15/16 points, offered better sensitivity across the different viral load threshold and is to be preferred for screening purposes;**
3. **Focus on men, as they are at higher risk of treatment failure due to poorer adherence.**

6 SIXTH CHAPTER - RENAL HEALTH AFTER LONG-TERM EXPOSURE TO TENOFOVIR DISOPROXIL FUMARATE (TDF) IN HIV/HBV POSITIVE ADULTS IN GHANA

6.1 INTRODUCTION

Tenofovir disoproxil fumarate (TDF), the prodrug of the nucleotide analogue reverse transcriptase inhibitor (NRTI) tenofovir, is active against both HIV and HBV. TDF use as part of antiretroviral therapy (ART) carries a risk of proximal tubular dysfunction and declining glomerular filtration rate (GFR),[265-267] and monitoring of renal function is recommended during treatment.[68,69] The risk is related to both level and length of TDF exposure and is enhanced by co-administration of pharmacological boosters (e.g., ritonavir), low body weight, and pre-existing chronic kidney disease (CKD).[268-272] Whilst TDF discontinuation is generally associated with improved renal function, longer exposure and lower GFR at TDF interruption predict a reduced likelihood of GFR recovery.[268]

Whilst in North America and Western Europe tenofovir alafenamide fumarate (TAF) provides a recommended alternative formulation with a reduced potential for renal toxicity, [68,69] TAF is not currently available in resource-limited settings. The World Health Organisation (WHO) recommends TDF as the preferred NRTI for the treatment of HIV and HIV/HBV positive individuals in sub-Saharan Africa (SSA).[67] There are limited data on the occurrence of TDF-related renal adverse events in African populations and only a few reported long-term data. A study from Malawi, South Africa, and Zimbabwe showed similar rates of renal events over 196 weeks in patients randomised to either TDF plus emtricitabine (FTC) or zidovudine (ZDV) plus lamivudine (3TC), each in combination with efavirenz (EFV).[273] A cross-sectional study among HIV-positive subjects who had received ART for a median of 9.3 years in Uganda similarly found no differences in renal function when comparing regimens with and without TDF.[274] Neither study assessed the influence of concomitant use of

ritonavir-boosted protease inhibitors (PI/r) or HBV co-infection on renal health. Short-term studies reported an increased risk of renal abnormalities with concomitant use of TDF and PI/r in SSA. In South Africa, HIV-1 positive adults on TDF experienced a small but significant decline in eGFR over a median of 13 months, and the decline was larger with concomitant PI use, older age, weight <60 kg, lower baseline eGFR, and CD4 counts <200 cells/mm^3.[272] In women receiving TDF in combination with either lopinavir/ritonavir (LPV/r) or nevirapine (NVP), renal events were predicted by LPV/r use, baseline HIV-1 RNA load, and baseline eGFR.[269]

By 2030, the number of patients requiring second-line ART in SSA is estimated to exceed 4 million, and an increasing number is likely to start therapy with PI/r.[275] At the same time, improved survival among people living with HIV in SSA is unmasking a substantial burden of co-morbidities, including HBV co-infection.[196] Limited data suggest that chronic hepatitis B may worsen renal outcomes. In one study in Zambia, HBV co-infection nearly doubled the odds of a reduced eGFR after adjusting for several factors, and the risk was higher among patients with raised serum transaminases.[276] Whilst the observation suggests a link between HBV disease activity and risk of renal dysfunction, published evidence has not been consistent.[277]

The aim of this study was to evaluate the renal function of HIV/HBV co-infected individuals receiving long-term TDF-containing ART in Ghana, integrating cross-sectionally measured tubular proteinuria (TuPr) and prospectively measured eGFR with markers of HIV and HBV status, and analysing the contributing role of PI/r use, hypertension, and diabetes.

6.2 METHODS

6.2.1 Setting

Eligible HIV/HBV co-infected positive adults (≥18 years) were drawn from the HEPIK (Hepatitis B Infection in Kumasi) prospective observational cohort based at the Komfo Anokye Teaching Hospital (KATH) in Kumasi, Ghana.[132,138] The cohort was established in 2010. Study visits took place at least once a year when participants underwent clinical assessment and sample collection. The last study visit occurred in November 2015. At study entry, upon detection of HBV co-infection, patients introduced TDF; subsequent management was at the discretion of the treating clinician. Between 2010 and 2015, monitoring for HIV-positive patients at KATH comprised routine measurements of haemoglobin, serum hepatic transaminases, and CD4 cell counts, and sporadic measurements of serum creatinine. Urine dipstick analysis, measurement of blood pressure in asymptomatic patients, and HIV/HBV virological monitoring were not part of routine care. No patient had access to TAF and TAF remains unavailable in Ghana in 2018. Ethical approval was granted by the Kwame Nkrumah University of Science and Technology, Ghana; all patients gave written informed consent.

6.2.2 Study population

The analysis comprised HEPIK participants who at the last study visit (November 2015) were on stable TDF-containing ART. At this time, patients underwent study-related clinical assessment and sample collection. Adherence to ART was self-reported through an ordinal visual scale graded from 0 to 100%, in 10% increments. Transient elastography was performed using Fibroscan (Ecosens, France) and interpretative cut-offs applied as previously reported.[138,278] Blood pressure (BP) was measured with a manual sphygmomanometer; abnormal findings were confirmed after the patient had rested for ≥20 minutes. Hypertension was graded as 1 (systolic 140-159 or diastolic 90-99 mmHg), 2 (systolic 160-179 or diastolic 100-109 mmHg) and 3 (systolic ≥180 or diastolic ≥110 mmHg).[279] Systolic BP <140 and/or diastolic

BP <90 mmHg on antihypertensive therapy was scored as grade 1. Diabetes was defined by glycated haemoglobin (HbA1c) ≥48 mmol/mol or receiving antidiabetic therapy.

6.2.3 Laboratory tests

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), haemoglobin, and CD4 cell counts were measured at the KATH diagnostic laboratory. Laboratory reference ranges are shown in Table 6-1.

Table 6-1 **Laboratory reference ranges**

Parameter	Reference
Haemoglobin (g/dl)	11.0-18.0
ALT (IU/l)	8-54
AST (IU/l)	17-60
Creatinine (µmol/l)	50-130
Hb1Ac (mmol/mol)	<42
CD4 count (cells/mm^3)	410-1590

Urine samples underwent dipstick analysis for proteinuria, haematuria, and glycosuria with Medi-Test Combi 5S (BHR Pharmaceuticals, Nuneaton, UK), and testing for *Schistosoma* circulating cathodic antigen (Ag) with the Urine-CCA Cassette test (Rapid Medical Diagnostics, Pretoria, South Africa).[280] Plasma was separated from whole blood in EDTA within one hour of collection and stored immediately at -80°C. Frozen aliquots of whole EDTA blood, plasma, serum, and urine were shipped to the UK for further testing. In the UK, plasma HIV-1 RNA and HBV DNA were quantified by the RealTime HIV-1 and HBV assays (Abbott Diagnostics, Maidenhead, UK), as previously described.[138] Hepatitis B e antigen (HBeAg) was measured by Architect (Abbott Diagnostics). Creatinine, urinary protein-to-creatinine ratio (uPCR), urinary albumin-to-creatinine ratio (uACR, performed if uPCR >20 mg/mmol), and HbA1c

were measured in the accredited diagnostic laboratory of the Royal Liverpool University Hospital, Liverpool, UK. TuPr was defined as uPCR >20 mg/mmol with uACR/uPCR ratio <0.4;[281] significant TuPr was defined by uPCR >30 mg/mmol with uACR/uPCR ratio <0.4. The eGFR was calculated using the CKD epidemiology collaboration-derived equation (CKD-EPI); the ethnicity factor was applied (x1.21).[282] Reduction in eGFR was classed as grade 2, 3, or 4 based on readings of 60-89, 30-59, and <30 ml/min/1.73m^2, respectively.[279] In addition to blood samples collected in November 2015, stored samples collected between 2010 and 2015 were retrieved and serum creatinine was measured retrospectively to calculate changes in eGFR over time. A rapid eGFR decline (RD-eGFR) was defined as a mean decline >5 ml/min/1.73m^2/year.[272]

6.2.4 Analysis

Patients' characteristics according to the presence or absence of TuPr or RD-eGFR were compared by the Fisher's, Chi-squared, or Mann-Whitney-Wilcoxon test, as appropriate. Factors associated with TuPr or RD-eGFR and factors associated with changes in eGFR over time were investigated in logistic and linear regression analyses, respectively. Each multivariable model explored factors associated with renal outcomes by stepwise selection. TuPr was not included in the analysis of factor associated with eGFR variation and vice-versa, as potential collinearity between the two could not be excluded. This approach resulted in the inclusion of gender and hypertension for TuPr; receipt of LPV/r and CD4 cell count for changes in eGFR; and receipt of LPV/r, duration of HIV diagnosis, and CD4 cell count for RD-eGFR. The robustness of the results was investigated in models that serially added all variables showing p<0.2 in the univariate analysis, including *Schistosoma* Ag test, liver stiffness, AST, and HbA1c for TuPr; ALT for changes in eGFR; and adherence for RD-eGFR. Haemoglobin was not included in the multivariable model for RD-eGFR as potentially part of the casual pathway of the outcome. The distribution of residuals was assessed for each linear regression, and indicated a good model fit. Performance of dipstick proteinuria (≥0.3 g/l), glycosuria (1.1 mmol/l) and haematuria (≥10 cells/µl) as predictors of TuPr was estimated through a receiver operating characteristic (ROC) analysis. Analyses were performed with STATA v.14.

6.3 RESULTS

6.3.1 Characteristics of the study population

The study population comprised 101 subjects that had received TDF for a median of 4.0 years (IQR 3.8-4.1) (Table 6-2). All subjects were also receiving 3TC and most were receiving EFV (87/101, 86.1%). Ten (9.9%) were on LPV/r, having received the PI/r for a median of 4.4 years (IQR 3.7-5.5). Plasma HIV-1 RNA was detected in 21/101 (20.8%) subjects at median levels of 4.2 $\log_{10}$ copies/ml (IQR 2.1-5.1). HBV DNA was detected in 15/101 (14.9%) subjects at median levels of 2.7 $\log_{10}$ IU/ml (IQR 1.7-3.8). Hypertension of any grade was diagnosed in 35/100 (35.0%) subjects. There were 9/101 (8.9%) patients on anti-hypertensive drugs (nifedipine, losartan, bendroflumethiazide) and five of these had elevated blood pressure of grade 1 (n=1), 2 (n=3), or 3 (n=1). Diabetes was diagnosed in 6/101 (5.9%) subjects. There were 3/101 (3.0%) patients on oral hypoglycaemic drugs (metformin, glibenclamide) and one of these had abnormal Hb1Ac levels (80 mmol/mol). The urinary *Schistosoma* Ag was reactive in 18/101 (17.8%) subjects. None of the participants was taking other regular medications.

		Total	With TuPr	Without TuPr	p	With RD-eGFR	Without RD-eGFR	p
		N=101	N=16	N=85		N=21	N=76	
Female gender, n (%)		67 (66.3)	15 (93.8)	52 (61.2)	0.01	16 (76.2)	48 (63.2)	0.31
Age, median years (IQR)		44 (39, 48)	47 (38, 52)	44 (39, 48)	0.63	44 (40, 47)	45 (39, 48)	0.45
BMI, median kg/m^2 (IQR)		23.5 (20.4, 27.3)	23.4 (20.7, 28.1)	23.5 (20.4, 27.1)	0.84	22.6 (20.8, 28.6)	23.3 (20.1, 27.1)	0.54
Duration HIV diagnosis, median years (IQR)		8.3 (6.6, 10.2)	8.2 (6.6, 9.4)	8.4 (6.6, 10.3)	0.96	7.5 (5.3, 9.4)	8.4 (6.9, 10.3)	0.16
ART duration, median years (IQR)		7.9 (6.0, 9.2)	7.9 (5.2, 8.8)	7.8 (6.3, 9.3)	0.71	7.4 (5.1, 8.8)	7.9 (6.2, 9.2)	0.40
TDF duration, median years (IQR)		4.0 (3.8, 4.1)	4.0 (3.8, 4.1)	4.0 (3.8, 4.1)	0.45	4.0 (3.8, 4.2)	4.0 (3.8, 4.1)	0.99
Prior ZDV, n (%)		89 (88.1)	14 (87.5)	75 (88.2)	1.00	19 (90.5)	67 (88.2)	1.00
Prior d4T, n (%)		48 (47.5)	8 (50.0)	40 (47.1)	1.00	7 (33.3)	39 (51.3)	0.22
Receiving EFV, n (%)		87 (86.1)	15 (93.8)	72 (84.7)	0.50	12 (57.1)	72 (94.7)	<0.001
Receiving NVP, n (%)		4 (4.0)	0 (0)	4 (4.7)	1.00	4 (19.1)	0 (0)	0.02
Receiving LPV/r, n (%)		10 (9.9)	1 (6.3)	9 (10.6)	1.00	5 (23.8)	4 (5.3)	0.02
Adherence ≥90%, n (%)		86 (86.9)	15 (93.8)	71 (83.5)	0.69	16 (76.2)	66 (86.8)	0.15
Haemoglobin, median g/dl (IQR)		13.2 (11.6, 14.6)	12.0 (11.1, 14.4)	13.2 (11.7, 14.7)	0.34	11.7 (11.4, 13.7)	13.4 (11.8, 14.7)	0.07
CD4 count, median cells/mm^3 (IQR)		572 (383, 716)	593 (302, 639)	565 (391, 717)	0.47	572 (391, 749)	559 (370, 711)	0.78
HIV-1 RNA >40 copies/ml, n (%)		21 (20.8)	4 (25.0)	17 (20.0)	0.74	6 (28.6)	14 (18.4)	0.36
HIV-1 RNA >1000 copies/ml, n (%)		14 (13.9)	3 (18.8)	11 (12.9)	0.69	2 (9.5)	12 (15.8)	0.73
HBV DNA >15 IU/ml, n (%)		15 (14.9)	2 (12.5)	13 (15.3)	1.00	3 (14.3)	11 (14.7)	1.00
HBV DNA >2000 IU/ml, n (%)		6 (5.9)	1 (6.3)	5 (5.9)	1.00	3 (14.3)	3 (4.0)	0.11
Liver stiffness, median kPa (IQR)		4.6 (3.8, 5.8)	4.8 (3.1, 5.5)	4.5 (3.8, 5.9)	0.86	4.6 (3.9, 6.1)	4.6 (3.8, 5.7)	0.87
Liver stiffness kPa >9.4, n (%)		7 (6.9)	2 (12.5)	5 (5.9)	0.29	1 (4.8)	5 (6.6)	1.00
AST, median IU/l (IQR)		31 (23, 38)	31 (22, 39)	27 (25, 35)	0.83	30 (24, 37)	31 (23, 39)	0.98
ALT, median IU/l (IQR)		24 (18, 33)	23 (16, 30)	24 (18, 36)	0.42	26 (17, 32)	24 (18, 36)	0.89
HBeAg positive, n (%)		11 (10.9)	1 (6.3)	10 (11.8)	0.63	3 (14.3)	8 (10.5)	0.61
Systolic BP, median mmHg (IQR)		122 (112, 145)	148 (122, 160)	121 (110, 137)	<0.01	126 (118, 130)	122 (110, 147)	0.64
Diastolic BP, median mmHg (IQR)		80 (71, 90)	90 (77, 110)	80 (70, 88)	0.02	78 (74, 86)	80 (70, 93)	0.90
Hypertension, n (%)	Any grade	35 (35.0)	10 (62.5)	25 (29.4)	0.02	6 (28.6)	28 (36.8)	0.61
	Grade 1	13 (13.0)[b]	2 (12.5)	11 (12.9)	-	2 (9.5)	11 (14.5)	-
	Grade 2	12 (12.0)	3 (18.8)	9 (10.6)	-	2 (9.5)	10 (13.2)	-
	Grade 3	10 (10.0)	5 (31.3)	5 (5.9)	-	2 (9.5)	7 (9.2)	-
	NA	1 (1.0)	0 (0)	1 (1.2)	-	0 (0)	0 (0)	-
Hb1Ac, median mmol/mol (IQR)		33 (31-38)	35 (31, 38)	33 (31, 38)	0.55	34 (32, 39)	33 (30, 38)	0.15
Diabetes, n (%)		6 (5.9)[c]	2 (12.5)	4 (4.7)	0.24	2 (9.5)	4 (5.3)	0.61
Schistosoma Ag positive, n (%)		18 (17.8)	5 (31.3)	13 (15.3)	0.16	5 (23.8)	12 (15.8)	0.52

Table 6-2 **Characteristics of HIV/HBV positive patients with long-term TDF exposure according to the detection of tubular proteinuria (TuPr; n=101 evaluated) and rapidly declining estimated glomerular filtration rate (RD-eGFR; n=97 evaluated)[a]**

[a]TuPr proteinuria was defined as uPCR ≥20 mg/mmol and uACR/uPCR <0.4; RD-eGFR was defined as ≥5 ml/min eGFR decline per year; [b]Comprising 4 subjects with normal readings while on anti-hypertensive medication; [c]Comprising 2 subjects with normal readings while on oral hypoglycaemic medication. uPCR=urinary protein/creatinine ratio; uACR=urinary albumin/creatinine ratio; IQR=interquartile range; BMI=body mass index; ART=antiretroviral therapy; TDF=tenofovir disoproxil fumarate; ZDV=zidovudine; d4T=stavudine; EFV=efavirenz; NVP=nevirapine; LPV/r=ritonavir-boosted lopinavir; AST=aspartate transaminase; ALT=alanine transaminase; BP=blood pressure; NA=not available; Hb1Ac=glycated haemoglobin; Ag=Antigen.

6.3.2 Markers of renal health

Tubular proteinuria

The uPCR was median 13 mg/mmol (IQR 13-20) and was >20 mg/mmol in 28/101 (27.7%) patients and >50 mg/mmol in 13/101 (12.9%) patients (Table 6-3). Among subjects with uPCR >20mg/mmol, the uACR was median 0.33 mg/mmol (IQR 0.17-0.49). TuPr was diagnosed in 16/101 (15.8%) subjects, including 9/101 (8.9%) with significant TuPr (Table 6-4). TuPr was significantly more prevalent in women and patients with hypertension and the association was confirmed after adjustment (Table 6-5). The univariate analysis showed trends for an association between TuPr and higher liver stiffness, higher HbA1c levels, and a positive *Schistosoma* Ag test. Separate models adjusting for these variables confirmed that gender and hypertension were each independently associated with TuPr (Table 6-6).

Table 6-3 **Relationship between markers of renal health in HIV/HBV positive patients with long-term TDF exposure**

		Total	With TuPr	Without TuPr	p	With RD-eGFR	Without RD-eGFR	p
		N=101	N=16	N=85		N=21	N=76	
Serum creatinine	median µmol/l (IQR)	78 (66, 87)	74 (64, 81)	79 (67, 90)	0.11	82 (75, 103)	77 (64, 85)	-
eGFR	median ml/min (IQR)	103 (92, 116)	105 (88, 124)	103 (93, 116)	0.97	92.3 (81.3, 105)	107 (94.1, 117)	-
≥90 ml/min	n (%)	79 (78.2)	12 (75.0)	67 (78.8)	0.75	12 (57.1)	63 (82.9)	-
60-89 ml/min	n (%)	18 (17.8)	3 (18.8)	15 (17.7)		7 (33.3)	11 (14.5)	-
30-60 ml/min	n (%)	4 (4.0)	1 (6.3)	3 (3.5)		2 (9.5)	2 (2.6)	-
change over time	median ml/min (IQR)	-1.8 (-4.4, -0.0)	-2.4 (-5.8, -1.0)	-1.7 (-4.3, +0.3)	0.49	-7.12 (-7.48, -5.79)	-1.20 (-2.82, +0.92)	-
rapid decline (n=97)	n (%)	21 (21.6)	4 (25.0)	17 (20.0)	0.12	21 (100)	0 (0)	-
uPCR	median mg/mmol (IQR)	13 (10, 20)	32 (24, 56)	12 (9, 17)	-	13 (9, 18)	14 (10, 21)	0.30
>20 mg/mmol	n (%)	28 (27.7)	16 (100)	12 (14.1)	-	5 (23.8)	21 (27.6)	1.00
≥50 mg/mmol	n (%)	13 (12.9)	5 (31.3)	8 (9.4)	-	3 (14.3)	10 (13.2)	1.00
Tubular proteinuria	n (%)	16 (15.8)	16 (100)	0 (0)	-	4 (19.1)	10 (13.2)	0.50
Dipstick protein	n (%)	15 (14.9)	4 (25.0)	11 (12.9)	0.25	3 (14.3)	12 (15.8)	1.00
Dipstick RBC	n (%)	12 (11.9)	5 (31.3)	7 (8.2)	0.02	3 (14.3)	8 (10.5)	0.70
Dipstick glucose	n (%)	17 (16.8)	4 (25.0)	13 (15.3)	0.46	5 (23.8)	11 (14.5)	0.33

TuPr=tubular proteinuria; RD-eGFR=rapid declining estimated glomerular filtration rate; IQR=inter-quartile range; uPCR=urinary protein/creatinine ratio; RBC=red blood cells.

Table 6-4 **HIV/HBV positive patients with significant tubular proteinuria (sTuPr)[a]**

		With sTuPr	Without sTuPr
		N=9	N=92
Female gender, n (%)		8 (88.9)	59 (64.1)
Age, median years (IQR)		42 (38, 48)	44 (39, 48)
BMI, median kg/m^2 (IQR)		22.6 (20.3, 24.2)	23.5 (20.5, 27.6)
Duration HIV diagnosis, median years (IQR)		8.0 (5.3, 9.6)	8.4 (6.7, 10.2)
ART duration, median years (IQR)		6.0 (5.0, 7.9)	8.0 (6.4, 9.2)
TDF duration, median years (IQR)		4.0 (3.8, 4.1)	4.0 (3.7, 4.1)
Prior ZDV, n (%)		7 (77.8)	82 (89.1)
Prior d4T, n (%)		4 (44.4)	44 (47.8)
Receiving EFV, n (%)		8 (88.9)	79 (85.9)
Receiving NVP, n (%)		0 (0)	4 (4.4)
Receiving LPV/r, n (%)		1 (11.1)	9 (9.8)
Adherence ≥90%, n (%)		9 (100)	77 (83.7)
Haemoglobin, median g/dl (IQR)		11.6 (10.5, 12.0)	13.4 (11.7, 14.7)
CD4 count, median cells/mm^3 (IQR)		463 (306, 628)	573 (390, 717)
HIV-1 RNA >40 copies/ml, n (%)		3 (33.3)	18 (19.6)
HIV-1 RNA >1000 copies/ml, n (%)		2 (22.2)	12 (13.0)
HBV DNA >15 IU/ml, n (%)		1 (11.1)	14 (15.4)
HBV DNA >2000 IU/ml, n (%)		0 (0)	6 (6.5)
Liver stiffness, median kPa (IQR)		4.8 (4.7, 8.8)	4.5 (3.8, 5.8)
Liver stiffness kPa >9.4, n (%)		2 (22.2)	5 (5.4)
AST, median IU/l (IQR)		26 (25, 37)	31 (23, 39)
ALT, median IU/l (IQR)		25 (14, 31)	24 (18, 35)
HBeAg positive, n (%)		1 (9.1)	10 (10.9)
Systolic BP, median mmHg (IQR)		140 (122, 160)	121 (110, 145)
Diastolic BP, median mmHg (IQR)		90 (76, 110)	80 (70, 90)
Hypertension, n (%)	Any grade	5 (55.6)	30 (32.6)
	Grade 1[b]	1 (11.1)	12 (13.0)
	Grade 2	1 (11.1)	11 (12.0)
	Grade 3	3 (33.3)	7 (7.6)
	NA	0 (0)	1 (1.1)
HB1Ac, median mmol/mol (IQR)		33 (32, 38)	33 (31, 38)
Diabetes, n (%)[c]		2 (22.2)	4 (4.5)
Schistosoma Ag positive, n (%)		3 (33.3)	15 (16.3)
Serum creatinine	median µmol/l (IQR)	77 (55, 82)	78 (67, 89)
eGFR	median ml/min (IQR)	112 (81.2, 130)	103 (92.6, 115)
≥90 ml/min	n (%)	6 (66.7)	73 (79.4)
60-89 ml/min	n (%)	2 (22.2)	16 (17.4)
30-60 ml/min	n (%)	1 (11.1)	3 (3.3)
change over time	median ml/min (IQR)	-2.77 (-7.93, -1.55)	-1.67 (-4.27, +0.29)
rapid decline (n=97)	n (%)	3 (37.5)	18 (20.2)
uPCR	median mg/mmol (IQR)	53 (39, 58)	13 (10, 19)
>20 mg/mmol	n (%)	9 (100)	19 (20.7)
≥50 mg/mmol	n (%)	5 (55.6)	8 (8.7)
Dipstick protein	n (%)	3 (33.3)	12 (13.0)
Dipstick RBC	n (%)	3 (33.3)	9 (9.8)
Dipstick glucose	n (%)	2 (22.2)	15 (16.3)

[a]Significant tubular proteinuria was defined by uPCR ≥30 mg/mmol and uACR/uPCR <0.4; [b]Comprising 4 subjects with normal readings while on anti-hypertensive medication; [c]Comprising 2 subjects with normal readings while on oral hypoglycaemic medication. IQR=inter-quartile range

BMI=body mass index; ART=antiretroviral therapy; TDF=tenofovir disoproxil fumarate; ZDV=zidovudine; d4T=stavudine; EFV=efavirenz; NVP=nevirapine; LPV/r=ritonavir-boosted lopinavir; cps=copies; AST=aspartate transaminase; ALT=alanine transaminase; BP=blood pressure; NA=not available; Hb1Ac=glycated haemoglobin; Ag=Antigen; RD-eGFR=rapid declining estimated glomerular filtration rate (≥5 ml/min eGFR decline per year); uPCR=urinary protein/creatinine ratio; uACR=urinary albumin/creatinine ratio; RBC=red blood cells.

Table 6-5 **Logistic regression analysis of factors associated with TuPr and RD-eGFR in HIV/HBV positive patients with long-term TDF exposure[a]**

		Factors associated with TuPr						Factors associated with RD-eGFR					
		Univariate analysis			Multivariable analysis[a]			Univariate analysis			Multivariable analysis[a]		
		OR	95% CI	p	OR	95% CI	p	OR	95% CI	p	OR	95% CI	p
Gender	female vs. male	9.52	1.20, 75.5	0.03	9.65	1.19, 78.5	0.03	1.87	0.62, 5.65	0.27			
Age	per 5 years older	1.14	0.81, 1.60	0.46				0.88	0.64, 1.21	0.43			
BMI	per kg/m^2 higher	1.00	0.90, 1.12	0.95				1.03	0.94, 1.14	0.50			
Duration HIV diagnosis	per year longer	0.96	0.77, 1.21	0.74				0.85	0.69, 1.06	0.15	0.83	0.66, 1.05	0.12
ART duration	per year longer	0.93	0.74, 1.17	0.53				0.90	0.73, 1.13	0.37			
TDF duration	per year longer	0.66	0.18, 2.42	0.53				0.80	0.26, 2.46	0.70			
Third antiretroviral	LPV/r vs. NNRTI	0.56	0.07, 4.78	0.60				5.63	1.36, 23.3	0.02	6.14	1.42, 26.5	0.02
Adherence	≥90% vs. <90%	2.54	0.31, 21.0	0.39				0.39	0.11, 1.35	0.14			
Haemoglobin	per g/dl higher	0.90	0.74, 1.10	0.30				0.84	0.68, 1.03	0.09			
CD4 count	per 50 $cells/mm^3$ higher	0.96	0.86, 1.06	0.42				1.02	0.93, 1.11	0.70			
HIV-1 RNA	per log_{10} copies/ml higher	1.10	0.70, 1.72	0.68				0.89	0.56, 1.42	0.63			
HBV DNA	per log_{10} IU/ml higher	0.88	0.45, 1.72	0.70				1.21	0.81, 1.82	0.36			
Liver stiffness	per one kPa higher	1.05	0.98, 1.14	0.16				1.04	0.97, 1.11	0.23			
AST	per 10 IU/l higher	0.75	0.49, 1.15	0.19				1.05	0.84, 1.31	0.64			
ALT	per 10 IU/l higher	1.06	0.83, 1.35	0.65				0.92	0.68, 1.25	0.61			
HBeAg	yes vs. no	0.52	0.06, 4.40	0.55				1.46	0.35, 6.08	0.61			
Hypertension	yes vs. no	3.93	1.29, 12.0	0.02	3.51	1.08, 11.4	0.04	0.69	0.24, 1.97	0.48			
Hb1Ac	per 5 mmol/mol higher	1.16	0.95, 1.42	0.14				1.06	0.89, 1.27	0.52			
Diabetes	yes vs. no	2.89	0.48, 17.3	0.25				1.89	0.32, 11.1	0.48			
Schistosoma Ag	positive vs. negative	2.52	0.75, 8.45	0.14				1.67	0.51, 5.42	0.40			

[a]Variables were identified for inclusion in the multivariable model using stepwise selection (p value entry and exit <0.2); the multivariable analysis of RD-eGFR excluded haemoglobin; OR=odds ratio; CI=confidence interval; BMI=body mass index; ART=antiretroviral therapy; TDF=tenofovir disoproxil fumarate; LPV/r=ritonavir-boosted lopinavir; NNRTI=non-nucleoside reverse transcriptase inhibitor; AST=aspartate transaminase; ALT=alanine transaminase; Hb1Ac=glycated haemoglobin; Ag=antigen.

Table 6-6 **Additional multivariable logistic regression analyses of factors associated with TuPr in HIV/HBV positive patients with long-term TDF exposure**

		Model 1[a]			Model 2[b]			Model 3[c]		
		OR	95% CI	p	OR	95% CI	p	OR	95% CI	p
Gender	female vs. male	13.4	1.28, 139	0.03	11.5	1.30, 101	0.03	9.19	1.21, 75.4	0.04
Liver stiffness	per kPa higher	1.09	0.94, 1.26	0.24	-	-	-	-	-	-
AST	per 10 IU/l higher	-	-	-	1.14	0.87, 1.50	0.34	-	-	-
Hypertension	yes vs. no	4.21	1.22, 14.5	0.02	4.26	1.30, 14.0	0.02	3.65	1.09, 12.3	0.04
HbA1c	per 5 mmol/mol higher	-	-	-	-	-	-	1.07	0.88, 1.30	0.52
Schistosoma Ag	positive vs. negative	1.18	0.26, 5.30	0.83	1.87	0.49, 7.14	0.36	1.95	0.51, 7.40	0.33

[a]Model 1 adjusted for gender, hypertension, *Schistosoma* Ag, and liver stiffness; [b]Model 2 adjusted for gender, hypertension, *Schistosoma* Ag, and AST level; [c]Model 3 adjusted for gender, hypertension, *Schistosoma* Ag, and HbA1c. OR=odds ratio; CI=confidence interval; AST=aspartate transaminase; Hb1Ac=glycated haemoglobin; Ag=antigen.

Changes in eGFR over time

At the last study visit, the eGFR was median 103 ml/min/1.73m^2 (IQR 92-116) and was <90 ml/min in 22/101 (21.8%) subjects, including 4/101 (4.0%) subjects with levels <30 ml/min (Table 6-2). The analysis of changes in eGFR over time comprised 90 subjects with data from three time points (T0, T1, T2) and seven subjects with data from two time points (T0 and T2). T0 occurred prior to TDF introduction (median -0.2 months; IQR -2.2, -0.1), whereas T1 and T2 occurred a median of 8.1 months (IQR 5.9-10.9) and 4.0 years (IQR 3.8-4.1) after TDF introduction, respectively. By univariate linear regression analysis, receiving LPV/r at T2 was associated with a larger eGFR decline, and the association persisted after adjusting for CD4 cell counts (Table 6-7). Of the total population on LPV/r, four subjects had started LPV/r prior to T0, five between T0 and T1, and one between T1 and T2. The univariate analysis indicated a trend for an association between changes in eGFR and ALT levels. A separate model adjusting for CD4 cell counts and ALT levels confirmed the independent association between LPV/r and eGFR decline (coefficient -3.31; 95% CI -5.87,-0.75; p=0.01); no other independent predictors were identified.

A diagnosis of RD-eGFR was made in 21/97 (21.6%) subjects. Patients with RD-eGFR were more likely to be receiving LPV/r, and the association persisted after adjustment (Table 6-5). Among the four subjects with eGFR <60 ml/min at T2, two had experienced RD-eGFR, whereas the other two had a low eGFR at T0. In the main logistic regression model, receiving LPV/r independently increased the risk of RD-eGFR after adjusting for duration of HIV diagnosis. A separate model also adjusting for adherence confirmed the findings with an odds ratio (OR) of 5.27 (95% CI 1.13-24.5; p=0.03).

Table 6-7 **Linear regression analysis of factors associated with changes in eGFR in HIV/HBV positive patients with long-term TDF exposure**

		Univariate analysis			Multivariable analysis[a]		
		Coefficient	95% CI	p	Coefficient	95% CI	p
Gender	female vs. male	-0.55	-2.14, +1.04	0.50			
Age	per 5 years older	+0.02	-0.07, +0.12	0.63			
BMI	per kg/m^2 higher	+0.04	-0.11, +0.20	0.57			
Duration HIV diagnosis	per year longer	-0.02	-0.36, +0.33	0.93			
ART duration	per year longer	-0.12	-0.46, +0.22	0.48			
TDF duration	per year longer	-1.16	-3.65, +1.33	0.36			
Third antiretroviral	LPV/r vs. NNRTI	-3.12	-5.64, -0.60	0.02	-3.51	-6.04, -0.98	0.01
Adherence	≥90% vs. <90%	+1.12	-1.10, +3.34	0.32			
Haemoglobin	per g/dl higher	+0.13	-0.15, +0.41	0.35			
CD4 count	per 50 cells/mm^3 higher	-0.09	-0.23, +0.05	0.20	-0.12	-0.26, +0.01	0.08
HIV-1 RNA	per log_{10} copies/ml higher	+1.02	-1.11, +3.16	0.34			
HBV DNA	per log_{10} IU/ml higher	-0.22	-0.95, +0.51	0.55			
Liver stiffness	per kPa higher	+0.10	-0.24, +0.44	0.56			
AST	per 10 IU/l higher	-0.10	-0.47, +0.27	0.61			
ALT	per 10 IU/l higher	+0.31	-0.13, +0.76	0.17			
HBeAg	yes vs. no	-0.52	-2.91, +1.87	0.67			
Hypertension	yes vs. no	-0.01	-1.59, +1.57	0.99			
Hb1Ac	per 5 mmol/mol higher	+0.02	-0.13, +0.16	0.83			
Diabetes	yes vs. no	-0.61	-3.74, +2.52	0.70			
Schistosoma Ag	positive vs. negative	-0.36	-2.35, +1.62	0.72			

[a]All variables with p<0.20 in the univariate analysis were considered for inclusion using a stepwise selection with p of exit <0.2; CI=confidence interval; BMI=body mass index; ART=antiretroviral therapy; LPV/r=ritonavir-boosted lopinavir; NNRTI=non-nucleoside reverse-transcriptase inhibitor; AST=aspartate transaminase; ALT=alanine transaminase; Hb1Ac=glycated haemoglobin.

6.3.3 Relationship between TuPr, eGFR, and urinary dipstick results

Median eGFR and prevalence of eGFR <90 ml/min were similar in subjects with and without TuPr (Table 6-3). Changes in eGFR were slightly greater in subjects with TuPr (median -2.4 ml/min/year) than in those without TuPr (median -1.7 ml/min/year). As a result, a diagnosis of RD-eGFR was made in a slightly larger proportion of subjects with TuPr (4/16; 25.0%) than in those without TuPr (17/85; 20.0%). Patients with TuPr showed increased prevalence of dipstick haematuria, and to a lesser extent increased prevalence of dipstick proteinuria. The ROC analysis indicated poor agreement between TuPr and dipstick proteinuria, glycosuria and haematuria, with an area under the curve (r) of 0.56, 0.55, and 0.61, respectively (Figure 6-1). A diagnosis of RD-eGFR was not associated with clear dipstick patterns, although prevalence of glycosuria was higher than in patients without RD-eGFR. A detailed summary of urinary dipstick results in relation to a diagnosis of TuPr, RD-GFR, hypertension, diabetes, or *Schistosoma* Ag positivity is presented in Table 6-8.

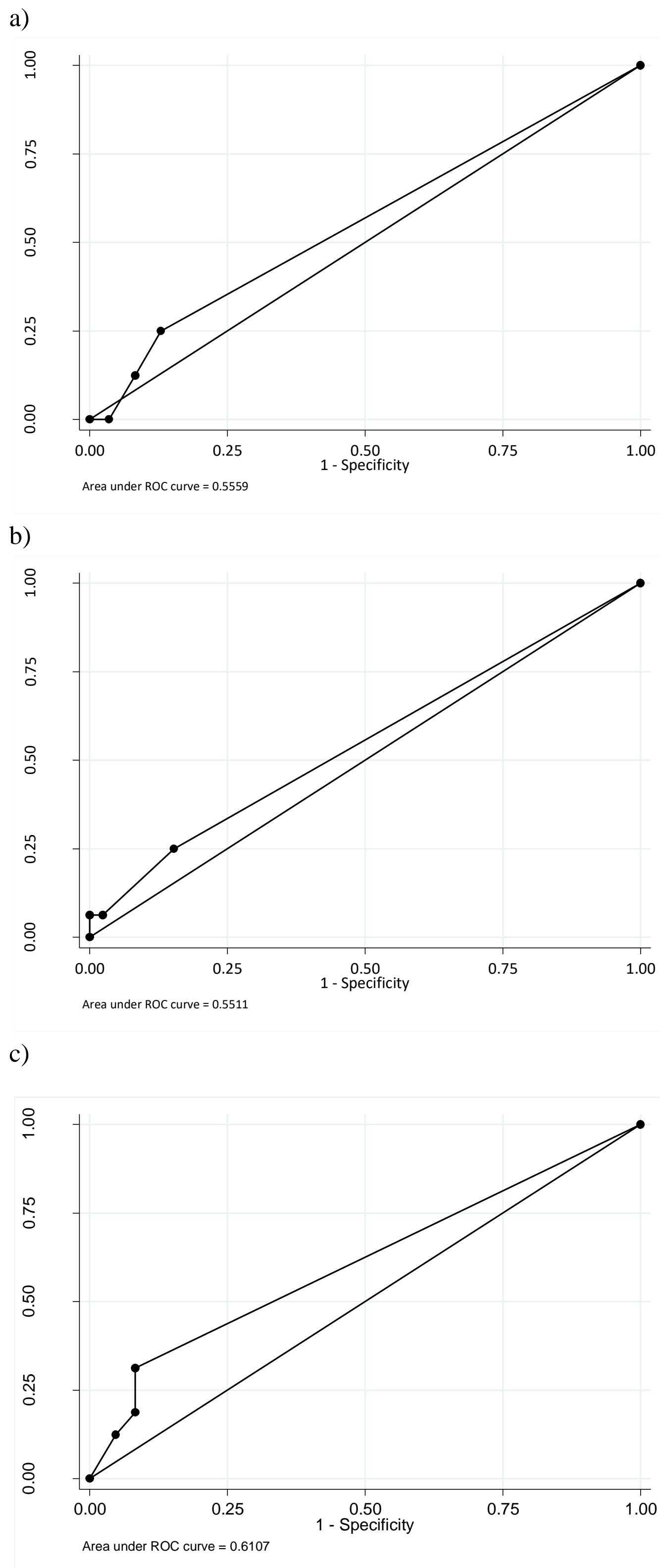


Figure 6-1 **ROC analysis on the performance of dipstick proteinuria (a), dipstick glycosuria (b) and dipstick haematuria (c) as predictors of TuPr**

Table 6-8 **Urinary dipstick in relation to a diagnosis of TuPr, RD-eGFR, hypertension, diabetes, and positive urinary Schistosoma antigen**

		TuPr	RD-eGFR	Hypertension	Diabetes	*Schistosoma* Ag$^+$
		N=16	N=21	N=35	N=6	N=18
Haematuria	trace	2 (12.5)	0 (0)	1 (2.9)	0 (0)	1 (5.6)
	+	1 (6.3)	1 (4.8)	2 (5.7)	0 (0)	2 (11.1)
	++	2 (12.5)	2 (9.5)	5 (14.3)	1 (16.7)	2 (11.1)
Glycosuria	trace	3 (18.8)	4 (19.1)	8 (22.9)	1 (16.7)	4 (22.2)
	+	0 (0)	1 (4.8)	2 (5.7)	2 (33.3)	1 (5.6)
	++	1 (6.3)	0 (0)	1 (2.9)	1 (16.7)	0 (0)
Proteinuria	trace	2 (12.5)	1 (4.8)	4 (11.4)	1 (16.7)	0 (0)
	+	2 (12.5)	1 (4.8)	4 (11.4)	0 (0)	4 (22.2)
	++	0 (0)	1 (4.8)	3 (8.6)	1 (16.7)	1 (5.6)

Haematuria (RBC/μL): Trace 5-10, + 50, ++ 250; glycosuria (mmol/L): Trace ≤1.1, + ≤2.8; ++ ≤ 55.5; proteinuria (g/L): Trace=≤0.3, +≤1, ++≤ 5. RBC=red blood cells; TuPr=tubular proteinuria; RD-eGFR=rapidly declining estimated glomerular filtration rate; Ag=antigen; RBC=red blood cells.

6.4 DISCUSSION

Among HIV/HBV co-infected subjects on long-term TDF-containing ART prevalence of TuPr was 15.8%, and the risk was highest among women and those with hypertension. There was an overall modest eGFR decline over time (1.8 ml/min per year of TDF), but 21.6% of participants experienced a more pronounced decline (≥5 ml/min per year). Despite the small number of patients on LPV/r, a strong association was detected between a larger eGFR decline and receiving LPV/r. There was limited overlap between TuPr and RD-eGFR, pointing at the different impact of promoting factors. In addition, there was no clear evidence of an effect of HBV disease activity on the two renal markers, as expressed by HBV DNA load, HBeAg status, transaminase levels, and liver stiffness. Urinary dipstick failed to accurately predict TuPr, although was able to point to important co-morbidities (i.e., proteinuria with hypertension and schistosomiasis, glycosuria with hypertension and diabetes).

Previous studies conducted predominantly in HIV-positive patients of white ethnicity reported tubular dysfunction in 7-22% of subjects receiving TDF-containing ART for up to nearly five years.[283-286] Prevalence of TuPr in the Kumasi cohort after a median of four years of TDF was close to the upper limit of the reported range, and hypertension increased the risk by over four-fold. There is a growing burden of hypertension across West Africa, and whilst the determinants remain to be fully established, the attributable mortality is estimated to have increased by over 100% between 1990 and 2015.[287] The prevalence of diagnosed hypertension can reach 54% in the general population[288] and hypertension is estimated to account for 32% of all cases of CKD in Ghana.[289] A third of patients in our study had hypertension, although only a few reported a previous diagnosis and fewer still were receiving anti-hypertensive medication. A similar high rate of untreated hypertension among HIV-positive patients has been reported from other regions of SSA.[290] While we found no evidence of related clinical events among patients still attending for care, the findings clearly highlight the urgent need to introduce routine blood pressure screening in African HIV care settings.

There was also a strong association between TuPr and female gender, although the large confidence interval prevented an accurate estimation of the magnitude of the

risk. Previous studies investigating factors associated with tubulopathy in HIV-positive subjects did not identify an effect of gender but included predominantly Caucasian males.[284,291,292] An association between female gender and risk of renal disease has been described, which may reflect the influence of sex hormones on several biological processes involved in kidney injury.[293] It could also be speculated that greater adherence or lower body weight among women may have increased TDF exposure relative to men, increasing the risk of tubulopathy. In SSA, women have been reported to have greater adherence to ART than men[232] and a similar trend was present in our cohort, with adherence rates ≥90% by visual scale reported in 91% of women and 79% of men respectively. The BMI however did not show an association with renal abnormalities, being higher in women than in men (24.2 vs. 21.4 km/m^2). Data from bigger cohorts are needed to confirm the role of gender in increasing the risk of tubular proteinuria among HIV-positive people on TDF-containing ART.

A previous study of the general population in the same region of Ghana showed that approximately 2% of adults (mean age 55 years) had an eGFR <60 ml/min.[294] This compares with a prevalence of 4% in our study, where the mean age was 45 years, suggesting a greater burden of renal disease. It should be noted that after a median of four years of exposure to TDF, there was only a modest decline in eGFR, which is in line with the reported overall good safety profile of TDF.[295] Importantly, and consistent with previous data,[269,271] there was evidence that the eGFR decline was greater in patients receiving LPV/r. Concomitant treatment with LPV/r may indirectly increase the risk of renal damage by boosting TDF exposure through reduced excretion or increased reabsorption, whilst other PIs may have a more direct nephrotoxic potential.[296] As the impact of concomitant PI may differ by type, it will be important to monitor the relative impact of atazanavir and darunavir, which are becoming available across SSA.[297]

CKD is estimated to have an overall prevalence of 14% across populations of SSA, although attention has been drawn to the poor quality of the data and the need for more information using validated measures of kidney function.[288] In addition to the direct effect of poorly controlled HIV, rising prevalence of CKD in SSA may be fuelled by increasing urbanisation, dietary changes, and growing rates of tobacco consumption, obesity, diabetes and hypertension acting on a background of longer life-expectancy[288] and genetic predisposition to renal disease.[298] There are limited data suggesting that

diabetes is a significant contributor to renal disease in HIV-positive African cohorts.[288,299] Although numbers were small, in our study there appeared to be a role for diabetes as a determinant of renal abnormalities, again pointing at the importance of screening for co-morbidities in HIV care settings.

A previous study suggested a role for HBV co-infection in increasing the risk of renal disease in Zambian HIV-positive adults.[276] Reassuringly, we detected no indication that HBV status increased the risk of renal function abnormalities. Among infectious co-morbidities with a potential impact on renal health, high rates of *Schistosoma* infection may play a role in Ghana. In Kumasi, prevalence rates of 21% have been described among hospital attendees, although varying considerably according to likelihood of exposure to contaminated water.[300] *S. haematobium,* which causes chronic infection of the urinary tract tends to prevail over *S. mansoni*,[301] although immunological-mediated impairment of glomerular and tubular function has also been associated with infection by *S. mansoni*.[302] Our data documented a reactive CCA-test in 17.8% of subjects, which suggest a high burden of infection. A reactive CCA-test was often accompanied by dipstick proteinuria and haematuria, and carried a 2.5-fold increase in the odds of tubular proteinuria. Data are needed to ascertain the impact of specific *Schistosoma* treatment on urinary findings.

There are limitations of this study. As it is often the case with cohorts in SSA, we observed a significant loss to follow-up (26%) and a documented mortality rate of at least 8% over five years. While some subjects may have moved to a different part of the country, most loss is believed to reflect undocumented mortality. Patients with severe renal impairment or complications of undiagnosed hypertension or diabetes might have died, leading to an underestimation of the burden of disease. Our data therefore should be interpreted as providing estimates for patients who continued to engage with HIV care. Tests not available in Kumasi were performed on frozen samples and sample volume restricted the number of tests. Ideally, additional measures of tubular function and explorative biomarkers of renal function might have been considered, while more frequent measurements of eGFR over time may have allowed increased confidence in the estimates. Further, a diagnosis of hypertension was based on two separate measurements on the same day, and repeated measurements over time would have improved diagnostic accuracy. Overall small study numbers meant that confidence intervals were wide, although associations were

controlled for carefully. Despite these limitations, the data have important implications for the management of HIV-positive patients in SSA, and identify several important research needs. Among subjects retained into care, no substantial toxicities caused by long-term TDF exposure were detected, especially in the context of EFV-containing ART. Attention should be paid to optimising blood pressure control, starting from the introduction of routine blood pressure monitoring. Regular measurements of eGFR should also be introduced, and prioritised for patients receiving TDF with a booster. The use of urinary dipstick was effective in detecting evidence of hypertension, diabetes and possible schistosomiasis, and should be included in routine care to improve diagnosis and management of prevalent co-morbidities. In our cohort, one in five individuals had detectable HIV viral load, typically coinciding with a detectable HBV DNA, and optimising control of virus replication remains a key priority. For patients with renal toxicity, where HBV co-infection and lack of entecavir make TDF discontinuation undesirable, reducing the dose of TDF could potentially improve renal safety.[303] The potential cost-benefits of enabling access to TAF for HIV-positive patients in SSA who are at risk of progressive renal dysfunction remain to be determined.

Box of recommendations

In HIV/HBV-positive cohorts from sub-Saharan Africa on long-term exposure to TDF:

1. **Prioritise screening for renal toxicity patients on second-line with boosted-PIs;**
2. **Implement use of urinary dipstick in routine clinical practise;**
3. **Screen routinely for hypertension and treat for hypertension.**

7 Seventh Chapter - Liver steatosis and fibrosis in HIV-positive adults with and without hepatitis B virus co-infection accessing programmatic care in sub-Saharan Africa

7.1 Introduction

Non-alcoholic fatty liver disease (NAFLD), defined as evidence of liver steatosis in the absence of known excess alcohol consumption, is predicted to become the leading cause of liver transplantation in the next decade.[304] Pooled data estimate a global prevalence of NAFLD in the general population of around 25%.[305] Estimates are higher for South America (31%) and the Middle East (32%) and lower for Africa (14%), mirroring the distribution of high body mass index (BMI)[306] and a range of metabolic disorders associated with NAFLD, including a high BMI, central obesity, insulin resistance, dyslipidaemia, and hypertension.[305] In addition to liver fibrosis and related complications, NAFLD is a predictor of cardiovascular disease and mortality.[307]

Multiple factors may contribute to an increased risk of liver steatosis in the context of HIV infection. A systematic review and meta-analysis of 10 cross-sectional studies from North America, Western Europe, China and Japan estimated that 35% of primarily male HIV-positive patients met the definition of NAFLD based on imaging or liver histology.[308] Both metabolic disorders and high CD4 cell counts were associated with NAFLD, whereas other HIV-related parameters including viral load, duration of HIV infection, duration of antiretroviral therapy (ART) and nadir CD4 cell count were not.[308] A study from Brazil similarly reported that 35% of HIV-positive patients had NAFLD and, independently of metabolic disorders, an association was detected between NAFLD and exposure to the antiretroviral agents zidovudine (AZT), stavudine (d4T), didanosine (ddI) and zalcitabine (ddC).[309] Despite growing rates of

liver-related mortality, there are scarce data from HIV-positive populations in sub-Saharan Africa.[24] Studies that analysed liver biopsies from HIV-positive patients in South Africa reported a prevalence of liver steatosis of around 19-28%,(7,8) and indicated that the prevalence of steatosis in HIV-positive patients was more than double that observed in HIV-negative controls.[310,311]

Transient elastography (TE) provides a valuable tool for the non-invasive assessment of liver fibrosis in resource-limited settings. Stockdale et al have already reported on the use of TE to assess variations in liver stiffness in HIV/HBV co-infected subjects on treatment with HBV at the same centre.[138] Measuring the controlled attenuation parameter (CAP) can be performed at the same time to provide a measure of liver steatosis.[312] In comparative studies, CAP showed good concordance with liver biopsies in both HIV-negative and HIV-positive populations.[312,313] The aim of this study was to assess the liver health of the HIV cohort attending for outpatient care by measuring prevalence and determinants of liver steatosis and fibrosis, and prevalence of viral hepatitis coinfections in HIV-positive individuals established on long-term ART in a programmatic care setting in Ghana. The analysis took into account life-style factors, HIV-related parameters, metabolic status, and evidence of co-infection with hepatitis B virus (HBV), hepatitis delta virus (HDV), or hepatitis C virus (HCV). Given the high prevalence of HBV in Ghana,[129,132] a specific focus was placed on investigating liver steatosis and fibrosis in HIV-positive patients with and without HBV co-infection.

7.2 METHODS

7.2.1 Study population

This cross-sectional study took place at the Komfo Anokye Teaching Hospital (KATH), a 1200-bed facility in the city of Kumasi and the second-largest hospital in Ghana, serving a population of around 10 million people in the Ashanti Region. The study received ethical approval from the Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. Participants provided written informed consent. During two weeks in February 2018, consecutive adult (≥18 years old) attendees of four HIV clinics were invited to participate. Whilst the prevalence of HBV co-infection is ~16% in the Kumasi HIV cohort,[132] enrichment for HBsAg positive patients was achieved by targeting a specific research clinic that offered point of care hepatitis B surface antigen (HBsAg) testing. Participants were administered a structured questionnaire by local trained assistants, which collected data on any smoking history (either current or past), alcohol intake, use of traditional or herbal remedies, and knowledge of any concomitant morbidity or treatment other than ART. Excessive alcohol consumption was defined as drinking more than once a week in moderate to large quantities. Clinical data were retrieved from the medical records. Height, weight and waist circumference were measured by standard methods.[314] Blood pressure (BP) was measured with a manual sphygmomanometer and abnormal findings were confirmed after the patient had rested for at least 20 minutes. CAP and TE were measured using Fibroscan (Ecosens, France) at least 2.5 hours after the last intake of food.

7.2.2 Laboratory investigations

Participants underwent collection of venous blood for local testing, and for storage of whole blood, serum and plasma at -80ºC. Full blood cell counts and CD4 cell counts were measured at the KATH diagnostic laboratory. Plasma HIV-1 RNA was quantified on site using Xpert HIV-1 Viral Load (Cepheid, Sunnyvale, US). Aliquots of plasma, serum, and whole blood were shipped frozen to the United Kingdom (UK) for further testing. HBsAg was measured in serum by Architect (Abbot Diagnostics,

Sligo, Ireland). HBV DNA was quantified in plasma using Xpert HBV Viral Load (Cepheid). Total HDV antibodies (anti-HDV) were measured in serum by enzyme immune assay (EIA) (LaunchDiagnostics Limited, Longfield, UK); HDV RNA was detected in plasma at the accredited diagnostic laboratory Micropathology (Coventy, UK) using a real-time assay that targets the ribozyme region. Pools of 10 plasma samples were prepared using 100 µL per samples and screened for HCV RNA with Xpert HCV Viral Load (Cepheid, Sunnyvale, US), followed by testing of individual samples of HCV RNA positive pools. Serum alanine and aspartate aminotransferases (ALT and AST), total cholesterol (TC), high-density and low-density lipoprotein cholesterol (HDL and LDL), non-HDL cholesterol, triglycerides, and creatinine were measured at the accredited diagnostic laboratory of the Royal Liverpool University Hospital (Liverpool, UK). The estimated glomerular filtration rate (eGFR) was calculated from serum creatinine with the Chronic Kidney Disease Epidemiology Collaboration (EPI-CKD) equation.[315] Glycated haemoglobin (HbA1c) was measured in whole blood in sodium fluoride in the same laboratory using either ion exchange high performance liquid chromatography or boronate affinity with fluorescence detection if the first test yielded invalid results.

7.2.3 Definitions and grading

Based on the BMI, patients were categorised as underweight (<18.5 kg/m^2), normal weight (18.5-24.9 kg/m^2), overweight (25.0-29.9 kg/m^2), or obese (>30.0 kg/m^2). Central (abdominal) obesity was defined as a waist circumference >94cm for men and >80cm for women.[316] Hypertension was graded as 1 (systolic 140-159 or diastolic 90-99 mmHg), 2 (systolic 160-179 or diastolic 100-109 mmHg), and 3 (systolic ≥180 or diastolic ≥110 mmHg).[279] A systolic BP <140 and diastolic BP <90 mmHg in a patient on antihypertensive therapy was graded as 1. Hypercholesterolaemia was graded as 1 (TC 5.18 to <6.19 mmol/L), 2 (6.19 to <7.77 mmol/L), and 3 (≥7.77 mmol/L). Raised LDL was graded as 1 (3.37 to <4.12 mmol/L), 2 (4.12 to <4.90 mmol/L), and 3 (≥4.90 mmol/L).[279] A low HDL was defined as a level <1.03 mmol/L in males and <1.29 mmol/L in females. Hypertriglyceridaemia was graded as 1 (1.71 to 3.42 mmol/L), 2 (>3.42 to 5.7 mmol/L), 3 (>5.7 to 11.4 mmol/L), and 4 (>11.4 mmol/L). Normal TC and triglycerides in patients that were receiving lipid-lowering therapy were graded

as 1. Diabetes was defined by HbA1c ≥48mmol/mol or a record of antidiabetic therapy; impaired glucose regulation was defined by HbA1c 42-47 mmol/mol. Metabolic syndrome was defined as central obesity or BMI ≥30 kg/m^2 plus ≥2 of [grade 1 or above hypertriglyceridaemia, low HDL cholesterol, increased systolic or diastolic BP ≥130/85 mmHg, respectively, or specific antihypertensive treatment, diabetes or impaired glucose regulation]. Based on CAP values, liver steatosis was graded as absent or S0 (<248 dB/m), mild or S1 (248-268 dB/m), moderate or S2 (268-280 dB/m) and severe S3 (>280 dB/m).[317] Based on TE values, fibrosis was graded as F0-F1, F2, F3 and F4; interpretative cut-offs for histologically-defined METAVIR scores were those previously determined for HIV/HBV co-infected patients (5.9 kPa, 7.6 kPa, and 9.4 kPa for F2, F3, and F4, respectively)[278] and HIV mono-infected patients (7.1 kPa, 9.4 kPa and 14.0 kPa).[318]

7.2.4 Statistical analysis

The characteristics of the study population according to HBsAg status were compared with X^2, Fisher's exact, or Mann Whitney U test, as appropriate. Factors associated with liver steatosis (expressed as CAP values) and liver fibrosis (expressed as TE values) were explored by linear regression analyses after natural log-transformation of the variable; coefficients were exponentiated to obtain odds ratios (OR). Robustness of the models was evaluated by standard post-estimation tests (variance inflation factors, residual-vs-fitted plots). A stepwise selection with $p<0.2$ for model entry and exit was used for inclusion in the multivariable model. The main model that explored factors associated with liver steatosis was adjusted for eGFR, cumulative exposure to stavudine, and metabolic syndrome. In separate sensitivity analysis, central obesity (or BMI), hypertension, HDL, triglycerides, and HbA1c replaced metabolic syndrome for the multivariable adjustment. Markers of liver health (ALT, AST, platelets) were not considered for inclusion in the multivariable models; liver stiffness was not included in the multivariable model of factors exploring liver steatosis as it lies in the causal pathway of the outcome. Statistical analyses were performed with STATA software, version 14 (StataCorp Inc, College Station, Texas, USA).

7.3 RESULTS

7.3.1 Study population

A total of 340 consecutive clinic attendees were invited to take part in the study and all consented. A valid CAP and TE was obtained in 329/340 (96.8%) subjects, which constituted the study population (Table 7-1). Eleven subjects (all females, median BMI 23.5 kg/m^2, median waist circumference 93.0 cm) were excluded from the analysis due to invalid TE measurements. Most participants were female (238/329, 72.3%) with a median age of 47 years (IQR 42-53), and only a small subset reported excessive alcohol consumption (6/329, 1.8%), cigarette smoking (20/329, 6.1%), or use of herbal or traditional remedies (11/329, 3.3%) (Table 7-1).

Table 7-1 **Participants' characteristics according to HBsAg status**

Characteristic		Total	HBsAg negative	HBsAg positive	p
Total, n (%)		329 (100)	239 (100)	90 (100)	-
Male gender, n (%)		91 (27.7)	60 (25.1)	31 (34.4)	0.09
Age, median years (IQR)		47 (42-53)	48 (41-54)	47 (42-52)	0.90
Cigarette smoking, n (%)		20 (6.1)	14 (5.9)	6 (6.7)	0.80
Excessive alcohol use, n (%)		6 (1.8)	4 (1.7)	2 (2.2)	0.67
Herbal or traditional remedies, n (%)		11 (3.3)	7 (2.9)	4 (4.4)	0.50
Time on ART, median years (IQR)		8.9 (5.7-11.3)	8.7 (4.9-11.2)	9.6 (6.9-11.3)	0.07
Current ART, n (%)	NNRTI-based	287 (87.2)	205 (85.8)	82 (91.1)	0.47
	PI-based	35 (10.6)	28 (11.7)	7 (7.8)	
	ART-naïve or off ART	7 (2.1)	6 (2.5)	1 (1.1)	
ART regimen, n (%)	TDF 3TC EFV	150 (45.6)	83 (34.7)	67 (74.4)	-
	TDF 3TC NVP	10 (3.0)	7 (2.9)	3 (3.3)	
	TDF 3TC ATV/r	2 (0.6)	2 (0.8)	0 (0)	
	TDF 3TC LPV/r	18 (5.5)	14 (5.9)	4 (4.4)	
	AZT 3TC EFV	58 (17.6)	54 (22.6)	4 (4.4)	
	AZT 3TC NVP	69 (21.0)	61 (25.5)	8 (8.9)	
	AZT 3TC ATV/r	1 (0.3)	1 (0.4)	0 (0)	
	AZT 3TC LPV/r	9 (2.7)	7 (2.9)	2 (2.2)	
	ABC 3TC AVT/r	1 (0.3)	1 (0.4)	0 (0)	
	Other	4 (1.2)	3 (1.3)	1 (1.1)	
Cumulative exposure, median years (IQR)	d4T	0 (0-1.13)	0 (0-0.71)	0 (0-1.78)	0.08
	AZT	3.14 (0-8.00)	5.41 (0-8.41)	2.05 (0-4.78)	<0.01
	TDF	0.84 (0-6.00)	0 (0-2.44)	6.12 (1.86-6.38)	<0.01
	EFV	3.12 (0-8.29)	1.78 (0-7.42)	5.88 (1.86-9.11)	<0.01
	NVP	0 (0-5.55)	0 (0-6.35)	0 (0-4.57)	0.58
HIV-1 RNA[1], median log_{10} copies/ml (IQR)		1.5 (1.3-2.6)	1.6 (1.3-2.7)	1.3 (1.3-2.4)	0.67
HIV-1 RNA <40 copies/ml, n (%)		162 (49.2)	114 (47.7)	48 (53.3)	0.46
CD4 cell count, median cells/mm^3 (IQR)		619 (358-830)	602 (349-829)	663 (390-840)	0.32
HBV DNA[2] median IU/ml (IQR)		-	2 (2-20)	-	
HBV DNA, IU/mL	<40	74 (22.5)	-	74 (82.2)	

	40-2,000	9 (2.7)	-	9 (10.0)	
	2,000-20,000	2 (0.6)	-	2 (2.2)	
	>20,000	5 (1.5)	-	5 (5.6)	
Metabolic syndrome, n (%)		79 (24.0)	61 (25.5)	18 (20.0)	0.30
BMI, median kg/m^2 (IQR)		23.9 (20.8-27.1)	23.9 (21.0-26.9)	23.8 (20.8-27.9)	0.96
Waist circumference, median cm (IQR)	male	82 (76-88)	82 (77-88)	82 (75-88)	0.94
	female	88 (80-96)	89 (80-96)	86 (77-98)	0.74
Central obesity, n (%)		198 (60.2)	150 (62.8)	48 (53.3)	0.12
Systolic blood pressure, median mmHg (IQR)		128 (113-144)	130 (113-147)	125 (110-140)	0.14
Diastolic blood pressure, median mmHg (IQR)		82 (71-91)	82 (72-92)	82 (71-90)	0.88
Total cholesterol, median mmol/L (IQR)		4.7 (4.1-5.4)	4.8 (4.1-5.5)	4.4 (4.0-5.1)	0.01
LDL, median mmol/L (IQR)		2.7 (2.2-3.4)	2.8 (2.2-3.4)	2.6 (2.2-3.2)	0.12
HDL, median mmol/L (IQR)		1.3 (1.1-1.6)	1.3 (1.0-1.6)	1.3 (1.1-1.6)	0.74
Total cholesterol:HDL ratio, median units (IQR)		3.6 (2.9-4.6)	3.7 (3.0-4.6)	3.4 (2.8-4.3)	0.07
Non-HDL cholesterol, median mmol/l (IQR)		3.4 (2.7-4.0)	3.4 (2.8-4.2)	3.1 (2.6-3.6)	0.01
Triglycerides, median mmol/L (IQR)		1.2 (0.9-1.6)	1.2 (0.9-1.7)	1.1 (0.8-1.4)	<0.01
Glycated haemoglobin, median mmol/mol (IQR)		33 (29-37)	33 (29-37)	34 (31-37)	0.20
Platelet count, median platelets x10^9/L (IQR)		231 (195-277)	237 (204-285)	209 (168-255)	<0.01
AST, median IU/L (IQR)		27 (23-34)	26 (22-33)	30 (25-37)	<0.01
ALT, median IU/L (IQR)		18 (14-24)	17 (13-22)	21 (15-29)	<0.01
Liver stiffness, median kPa (IQR)		4.9 (4.0-6.0)	4.9 (3.9-5.9)	4.9 (4.2-6.4)	0.18
Fibrosis grade, n (%)	F0-F1	274 (83.3)	217 (90.8)	57 (63.3)	<0.01
	F2	38 (11.6)	17 (7.1)	21 (23.3)	
	F3	12 (3.7)	4 (1.7)	8 (8.9)	
	F4	5 (1.5)	1 (0.4)	4 (4.4)	
CAP, median dB/m (IQR)		207 (175-240)	206 (172-239)	214 (181-242)	0.33
Steatosis grade, n (%)	S0	260 (79.0)	189 (79.1)	71 (78.9)	0.93
	S1	26 (7.9)	20 (8.4)	6 (6.7)	
	S2	20 (6.1)	14 (5.9)	6 (6.7)	
	S3	23 (7.0)	16 (6.7)	7 (7.8)	

[1]A value of 5 copies/ml was assigned for patients with HIV RNA <LLD and a value of 20 copies/ml for patients with HIV RNA <LLQ; HIV RNA was quantified for patients on ART only; [2]a value of 2 IU/ml was assigned for patients with HBV DNA <LLD and a value of 20 copies/ml for patients with HBV DNA <LLQ

HBsAg=hepatitis B virus surface antigen; BMI=body mass index; LDL=low density lipoprotein; HDL=high density lipoprotein; $eGFR_{EPI-CKD}$=estimated glomerular filtration rate according to the EPI-CKD formula; HCV=hepatitis C virus; HBV=hepatitis B virus; LLD=lower limit of detection; LLQ=lower limit of quantification; AST=aspartate aminotransferase; ALT=alanine aminotransferase; CAP=controlled attenuation parameter; ART=antiretroviral treatment; NRTI=nucleotide reverse transcriptase inhibitor; PI=protease inhibitor; TDF=tenofovir disoproxil fumarate; 3TC=lamivudine; AZT=zidovudine; ABC=abacavir; EFV=Efavirenz; NVP=Nevirapine; LPV/r=ritonavir-boosted lopinavir; ATV/r=ritonavir-boosted atazanavir; d4T=stavudine; NA=not applicable

7.3.2 HIV status

Participants had received ART for a median of 8.9 years and were mainly on a first-line regimen with two NRTIs and an NNRTI (288/330, 87.3%) (Table 7-1). The NRTIs comprised predominantly TDF/3TC in 180/329 (54.7%) and AZT/3TC in 137/329 (41.6%); 114/329 (34.7%) had a history of previous exposure to stavudine. Virological suppression was suboptimal; of those on ART (322/329, 97.9%), nearly half (160/322, 49.7%) showed a detectable plasma HIV-1 RNA (>40 copies/ml), with a median viral load in this group of 2.6 log_{10} copies/ml (IQR 2.0-4.4). CD4 counts were relatively preserved in the study population with a median 619 cells/mm^3.

7.3.3 Viral hepatitis co-infection

Overall, 90/329 (27.4%) patients tested HBsAg positive, including 61/90 (88.4%) with a previous HBV diagnosis. Of these, 14/90 (15.6%) were receiving lamivudine alone and 75/90 (83.3%) were receiving tenofovir disoproxil fumarate (TDF) plus lamivudine as part of their ART regimen, whereas 1/90 (1.1%) had discontinued all ART more than 3 months prior to the study visit. Plasma HBV DNA was quantified (>40 IU/ml) in 16/90 (17.8%) individuals, with a median viral load in this group of 1244 IU/ml (IQR 216-71920). There were 4/90 (4.4%) HBsAg-positive patients with a positive (n=3) or indeterminate (n=1) anti-HDV result; all however showed undetectable (<50 copies/ml) HDV RNA. HCV RNA was detected in 1/329 (0.3%) patients with a viral load of 18,700,000 IU/ml.

7.3.4 Metabolic syndrome

Overall, 79/329 (24.0%) patients met a diagnosis of metabolic syndrome. Based on BMI, 92/329 (28.0%) and 41/329 (12.5%) patients were either overweight or obese, whereas 198/329 (60.2%) had central obesity based on waist circumference (Tables 7-1 and 7-2).

Table 7-2 **Comorbidities and their grading of severity in the study population**

n (%)										
	Hypertension	TC	LDL	Triglycerides	Fibrosis	Steatosis	BMI		Glucose regulation	
Grade 0	182 (55.3)	218 (66.3)	242 (73.6)	261 (79.3)	274 (83.0)	260 (79.0)	Underweight	31 (9.4)	Normal	295 (89.7)
Grade 1	80 (24.3)	78 (23.7)	54 (16.4)	55 (16.7)	38 (11.6)	26 (7.9)	Normal	165 (50.2)	Impaired	15 (4.6)
Grade 2	33 (10.1)	27 (8.2)	18 (5.5)	10 (3.0)	12 (3.70	20 (6.1)	Overweight	92 (28.0)		
Grade 3/4	27 (8.2)	6 (1.8)	9 (2.7)	2[1] (0.6)	5 (1.5)	23 (7.0)	Obese	41 (12.5)	Diabetes	17 (5.2)
No data	7 (2.1)	0 (0)	6 (1.8)	1 (0.3)	0 (0)	0 (0)	No data	0 (0)	No data	2 (0.6)

[1]no patient presented grade 4; [2]1 patient presented grade 4

Hypertension of any grade was diagnosed in nearly half of the cohort (140/329, 42.6%), and 57/329 (17.3%) subjects were taking antihypertensive medication (nifedipine, losartan, amlodipine, bendroflumethiazide, lisinopril, methyldopa, or valsartan). Nearly half of the cohort (159/329, 48.3%) had either hypercholesterolemia and/or hypertriglyceridaemia; only one patient was on therapy with atorvastatin. Overall, 15/329 (4.6%) subjects showed impaired glucose regulation, whereas 17/329 (5.2%) had diabetes (Table 7-2).

7.3.5 Liver steatosis and fibrosis status

The median CAP was 207 dB/m (IQR 175-240), whereas the median TE was 4.9 (IQR 4.0-6.0) (Table 1). Liver steatosis and liver fibrosis of any grade were diagnosed in 69/329 (21.2%) and 55/329 (16.7%) subjects, respectively (Table 7-2). None of the patients with liver cirrhosis showed signs of clinical decompensation on examination. Prevalence of liver steatosis in the absence of excessive alcohol consumption, with or without fibrosis, and according to HBsAg status, is reported in Figure 7-1.

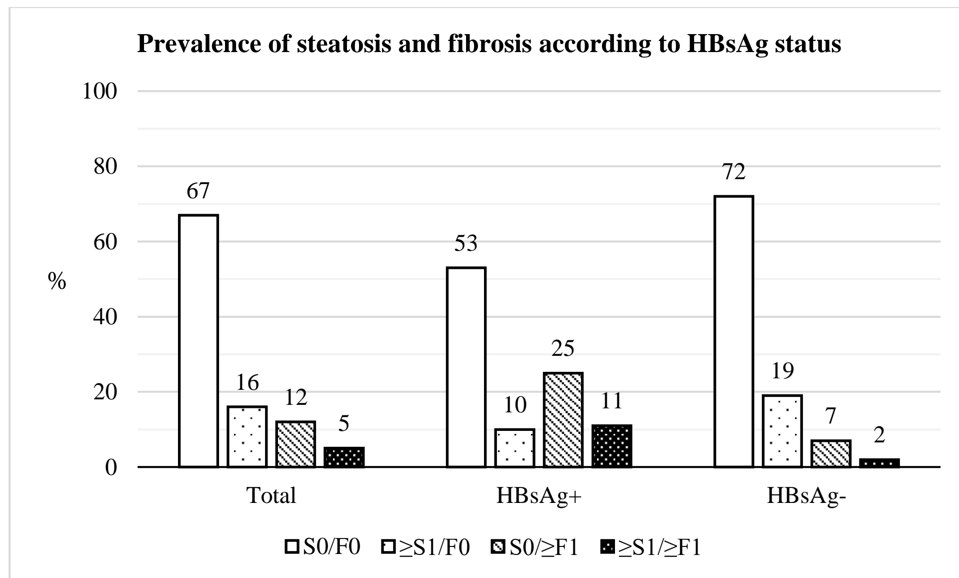


Figure 7-1 **Prevalence of liver steatosis and fibrosis after exclusion of patient with excessive alcohol consumption**

HBsAg=hepatitis B surface antigen

When comparing patients according to HBsAg-status, median CAP levels did not differ between HBsAg positive and negative subjects. In contrast, there was a higher prevalence of fibrosis in the HBsAg-positive group (Table 7-1). In addition, HBsAg-positive patients showed lower TC and triglycerides. They also showed lower platelets, higher hepatic transaminases, longer cumulative exposure to TDF and efavirenz ($p<0.01$), and shorter cumulative exposure to zidovudine ($p=0.02$) (Table 7-1).

Factors associated with liver steatosis

In the univariable analysis, factors associated with higher CAP values were longer cumulative exposure to stavudine, metabolic syndrome, central obesity, and higher BMI, hypertension grade, platelets counts and plasmatic concentrations of TC, LDL, triglycerides, and HbA1c. After adjustment, metabolic syndrome independently predicted higher CAP values (Table 7-3).

Table 7-3 **Univariate and multivariable linear regression analysis of factors associated with hepatic steatosis**

		Univariable analysis			Multivariable analysis		
Demographics		OR	95% CI	p	OR	95% CI	p
Gender	female vs male	1.05	0.99-1.12	0.12			
Age	per 5-years older	1.01	0.99-1.03	0.23			
Cigarette smoking	yes vs no	0.96	0.86-1.08	0.54			
Regular alcohol use	yes vs no	1.07	0.87-1.32	0.51			
Herbal or traditional remedies	yes vs no	1.10	0.95-1.29	0.21			
Time on ART	per year longer	1.01	1.00-1.01	0.09			
Stavudine	per year longer	1.02	1.00-1.04	0.02	1.02	1.00-1.04	0.06
Zidovudine	per year longer	1.00	0.99-1.01	0.75			
Tenofovir disoproxil fumarate	per year longer	1.00	1.00-1.00	0.72			
Efavirenz	per year longer	1.00	1.00-1.01	0.45			
Nevirapine	per year longer	1.00	1.00-1.01	0.37			
HIV RNA	per 1 $\log_{10}$ higher	0.99	0.97-1.01	0.23			
Current CD4 cell count	per 100 cells/mm^3 higher	1.01	1.00-1.02	0.06			
HBsAg	positive vs negative	1.02	0.96-1.09	0.44			
HBV DNA (n=90)	per 1 $\log_{10}$ IU/mL higher	1.00	0.96-1.03	0.90			
Metabolic syndrome	yes vs no	1.20	1.29-1.28	<0.01	1.19	1.12-1.26	<0.01
BMI	per kg/m^2 higher	1.02	1.01-1.02	<0.01			
Central obesity	yes vs no	1.14	1.08-1.20	<0.01			
Hypertension	per grade higher	1.05	1.02-1.08	<0.01			
Total cholesterol	per mmol/L higher	1.05	1.02-1.07	<0.01			
LDL	per mmol/L higher	1.05	1.02-1.09	<0.01			
HDL	per 0.1 mmol/L higher	1.00	0.99-1.00	0.26			
Triglycerides	per 0.1 mmol/L higher	1.01	1.00-1.01	<0.01			
Glycated haemoglobin	per 5 mmol/mol higher	1.04	1.02-1.06	<0.01			
Platelet count	per 50,000/µL higher	1.02	1.00-1.04	0.02			
AST	per 10 IU/L higher	0.99	0.97-1.01	0.28			
ALT	per 10 IU/L higher	0.99	0.97-1.01	0.45			
Liver stiffness	per kPa higher	1.01	1.00-1.03	0.10			

OR=odds ratio; CI=confidence interval; eGFR=estimated-glomerular filtration rate; ART=antiretroviral treatment; RNA=ribonucleic acid; HBsAg=hepatitis B surface antigen; BMI=body mass index; LDL=low density lipoprotein; HDL=high density lipoprotein; AST=aspartate aminotransferase; ALT=alanine aminotransferase

In separate sensitivity analyses where components of metabolic syndrome were analysed separately, longer cumulative exposure to stavudine, presence of central obesity or higher BMI, and higher plasmatic concentration of HbA1c were independently associated with higher CAP values (Table 7-4).

7.3.6 Factors associated with liver fibrosis

Factors associated with liver fibrosis were investigated by linear regression analysis (Table 7-5). In the univariable analysis, male gender, absence of central obesity, higher ALT and AST levels, higher HIV viral load and lower CD4 cell count were associated with higher liver stiffness. After adjustment for gender, HIV viral load, HBsAg status, central obesity and CAP values, higher TE values were independently associated with male gender, higher HIV RNA and higher CAP values (Table 7-5).

Table 7-4 **Univariable and multivariable linear regression analysis of predictors of hepatic steatosis grade**

		Multivariable analysis					
		Model a[1]			Model b[2]		
Demographics		OR	95% CI	p	OR	95% CI	p
Gender	female vs male						
Age	per 5-years older						
Cigarette smoking	yes vs no						
Regular alcohol use	yes vs no						
Herbal or traditional remedies	yes vs no						
Time on ART	per year longer						
Stavudine	per year longer	1.02	1.00-1.04	0.05	1.02	1.00-1.04	0.04
Zidovudine	per year longer						
Tenofovir disoproxil fumarate	per year longer						
Efavirenz	per year longer						
Nevirapine	per year longer						
HIV RNA	per 1 log_{10} higher						
Current CD4 cell count	per 100 cells/mm^3 higher						
HBsAg	positive vs negative						
HBV DNA (n=90)	per 1 log_{10} IU/ml higher						
Metabolic syndrome	yes vs no						
BMI	per kg/m^2 higher				1.02	1.01-1.02	<0.01
Central obesity	yes vs no	1.12	1.06-1.18	<0.01			
Hypertension	per grade higher	1.03	1.00-1.06	0.06	1.02	0.99-1.05	0.15
Total cholesterol	per mmol/L higher						
LDL	per mmol/L higher						
HDL	per 0.1 mmol/L higher						
Triglycerides	per 0.1 mmol/L higher	1.00	1.00-1.01	0.07	1.00	1.00-1.01	0.07
Glycated haemoglobin	per 5 mmol/mol higher	1.03	1.01-1.05	<0.01	1.03	1.01-1.05	0.01
Platelet count	per 50,000/µL higher						
AST	per 10 IU/L higher						
ALT	per 10 IU/L higher						
Liver stiffness	per kPa higher						

[1]Model a includes components of metabolic syndrome: central obesity, hypertension, HDL, trglycerides, and glycated haemoglobin; model b[2] includes: BMI, hypertension, HDL, trglycerides, and glycated haemoglobin.
OR=odds ratio; CI=confidence interval; eGFR=estimated-glomerular filtration rate; ART=antiretroviral treatment; RNA=ribonucleic acid; HBsAg=hepatitis B surface antigen; BMI=body mass index; LDL=low density lipoprotein; HDL=high density lipoprotein; AST=aspartate aminotransferase; ALT=alanine aminotransferase

Table 7-5 **Univariable and multivariable linear regression analysis of factors associated with hepatic fibrosis**

Characteristics			Univariable analysis			Multivariable analysis		
			OR[1]	95% CI	p	OR[1]	95% CI	p
Gender		female vs male	0.86	0.80-0.93	<0.01	0.89	0.81-0.97	0.01
Age		per 5-years older	1.01	0.99-1.03	0.26			
Cigarette smoking		yes vs no	1.12	0.97-1.29	0.11			
Herbal or traditional remedies		yes vs no	1.14	0.94-1.37	0.18			
Regular alcohol use		yes vs no	1.06	0.83-1.37	0.63			
Time on ART		per year longer	1.00	0.99-1.01	0.91			
Stavudine		per year longer	0.99	0.97-1.02	0.63			
Zidovudine		per year longer	1.00	0.99-1.01	1			
Tenofovir disoproxil fumarate	all	per year longer	1.00	1.00-1.00	0.88			
	HBsAg+	per year longer	0.97	0.94-1.00	0.03			
	HBsAg-	per year longer	1.00	1.00-1.00	0.77			
Efavirenz		per year longer	1.00	0.99-1.01	0.52			
Nevirapine		per year longer	1.00	0.99-1.01	0.96			
HIV RNA		per 1 $\log_{10}$ copies/ml higher	1.03	1.01-1.05	0.01	1.03	1.00-1.05	0.02
Current CD4 cell count		per 100 cells/mm^3 higher	0.99	0.98-1.00	0.02			
HBsAg		positive vs negative	1.06	0.99-1.15	0.11	1.05	0.97-1.13	0.23
HBV DNA (n=90)		per 1 $\log_{10}$ higher	1.02	0.97-1.06	0.53			
Metabolic syndrome		yes vs no	0.99	0.92-1.07	0.84			
BMI		per kg/m^2 higher	1.00	0.99-1.01	0.90			
Central obesity		yes vs no	0.91	0.85-0.98	0.01	0.96	0.88-1.04	0.29
Hypertension		per grade higher	1.03	0.99-1.06	0.14			
Total cholesterol		per mmol/L higher	0.99	0.96-1.02	0.34			
LDL		per mmol/L higher	0.97	0.94-1.01	0.11			
HDL		per 0.1 mmol/L higher	1.00	0.99-1.01	0.59			
Triglycerides		per 0.1 mmol/L higher	1.00	1.00-1.01	0.33			
Glycated haemoglobin		per 5 mmol/mol higher	1.01	0.99-1.03	0.32			
Platelet count		per 50,000/µL higher	1.00	0.98-1.03	0.76			
ALT		per 10 IU/L higher	1.03	1.01-1.05	0.01			
AST		per 10 IU/L higher	1.05	1.03-1.07	<0.01			
CAP		per 50 db/m higher	1.03	1.00-1.07	0.09	1.04	1.01-1.08	0.01

Stepwise selection for the multivariable model ($p<0.2$ for model entry and exit). The stepwise approach selected gender, CAP, HIV, and central obesity. HBsAg was subsequently forced into the model.
OR=odds ratio; CI=confidence interval; eGFR=estimated-glomerular filtration rate; ART=antiretroviral treatment; RNA=ribonucleic acid; HBsAg=hepatitis B surface antigen; BMI=body mass index; LDL=low density lipoprotein; HDL=high density lipoprotein; AST=aspartate aminotransferase; ALT=alanine aminotransferase

7.4 DISCUSSION

This cross-sectional investigation assessed prevalence and factors associated with hepatic steatosis and increased liver stiffness in a cohort of HIV-positive subjects on long-term antiretroviral treatment and receiving care in a typical programmatic setting in sub-Saharan Africa. Given the high prevalence of HBV coinfection in the cohort, differences between HBsAg positive and negative subjects were explored. The study found that one in five individuals in the cohort was affected by hepatic steatosis of any grade: the condition was independently associated with a diagnosis of metabolic syndrome, which affected almost one in every four individuals, and it was independent from HBsAg status. Prevalence of HBV co-infection was particularly high (27%) and prevalence of higher fibrosis grades was higher among HBsAg-positive subjects. Independent predictors of hepatic fibrosis included male gender, higher CAP values and higher HIV viral loads

A previous systematic review and meta-analysis on the prevalence of NAFLD in HIV mono-infected subjects based on radiological criteria documented a prevalence of 35%,[308] a higher figure than the 25% estimated for the general population.[305] This analysis, however, included studies from high income countries of the northern hemisphere only, with a proportion of individuals of black ethnicity of 8-40% from studies limited uniquely to the USA and Canada. In our investigation, liver steatosis of any grade affected 21% of the cohort, lower than the data reported in the meta-analysis[308] and lower than the 35% found in a Brazilian cohort of HIV mono-infected individuals,[309] and more in line with data from African studies, which place the prevalence of NAFLD in HIV cohorts between 19-28%.[310,311]

Associations with higher grades of hepatic steatosis and metabolic comorbidities is in line with findings from other studies.[308] Metabolic syndrome was diagnosed in 24% of individuals in our cohorts. Recent systematic reviews and meta-analyses on the prevalence of metabolic syndrome in HIV cohorts in sub-Saharan Africa estimated this figure to be between 16-31%,[319-321] which is in agreement with our findings. Metabolic syndrome is an important predictor of future cardiovascular disease.[322] Our data suggest that metabolic abnormalities, hypertension and more advanced grades of hepatic steatosis tend to cluster; this represents a worrying phenomenon, as with the

ageing of HIV-positive cohorts we are destined to witness an increase in cardiovascular events if large scale preventive measures are not urgently put in place.

Fibrosis of any grade was documented in 15% of our study population. Higher CAP values, higher HIV viral load and male gender were independently associated with liver fibrosis. In addition, longer cumulative exposure to TDF was associated with lower liver stiffness in HBsAg-positive subjects. This is in line with previous data from HBsAg-positive individuals from the same centre in Kumasi, as we have already documented on the protective role of TDF on hepatic fibrosis in this setting.[138] Data from HIV mono-infected and HIV/HBV co-infected patients from Zambia have described improvements in liver stiffness after ART initiation in both groups, suggesting a role of HIV alone in promoting liver inflammation and fibrosis.[323] We observed an association between higher HIV viral loads and increased liver stiffness. Despite the cross sectional design for which causality cannot be inferred, the role of HIV in promoting liver damage and progressive liver fibrosis is known: HIV infection can mediate its damage to the liver by an increased oxidative stress and immune activation, with increased synthesis of profibrotic mediators.[324] A better control of HIV replication in this setting is therefore mandatory to avoid onset of liver damage along with the decline in CD4 count and immune function. Screening of HBV coinfection in HIV cohorts, along with optimal control of HIV viral replication, are fundamental in preventing onset of liver disease and its progression. Finally, higher CAP values were associated with higher stiffness, suggesting a role of lipotoxicity and potentially of non-alcoholic steato-hepatitis (NASH), given the independent association from HBsAg status. Liver steatosis can evolve into fibrosis, cirrhosis and hepatocellular carcinoma, hence the importance of early diagnosis and preventive strategies.

Estimating CAP along with TE has proven to be easily implementable with portable Fibroscan equipment. Measurement of CAP and TE are reliable estimates when biopsies are not easily available,[325] and if implemented on a large scale can represent useful screening tools to unmask liver disease. Routine CAP and TE measurements should be implemented in routine medical practice, along with measurements of BP. Prevalence of non-communicable diseases in low and middle income countries among people living with HIV were recently explored by a systematic review and meta-analysis, which estimated a prevalence of hypertension around 21%,[326] whereas data

from our cohort confirm previously reported higher figures (35% in [142], 43% in the present study), affecting both systolic and diastolic measurements. In addition, we observed a higher prevalence of hypercholesterolemia (34% vs 22%), raised LDL (25% vs 23%) and obesity (12% vs 8%) and lower prevalence of hypertriglyceridaemia (21% vs 27%) and low HDL (15% vs 52%).[326] High systolic BP and LDL are the leading risk factors for all-cause risk attributable burden of disease globally and their prevalence is increasing in low-income countries, fuelled by an increase in the prevalence of obesity.[327] Counselling on life-style corrections, such as on regular exercise, low-salt intake, avoidance of tobacco and alcohol consumption, represents therefore a priority in this setting. Early detection and treatment of hypertension is mandatory, as it is still largely undiagnosed and undertreated in our setting, as only one in every two patients received specific antihypertensive drugs. Even more neglected appears the diagnosis and treatment of dyslipidaemias, and introduction of lipid-lowering agents in routine clinical practice represents a priority.

In conclusion, despite the limitations given by the cross-sectional design, our study sheds light on the prevalence of a number of non-communicable diseases in this HIV positive cohort in sub-Saharan Africa. Liver steatosis in this settings represents the epiphenomenon of multiple concurrent metabolic conditions, including obesity, metabolic syndrome, and dyslipidaemias. Their high prevalence, along with the poorly controlled hypertension figures of the region, represent worrying public health concerns given the risk of long-term cardiovascular complications that are likely to elicit with time. In this setting of high HBV endemicity, HBV plays a fundamental role in the development of liver fibrosis; screening for HBsAg is of paramount important, as universal treatment with TDF of HBV/HIV co-infected individuals, to prevent the onset of fibrosis in these patients. Furthermore, HIV control and aggressive preventive measures to reduce the prevalence of NAFLD are likely to improve the liver health of HIV cohorts in this setting.

Box of recommendations

In HIV-positive cohorts from sub-Saharan Africa:

1. **Screen for HBV-coinfection;**
2. **Screen for metabolic syndrome;**
3. **Treat metabolic syndrome and its determinants as appropriate (i.e. life-style corrections, anti-hypertensive agents; lipid-lowering drugs; anti-diabetic medications)**

8 EIGHTH CHAPTER – GENERAL DISCUSSION

This research project found that in a mature HIV cohort accessing treatment in a real-life setting in Ghana, after long-term, mainly NNRTI-based ART, nearly half of the patients had a detectable viral load, and 1 in 5 had a viral load ≥1000 copies/mL. POC viral load testing was used to measure the viral load and results allowed immediate adherence counselling for patients with viraemia. After eight weeks from the adherence intervention, retention into follow-up was high and there was evidence of improved adherence, but resuppression rates were poor, and none of the patients with a viral load ≥1000 copies/mL achieved resuppression while continuing NNRTI-based ART. Good viral load responses were seen in the few patients who switched to second-line PI/r-based ART while in the study, whereas co-existence of high viral loads and complex drug resistance patterns was common among patients failing to suppress.

When tenofovir was introduced in the ART regimen in place of zidovudine or stavudine (i) in the presence of a detectable viraemia and (ii) with the evidence of NNRTI resistance, it was predictive of a lack of viral load suppression after four years. Patients who experienced viraemia while on TDF/3TC+NNRTI acquired discriminatory NRTI RAMs, including well recognised tenofovir RAMs (K65R, K70E/T) as well as RAMs not typically associated with tenofovir (L74I/V, Y115F), alongside M184V and with or without TAMs. There was no suggestion of impaired viral fitness based on viral load and CD4 cell counts. In this setting in Ghana, self-reported adherence was predictive of virological outcomes, and composite self-reported adherence grading systems showed good specificity and sensitivity, and should be implemented in routine clinical practice. Despite reporting greater socio-economic disadvantage and worse physical and psychological wellbeing, women had a >3-fold greater likelihood of reporting optimal adherence than men.

In terms of long-term clinical outcomes, among HIV/HBV co-infected subjects on long-term TDF-containing ART prevalence of tubular proteinuria was 15.8%, and the risk was highest among women and those with hypertension. A strong association was detected between a larger eGFR decline and receiving LPV/r. Finally, our study found that 13% of patients had CAP values consistent with at least moderate hepatic steatosis

of any grade and they were independently associated with a diagnosis of metabolic syndrome, which affected almost one in four subjects, and specifically with central obesity, high BMI, and higher triglyceride and HbA1c levels. TE values consistent with ≥F2 fibrosis were measured in 16.7% and higher TE values were observed more commonly in HBsAg positive patients. Overall however, higher TE value were associated with male gender and higher CAP values. Thus, regardless of HBsAg status, controlling factors associated with steatosis is required to prevent progressive liver disease in the HIV-positive population of Ghana.

Our study provides evidence that virological monitoring should be integrated into routine practice in Ghana to guide treatment changes and prevent the onset of drug resistance, as recommended by the World Health Organisation.[67] Availability of viral load testing at point of care may help implementation. In our work, the use of a point-of-care platform delivered viral load measurements that guided immediate adherence reviews and ART management. The key findings was that in this cohort lacking routine access to virological monitoring, most patients with a viral load >1000 copies/mL harboured extensive drug resistance affecting both the NRTIs and the NNRTIs. In patients with viral load >1000 copies/mL, adherence counselling was ineffective, and a switch to second-line ART should be prioritised without delays. It is especially important to highlight that the patients experienced both drug resistance and a high viral load, with significant implications in terms of risk of clinical progression and onward transmission.

In HIV-positive cohorts in sub-Saharan Africa on long-term NNRTI-based ART regimes, we advocate for a prompt switch to second-line ART upon detection of a viral load >1000 copies/ml. However, this means an increment in the use of protease inhibitors, which are currently the only available agents for second line treatment in Kumasi, whereas DTG has yet to be introduced. While DTG use is currently endorsed by the World Health Organisation as the preferred third agent in first-line and second-line ART regimens,[40] for patients with pre-existing NRTI resistance it remains to be demonstrated that DTG in combination with 2 NRTIs will be as effective as use of boosted PIs. Furthermore, in the absence of virological monitoring, substituting the NNRTI with DTG retaining the same backbone require very careful consideration: patients failing NNRTI-based first line treatment are likely to harbour dual-class resistance, as showed in this research. In these circumstances, DTG might act as the

sole active agent, albeit some residual activity of the backbone cannot be excluded depending on the resistance profile. We demonstrated that drug-resistance decreased the odds of virological suppression when AZT or D4T were switched "blindly" in favour of TDF.[140] Switch from first-line TDF/3TC/EFV to TDF/3TC/DTG in patients with complex NRTI resistance patterns will require close monitoring. Surveillance on emergence of integrase drug-resistance will be needed after introduction of DTG in the region.

In patients on ART, adherence is one of the most important determinants of virological failure,[109] hence reliable measures of adherence are needed in routine clinical practice to detect potential gaps in compliance and address them before the onset of drug resistance and a compromised immunological and clinical status. In our cohort in Kumasi, self-reported adherence correlated with the viral load, indicating that this measure can be used as a predictor of virological detectability. Further studies evaluating the performance of direct measures of adherence, such as electronic pill monitoring, or relationship between adherence, viral load and plasma drug concentration, could provide additional characterisation of this population. However, it is unlikely that more sophisticated adherence-measure tools than self-reported ones could be implemented easily in daily practice, owing to high cost and overburdened health care services. In our study, a composite adherence measure that incorporated different recall times and assessments (i.e., number of doses missed, a visual analogue scale) outperformed individual adherence measures, reflecting how different modalities capture the multidimensional aspects of adherence. Adherence can be part of more complex scores with better predictive ability for virological failure. These scores were designed by combining adherence, CD4 cell counts, ART history and clinical data. This approach has been endorsed for use in settings like Malawi,[328] Cambodia,[329,330] Lesotho,[331] and diverse low-middle income settings,[332] with good discriminatory ability. We believe that this approach should be also explored in the Kumasi cohort, as it could be useful in fast-tracking patients at high risk of failure to virological monitoring.

In line with the pooled estimates from sub-Saharan Africa,[326] depression was common in Kumasi, with estimates of moderate to severe depression in the HEPIK cohort of

25% in men and women. Depression can affect treatment outcomes in people with HIV and reduce adherence to treatment.[333] An association between adherence and depression was not observed in our setting, however our analysis was centred on patients attending the HIV clinics and retained in care, which might have underestimated the magnitude of the disease in those undiagnosed or disengaged from care. Our study unmasked a burden of mental health issues that are currently neglected in the busy HIV clinic, and resources should be put in place to integrate mental health assessment in routine practice.[326]

We observed higher grades of hepatic fibrosis in HBV/HIV co-infected individuals in the Kumasi cohort. In addition to HBV co-infection, poor HIV control appears to be a contributing factor, highlighting the importance of integrated management strategies in this setting. Furthermore, in Ghana, environmental causes might play an additional role in the development of fibrosis: potential exposure to aflatoxins for example or high rates of schistosomiasis,[334] not investigated in our study, along with alcohol consumption in the male population and potential use of traditional medicine. The overall contribution of these multiple factors remains to be explored in the Kumasi cohort, whereas we were reassured by the observation that HCV and HDV co-infection were rare. Finally, in our cohort, one in five participants showed liver steatosis, whose main determinant was a diagnosis of metabolic syndrome. Given the high prevalence of liver disease in this cohort, implementation of Fibroscan monitoring for assessment of transient elastography and controlled attenuation parameter should be available. This technology is portable, does not require extensive training and can be used at the point of care.

We found a high prevalence of hypertension in Kumasi, with rates of 43% in the OPTIMISE cohort and 35% in the HEPIK cohort. A meta-analysis of studies from sub-Saharan Africa estimated a pooled prevalence of hypertension of 30% in the region, lower than in our cohorts.[335] We also observed an association between hypertension and renal abnormalities in Kumasi, indicating that routine monitoring of blood pressure and more systematic monitoring of serum creatinine and urinary dipstick should be implemented in routine practice. This is particularly important in

light of the fact that people living with HIV have two-fold higher risk of developing cardiovascular disease than HIV-negative populations.[336]

We detected a lower, but nonetheless significant prevalence of diabetes, which affected 5% of individuals from our cohort, with an additional 5% showing evidence of impaired glucose regulation. Additional metabolic factors such as hypercholesterolemia, elevated LDL, hypertriglyceridemia, low HDL and obesity play a known role in the onset of cardiovascular disease. The pooled data from the meta-analysis by Patel et al. estimated prevalence of these abnormalities in sub-Saharan Africa to be 22%, 23%, 27%, 52% and 8%, respectively.[326] In our setting in Kumasi, some findings were even more alarming: 34% showed hypercholesterolemia, 25% elevated LDL, and 41% were classed as overweight or obese. This highlights the need for urgent interventions, including nutritional advice, physical exercise, and detection and pharmacological control of dyslipidaemias. Validation of predictive tools for cardiovascular disease, such as the D:A:D CVD risk equation in these population might be beneficial to stratify the risk and prioritise patients for treatment.[337] It should be noted that extensive use of protease inhibitors is also likely to worsen the metabolic profile, strengthening the case of making DTG available to the population.[338] Non-communicable diseases are likely to attract greater attention in coming years in HIV cohorts in sub-Saharan Africa, following trends seen in the general population, and reflecting reduced rates of HIV mortality alongside economic growth and changing life-styles.[339,340]

Before the advent of ART, the HIV epidemic had dramatically reduced life-expectancy in sub-Saharan Africa, where mean reductions in life expectancy reached 20 years.[341] The introduction of ART in the mid-1990s dramatically changed the course of the infection, reducing HIV-related morbidity and mortality. However, disparities persist relative to high income countries, and the disease impacts men and women diffentely.[56] In middle/low income countries, women with HIV have an overall better prognosis than HIV-positive men. This could be attributed to better access to testing (e.g., at prenatal clinic) and better enagagment and retention into

care.[342] Different adherence behaviours has also been reported in sub-Saharan Africa, where women show better overall adherence to ART than men.[232]

The better outcomes of women on ART in sub-Saharan Africa are in contrast with the gender inequalities that are present in the region, characterised by a dominant male role.[343] The findings of this research work are in agreement with these observations: women with HIV receiving care in Kumasi were 3-fold more likely to have optimal adherence to ART than men, and showed a better control of HIV replication and superior immunological outcomes. However, interestingly women were less likely to be employed, travelled longer journeys to attend the HIV clinic, and suffered from poorer mental health and worse distress caused by physical and psychological symptoms.

Further research is needed to better define what the drivers of poorer adherence are in men in this region and improve their HIV-related outcome. A better understanding of the role of stigma in influencing adherence is needed in this context, as a detailed characterisation was not conducted in this project and its presence only inferred from the extent of the reported HIV disclosure. HIV and stigma, and fear of stigma, are deeply connected in the health care practice and might influence presentation and retention in care. Segregation of HIV-positive individuals, confidentiality violations, and moral judgements are a few examples of obstacles that individuals with HIV could face and fears of these events could have a negative influence on health seeking behaviours.[344] A qualitative approach with in-depth interviews could provide additional insights on gender differences in adherence to ART in this setting.

A higher prevalence of tubular proteinuria was observed in women in our study. An effect of gender in previous studies investigating factors associated with tubulopathy or chronic kidney disease in HIV-positive subjects was not found,[284,291,292,345] albeit an association between female gender and risk of renal disease has been described.[293] It could also be speculated that greater adherence among women may have increased TDF exposure relative to men, increasing the risk of tubulopathy. While greater adherence, hence higher plasmatic concentration of TDF, could be an explanation for the finding, more studies on larger cohorts are needed to further characterise the gender differences that we observed in renal health.

The association between male gender and liver fibrosis has already been described in HIV-positive cohorts from sub-Saharan Africa.[323] In a cohort from Zambia investigating the evolution of liver fibrosis in HIV-monoinfected and HIV/HBV co-infected patients, male gender was associate with an independent 3-fold higher odd of significant fibrosis and cirrhosis at one-year follow-up.[323,346] The same finding was also documented from an Ugandan cohort, where alcohol consumption was highly associated with male gender,[347] and a Swiss cohort in a western setting.[347,348]

This research project faced several limitations. In terms of study design, the presence of a control arm and randomisation would have added strength to our findings, if confirmed in a randomised fashion. In our study, the evaluation of the POC viral load monitoring did not allow for a comparison with the current standard of care, which is lack of any viral load testing, nor with a laboratory-based arm. The same consideration applies for the analysis of TDF-mediated renal toxicity, as a control arm of patients not on TDF was not in place. The cross-sectional design of the study that explored prevalence and predictors of liver steatosis and cirrhosis in the OPTIMISE cohort did not allow to investigate proper causality, as a longitudinal observation would have been necessary to assess the direction of causality between the predictors and the outcome of interest. Furthermore, all the work presented in this research project did not have a focus on key populations, namely MSMs, people in prisons and other closed settings, FSWs, IDUs and transgender people. Even in the Ghanaian setting, where the HIV epidemics is generalised, the proportion of new infections in key populations is high and estimated to be around 43% of the total.[349] Poor adherence to ART and poor retention in care, along with a great burden of mental health issues, disproportionally affect this group, and their underrepresentation in this study might have biased the findings. Patients with other co-infections were not included in the study cohorts. Most importantly, a proper assessment of tuberculosis (TB) co-infection was not performed. A study conducted in Accra documented that TB co-infection had a prevalence of 13% in subjects attending for out-patient HIV care, and that 60% had no suggestive symptoms.[350] The odds of having extra-pulmonary TB were 3-fold higher in HIV-positive individuals in another large cohort study conducted in Accra, and extra-pulmonary TB was associated with a greater than 3-fold higher odds of mortality in this group.[351]At KATH, the most common

comorbidity in HIV-positive inpatients admitted in the year 2018 was TB (40% of the total).[352] TB might affect the liver as an extra-pulmonary site, especially in HIV-positive subjects;[353] in addition, HIV-positive subjects might have paradoxical reactions or drug-induced liver injury during or after TB-treatment in the form of granulomas or increased steatosis.[311,354,355] Screening for TB would have provided a better evaluation of the liver health of these patients. In addition, other potential environmental causes of liver toxicity were not explored, such as environmental aflatoxins. Aflatoxins are food contaminants produced by *Aspergillus spp.*; chronic exposure to aflatoxins is associated with the development of hepatic cancer, and both epidemiological and animal studies show that HBV and aflatoxin act synergistically on the development of liver malignancies.[356] It has been already reported on the exposure of aflatoxins and liver disease in patients attending KATH.[357] Again, screening for exposure to these toxins would have improved the assessment of the liver health of our cohort.

8.1 RECOMMENDATIONS

Based on this research work conducted in Kumasi, we recommend:

- Implementation of routine virological monitoring and immediate switch to second-line ART if the viral load is ≥1000 copies/mL in patients on long-term exposure to NNRTI;
- POC molecular platforms to be considered for the scale-up of viral load monitoring as they offer
  - the potential for immediate treatment optimisation and adherence support upon detection of viraemia;
  - task shifting to non-laboratory personnel;
  - decentralisation of the testing;
- Treatment changes to be guided by viral load testing, especially if they involve the substitution of a single agent;
- Urgent introduction of dolutegravir in the ART armamentarium;
- Continuous uptake of HIV drug resistance surveillance in the Ashanti region;
- Routine screening for HBV infection;

- Routine screening for non-infectious comorbidities, namely hypertension (via routine blood pressure measurements at the clinical assessments), diabetes and metabolic syndrome;
- Implementation of Fibroscan testing for the assessment of liver health.

8.2 FUTURE DIRECTIONS

While universal viral load monitoring will be most likely preceded by incomplete coverage, strategies to optimise selection of patients at greatest risk of virological failure are needed. I am currently developing a predictive score by using adherence data along with clinical and socio-demographics characteristics of these patients to use in routine practise to fast-track patients at higher risk of failure to virological monitoring. My plan is to split the OPTIMISE dataset into a training set and validation set (ratio: 70/30) and develop a model on the significant findings from the multivariable logistic regression model and additional variables of interest in keeping with the lowest Akaike's information criterion. Goodness of fit of the model will be subsequently tested with the Hosmer-Lemeshow test. Based on the area under the ROC curve, a cut-off between sensitivity and specificity will be chosen and the score developed accordingly. The cut-off will be chosen to maximise sensitivity, whilst also considering a reasonable value for the specificity and ease of implementation in the busy clinical centre. If performance is satisfactory in both training and validation sets, the tool will be tested in a sample of the cohort in Ghana.

REFERENCES

1. Azevedo-Pereira JM, Santos-Costa Q. HIV Interaction With Human Host: HIV-2 As a Model of a Less Virulent Infection. *AIDS reviews* 2016; **18**(1): 44-53.

2. Peeters M, D'Arc M, Delaporte E. Origin and diversity of human retroviruses. *AIDS reviews* 2014; **16**(1): 23-34.

3. Faria NR, Rambaut A, Suchard MA, et al. The early spread and epidemic ignition of HIV-1 in human populations. *Science* 2014; **346**(6205): 56-61.

4. Simon-Loriere E, Rossolillo P, Negroni M. RNA structures, genomic organization and selection of recombinant HIV. *RNA Biology* 2011; **8**(2): 280-6.

5. De Oliveira F, Mourez T, Vessiere A, et al. Multiple HIV-1/M + HIV-1/O dual infections and new HIV-1/MO inter-group recombinant forms detected in Cameroon. *Retrovirology* 2017; **14**(1): 1.

6. McCutchan FE. Global epidemiology of HIV. *Journal of Medical Virology* 2006; **78**(S1): S7-S12.

7. Geretti AM. HIV-1 subtypes: epidemiology and significance for HIV management. *Current Opinion in Infectious Diseases* 2006; **19**(1): 1-7.

8. Foster GM, Ambrose JC, Hué S, et al. Novel HIV-1 Recombinants Spreading across Multiple Risk Groups in the United Kingdom: The Identification and Phylogeography of Circulating Recombinant Form (CRF) 50_A1D. *PLOS ONE* 2014; **9**(1): e83337.

9. Geretti AM. Human Immunodeficiency Virus (HIV). eLS; 2017.

10. Briggs JAG, Kräusslich H-G. The Molecular Architecture of HIV. *Journal of Molecular Biology* 2011; **410**(4): 491-500.

11. Engelman A, Cherepanov P. The structural biology of HIV-1: mechanistic and therapeutic insights. *Nature Reviews Microbiology* 2012; **10**: 279.

12. Deeks SG, Overbaugh J, Phillips A, Buchbinder S. HIV infection. *Nature Reviews Disease Primers* 2015; **1**: 15035.

13. Iordanskiy S, Santos S, Bukrinsky M. Nature, nurture and HIV: The effect of producer cell on viral physiology. *Virology* 2013; **443**(2): 208-13.

14. Smyth RP, Davenport MP, Mak J. The origin of genetic diversity in HIV-1. *Virus Research* 2012; **169**(2): 415-29.

15. Delviks-Frankenberry K, Galli A, Nikolaitchik O, Mens H, Pathak VK, Hu W-S. Mechanisms and factors that influence high frequency retroviral recombination. *Viruses* 2011; **3**(9): 1650-80.

16. Le Grice SF. Human immunodeficiency virus reverse transcriptase: 25 years of research, drug discovery, and promise. *J Biol Chem* 2012; **287**(49): 40850-7.

17. Hamid FB, Kim J, Shin C-G. Distribution and fate of HIV-1 unintegrated DNA species: a comprehensive update. *AIDS research and therapy* 2017; **14**(1): 9.

18. Freed EO. HIV-1 assembly, release and maturation. *Nature Reviews Microbiology* 2015; **13**: 484.

19. Patel P, Borkowf CB, Brooks JT, Lasry A, Lansky A, Mermin J. Estimating per-act HIV transmission risk: a systematic review. *AIDS (London, England)* 2014; **28**(10): 1509-19.

20. Hughes JP, Baeten JM, Lingappa JR, et al. Determinants of per-coital-act HIV-1 infectivity among African HIV-1-serodiscordant couples. *The Journal of infectious diseases* 2012; **205**(3): 358-65.

21. Wawer MJ, Gray RH, Sewankambo NK, et al. Rates of HIV-1 transmission per coital act, by stage of HIV-1 infection, in Rakai, Uganda. *The Journal of infectious diseases* 2005; **191**(9): 1403-9.

22. Hollingsworth TD, Anderson RM, Fraser C. HIV-1 transmission, by stage of infection. *The Journal of infectious diseases* 2008; **198**(5): 687-93.

23. Panel on Treatment of Pregnant Women with HIV Infection and Prevention of Perinatal Transmission. Recommendations for the Use of Antiretroviral Drugs in Pregnant Women with HIV Infection and Interventions to Reduce Perinatal HIV Transmission in the United States. 2018 (accessed 17/03/19).

24. Joint United Nations Programme on HIV/AIDS (UNAIDS). UNAIDS 2018 estimates; Global AIDS Monitoring 2018. 2018. http://aidsinfo.unaids.org/ (accessed 28/03/19).

25. Myer L, Phillips TK. Beyond "Option B+": Understanding Antiretroviral Therapy (ART) Adherence, Retention in Care and Engagement in ART Services Among Pregnant and Postpartum Women Initiating Therapy in Sub-Saharan Africa. *J Acquir Immune Defic Syndr* 2017; **75 Suppl 2**: S115-S22.

26. Anglemyer A, Rutherford GW, Horvath T, Baggaley RC, Egger M, Siegfried N. Antiretroviral therapy for prevention of HIV transmission in HIV-discordant couples. *The Cochrane database of systematic reviews* 2013; (4): Cd009153.

27. Rodger AJ, Cambiano V, Bruun T, et al. Sexual Activity Without Condoms and Risk of HIV Transmission in Serodifferent Couples When the HIV-Positive Partner Is Using Suppressive Antiretroviral Therapy. *JAMA* 2016; **316**(2): 171-81.

28. Cohen MS, Chen YQ, McCauley M, et al. Antiretroviral Therapy for the Prevention of HIV-1 Transmission. *New England Journal of Medicine* 2016; **375**(9): 830-9.

29. Baeten JM, Donnell D, Ndase P, et al. Antiretroviral prophylaxis for HIV prevention in heterosexual men and women. *The New England journal of medicine* 2012; **367**(5): 399-410.

30. Grant RM, Lama JR, Anderson PL, et al. Preexposure chemoprophylaxis for HIV prevention in men who have sex with men. *The New England journal of medicine* 2010; **363**(27): 2587-99.

31. McCormack S, Dunn DT, Desai M, et al. Pre-exposure prophylaxis to prevent the acquisition of HIV-1 infection (PROUD): effectiveness results from the pilot phase of a pragmatic open-label randomised trial. *Lancet (London, England)* 2016; **387**(10013): 53-60.

32. Molina JM, Capitant C, Spire B, et al. On-Demand Preexposure Prophylaxis in Men at High Risk for HIV-1 Infection. *The New England journal of medicine* 2015; **373**(23): 2237-46.

33. Marrazzo JM, Ramjee G, Richardson BA, et al. Tenofovir-based preexposure prophylaxis for HIV infection among African women. *The New England journal of medicine* 2015; **372**(6): 509-18.

34. World Health Organisation. Guideline on when to start antiretroviral therapy and on pre-exposure prophylaxis for HIV. Geneva, Switzerland; 2015.

35. British HIV Association/British Association for Sexual Health and HIV. BHIVA/BASHH guidelines on the use of HIV pre-exposure prophylaxis (PrEP) 2018. 2018.

36. Edmundson C, Heinsbroek E, Glass R, et al. Sexualised drug use in the United Kingdom (UK): A review of the literature. *International Journal of Drug Policy* 2018; **55**: 131-48.

37. Tsai CC, Follis KE, Sabo A, et al. Prevention of SIV infection in macaques by (R)-9-(2-phosphonylmethoxypropyl)adenine. *Science* 1995; **270**(5239): 1197-9.

38. Tsai CC, Emau P, Sun JC, et al. Post-exposure chemoprophylaxis (PECP) against SIV infection of macaques as a model for protection from HIV infection. *J Med Primatol* 2000; **29**(3-4): 248-58.

39. British Association for Sexual Health and HIV. UK Guideline for the use of HIV Post-Exposure Prophylaxis Following Sexual Exposure (PEPSE) 2015. 2015.

40. World Health Organisation. Updated recommendations on first-line and second-line antiretroviral regimens and post-exposure prophylaxis and recommendations on early infant diagnosis of HIV. Geneva; 2018.

41. Charania MR, Crepaz N, Guenther-Gray C, et al. Efficacy of Structural-Level Condom Distribution Interventions: A Meta-Analysis of U.S. and International Studies, 1998–2007. *AIDS and Behavior* 2011; **15**(7): 1283-97.

42. Weller S, Davis K. Condom effectiveness in reducing heterosexual HIV transmission. *The Cochrane database of systematic reviews* 2002; (1): CD003255.

43. Gray RH, Kigozi G, Serwadda D, et al. Male circumcision for HIV prevention in men in Rakai, Uganda: a randomised trial. *The Lancet* 2007; **369**(9562): 657-66.

44. Quinn TC, Wawer MJ, Sewankambo N, et al. Viral Load and Heterosexual Transmission of Human Immunodeficiency Virus Type 1. *New England Journal of Medicine* 2000; **342**(13): 921-9.

45. Goodreau SM, Carnegie NB, Vittinghoff E, et al. Can male circumcision have an impact on the HIV epidemic in men who have sex with men? *PLoS One* 2014; **9**(7): e102960.

46. Wiysonge CS, Kongnyuy EJ, Shey M, et al. Male circumcision for prevention of homosexual acquisition of HIV in men. *Cochrane Database of Systematic Reviews* 2011; (6).

47. Fiebig EW, Wright DJ, Rawal BD, et al. Dynamics of HIV viremia and antibody seroconversion in plasma donors: implications for diagnosis and staging of primary HIV infection. *AIDS* 2003; **17**(13): 1871-9.

48. Keele BF, Giorgi EE, Salazar-Gonzalez JF, et al. Identification and characterization of transmitted and early founder virus envelopes in primary HIV-1 infection. *Proceedings of the National Academy of Sciences* 2008; **105**(21): 7552-7.

49. Hollingsworth TD, Pilcher CD, Hecht FM, Deeks SG, Fraser C. High Transmissibility During Early HIV Infection Among Men Who Have Sex With Men-San Francisco, California. *The Journal of infectious diseases* 2015; **211**(11): 1757-60.

50. Ananworanich J, Chomont N, Eller LA, et al. HIV DNA Set Point is Rapidly Established in Acute HIV Infection and Dramatically Reduced by Early ART. *EBioMedicine* 2016; **11**: 68-72.

51. Vidya Vijayan KK, Karthigeyan KP, Tripathi SP, Hanna LE. Pathophysiology of CD4+ T-Cell Depletion in HIV-1 and HIV-2 Infections. *Frontiers in immunology* 2017; **8**: 580.

52. Maartens G, Celum C, Lewin SR. HIV infection: epidemiology, pathogenesis, treatment, and prevention. *The Lancet* 2014; **384**(9939): 258-71.

53. Ghosn J, Taiwo B, Seedat S, Autran B, Katlama C. HIV. *The Lancet* 2018; **392**(10148): 685-97.

54. Younas M, Psomas C, Reynes J, Corbeau P. Immune activation in the course of HIV-1 infection: Causes, phenotypes and persistence under therapy. *HIV medicine* 2016; **17**(2): 89-105.

55. Pantaleo G, Graziosi C, Fauci AS. The Immunopathogenesis of Human Immunodeficiency Virus Infection. *New England Journal of Medicine* 1993; **328**(5): 327-35.

56. Teeraananchai S, Kerr S, Amin J, Ruxrungtham K, Law M. Life expectancy of HIV-positive people after starting combination antiretroviral therapy: a meta-analysis. *HIV medicine* 2017; **18**(4): 256-66.

57. Manzardo C, Guardo AC, Letang E, Plana M, Gatell JM, Miro JM. Opportunistic infections and immune reconstitution inflammatory syndrome in HIV-1-infected adults in the combined antiretroviral therapy era: a comprehensive review. *Expert Rev Anti Infect Ther* 2015; **13**(6): 751-67.

58. Avila D, Althoff KN, Mugglin C, et al. Immunodeficiency at the start of combination antiretroviral therapy in low-, middle-, and high-income countries. *J Acquir Immune Defic Syndr* 2014; **65**(1): e8-16.

59. Mzingwane ML, Tiemessen CT. Mechanisms of HIV persistence in HIV reservoirs. *Reviews in medical virology* 2017; **27**(2).

60. Ruggiero A, De Spiegelaere W, Cozzi-Lepri A, et al. During Stably Suppressive Antiretroviral Therapy Integrated HIV-1 DNA Load in Peripheral Blood is Associated with the Frequency of CD8 Cells Expressing HLA-DR/DP/DQ. *EBioMedicine* 2015; **2**(9): 1153-9.

61. Chun T-W, Moir S, Fauci AS. HIV reservoirs as obstacles and opportunities for an HIV cure. *Nature Immunology* 2015; **16**: 584.

62. Fletcher CV, Staskus K, Wietgrefe SW, et al. Persistent HIV-1 replication is associated with lower antiretroviral drug concentrations in lymphatic tissues. *Proceedings of the National Academy of Sciences of the United States of America* 2014; **111**(6): 2307-12.

63. Cory TJ, Schacker TW, Stevenson M, Fletcher CV. Overcoming pharmacologic sanctuaries. *Current opinion in HIV and AIDS* 2013; **8**(3): 190-5.

64. Lorenzo-Redondo R, Fryer HR, Bedford T, et al. Persistent HIV-1 replication maintains the tissue reservoir during therapy. *Nature* 2016; **530**: 51.

65. Perelson AS, Ribeiro RM. Modeling the within-host dynamics of HIV infection. *BMC Biology* 2013; **11**(1): 96.

66. Clutter DS, Jordan MR, Bertagnolio S, Shafer RW. HIV-1 drug resistance and resistance testing. *Infection, Genetics and Evolution* 2016; **46**: 292-307.

67. World Health Organisation. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: recommendations for a public health approach – 2nd ed. Geneva, Switzerland; 2016.

68. Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents Living with HIV. Department of Health and Human Services. 2018. http://aidsinfo.nih.gov/contentfiles/lvguidelines/AdultandAdolescentGL.pdf (accessed 21/03/19 2019).

69. European AIDS Clinical Society. Guidelines version 9.1. 2019. http://www.eacsociety.org/files/2018_guidelines-9.1-english.pdf (accessed 21/03/19).

70. Marcelin A-G, Delaugerre C, Wirden M, et al. Thymidine analogue reverse transcriptase inhibitors resistance mutations profiles and association to other nucleoside reverse transcriptase inhibitors resistance mutations observed in the context of virological failure. *Journal of Medical Virology* 2004; **72**(1): 162-5.

71. Stockdale AJ, Saunders MJ, Boyd MA, et al. Effectiveness of Protease Inhibitor/Nucleos(t)ide Reverse Transcriptase Inhibitor-Based Second-line Antiretroviral Therapy for the Treatment of Human Immunodeficiency Virus Type 1 Infection in Sub-Saharan Africa: A Systematic Review and Meta-analysis. *Clin Infect Dis* 2018; **66**(12): 1846-57.

72. Gregson J, Tang M, Ndembi N, et al. Global epidemiology of drug resistance after failure of WHO recommended first-line regimens for adult HIV-1 infection: a multicentre retrospective cohort study. *The Lancet Infectious Diseases* 2016; **16**(5): 565-75.

73. Melikian GL, Rhee SY, Taylor J, et al. Standardized comparison of the relative impacts of HIV-1 reverse transcriptase (RT) mutations on nucleoside RT inhibitor susceptibility. *Antimicrob Agents Chemother* 2012; **56**(5): 2305-13.

74. Israelski D, Melikian GL, Shafer RW, et al. Non-nucleoside reverse transcriptase inhibitor (NNRTI) cross-resistance: implications for preclinical evaluation of novel NNRTIs and clinical genotypic resistance testing. *Journal of Antimicrobial Chemotherapy* 2013; **69**(1): 12-20.

75. Akil B, Blick G, Hagins DP, et al. Dolutegravir versus placebo in subjects harbouring HIV-1 with integrase inhibitor resistance associated substitutions: 48-week results from VIKING-4, a randomized study. *Antivir Ther* 2015; **20**(3): 343-8.

76. Castagna A, Maggiolo F, Penco G, et al. Dolutegravir in antiretroviral-experienced patients with raltegravir- and/or elvitegravir-resistant HIV-1: 24-week results of the phase III VIKING-3 study. *The Journal of infectious diseases* 2014; **210**(3): 354-62.

77. Eron JJ, Clotet B, Durant J, et al. Safety and efficacy of dolutegravir in treatment-experienced subjects with raltegravir-resistant HIV type 1 infection: 24-week results of the VIKING Study. *The Journal of infectious diseases* 2013; **207**(5): 740-8.

78. Geretti AM, Armenia D, Ceccherini-Silberstein F. Emerging patterns and implications of HIV-1 integrase inhibitor resistance. *Curr Opin Infect Dis* 2012; **25**(6): 677-86.

79. Doyle T, Dunn DT, Ceccherini-Silberstein F, et al. Integrase inhibitor (INI) genotypic resistance in treatment-naive and raltegravir-experienced patients infected with diverse HIV-1 clades. *J Antimicrob Chemother* 2015; **70**(11): 3080-6.

80. Sharma PL, Nurpeisov V, Lee K, Skaggs S, di San Filippo CA, Schinazi RF. Replication-dependent 65R→K reversion in human immunodeficiency virus type 1 reverse transcriptase double mutant K65R + L74V. *Virology* 2004; **321**(2): 222-34.

81. Chunduri H, Rimland D, Nurpeisov V, Crumpacker CS, Sharma PL. A Leu to Ile but not Leu to Val change at HIV-1 reverse transcriptase codon 74 in the background of K65R mutation leads to an increased processivity of K65R+L74I enzyme and a replication competent virus. *Virology journal* 2011; **8**: 33.
82. Wirden M, Lambert-Niclot S, Marcelin AG, et al. Antiretroviral combinations implicated in emergence of the L74I and L74V resistance mutations in HIV-1-infected patients. *Aids* 2009; **23**(1): 95-9.
83. Hu Z, Kuritzkes DR. Interaction of Reverse Transcriptase (RT) Mutations Conferring Resistance to Lamivudine and Etravirine: Effects on Fitness and RT Activity of Human Immunodeficiency Virus Type 1. *Journal of Virology* 2011; **85**(21): 11309-14.
84. Tseng A, Seet J, Phillips EJ. The evolution of three decades of antiretroviral therapy: challenges, triumphs and the promise of the future. *British Journal of Clinical Pharmacology* 2015; **79**(2): 182-94.
85. World Health Organisation. Updated recommendations on first-line and second-line antiretroviral regimens and post-exposure prophylaxis and recommendations on early infant diagnosis of HIV: interim guidelines. Supplement to the 2016 consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection. Geneva; 2018.
86. The INSIGHT START Study Group. Initiation of Antiretroviral Therapy in Early Asymptomatic HIV Infection. *New England Journal of Medicine* 2015; **373**(9): 795-807.
87. World Health Organisation. Updated recommendations on first-line and second-line antiretroviral regimens and post-exposure prophylaxis and recommendations on early infant diagnosis of HIV. Geneva, Switzerland; 2018.
88. Shoko C, Chikobvu D. A superiority of viral load over CD4 cell count when predicting mortality in HIV patients on therapy. *BMC infectious diseases* 2019; **19**(1): 169.
89. Working Group on Modelling of Antiretroviral Therapy Monitoring Strategies in Sub-Saharan A, Phillips A, Shroufi A, et al. Sustainable HIV treatment in Africa through viral-load-informed differentiated care. *Nature* 2015; **528**(7580): S68-76.
90. World Health Organisation. What's new in treatment monitoring: viral load and CD4 testing. Geneva; 2017.
91. Nash S DS, Croxford S, Guerra L, Lowndes C, Connor N, Gill ON,. Progress towards ending the HIV epidemic in the United Kingdom: 2018 report. . London: Public Health England; 2018.
92. Baxter JD, Dunn D, White E, et al. Global HIV-1 transmitted drug resistance in the INSIGHT Strategic Timing of AntiRetroviral Treatment (START) trial. *HIV Medicine* 2015; **16**: 77-87.

93. Bertagnolio S, Inzaule S.,. Global action plan on HIV drug resistance 2017-2021: 2018 progress report. In: Department of HIV and Global Hepatitis Programme WHO, editor.; 2018.

94. Goodall RL, Dunn DT, Nkurunziza P, et al. Rapid accumulation of HIV-1 thymidine analogue mutations and phenotypic impact following prolonged viral failure on zidovudine-based first-line ART in sub-Saharan Africa. *J Antimicrob Chemother* 2017; **72**(5): 1450-5.

95. De Luca A, Sidumo ZJ, Zanelli G, et al. Accumulation of HIV-1 drug resistance in patients on a standard thymidine analogue-based first line antiretroviral therapy after virological failure: implications for the activity of next-line regimens from a longitudinal study in Mozambique. *BMC infectious diseases* 2017; **17**(1): 605.

96. Drain PK, Dorward J, Bender A, et al. Point-of-Care HIV Viral Load Testing: an Essential Tool for a Sustainable Global HIV/AIDS Response. *Clinical microbiology reviews* 2019; **32**(3).

97. Smit PW, Sollis KA, Fiscus S, et al. Systematic Review of the Use of Dried Blood Spots for Monitoring HIV Viral Load and for Early Infant Diagnosis. *PLOS ONE* 2014; **9**(3): e86461.

98. World Health Organisation. Global action plan on HIV drug resistance 2017–2021. Geneva; 2017.

99. Gupta RK, Gregson J, Parkin N, et al. HIV-1 drug resistance before initiation or re-initiation of first-line antiretroviral therapy in low-income and middle-income countries: a systematic review and meta-regression analysis. *Lancet Infect Dis* 2018; **18**(3): 346-55.

100. Rhee S-Y, Blanco JL, Jordan MR, et al. Geographic and Temporal Trends in the Molecular Epidemiology and Genetic Mechanisms of Transmitted HIV-1 Drug Resistance: An Individual-Patient- and Sequence-Level Meta-Analysis. *PLOS Medicine* 2015; **12**(4): e1001810.

101. Hamers RL, Schuurman R, Sigaloff KCE, et al. Effect of pretreatment HIV-1 drug resistance on immunological, virological, and drug-resistance outcomes of first-line antiretroviral treatment in sub-Saharan Africa: a multicentre cohort study. *The Lancet Infectious Diseases* 2012; **12**(4): 307-17.

102. Phillips AN, Stover J, Cambiano V, et al. Impact of HIV Drug Resistance on HIV/AIDS-Associated Mortality, New Infections, and Antiretroviral Therapy Program Costs in Sub-Saharan Africa. *The Journal of infectious diseases* 2017; **215**(9): 1362-5.

103. Boender TS, Sigaloff KCE, McMahon JH, et al. Long-term Virological Outcomes of First-Line Antiretroviral Therapy for HIV-1 in Low- and Middle-Income Countries: A Systematic Review and Meta-analysis. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2015; **61**(9): 1453-61.

104. Inzaule SC, Hamers RL, Noguera-Julian M, et al. Primary resistance to integrase strand transfer inhibitors in patients infected with diverse HIV-1 subtypes in sub-Saharan Africa. *Journal of Antimicrobial Chemotherapy* 2018; **73**(5): 1167-72.

105. World Health Organisation. Guidelines on the public health response to pretreatment HIV drug resistance. Geneva; 2017.

106. Haberer JE, Musinguzi N, Boum Y, 2nd, et al. Duration of Antiretroviral Therapy Adherence Interruption Is Associated With Risk of Virologic Rebound as Determined by Real-Time Adherence Monitoring in Rural Uganda. *J Acquir Immune Defic Syndr* 2015; **70**(4): 386-92.

107. Paterson DL, Swindells S, Mohr J, et al. Adherence to protease inhibitor therapy and outcomes in patients with HIV infection. *Ann Intern Med* 2000; **133**(1): 21-30.

108. Wood E, Hogg RS, Yip B, Harrigan PR, O'Shaughnessy MV, Montaner JS. Effect of medication adherence on survival of HIV-infected adults who start highly active antiretroviral therapy when the CD4+ cell count is 0.200 to 0.350 x 10(9) cells/L. *Ann Intern Med* 2003; **139**(10): 810-6.

109. Wood E, Hogg RS, Yip B, Harrigan PR, O'Shaughnessy MV, Montaner JS. The impact of adherence on CD4 cell count responses among HIV-infected patients. *J Acquir Immune Defic Syndr* 2004; **35**(3): 261-8.

110. Begley K, McLaws M-L, Ross MW, Gold J. Cognitive and behavioural correlates of non-adherence to HIV anti-retroviral therapy: Theoretical and practical insight for clinical psychology and health psychology. *Clinical Psychologist* 2008; **12**(1): 9-17.

111. Bolsewicz K, Debattista J, Vallely A, Whittaker A, Fitzgerald L. Factors associated with antiretroviral treatment uptake and adherence: a review. Perspectives from Australia, Canada, and the United Kingdom. *AIDS Care* 2015; **27**(12): 1429-38.

112. Mills EJ, Nachega JB, Buchan I, et al. Adherence to antiretroviral therapy in sub-saharan africa and north america: A meta-analysis. *JAMA* 2006; **296**(6): 679-90.

113. Kagee A, Remien RH, Berkman A, Hoffman S, Campos L, Swartz L. Structural barriers to ART adherence in Southern Africa: Challenges and potential ways forward. *Glob Public Health* 2011; **6**(1): 83-97.

114. Stirratt MJ, Remien RH, Smith A, Copeland OQ, Dolezal C, Krieger D. The Role of HIV Serostatus Disclosure in Antiretroviral Medication Adherence. *AIDS and Behavior* 2006; **10**(5): 483-93.

115. Treffry-Goatley A, Lessells R, Sykes P, et al. Understanding Specific Contexts of Antiretroviral Therapy Adherence in Rural South Africa: A Thematic Analysis of Digital Stories from a Community with High HIV Prevalence. *PLoS ONE* 2016; **11**(2): e0148801.

116. Haberer JE, Sabin L, Amico KR, et al. Improving antiretroviral therapy adherence in resource-limited settings at scale: a discussion of interventions and recommendations. *Journal of the International AIDS Society* 2017; **20**(1): 21371.

117. Ghana AIDS Commission. National and Sub-National HIV and AIDS Estimates and Projections. 2017 Report. 2017.

118. Ghana Health Service National AIDS/STI Control Programme. Guidelines for Antiretroviral Therapy in Ghana. 2016.

119. Ali H AF, Baden D, Durand L, Bronson M, Kim A, Grant-Greene Y, Imtiaz R, Swaminathan M. Ghana's HIV epidemic and PEPFAR's contribution towards epidemic control. *Ghana Med J* 2019; **53**(1): 59-62.

120. Ghana Health Service National AIDS/STI Control Programme. Guidelines for antiretroviral therapy in Ghana. 2010.

121. Poku RA, Owusu AY, Mullen PD, Markham C, McCurdy SA. HIV antiretroviral medication stock-outs in Ghana: contributors and consequences. *Afr J AIDS Res* 2017; **16**(3): 231-9.

122. Diabate S, Alary M, Koffi CK. Determinants of adherence to highly active antiretroviral therapy among HIV-1-infected patients in Cote d'Ivoire. *Aids* 2007; **21**(13): 1799-803.

123. Eholie SP, Tanon A, Polneau S, et al. Field adherence to highly active antiretroviral therapy in HIV-infected adults in Abidjan, Cote d'Ivoire. *J Acquir Immune Defic Syndr* 2007; **45**(3): 355-8.

124. Moriarty K, Genberg B, Norman B, Reece R. The Effect of Antiretroviral Stock-Outs on Medication Adherence Among Patients Living With HIV in Ghana: A Qualitative Study. *J Assoc Nurses AIDS Care* 2018; **29**(2): 231-40.

125. Bonney EY, Addo NA, Ntim NAA, et al. Low level of transmitted HIV Drug resistance at two HIV care centres in Ghana: a threshold survey. *Ghana Med J* 2013; **47**(2): 82-6.

126. Nii-Trebi NI, Ibe S, Barnor JS, et al. HIV-1 drug-resistance surveillance among treatment-experienced and -naive patients after the implementation of antiretroviral therapy in Ghana. *PLoS One* 2013; **8**(8): e71972.

127. Martin-Odoom A, Brown CA, Odoom JK, et al. Emergence of HIV-1 drug resistance mutations in mothers on treatment with a history of prophylaxis in Ghana. *Virology journal* 2018; **15**(1): 143.

128. Martin-Odoom A, Adiku T, Delgado E, Lartey M, Ampofo WK. Occurrence of transmitted HIV-1 drug resistance among Drug-naïve pregnant women in selected HIV-care centres in Ghana. *Ghana Med J* 2017; **51**(1): 20-3.

129. Agyeman AA, Ofori-Asenso R. Prevalence of HIV and hepatitis B coinfection in Ghana: a systematic review and meta-analysis. *AIDS Res Ther* 2016; **13**: 23.

130. Pappoe F, Hagan CKO, Obiri-Yeboah D, Nsiah P. Sero-prevalence of hepatitis B and C viral infections in Ghanaian HIV positive cohort: a consideration for their health care. *BMC infectious diseases* 2019; **19**(1): 380.

131. Boateng R, Mutocheluh M, Dompreh A, et al. Sero-prevalence of Hepatitis B and C viral co-infections among HIV-1 infected ART-naive individuals in Kumasi, Ghana. *PLoS One* 2019; **14**(4): e0215377.

132. Geretti AM, Patel M, Sarfo FS, et al. Detection of Highly Prevalent Hepatitis B Virus Coinfection among HIV-Seropositive Persons in Ghana. *Journal of Clinical Microbiology* 2010; **48**(9): 3223-30.

133. Luo A, Jiang X, Ren H. Lamivudine plus tenofovir combination therapy versus lamivudine monotherapy for HBV/HIV coinfection: a meta-analysis. *Virology journal* 2018; **15**(1): 139.

134. King S, Adjei-Asante K, Appiah L, et al. Antibody screening tests variably overestimate the prevalence of hepatitis C virus infection among HIV-infected adults in Ghana. *J Viral Hepat* 2015; **22**(5): 461-8.

135. Stockdale AJ, Chaponda M, Beloukas A, et al. Prevalence of hepatitis D virus infection in sub-Saharan Africa: a systematic review and meta-analysis. *The Lancet Global Health* 2017; **5**(10): e992-e1003.

136. Lai Y-S, Biedermann P, Ekpo UF, et al. Spatial distribution of schistosomiasis and treatment needs in sub-Saharan Africa: a systematic review and geostatistical analysis. *The Lancet Infectious Diseases* 2015; **15**(8): 927-40.

137. World Health Organisation. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing hiv infection. Recommendations for a public health approach. Geneva, Switzerland.; 2013.

138. Stockdale AJ, Phillips RO, Beloukas A, et al. Liver Fibrosis by Transient Elastography and Virologic Outcomes After Introduction of Tenofovir in Lamivudine-Experienced Adults With HIV and Hepatitis B Virus Coinfection in Ghana. *Clin Infect Dis* 2015; **61**(6): 883-91.

139. Osei FB, Duker AA. Spatial and demographic patterns of cholera in Ashanti region - Ghana. *Int J Health Geogr* 2008; **7**: 44.

140. Villa G, Phillips RO, Smith C, et al. Drug resistance outcomes of long-term ART with tenofovir disoproxil fumarate in the absence of virological monitoring. *J Antimicrob Chemother* 2018; **73**(11): 3148-57.

141. International Congress of Drug Therapy in HIV Infection 23-26 October 2016, Glasgow, UK. *Journal of the International AIDS Society* 2016; **19**(8S7): 21487.

142. Villa G, Phillips RO, Smith C, et al. Renal health after long-term exposure to tenofovir disoproxil fumarate (TDF) in HIV/HBV positive adults in Ghana. *Journal of Infection* 2018; **76**(6): 515-21.

143. Amico KR, Fisher WA, Cornman DH, et al. Visual analog scale of ART adherence: association with 3-day self-report and adherence barriers. *J Acquir Immune Defic Syndr* 2006; **42**(4): 455-9.

144. Speakman A, Rodger A, Phillips AN, et al. The 'Antiretrovirals, Sexual Transmission Risk and Attitudes' (ASTRA) study. Design, methods and participant characteristics. *PLoS One* 2013; **8**(10): e77230.

145. Burch LS, Smith CJ, Anderson J, et al. Socioeconomic status and treatment outcomes for individuals with HIV on antiretroviral treatment in the UK: cross-sectional and longitudinal analyses. *Lancet Public Health* 2016; **1**(1): e26-e36.

146. Kroenke K, Spitzer RL, Williams JB. The PHQ-9: validity of a brief depression severity measure. *J Gen Intern Med* 2001; **16**(9): 606-13.

147. Spitzer RL, Kroenke K, Williams JB, Lowe B. A brief measure for assessing generalized anxiety disorder: the GAD-7. *Arch Intern Med* 2006; **166**(10): 1092-7.

148. Webber K, Davies AN. Validity of the memorial symptom assessment scale-short form psychological subscales in advanced cancer patients. *J Pain Symptom Manage* 2011; **42**(5): 761-7.

149. Rabin R, de Charro F. EQ-5D: a measure of health status from the EuroQol Group. *Ann Med* 2001; **33**(5): 337-43.

150. Jelsma J, Hansen K, De Weerdt W, De Cock P, Kind P. How do Zimbabweans value health states? *Popul Health Metr* 2003; **1**(1): 11.

151. Bravo AA, Sheth SG, Chopra S. Liver biopsy. *The New England journal of medicine* 2001; **344**(7): 495-500.

152. Tsochatzis EA, Gurusamy KS, Ntaoula S, Cholongitas E, Davidson BR, Burroughs AK. Elastography for the diagnosis of severity of fibrosis in chronic liver disease: a meta-analysis of diagnostic accuracy. *Journal of hepatology* 2011; **54**(4): 650-9.

153. Talwalkar JA, Kurtz DM, Schoenleber SJ, West CP, Montori VM. Ultrasound-based transient elastography for the detection of hepatic fibrosis: systematic review and meta-analysis. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association* 2007; **5**(10): 1214-20.

154. Armstrong MJ, Corbett C, Hodson J, et al. Operator training requirements and diagnostic accuracy of Fibroscan in routine clinical practice. *Postgraduate Medical Journal* 2013; **89**(1058): 685-92.

155. Arena U, Vizzutti F, Corti G, et al. Acute viral hepatitis increases liver stiffness values measured by transient elastography. *Hepatology* 2008; **47**(2): 380-4.

156. Fung J, Lai C-L, Cheng C, Wu R, Wong DK-H, Yuen M-F. Mild-to-Moderate Elevation of Alanine Aminotransferase Increases Liver Stiffness Measurement by Transient Elastography in Patients With Chronic Hepatitis B. *American Journal of Gastroenterology* 2011; **106**(3): 492-6.

157. Millonig G, Reimann FM, Friedrich S, et al. Extrahepatic cholestasis increases liver stiffness (FibroScan) irrespective of fibrosis. *Hepatology* 2008; **48**(5): 1718-23.

158. Hopper I, Kemp W, Porapakkham P, et al. Impact of heart failure and changes to volume status on liver stiffness: non-invasive assessment using transient elastography. *European Journal of Heart Failure* 2012; **14**(6): 621-7.

159. Mederacke I, Wursthorn K, Kirschner J, et al. Food intake increases liver stiffness in patients with chronic or resolved hepatitis C virus infection. *Liver International* 2009; **29**(10): 1500-6.

160. Petta S, Maida M, Macaluso FS, et al. The severity of steatosis influences liver stiffness measurement in patients with nonalcoholic fatty liver disease. *Hepatology* 2015; **62**(4): 1101-10.

161. Castéra L, Foucher J, Bernard P-H, et al. Pitfalls of liver stiffness measurement: A 5-year prospective study of 13,369 examinations. *Hepatology* 2010; **51**(3): 828-35.

162. Roca B, Resino E, Torres V, Herrero E, Penades M. Interobserver discrepancy in liver fibrosis using transient elastography. *Journal of Viral Hepatitis* 2012; **19**(10): 711-5.

163. Cepheid. Xpert® HIV-1 Viral Load. 2018. http://www.cepheid.com/en/cepheid-solutions/clinical-ivd-tests/virology/xpert-hiv-1-viral-load (accessed 16/09/2018 2018).

164. WHO Prequalification of In Vitro Diagnostics. Product: Xpert® HIV-1 Viral Load with GeneXpert® Dx, GeneXpert® Infinity48, GeneXpert® Infinity-48s and GeneXpert® Infinity-80. WHO reference numbers: PQDx 0192-070-00, PQDx 0193-070-00, PQDx 0194-070-00, PQDx 0195-070-00. In: Report WPP, editor.; 2017.

165. Popp C, Krams D, Beckert C, et al. HBsAg blood screening and diagnosis: performance evaluation of the ARCHITECT HBsAg qualitative and ARCHITECT HBsAg qualitative confirmatory assays. *Diagnostic Microbiology and Infectious Disease* 2011; **70**(4): 479-85.

166. Cepheid. XPERT® HBV VIRAL LOAD. Datasheet. 2018.

167. Cepheid. XPERT® HCV VIRAL LOAD. Datasheet. 2018.

168. DIA.PRO. HDV Ab - Competitive Enzyme Immunoassay for the determination of antibodies to Hepatitis Delta Virus in human serum and plasma. 2011.

169. RapidMedical Diagnostics. For qualitative detection of: Bilharzia (Schistosoma).

170. Ford N, Meintjes G, Pozniak A, et al. The future role of CD4 cell count for monitoring antiretroviral therapy. *Lancet Infect Dis* 2015; **15**(2): 241-7.

171. Bertagnolio S, De Luca A, Vitoria M, et al. Determinants of HIV drug resistance and public health implications in low- and middle-income countries. *Antivir Ther* 2012; **17**(6): 941-53.

172. Hoffmann CJ, Charalambous S, Grant AD, Morris L, Churchyard GJ, Chaisson RE. Durable HIV RNA resuppression after virologic failure while remaining on a first-line regimen: a cohort study. *Trop Med Int Health* 2014; **19**(2): 236-9.

173. Gupta RK, Goodall RL, Ranopa M, et al. High rate of HIV resuppression after viral failure on first-line antiretroviral therapy in the absence of switch to second-line therapy. *Clin Infect Dis* 2014; **58**(7): 1023-6.

174. McCluskey SM, Boum Y, 2nd, Musinguzi N, et al. Brief Report: Appraising Viral Load Thresholds and Adherence Support Recommendations in the World Health Organization Guidelines for Detection and Management of Virologic Failure. *J Acquir Immune Defic Syndr* 2017; **76**(2): 183-7.

175. Joint United Nations Programme on HIV/AIDS. 90-90-90 An ambitious treatment target to help end the AIDS epidemic. 2014.

176. Roberts T, Cohn J, Bonner K, Hargreaves S. Scale-up of Routine Viral Load Testing in Resource-Poor Settings: Current and Future Implementation Challenges. *Clinical Infectious Diseases* 2016; **62**(8): 1043-8.

177. Mutasa-Apollo T, Ford N, Wiens M, et al. Effect of frequency of clinic visits and medication pick-up on antiretroviral treatment outcomes: a systematic literature review and meta-analysis. *J Int AIDS Soc* 2017; **20**(Suppl 4): 21647.

178. World Health Organisation. HIV diagnostic tests in low and middle-income countries: forecasts of global demand for 2014-2018. Geneva; 2015.

179. Albert H, Nathavitharana RR, Isaacs C, Pai M, Denkinger CM, Boehme CC. Development, roll-out and impact of Xpert MTB/RIF for tuberculosis: what lessons have we learnt and how can we do better? *Eur Respir J* 2016; **48**(2): 516-25.

180. Dorward J, Drain PK, Garrett N. Point-of-care viral load testing and differentiated HIV care. *The Lancet HIV* 2018; **5**(1): e8-e9.

181. Moyo S, Mohammed T, Wirth KE, et al. Point-of-Care Cepheid Xpert HIV-1 Viral Load Test in Rural African Communities Is Feasible and Reliable. *Journal of Clinical Microbiology* 2016; **54**(12): 3050.

182. Gous N, Scott L, Berrie L, Stevens W. Options to Expand HIV Viral Load Testing in South Africa: Evaluation of the GeneXpert(R) HIV-1 Viral Load Assay. *PLoS One* 2016; **11**(12): e0168244.

183. Ceffa S, Luhanga R, Andreotti M, et al. Comparison of the Cepheid GeneXpert and Abbott M2000 HIV-1 real time molecular assays for monitoring HIV-1 viral load and detecting HIV-1 infection. *Journal of virological methods* 2016; **229**: 35-9.

184. Doyle T, Smith C, Vitiello P, et al. Plasma HIV-1 RNA detection below 50 copies/ml and risk of virologic rebound in patients receiving highly active antiretroviral therapy. *Clin Infect Dis* 2012; **54**(5): 724-32.

185. Konou AA, Dagnra AY, Vidal N, et al. Alarming rates of virological failure and drug resistance in patients on long-term antiretroviral treatment in routine HIV clinics in Togo. *AIDS* 2015; **29**(18): 2527-30.

186. Ndlovu Z, Fajardo E, Mbofana E, et al. Multidisease testing for HIV and TB using the GeneXpert platform: A feasibility study in rural Zimbabwe. *PLOS ONE* 2018; **13**(3): e0193577.

187. Rupérez M, Pou C, Maculuve S, et al. Determinants of virological failure and antiretroviral drug resistance in Mozambique. *Journal of Antimicrobial Chemotherapy* 2015; **70**(9): 2639-47.

188. Kiweewa F, Esber A, Musingye E, et al. HIV virologic failure and its predictors among HIV-infected adults on antiretroviral therapy in the African Cohort Study. *PLOS ONE* 2019; **14**(2): e0211344.

189. Bonner K, Mezochow A, Roberts T, Ford N, Cohn J. Viral Load Monitoring as a Tool to Reinforce Adherence: A Systematic Review. *JAIDS Journal of Acquired Immune Deficiency Syndromes* 2013; **64**(1): 74-8.

190. Guichet E, Aghokeng A, Serrano L, et al. Short Communication: High Viral Load and Multidrug Resistance Due to Late Switch to Second-Line Regimens Could Be a Major Obstacle to Reach the 90-90-90 UNAIDS Objectives in Sub-Saharan Africa. *AIDS Res Hum Retroviruses* 2016; **32**(12): 1159-62.

191. Labhardt ND, Ringera I, Lejone TI, et al. When patients fail UNAIDS' last 90 - the "failure cascade" beyond 90-90-90 in rural Lesotho, Southern Africa: a prospective cohort study. *Journal of the International AIDS Society* 2017; **20**(1): 21803.

192. Marcelin AG, Soulié C, Fofana DB, et al. High level of HIV-1 resistance in patients failing long-term first-line antiretroviral therapy in Mali. *Journal of Antimicrobial Chemotherapy* 2014; **69**(9): 2531-5.

193. Ciaffi L, Koulla-Shiro S, Sawadogo AB, et al. Boosted protease inhibitor monotherapy versus boosted protease inhibitor plus lamivudine dual therapy as second-line maintenance treatment for HIV-1-infected patients in sub-Saharan Africa (ANRS12 286/MOBIDIP): a multicentre, randomised, parallel, open-label, superiority trial. *Lancet HIV* 2017; **4**(9): e384-e92.

194. Joint United Nations Programme on HIV/AIDS (UNAIDS). Global AIDS update 2016. Geneva: UNAIDS; 2016.

195. World Health Organisation. Rapid advice: antiretroviral therapy for HIV infection in adults and adolescents -November 2009 [electronic version]. 2009.

196. Matthews PC, Geretti AM, Goulder PJ, Klenerman P. Epidemiology and impact of HIV coinfection with hepatitis B and hepatitis C viruses in Sub-Saharan Africa. *J Clin Virol* 2014; **61**(1): 20-33.

197. Clinton Health Access Initiative. ARV Market Report: The state of the antiretroviral drug market in low- and middle-income countries, 2015-2020. 2016.

198. Haas AD, Keiser O, Balestre E, et al. Monitoring and switching of first-line antiretroviral therapy in adult treatment cohorts in sub-Saharan Africa: collaborative analysis. *Lancet HIV* 2015; **2**(7): e271-8.

199. Madec Y, Leroy S, Rey-Cuille MA, Huber F, Calmy A. Persistent difficulties in switching to second-line ART in sub-saharan Africa--a systematic review and meta-analysis. *PLoS One* 2013; **8**(12): e82724.

200. Rohr JK, Ive P, Horsburgh CR, et al. Marginal Structural Models to Assess Delays in Second-Line HIV Treatment Initiation in South Africa. *PLoS One* 2016; **11**(8): e0161469.

201. Ambia J, Renju J, Wringe A, et al. From policy to practice: exploring the implementation of antiretroviral therapy access and retention policies between 2013 and 2016 in six sub-Saharan African countries. *BMC Health Serv Res* 2017; **17**(1): 758.

202. Garfield S, Clifford S, Eliasson L, Barber N, Willson A. Suitability of measures of self-reported medication adherence for routine clinical use: a systematic review. *BMC Med Res Methodol* 2011; **11**: 149.

203. Oyugi JH, Byakika-Tusiime J, Charlebois ED, et al. Multiple validated measures of adherence indicate high levels of adherence to generic HIV antiretroviral therapy in a resource-limited setting. *J Acquir Immune Defic Syndr* 2004; **36**(5): 1100-2.

204. Cunningham E, Chan YT, Aghaizu A, et al. Enhanced surveillance of HIV-1 drug resistance in recently infected MSM in the UK. *J Antimicrob Chemother* 2017; **72**(1): 227-34.

205. Beloukas A, King S, Childs K, et al. Detection of the NS3 Q80K polymorphism by Sanger and deep sequencing in hepatitis C virus genotype 1a strains in the UK. *Clin Microbiol Infect* 2015; **21**(11): 1033-9.

206. Geretti AM, Paredes R, Kozal MJ. Transmission of HIV drug resistance: lessons from sensitive screening assays. *Curr Opin Infect Dis* 2015; **28**(1): 23-30.

207. Kityo C, Thompson J, Nankya I, et al. HIV Drug Resistance Mutations in Non-B Subtypes After Prolonged Virological Failure on NNRTI-Based First-Line Regimens in Sub-Saharan Africa. *J Acquir Immune Defic Syndr* 2017; **75**(2): e45-e54.

208. The TenoRes Study G. Global epidemiology of drug resistance after failure of WHO recommended first-line regimens for adult HIV-1 infection: a multicentre retrospective cohort study. *The Lancet Infectious Diseases* 2016; **16**(5): 565-75.

209. Hunt GM, Dokubo EK, Takuva S, et al. Rates of virological suppression and drug resistance in adult HIV-1-positive patients attending primary healthcare facilities in KwaZulu-Natal, South Africa. *J Antimicrob Chemother* 2017; **72**(11): 3141-8.

210. Boender TS, Kityo CM, Boerma RS, et al. Accumulation of HIV-1 drug resistance after continued virological failure on first-line ART in adults and children in sub-Saharan Africa. *J Antimicrob Chemother* 2016; **71**(10): 2918-27.

211. Sigaloff KC, Hamers RL, Wallis CL, et al. Unnecessary antiretroviral treatment switches and accumulation of HIV resistance mutations; two arguments for viral load monitoring in Africa. *J Acquir Immune Defic Syndr* 2011; **58**(1): 23-31.

212. Barth RE, Aitken SC, Tempelman H, et al. Accumulation of drug resistance and loss of therapeutic options precede commonly used criteria for treatment failure in HIV-1 subtype-C-infected patients. *Antivir Ther* 2012; **17**(2): 377-86.

213. Hamers RL, Sigaloff KC, Wensing AM, et al. Patterns of HIV-1 drug resistance after first-line antiretroviral therapy (ART) failure in 6 sub-Saharan African countries: implications for second-line ART strategies. *Clin Infect Dis* 2012; **54**(11): 1660-9.

214. Lam EP, Moore CL, Gotuzzo E, et al. Antiretroviral Resistance After First-Line Antiretroviral Therapy Failure in Diverse HIV-1 Subtypes in the SECOND-LINE Study. *AIDS Res Hum Retroviruses* 2016; **32**(9): 841-50.

215. Rhee S-Y, Varghese V, Holmes SP, et al. Mutational Correlates of Virological Failure in Individuals Receiving a WHO-Recommended Tenofovir-Containing First-Line Regimen: An International Collaboration. *EBioMedicine* 2017; **18**: 225-35.

216. Vitoria M, Hill A, Ford N, et al. The transition to dolutegravir and other new antiretrovirals in low-income and middle-income countries: what are the issues? *AIDS* 2018; **32**(12): 1551-61.

217. World Health Organisation. Transition to new antiretrovirals in HIV programmes. Geneva, Switzerland; 2017.

218. Moriarty K RR, Norman B. Saving up for the future: HAART stock-outs as a contributor to treatment non-compliance among HIV-positive patients in Kumasi, Ghana. *Annals of Global Health* 2016; **82**(3): 515-6.

219. Pasquet A, Messou E, Gabillard D, et al. Impact of drug stock-outs on death and retention to care among HIV-infected patients on combination antiretroviral therapy in Abidjan, Cote d'Ivoire. *PLoS One* 2010; **5**(10): e13414.

220. Mukherjee JS, Barry D, Weatherford RD, Desai IK, Farmer PE. Community-Based ART Programs: Sustaining Adherence and Follow-up. *Current HIV/AIDS reports* 2016; **13**(6): 359-66.

221. Nachega JB, Adetokunboh O, Uthman OA, et al. Community-Based Interventions to Improve and Sustain Antiretroviral Therapy Adherence, Retention in HIV Care and Clinical

Outcomes in Low- and Middle-Income Countries for Achieving the UNAIDS 90-90-90 Targets. *Current HIV/AIDS reports* 2016; **13**(5): 241-55.
222. Bor J, Fox MP, Rosen S, et al. Treatment eligibility and retention in clinical HIV care: A regression discontinuity study in South Africa. *PLoS Med* 2017; **14**(11): e1002463.
223. Geretti AM, Fox Z, Johnson JA, et al. Sensitive assessment of the virologic outcomes of stopping and restarting non-nucleoside reverse transcriptase inhibitor-based antiretroviral therapy. *PLoS One* 2013; **8**(7): e69266.
224. Hermans LE, Moorhouse M, Carmona S, et al. Effect of HIV-1 low-level viraemia during antiretroviral therapy on treatment outcomes in WHO-guided South African treatment programmes: a multicentre cohort study. *Lancet Infect Dis* 2018; **18**(2): 188-97.
225. Paton NI, Kityo C, Thompson J, et al. Nucleoside reverse-transcriptase inhibitor cross-resistance and outcomes from second-line antiretroviral therapy in the public health approach: an observational analysis within the randomised, open-label, EARNEST trial. *Lancet HIV* 2017.
226. Miller MD, Margot N, Lu B, et al. Genotypic and phenotypic predictors of the magnitude of response to tenofovir disoproxil fumarate treatment in antiretroviral-experienced patients. *The Journal of infectious diseases* 2004; **189**(5): 837-46.
227. Nichols BE, Sigaloff KC, Kityo C, et al. Increasing the use of second-line therapy is a cost-effective approach to prevent the spread of drug-resistant HIV: a mathematical modelling study. *J Int AIDS Soc* 2014; **17**: 19164.
228. Vrijens B, De Geest S, Hughes DA, et al. A new taxonomy for describing and defining adherence to medications. *Br J Clin Pharmacol* 2012; **73**(5): 691-705.
229. Kaufman MR, Cornish F, Zimmerman RS, Johnson BT. Health behavior change models for HIV prevention and AIDS care: practical recommendations for a multi-level approach. *J Acquir Immune Defic Syndr* 2014; **66 Suppl 3**: S250-8.
230. Daskalopoulou M, Lampe FC, Sherr L, et al. Non-Disclosure of HIV Status and Associations with Psychological Factors, ART Non-Adherence, and Viral Load Non-Suppression Among People Living with HIV in the UK. *AIDS Behav* 2017; **21**(1): 184-95.
231. Miners A, Phillips A, Kreif N, et al. Health-related quality-of-life of people with HIV in the era of combination antiretroviral treatment: a cross-sectional comparison with the general population. *Lancet HIV* 2014; **1**(1): e32-40.
232. Heestermans T, Browne JL, Aitken SC, Vervoort SC, Klipstein-Grobusch K. Determinants of adherence to antiretroviral therapy among HIV-positive adults in sub-Saharan Africa: a systematic review. *BMJ Glob Health* 2016; **1**(4): e000125.
233. Orrell C, Cohen K, Leisegang R, Bangsberg DR, Wood R, Maartens G. Comparison of six methods to estimate adherence in an ART-naive cohort in a resource-poor setting: which best predicts virological and resistance outcomes? *AIDS Res Ther* 2017; **14**(1): 20.

234. Feldman BJ, Fredericksen RJ, Crane PK, et al. Evaluation of the Single-Item Self-Rating Adherence Scale for Use in Routine Clinical Care of People Living with HIV. *AIDS and Behavior* 2013; **17**(1): 307-18.

235. Mekuria LA, Prins JM, Yalew AW, Sprangers MA, Nieuwkerk PT. Sub-optimal adherence to combination anti-retroviral therapy and its associated factors according to self-report, clinician-recorded and pharmacy-refill assessment methods among HIV-infected adults in Addis Ababa. *AIDS Care* 2017; **29**(4): 428-35.

236. Wilson IB, Lee Y, Michaud J, Fowler FJ, Jr., Rogers WH. Validation of a New Three-Item Self-Report Measure for Medication Adherence. *AIDS Behav* 2016; **20**(11): 2700-8.

237. Ochieng W, Kitawi RC, Nzomo TJ, et al. Implementation and Operational Research: Correlates of Adherence and Treatment Failure Among Kenyan Patients on Long-term Highly Active Antiretroviral Therapy. *J Acquir Immune Defic Syndr* 2015; **69**(2): e49-56.

238. Sangeda RZ, Mosha F, Prosperi M, et al. Pharmacy refill adherence outperforms self-reported methods in predicting HIV therapy outcome in resource-limited settings. *BMC Public Health* 2014; **14**: 1035.

239. Bezabhe WM, Chalmers L, Bereznicki LR, Peterson GM. Adherence to Antiretroviral Therapy and Virologic Failure: A Meta-Analysis. *Medicine (Baltimore)* 2016; **95**(15): e3361.

240. Ortego C, Huedo-Medina TB, Llorca J, et al. Adherence to highly active antiretroviral therapy (HAART): a meta-analysis. *AIDS Behav* 2011; **15**(7): 1381-96.

241. Obirikorang C, Selleh PK, Abledu JK, Fofie CO. Predictors of Adherence to Antiretroviral Therapy among HIV/AIDS Patients in the Upper West Region of Ghana. *ISRN AIDS* 2013; **2013**: 7.

242. Bijker R, Jiamsakul A, Kityo C, et al. Adherence to antiretroviral therapy for HIV in sub-Saharan Africa and Asia: a comparative analysis of two regional cohorts. *J Int AIDS Soc* 2017; **20**(1): 21218.

243. Puskas CM, Forrest JI, Parashar S, et al. Women and vulnerability to HAART non-adherence: a literature review of treatment adherence by gender from 2000 to 2011. *Current HIV/AIDS reports* 2011; **8**(4): 277-87.

244. Barber TJ, Geretti AM, Anderson J, et al. Outcomes in the first year after initiation of first-line HAART among heterosexual men and women in the UK CHIC Study. *Antivir Ther* 2011; **16**(6): 805-14.

245. Denton M, Walters V. Gender differences in structural and behavioral determinants of health: an analysis of the social production of health. *Soc Sci Med* 1999; **48**(9): 1221-35.

246. Memiah P, Shumba C, Etienne-Mesubi M, et al. The effect of depressive symptoms and CD4 count on adherence to highly active antiretroviral therapy in sub-Saharan Africa. *J Int Assoc Provid AIDS Care* 2014; **13**(4): 346-52.

247. Wroe EB, Hedt-Gauthier BL, Franke MF, Nsanzimana S, Turinimana JB, Drobac P. Depression and patterns of self-reported adherence to antiretroviral therapy in Rwanda. *Int J STD AIDS* 2015; **26**(4): 257-61.

248. Ciesla JA, Roberts JE. Meta-analysis of the relationship between HIV infection and risk for depressive disorders. *Am J Psychiatry* 2001; **158**(5): 725-30.

249. Bernard C, Dabis F, de Rekeneire N. Prevalence and factors associated with depression in people living with HIV in sub-Saharan Africa: A systematic review and meta-analysis. *PLoS One* 2017; **12**(8): e0181960.

250. Vosvick M, Martin LA, Smith NG, Jenkins SR. Gender differences in HIV-related coping and depression. *AIDS Behav* 2010; **14**(2): 390-400.

251. Viswanathan S, Justice AC, Alexander GC, et al. Adherence and HIV RNA Suppression in the Current Era of Highly Active Antiretroviral Therapy. *J Acquir Immune Defic Syndr* 2015; **69**(4): 493-8.

252. Geretti AM, Loutfy M, D'Arminio Monforte A, et al. Out of focus: tailoring the cascade of care to the needs of women living with HIV. *HIV Med* 2017; **18 Suppl 2**: 3-17.

253. Weiser SD, Palar K, Frongillo EA, et al. Longitudinal assessment of associations between food insecurity, antiretroviral adherence and HIV treatment outcomes in rural Uganda. *AIDS* 2014; **28**(1): 115-20.

254. Wu P, Johnson BA, Nachega JB, et al. The combination of pill count and self-reported adherence is a strong predictor of first-line ART failure for adults in South Africa. *Curr HIV Res* 2014; **12**(5): 366-75.

255. Meresse M, Carrieri MP, Laurent C, et al. Time patterns of adherence and long-term virological response to non-nucleoside reverse transcriptase inhibitor regimens in the Stratall ANRS 12110/ESTHER trial in Cameroon. *Antiviral therapy* 2013; **18**(1): 29-37.

256. Ford N, Darder M, Spelman T, Maclean E, Mills E, Boulle A. Early Adherence to Antiretroviral Medication as a Predictor of Long-Term HIV Virological Suppression: Five-Year Follow Up of an Observational Cohort. *PLOS ONE* 2010; **5**(5): e10460.

257. Kagee A, Nel A. Assessing the association between self-report items for HIV pill adherence and biological measures. *AIDS Care* 2012; **24**(11): 1448-52.

258. Twumasi PA. A social history of the Ghanaian pluralistic medical system. *Soc Sci Med Med Anthropol* 1979; **13B**(4): 349-56.

259. Fosu GB. Disease classification in rural Ghana: framework and implications for health behaviour. *Soc Sci Med B* 1981; **15**(4): 471-82.

260. Mill JE. I'm Not a "Basabasa" Woman: An Explanatory Model of HIV Illness in Ghanaian Women. *Clinical Nursing Research* 2001; **10**(3): 254-74.

261. Awusabo-Asare K; Abane AM; Badasu DM; Anarfi JK. All die be die: obstacles to change in the face of HIV infection in Ghana. In: John C. Caldwell PC, John Anarfi, Kofi

Awusabo-Asare, James Ntozi, I.O. Orubuloye, Jeff Marck, Wendy Cosford, Rachel Colombo and Elaine Hollings,, ed. Resistances to behavioural change to reduce HIV / AIDS infection in predominantly heterosexual epidemics in Third World countries. Camberra, Australia: Australian National University, National Centre for Epidemiology and Population Health, Health Transition Centre; 1999: 125-32.

262. Takyi BK. Religion and women's health in Ghana: insights into HIV/AIDs preventive and protective behavior. *Social Science & Medicine* 2003; **56**(6): 1221-34.

263. Ogunbajo A, Kershaw T, Kushwaha S, Boakye F, Wallace-Atiapah N-D, Nelson LE. Barriers, Motivators, and Facilitators to Engagement in HIV Care Among HIV-Infected Ghanaian Men Who have Sex with Men (MSM). *AIDS and behavior* 2018; **22**(3): 829-39.

264. Eshun-Wilson I, Rohwer A, Hendricks L, Oliver S, Garner P. Being HIV positive and staying on antiretroviral therapy in Africa: A qualitative systematic review and theoretical model. *PLOS ONE* 2019; **14**(1): e0210408.

265. Scherzer R, Estrella M, Li Y, et al. Association of tenofovir exposure with kidney disease risk in HIV infection. *Aids* 2012; **26**(7): 867-75.

266. Milian L, Peris JE, Gandia P, et al. Tenofovir-induced toxicity in renal proximal tubular epithelial cells: involvement of mitochondria. *AIDS* 2017; **31**(12): 1679-84.

267. Ryom L, Mocroft A, Kirk O, et al. Predictors of estimated glomerular filtration rate progression, stabilization or improvement after chronic renal impairment in HIV-positive individuals. *AIDS* 2017; **31**(9): 1261-70.

268. Jose S, Hamzah L, Campbell LJ, et al. Incomplete reversibility of estimated glomerular filtration rate decline following tenofovir disoproxil fumarate exposure. *The Journal of infectious diseases* 2014; **210**(3): 363-73.

269. Mwafongo A, Nkanaunena K, Zheng Y, et al. Renal events among women treated with tenofovir/emtricitabine in combination with either lopinavir/ritonavir or nevirapine. *AIDS* 2014; **28**(8): 1135-42.

270. Bedimo R, Rosenblatt L, Myers J. Systematic review of renal and bone safety of the antiretroviral regimen efavirenz, emtricitabine, and tenofovir disoproxil fumarate in patients with HIV infection. *HIV Clin Trials* 2016; **17**(6): 246-66.

271. De Waal R, Cohen K, Fox MP, et al. Changes in estimated glomerular filtration rate over time in South African HIV-1-infected patients receiving tenofovir: a retrospective cohort study. *J Int AIDS Soc* 2017; **20**(1): 1-8.

272. Hamzah L, Jose S, Booth JW, et al. Treatment-limiting renal tubulopathy in patients treated with tenofovir disoproxil fumarate. *J Infect* 2017.

273. Touzard Romo F, Smeaton LM, Campbell TB, et al. Renal and Metabolic Toxicities Following Initiation of HIV-1 Treatment Regimen in a Diverse, Multinational Setting: A

Focused Safety Analysis of ACTG PEARLS (A5175). *HIV Clinical Trials* 2014; **15**(6): 246-60.

274. Salome T, Kasamba I, Mayanja BN, et al. The effect of Tenofovir on renal function among Ugandan adults on long-term antiretroviral therapy: a cross-sectional enrolment analysis. *AIDS Res Ther* 2016; **13**(1): 28.

275. Estill J, Ford N, Salazar-Vizcaya L, et al. The need for second-line antiretroviral therapy in adults in sub-Saharan Africa up to 2030: a mathematical modelling study. *Lancet Hiv* 2016; **3**(3): e132-9.

276. Mweemba A, Zanolini A, Mulenga L, et al. Chronic Hepatitis B Virus Coinfection Is Associated With Renal Impairment Among Zambian HIV-Infected Adults. *Clinical Infectious Diseases* 2014; **59**(12): 1757-60.

277. Mocroft A, Neuhaus J, Peters L, et al. Hepatitis B and C Co-Infection Are Independent Predictors of Progressive Kidney Disease in HIV-Positive, Antiretroviral-Treated Adults. *PLOS ONE* 2012; **7**(7): e40245.

278. Miailhes P, Pradat P, Chevallier M, et al. Proficiency of transient elastography compared to liver biopsy for the assessment of fibrosis in HIV/HBV-coinfected patients. *J Viral Hepat* 2011; **18**(1): 61-9.

279. U.S. Department of Health and Human Services. National Institute of Allergy and Infectious Diseases. Division of AIDS. Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1. 2017.

280. Sousa-Figueiredo JC, Betson M, Kabatereine NB, Stothard JR. The urine circulating cathodic antigen (CCA) dipstick: a valid substitute for microscopy for mapping and point-of-care diagnosis of intestinal schistosomiasis. *PLoS Negl Trop Dis* 2013; **7**(1): e2008.

281. Campbell LJ, Dew T, Salota R, et al. Total protein, albumin and low-molecular-weight protein excretion in HIV-positive patients. *BMC Nephrol* 2012; **13**: 85.

282. Booysen HL, Woodiwiss AJ, Raymond A, et al. Chronic kidney disease epidemiology collaboration-derived glomerular filtration rate performs better at detecting preclinical end-organ changes than alternative equations in black Africans. *J Hypertens* 2016; **34**(6): 1178-85.

283. Rodriguez-Novoa S, Labarga P, Soriano V, et al. Predictors of kidney tubular dysfunction in HIV-infected patients treated with tenofovir: a pharmacogenetic study. *Clin Infect Dis* 2009; **48**(11): e108-16.

284. Reynes J, Cournil A, Peyriere H, et al. Tubular and glomerular proteinuria in HIV-infected adults with estimated glomerular filtration rate >/= 60 ml/min per 1.73 m2. *Aids* 2013; **27**(8): 1295-302.

285. Dauchy FA, Lawson-Ayayi S, de La Faille R, et al. Increased risk of abnormal proximal renal tubular function with HIV infection and antiretroviral therapy. *Kidney Int* 2011; **80**(3): 302-9.

286. Labarga P, Barreiro P, Martin-Carbonero L, et al. Kidney tubular abnormalities in the absence of impaired glomerular function in HIV patients treated with tenofovir. *Aids* 2009; **23**(6): 689-96.

287. Forouzanfar MH, Liu P, Roth GA, et al. Global burden of hypertension and systolic blood pressure of at least 110 to 115 mm hg, 1990-2015. *JAMA* 2017; **317**(2): 165-82.

288. Stanifer JW, Jing B, Tolan S, et al. The epidemiology of chronic kidney disease in sub-Saharan Africa: a systematic review and meta-analysis. *The Lancet Global Health* 2014; **2**(3): e174-e81.

289. Bamgboye EL. The challenges of ESRD care in developing economies: sub-Saharan African opportunities for significant improvement. *Clin Nephrol* 2016; **86 (2016)**(13): 18-22.

290. Wensink GE, Schoffelen AF, Tempelman HA, Rookmaaker MB, Hoepelman AI, Barth RE. Albuminuria Is Associated with Traditional Cardiovascular Risk Factors and Viral Load in HIV-Infected Patients in Rural South Africa. *PLoS One* 2015; **10**(8): e0136529.

291. Sheets KM, Atta MG, Fine DM, et al. Longitudinal Assessment of Proximal Tubular Dysfunction in HIV Seropositive and Seronegative Persons: Correlates and Implications. *J Acquir Immune Defic Syndr* 2017; **75**(1): 45-51.

292. Casado JL, Banon S, Santiuste C, et al. Prevalence and significance of proximal renal tubular abnormalities in HIV-infected patients receiving tenofovir. *Aids* 2016; **30**(2): 231-9.

293. Goldberg I., I. K. The role of gender in chronic kidney disease. *European Medical Journal* 2016; **1**(2): 58-64.

294. Eastwood JB, Kerry SM, Plange-Rhule J, et al. Assessment of GFR by four methods in adults in Ashanti, Ghana: the need for an eGFR equation for lean African populations. *Nephrology Dialysis Transplantation* 2010; **25**(7): 2178-87.

295. Cooper RD, Wiebe N, Smith N, Keiser P, Naicker S, Tonelli M. Systematic Review and Meta-analysis: Renal Safety of Tenofovir Disoproxil Fumarate in HIV-Infected Patients. *Clinical Infectious Diseases* 2010; **51**(5): 496-505.

296. Yombi JC, Pozniak A, Boffito M, et al. Antiretrovirals and the kidney in current clinical practice: renal pharmacokinetics, alterations of renal function and renal toxicity. *Aids* 2014; **28**(5): 621-32.

297. Jose S, Nelson M, Phillips A, et al. Improved kidney function in patients who switch their protease inhibitor from atazanavir or lopinavir to darunavir. *AIDS* 2017; **31**(4): 485-92.

298. Kasembeli AN, Duarte R, Ramsay M, Naicker S. African origins and chronic kidney disease susceptibility in the human immunodeficiency virus era. *World J Nephrol* 2015; **4**(2): 295-306.

299. Kalyesubula R, Wearne N, Semitala FC, Bowa K. HIV-associated renal and genitourinary comorbidities in Africa. *J Acquir Immune Defic Syndr* 2014; **67 Suppl 1**: S68-78.

300. Sammy Crowther Kofi Tay, Richcane Amankwa, Gbedema. SY. Prevalence of Schistosoma haematobium infection in Ghana: a retrospective case study in Kumasi. *International Journal of Parasitology Research* 2011; **3**(2): 48-52.

301. Soares Magalhães RJ, Biritwum N-K, Gyapong JO, et al. Mapping Helminth Co-Infection and Co-Intensity: Geostatistical Prediction in Ghana. *PLOS Neglected Tropical Diseases* 2011; **5**(6): e1200.

302. Duarte DB, Vanderlei LA, de Azevêdo Bispo RK, et al. Renal Function in Hepatosplenic Schistosomiasis – An Assessment of Renal Tubular Disorders. *PLoS ONE* 2014; **9**(12): e115197.

303. Hill A, Khoo S, Back D, Pozniak A, Boffito M. Should the dose of tenofovir be reduced to 200-250 mg/day, when combined with protease inhibitors? *J Int AIDS Soc* 2014; **17**(4 Suppl 3): 19583.

304. Charlton MR, Burns JM, Pedersen RA, Watt KD, Heimbach JK, Dierkhising RA. Frequency and outcomes of liver transplantation for nonalcoholic steatohepatitis in the United States. *Gastroenterology* 2011; **141**(4): 1249-53.

305. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease—Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 2016; **64**(1): 73-84.

306. The GBD 2015 Obesity Collaborators. Health Effects of Overweight and Obesity in 195 Countries over 25 Years. *New England Journal of Medicine* 2017; **377**(1): 13-27.

307. Younossi ZM. The epidemiology of nonalcoholic steatohepatitis. *Clinical Liver Disease* 2018; **11**(4): 92-4.

308. Maurice JB, Patel A, Scott AJ, Patel K, Thursz M, Lemoine M. Prevalence and risk factors of nonalcoholic fatty liver disease in HIV-monoinfection. *AIDS (London, England)* 2017; **31**(11): 1621-32.

309. Perazzo H, Cardoso SW, Yanavich C, et al. Predictive factors associated with liver fibrosis and steatosis by transient elastography in patients with HIV mono-infection under long-term combined antiretroviral therapy. *Journal of the International AIDS Society* 2018; **21**(11): e25201.

310. Sonderup MW, Wainwright H, Hall P, Hairwadzi H, Spearman CW. A clinicopathological cohort study of liver pathology in 301 patients with human immunodeficiency virus/acquired immune deficiency syndrome. *Hepatology* 2015; **61**(5): 1721-9.

311. Hoffmann CJ, Hoffmann JD, Kensler C, et al. Tuberculosis and hepatic steatosis are prevalent liver pathology findings among HIV-infected patients in South Africa. *PLoS One* 2015; **10**(2): e0117813.

312. Lemoine M, Assoumou L, De Wit S, et al. Diagnostic Accuracy of Noninvasive Markers of Steatosis, NASH, and Liver Fibrosis in HIV-Monoinfected Individuals at Risk of Nonalcoholic Fatty Liver Disease (NAFLD): Results From the ECHAM Study. *J Acquir Immune Defic Syndr* 2019; **80**(4): e86-e94.

313. Mi YQ, Shi QY, Xu L, et al. Controlled attenuation parameter for noninvasive assessment of hepatic steatosis using Fibroscan(R): validation in chronic hepatitis B. *Dig Dis Sci* 2015; **60**(1): 243-51.

314. World Health Organisation. Waist circumference and waist–hip ratio: report of a WHO expert consultation, Geneva, 8–11 December 2008. 2011.

315. Levey AS, Stevens LA, Schmid CH, et al. A New Equation to Estimate Glomerular Filtration RateDevelopment and Comparison of a New Equation to Estimate GFR. *Annals of Internal Medicine* 2009; **150**(9): 604-12.

316. International Diabetes Federation. The IDF worldwide definition of the metabolic syndrome. 04/02/2019 2006. https://www.idf.org/e-library/consensus-statements/60-idfconsensus-worldwide-definitionof-the-metabolic-syndrome.html.

317. Karlas T, Petroff D, Sasso M, et al. Individual patient data meta-analysis of controlled attenuation parameter (CAP) technology for assessing steatosis. *Journal of Hepatology* 2017; **66**(5): 1022-30.

318. Matthews GV, Neuhaus J, Bhagani S, et al. Baseline prevalence and predictors of liver fibrosis among HIV-positive individuals: a substudy of the INSIGHT Strategic Timing of AntiRetroviral Treatment (START) trial. *HIV Med* 2015; **16 Suppl 1**: 129-36.

319. Todowede OO, Mianda SZ, Sartorius B. Prevalence of metabolic syndrome among HIV-positive and HIV-negative populations in sub-Saharan Africa—a systematic review and meta-analysis. *Systematic Reviews* 2019; **8**(1): 4.

320. Sivaraj N, Sasheela P, Bahyah KS, Adeeba K. Prevalence of Metabolic Syndrome Among People Living with HIV in Developing Countries: A Systematic Review. *AIDS Patient Care and STDs* 2017; **31**(1): 1-13.

321. Nguyen KA, Peer N, Mills EJ, Kengne AP. A Meta-Analysis of the Metabolic Syndrome Prevalence in the Global HIV-Infected Population. *PLOS ONE* 2016; **11**(3): e0150970.

322. Gami AS, Witt BJ, Howard DE, et al. Metabolic syndrome and risk of incident cardiovascular events and death: a systematic review and meta-analysis of longitudinal studies. *Journal of the American College of Cardiology* 2007; **49**(4): 403-14.

323. Vinikoor MJ, Sinkala E, Chilengi R, et al. Impact of Antiretroviral Therapy on Liver Fibrosis Among Human Immunodeficiency Virus-Infected Adults With and Without HBV Coinfection in Zambia. *Clin Infect Dis* 2017; **64**(10): 1343-9.

324. Kaspar MB, Sterling RK. Mechanisms of liver disease in patients infected with HIV. *BMJ open gastroenterology* 2017; **4**(1): e000166-e.

325. Eddowes PJ, Sasso M, Allison M, et al. Accuracy of FibroScan Controlled Attenuation Parameter and Liver Stiffness Measurement in Assessing Steatosis and Fibrosis in Patients With Non-alcoholic Fatty Liver Disease. *Gastroenterology* 2019.

326. Patel P, Rose CE, Collins PY, et al. Noncommunicable diseases among HIV-infected persons in low-income and middle-income countries: a systematic review and meta-analysis. *AIDS (London, England)* 2018; **32 Suppl 1**(Suppl 1): S5-S20.

327. Stanaway JD, Afshin A, Gakidou E, et al. Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *The Lancet* 2018; **392**(10159): 1923-94.

328. Mungwira RG, Divala TH, Nyirenda OM, et al. A targeted approach for routine viral load monitoring in Malawian adults on antiretroviral therapy. *Tropical Medicine & International Health* 2018; **23**(5): 526-32.

329. Phan V, Thai S, Koole O, et al. Validation of a Clinical Prediction Score to Target Viral Load Testing in Adults With Suspected First-Line Treatment Failure in Resource-Constrained Settings. *JAIDS Journal of Acquired Immune Deficiency Syndromes* 2013; **62**(5): 509-16.

330. van Griensven J, Phan V, Thai S, Koole O, Lynen L. Simplified Clinical Prediction Scores to Target Viral Load Testing in Adults with Suspected First Line Treatment Failure in Phnom Penh, Cambodia. *PLOS ONE* 2014; **9**(2): e87879.

331. Labhardt ND, Lejone T, Setoko Ml, et al. A Clinical Prediction Score in Addition to WHO Criteria for Anti-Retroviral Treatment Failure in Resource-Limited Settings - Experience from Lesotho. *PLOS ONE* 2012; **7**(10): e47937.

332. Rutstein SE, Hosseinipour MC, Weinberger M, et al. Predicting resistance as indicator for need to switch from first-line antiretroviral therapy among patients with elevated viral loads: development of a risk score algorithm. *BMC Infectious Diseases* 2016; **16**: 280.

333. Mayston R, Kinyanda E, Chishinga N, Prince M, Patel V. Mental disorder and the outcome of HIV/AIDS in low-income and middle-income countries: a systematic review. *Aids* 2012; **26 Suppl 2**: S117-35.

334. Kumar P, Mahato DK, Kamle M, Mohanta TK, Kang SG. Aflatoxins: A Global Concern for Food Safety, Human Health and Their Management. *Frontiers in microbiology* 2017; **7**: 2170-.

335. Ataklte F, Erqou S, Kaptoge S. 147 Burden of Undiagnosed Hypertension in Sub-Saharan Africa: A Systematic Review and Meta-Analysis. *Heart* 2015; **101**(Suppl 4): A84-A.

336. Shah ASV, Stelzle D, Lee KK, et al. Global Burden of Atherosclerotic Cardiovascular Disease in People Living With HIV. *Circulation* 2018; **138**(11): 1100-12.

337. Friis-Moller N, Ryom L, Smith C, et al. An updated prediction model of the global risk of cardiovascular disease in HIV-positive persons: The Data-collection on Adverse Effects of Anti-HIV Drugs (D:A:D) study. *Eur J Prev Cardiol* 2016; **23**(2): 214-23.

338. Echecopar-Sabogal J, D'Angelo-Piaggio L, Chanamé-Baca DM, Ugarte-Gil C. Association between the use of protease inhibitors in highly active antiretroviral therapy and incidence of diabetes mellitus and/or metabolic syndrome in HIV-infected patients: A systematic review and meta-analysis. *International Journal of STD & AIDS* 2018; **29**(5): 443-52.

339. Mendenhall E, Kohrt BA, Norris SA, Ndetei D, Prabhakaran D. Non-communicable disease syndemics: poverty, depression, and diabetes among low-income populations. *The Lancet* 2017; **389**(10072): 951-63.

340. Angkurawaranon C, Nitsch D, Larke N, Rehman AM, Smeeth L, Addo J. Ecological Study of HIV Infection and Hypertension in Sub-Saharan Africa: Is There a Double Burden of Disease? *PLoS One* 2016; **11**(11): e0166375.

341. United Nations Development Programme. Human development report: The Rise of the South: Human Progress in a Diverse World.; 2013.

342. Tsai AC, Siedner MJ. The Missing Men: HIV Treatment Scale-Up and Life Expectancy in Sub-Saharan Africa. *PLOS Medicine* 2015; **12**(11): e1001906.

343. Jewkes R, Morrell R. Gender and sexuality: emerging perspectives from the heterosexual epidemic in South Africa and implications for HIV risk and prevention. *Journal of the International AIDS Society* 2010; **13**: 6-.

344. Chambers LA, Rueda S, Baker DN, et al. Stigma, HIV and health: a qualitative synthesis. *BMC public health* 2015; **15**: 848-.

345. Ekrikpo UE, Kengne AP, Bello AK, et al. Chronic kidney disease in the global adult HIV-infected population: A systematic review and meta-analysis. *PLOS ONE* 2018; **13**(4): e0195443.

346. Vinikoor MJ, Mulenga L, Siyunda A, et al. Association between hepatitis B co-infection and elevated liver stiffness among HIV-infected adults in Lusaka, Zambia. *Trop Med Int Health* 2016; **21**(11): 1435-41.

347. Stabinski L, Reynolds SJ, Ocama P, et al. High prevalence of liver fibrosis associated with HIV infection: a study in rural Rakai, Uganda. *Antivir Ther* 2011; **16**(3): 405-11.

348. Wandeler G, Mulenga L, Vinikoor MJ, et al. Liver fibrosis in treatment-naive HIV-infected and HIV/HBV co-infected patients: Zambia and Switzerland compared. *International*

journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases 2016; **51**: 97-102.

349. World Health Organisation. Policy brief: Consolidated guidelines on HIV prevention, diagnosis, treatment and care for key populations, 2016 update. Geneva; 2017.

350. Bjerrum S, Oliver-Commey J, Kenu E, et al. Tuberculosis and non-tuberculous mycobacteria among HIV-infected individuals in Ghana. *Tropical Medicine & International Health* 2016; **21**(9): 1181-90.

351. Ohene SA, Bakker MI, Ojo J, Toonstra A, Awudi D, Klatser P. Extra-pulmonary tuberculosis: A retrospective study of patients in Accra, Ghana. *PLoS One* 2019; **14**(1): e0209650.

352. Phillips RO, Steinmetz A, Nichols J, et al. Spectrum of disease in HIV-positive patients presenting to a tertiary care hospital: a retrospective, cross-sectional review in Kumasi, Ghana. *BMC infectious diseases* 2018; **18**(1): 419.

353. Hickey AJ, Gounder L, Moosa MY, Drain PK. A systematic review of hepatic tuberculosis with considerations in human immunodeficiency virus co-infection. *BMC infectious diseases* 2015; **15**: 209.

354. Ngouleun W, Biapa Nya PC, Pieme AC, Telefo PB. Risk assessment of hepatotoxicity among tuberculosis and human immunodeficiency virus/AIDS-coinfected patients under tuberculosis treatment. *International journal of mycobacteriology* 2016; **5**(4): 482-8.

355. Tweed CD, Wills GH, Crook AM, et al. Liver toxicity associated with tuberculosis chemotherapy in the REMoxTB study. *BMC Med* 2018; **16**(1): 46.

356. Peers F, Bosch X, Kaldor J, Linsell A, Pluijmen M. Aflatoxin exposure, hepatitis B virus infection and liver cancer in Swaziland. *International journal of cancer* 1987; **39**(5): 545-53.

357. Afum C, Cudjoe L, Hills J, et al. Association between Aflatoxin M1 and Liver Disease in HBV/HCV Infected Persons in Ghana. *International Journal of Environmental Research and Public Health* 2016; **13**(4): 377.

APPENDIX 1: NON-COMMERCIAL MATERIAL TRANSFER AGREEMENT

NON-COMMERICAL MATERIAL TRANSFER AGREEMENT

BETWEEN: The University of Liverpool, The Foundation Building, 765 Brownlow Hill, Liverpool, L69 7ZX (the **"University")**;

AND: Kwame Nkrumah University of Science and Technology College of Health Sciences (the **"Provider"**);

Upon: Professor Anna Maria Geretti (the "**Principal Investigator**"), an employee of the University, requesting Serum and Plasma (the "**Material**") for use at the University's premises in the project entitled "A study of Hepatitis B Co-infection Among HIV-Positive Patients in KATH" (the "**Purpose**"). The Provider confirms it is willing to supply the Material subject to the following terms.

It is Agreed by the parties as follows:

1. DEFINITIONS AND INTERPRETATIONS

1.1. **"Confidential Information"** means all information including without limitation all ideas, techniques, processes, know-how, routines, specifications, formulae, drawings, methods and other knowledge concerning the Material and the use of the Material in any Replicates or Derivates.

1.2. **"Derivative"** means any material created from the Material that is substantially modified but still represents a non-severable improvement to or amendment of the Material.

1.3. **"Intellectual Property"** shall mean patent applications, patents, trademarks, service marks, registered designs, domain names, applications for any of the foregoing, trade and business names, unregistered trademarks and service marks, know-how, copyrights, rights in designs, rights in databases, rights in inventions, rights in improvements and rights of the same or similar effect or nature, in any part of the world.

1.4. **"Replicate"** means any biological or chemical material representing substantially unmodified copy of all or part of the Material.

2. USE OF THE MATERIALS

2.1. The Material is to be used solely for the Purpose.

2.2. The Provider shall retain all right and title in and to the Material supplied under this Agreement which shall not be distributed or released to any person other than the Principal Investigator and co-workers working under the supervision of the Principal Investigator.

2.3. The Provider agrees that ownership of any Intellectual Property rights arising from use of the Material or any Replicates or Derivatives thereof by the University shall vest in the University. The Provider shall execute all such documents and do all such things as the University may request, at the University's expense, to ensure that such Intellectual Property rights vest in the University.

2.4. The University acknowledges and agrees that the Material or Replicates or Derivatives are not intended for use in humans or animals.

2.5. The University agrees to comply with all restrictions on export from the United Kingdom and re-export from other countries as required by law for the Material, Replicates and Derivatives. Regarding transfers of Material, Replicates, or Derivatives to destinations outside the United Kingdom:

2.5.1. The University assumes all risk and responsibility in connection with complying with applicable foreign law and regulations concerning the import, handling, transportation, storage, use, and misuse or other wrongdoing with respect to the Material, Replicates or Derivatives.

2.6. The Provider may at its discretion provide technical assistance and information with respect to the Material, Replicates or Derivatives as well as other products and procedures associated with use of the Material, Replicates or Derivatives.

3. PUBLICATIONS

3.1. The University will acknowledge the Provider as the source of the Material, Replicates or Derivatives in any and all publications that reference their use.

4. CONFIDENTIALITY

4.1. The University shall not disclose any part of the Confidential Information to any third party without the written permission of the Provider, except where the Confidential Information comes into the public domain without breach of this Agreement by the University; the University was lawfully in possession of the Confidential Information prior to the date of this Agreement; is disclosed with the prior written approval of the Provider; is obtained lawfully from a third party entitled to disclose the same; is required to be disclosed by law or by order of a court of competent jurisdiction.

4.2. The University shall only disclose the Confidential Information to its employees or agents who need to know the same for the Purpose and the University maintains that such employees and agents are obliged by obligations of confidentiality no less stringent than those contained in this Agreement.

4.3. The Provider acknowledges that this Agreement does not intend to prevent publication by the University of the results produced through completion of the Purpose, and the Provider may only reasonably object to publication by the University if the proposed publication contains Confidential Information belonging to it.

4.4. The University shall apply no lesser security or degree of care than that which it applies to its own confidential or proprietary information.

5. INDEMNITY AND LIABILITY

5.1. The University acknowledges that the Material is experimental in nature and the Material and Confidential Information is provided by the Provider without any representation, condition or warranty whatsoever. There is no representation, condition or warranty given by the Provider that the Material or Confidential Information will be fit for the particular purpose required by the Recipient.

5.2. The University shall indemnify the Provider, its directors, employees and representatives from any reasonable loss (with the exception of consequential and/or indirect losses), claim, damage, injury, expense or other liability resulting from the University's possession, use, storage, transport or disposal of the Material except to the

extent that such reasonable loss, claim, damage, injury, expense or other liability is caused or contributed to by the negligence of the Provider.

6. GENERAL

6.1. The Provider warrants that it has received the relevant ethical approval to obtain the Material and that the subjects who have provided the Material did so with informed consent.

6.2. This Agreement shall come into force on the date of the last signature to the Agreement, and shall expire 10 years from that date. Either party may terminate this Agreement upon 30 days written notice to the other.

6.3. The University may terminate this Agreement immediately upon written notice to the Provider if the Provider breaches any of the terms of this Agreement. Failure by the University to immediately terminate the Agreement upon notice of such a breach shall not constitute an acceptance of the breach or a waiver of the University's right to terminate the Agreement for the breach in future.

6.4. If any provision of this Agreement is declared void or unenforceable by a Court of competent jurisdiction it shall be severed from the Agreement and the remaining provisions shall continue to the fullest extent permitted by law.

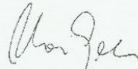
6.5. Nothing in this Agreement creates a relationship of employment, agency or partnership between the parties.

6.6. The Provider shall not be entitled to assign this Agreement without the express written permission of the University.

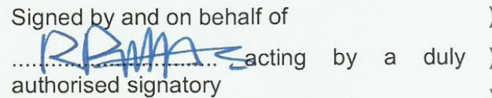
6.7. This Agreement contains the entire agreement between the parties. No amendments or modifications to this agreement will be of any effect unless in writing signed by authorised representatives of both parties.

6.8. This Agreement is subject to the laws of England and both parties hereby submit to the exclusive jurisdiction of the English Courts.

Signed by and on behalf of The University of Liverpool acting by a duly authorised signatory:)
)
Name: Professor Anna Maria Geretti)



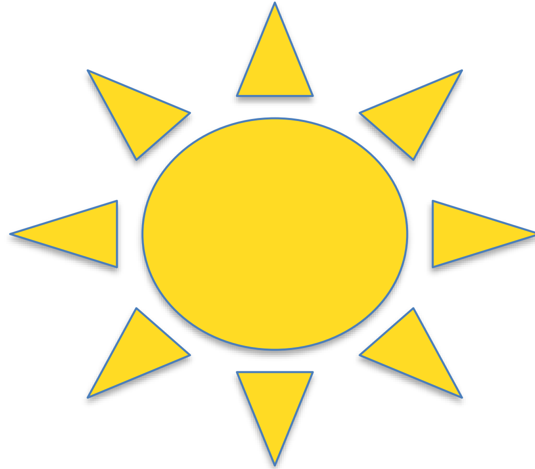
Date:12/12/2011

Signed by and on behalf of)

..........................acting by a duly)
authorised signatory)
Name: Dr. Richard Odame Phillips

Date: 12.12.2011

APPENDIX 2: HEPIK-2 STUDY QUESTIONNAIRE

HEPIK 2



Questionnaire

Thank you for agreeing to complete this confidential questionnaire. Please answer all the questions as fully as you can. We hope that you will answer all the questions, however you are free to leave any question you do not want to answer.

Please do NOT write your name on this questionnaire. The doctors and nurses in the clinic will NOT see your answers, and your answers will NEVER be recorded in your clinic notes.

If you have any questions or need any help, please ask the person who gave you this questionnaire. Once you have finished, please place the questionnaire in the envelope, seal the envelope, and give it back to the person who gave it to you. Thank you for your help!

Study No.	HEPIK				Date

SECTION A: GENERAL INFORMATION ABOUT YOU

A1. What is your age? ____________ years **Are you?** Male ☐ Female ☐

A2. Where do you come from, which is your ethnicity?

Akan ☐ Dagomba ☐ Ewe ☐ Ga ☐ Frafra ☐ Other (specify) ☐ ____________

A3. What is your current work situation?

In paid work full-time (30 hours or more per week)	☐	**go to A4**
In paid work part-time (less than 30 hours per week)	☐	**go to A4**
Looking after home / family	☐	**go to A5**
Student or trainee	☐	**go to A5**
Unemployed, able to work	☐	**go to A5**
Unemployed, unable to work	☐	**go to A5**
Retired	☐	**go to A5**

A4. If you are working currently, what type of work do you?

Manual work (e.g., farmer) ☐ Semiskilled worker (e.g., artisan, trader) ☐

Skilled worker (e.g., teacher) ☐ Other (specify) ☐ ____________

A5. What is your current housing situation?

Own my own home ☐ Renting a home ☐ Staying with family or friends ☐

Homeless ☐ Other (specify) ☐ ____________

A6. How do you travel to the HIV clinic?

Walk ☐ Own car ☐ Public transport (e.g., bus, trotro) ☐

Taxi ☐ Other (specify) ☐ ____________

A7. How long does it take you to travel to the clinic? ____________ hour(s)

A8. Do you have enough money to cover your basic needs (e.g., food, clothes)?

Yes, all of the time ☐ Yes, most of the time ☐ Yes, some of the time ☐ No ☐

A9. What is your level of schooling?

No formal education ☐ Up to primary level ☐ Up to secondary level ☐

University ☐ Postgraduate ☐

A10. What is your marital status?

Single ☐ Separated ☐ Divorced ☐ Widow ☐ **For any of these go to A13**

Married ☐ Cohabitant ☐ **For either of these go to A11**

A11. If you have a partner, how long have you been together? ____________

A12. If you have a partner, does your partner have HIV? Yes ☐ No ☐ Don't know ☐

A13. Do you have any children? Yes ☐ **go to A14** No ☐ **go to section B**

A14. If YES, how many children do you have? ____________

A15. If YES, what is the age of the youngest child? ____________

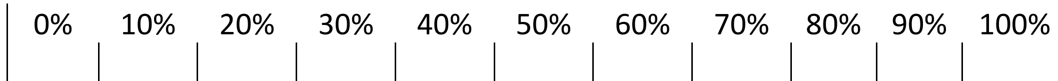
SECTION B: INFORMATION ABOUT HIV and TREATMENT				
B1. Apart from clinic/hospital staff, whom have you told that you have HIV?				
I told a partner / wife / husband		Yes ☐	No ☐	Not applicable ☐
I told other family members		None ☐	Some ☐	Most/all ☐
I told my friends		None ☐	Some ☐	Most/all ☐
I told work colleagues	None ☐	Some ☐	Most/all ☐	Not applicable ☐
I told someone else (specify) ☐				
B2. In which year did you first start taking HIV treatment?				
B3. Did you start treatment because HIV was making you ill?			Yes ☐	No ☐
B4. Which response is closest to your view? "Compared to what I expected before starting HIV treatment, taking treatment was..."				
Worse than I expected ☐	About the same as I expected ☐	Better than I expected ☐	Don't know / can't remember ☐	
B5. After you started the HIV treatment, did you ever stop the treatment for more than 3 days?			Yes ☐ **go to B6**	No ☐ **go to B7**
B6. How many times did you stop the HIV treatment?		1 ☐	2 ☐	3 or more ☐
B7. What did you do when the KATH HIV clinic closed (mid 2013 to end 2014?				
Stopped HIV treatment ☐		Got HIV treatment from KATH ☐		
Got the HIV treatment from another clinic ☐		Used alternative medicine ☐		
Other (specify) ☐				
B8. Are you taking HIV treatment currently?			NO ☐ **go to B9**	Yes ☐ **go to B10**
B9. If NO, when did you stop?				
B10. If YES, when did you take your last dose?				
B11. How often do you take your treatment?			Once a day ☐	Twice a day ☐
B12. In the LAST WEEK, how many doses of HIV treatment have you missed?				
I missed no doses in the last week ☐		I missed 1 dose ☐		
I missed 2 or 3 doses ☐		I missed more than 2 or 3 doses ☐		
B13. If you missed one or more doses in the LAST WEEK, what were the reasons?				
Treatment was making me feel ill			Yes ☐	No ☐
I forgot to take the pills			Yes ☐	No ☐
I was away from home and forgot to bring my pills			Yes ☐	No ☐
I ran out of pills			Yes ☐	No ☐
I was in a place where people could see me			Yes ☐	No ☐
I was with people who did not know I had HIV			Yes ☐	No ☐
I was fed up with taking pills			Yes ☐	No ☐
I was feeling depressed / low			Yes ☐	No ☐
Other (specify)			Yes ☐	No ☐
B14. In the PAST 3 MONTHS, did you miss the HIV treatment for 3 or more days in a row?				
Yes ☐ **go to B15**	No ☐ **go to B16**	Don't know/don't remember ☐ **go to B16**		

B15. How many times did you miss the HIV treatment for 3 or more days in a row?

Once ☐ 2 to 3 times ☐ More than 3 times ☐

B16. People don't always manage to take 100% of their HIV pills. What is your best guess about how much of the HIV treatment you took in the PAST THREE MONTHS?

For example, 0% means you took none of the pills, 50% means you took half of the pills, and 100% means you took every single pill. **Please put an "X" below** at the point showing your guess:



SECTION C: INFORMATION ABOUT LIFESTYLE

C1. Do you drink alcohol?

Yes, currently ☐ **go to C2**
Yes in the past, but not currently ☐ **go to C4**
No, never ☐ **go to Section D**

C2. If YES currently, how often do you have a drink that contains alcohol?

Every day ☐ 3 to 4 times each week ☐ 1 to 2 times each week ☐
1 or 2 times each month ☐ Less than once a month ☐

C3. If YES, currently, which type of alcohol do you drink?

Beer ☐ Wine ☐ Spirits ☐ Local brew ☐

C4. If YES in the past but not currently, when did you stop drinking alcohol?

In the last year ☐ In the last 2 years ☐ In the last 5 years ☐ More than 5 years ago ☐

C5. Have you ever felt or been told that you should cut down on your drinking?

Yes, felt I should ☐ Yes, was told I should ☐ No ☐

SECTION D: INFORMATION ABOUT YOUR HEALTH AND WELLBEING

In this section, we use some questions to ask you about your health. If you are worried about any symptoms, please talk to your doctor. Your doctor or nurse will not see the answers from this questionnaire.

D1. During the PAST WEEK, have you had any of the following symptoms?

	Most of the time	Some of the time	Rarely or never
Difficulty concentrating	☐	☐	☐
Sleep problems	☐	☐	☐
Lack of energy, feeling tired	☐	☐	☐
Trouble remembering things	☐	☐	☐
Headache	☐	☐	☐
Pain	☐	☐	☐
Lack of appetite	☐	☐	☐
Nausea	☐	☐	☐
Vomiting	☐	☐	☐
Diarrhoea	☐	☐	☐
Constipation	☐	☐	☐
Feeling bloated/gas/wind	☐	☐	☐

Muscle aches or joint pains	☐	☐	☐
Dizziness	☐	☐	☐
Sweat or fever	☐	☐	☐
Cough	☐	☐	☐
Short of breath	☐	☐	☐
Sexual problems	☐	☐	☐
Skin problems (e.g., rash, itching)	☐	☐	☐
Dry mouth	☐	☐	☐
Mouth sores	☐	☐	☐
Changes in the way food tastes	☐	☐	☐
Weight loss	☐	☐	☐
Weight gain	☐	☐	☐
Changes in fat in face or body	☐	☐	☐
Bleeding from the mouth	☐	☐	☐
Tremor (for example in your hands)	☐	☐	☐
Numbness, tingling, in hands/ feet	☐	☐	☐

D2. During the PAST WEEK, how often have you been bothered by any of the following problems?

	Most of the time	Some of the time	Rarely or never
Feeling sad	☐	☐	☐
Feeling nervous or anxious	☐	☐	☐
Little interest or pleasure in doing things	☐	☐	☐
I don't enjoy life	☐	☐	☐
Feeling down	☐	☐	☐
Feeling depressed	☐	☐	☐
I have no hope in the future	☐	☐	☐
Feeling worried	☐	☐	☐
Feeling irritable, get angry easily	☐	☐	☐
Feeling afraid	☐	☐	☐
Feeling bad, feeling I have let people down	☐	☐	☐
Thinking I would be better off dead	☐	☐	☐
Wanting to harm myself	☐	☐	☐
Having trouble relaxing	☐	☐	☐
Feeling restless	☐	☐	☐

If you have had any of these problems, have they made it difficult for you to do your work, take care of things at home, or get along with other people?

No ☐ A little ☐ A lot ☐ Extremely ☐

D3. About TODAY, which statements best describe your state of health TODAY

Mobility I have no problems in walking about ☐

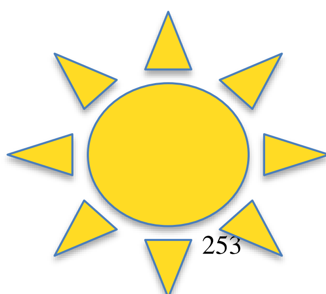
	I have some problems in walking about ☐ I have to stay in bed most of the time ☐
Self-care	I have no problems with self-care ☐ I have some problems washing and dressing myself ☐ I am unable to was and dress myself ☐
Usual activities (work, study, housework, family or leisure activities)	I have no problems with performing my usual activities ☐ I have some problems with performing my usual activities ☐ I am unable to perform my usual activities ☐
Pain/discomfort	I have no pain or discomfort ☐ I have moderate pain or discomfort ☐ I have extreme pain or discomfort ☐
Anxiety/depression	I am not anxious or depressed ☐ I am moderately anxious or depressed ☐ I am extremely anxious or depressed ☐

D4. Here is a list of some things that other people do for us that may be helpful or supportive. Please place a tick in the column that is closest to your situation.

	As much as I would like	Some, but would like more	Much less than I would like
I have people who care about me	☐	☐	☐
I get love and affection	☐	☐	☐
I get chances to talk to someone I trust about personal problems	☐	☐	☐
I get invitations to go out and do things with other people	☐	☐	☐
I get help when I am sick in bed	☐	☐	☐

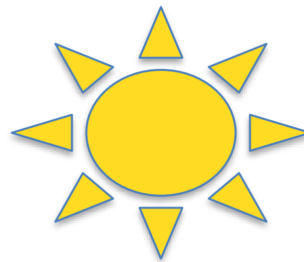
This is the end of the questionnaire. Please use this space for any comment you wish to make.

Thank you for completing the questionnaire. Please seal the questionnaire in the envelope provided and give it back to the person who gave it to you.



APPENDIX 3: OPTIMISE STUDY QUESTIONNAIRE 1

OPTIMISE



QUESTIONNAIRE

Thank you for agreeing to complete this confidential questionnaire. We hope that you feel able to answer all the questions sincerely, however you are free to leave out any question you do not want to answer. Your answers will not be linked to your name. Therefore please do NOT write your name on the questionnaire. The doctors and nurses in the clinic will not see your answers, and your answers will not be recorded in your clinic notes. The researchers will use the answers to understand how to improve the care of people coming to clinic.

Study No.	OPTIMISE				Date

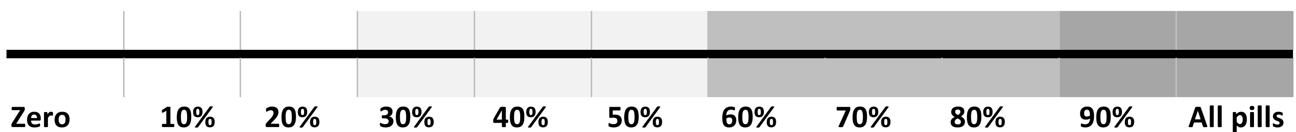
SECTION A: GENERAL INFORMATION			
A1. Are you?	Male ☐	Female ☐	
A2. How long was your travel to the clinic today?	Less than 2 hours ☐	More than 2 hours ☐	
A3. How do you feel about coming to the HIV clinic?			
It's good, it's important for me	☐		
It's not good and it's not bad	☐		
I don't like it, it's something I have to do	☐		
A4. When did you finish school?			
I did not go to school	☐	I left school after primary	☐
I left school after secondary	☐	I went further than secondary	☐
A5. Do you have a regular partner?	Yes ☐	No ☐	
A6. Do you have children?	Yes ☐	No ☐	
A7. If YES, how many children do you have?			
A8. Do you have enough food to eat?			
Yes, every day	☐	Yes, sometimes	☐
Yes, most days	☐	No, I don't have enough food most days	☐

SECTION B: INFORMATION ABOUT LIFESTYLE			
B1. Do you drink alcohol?	No, never ☐	*Please go to section C*	
Yes, once a week or less ☐	Yes, a few times a week ☐	Yes, everyday ☐	
B2. If you drink alcohol, what do you usually drink?			
Beer ☐	Wine ☐	Spirits (e.g. whiskey) ☐	Other ☐ ________________
B3. If you drink alcohol, on days when you drink, do you drink			
A little (e.g. a small bottle of beer) ☐	In moderation (e.g. 2-3 bottles of beer) ☐		
A lot (e.g. more than 3 bottles of beer) ☐			
B4. How often do you feel the need for a drink in the morning?			
Never ☐	Once a month or less ☐	Once a week ☐	Every day ☐
B5. How often do you forget to take your HIV medicines because of drinking?			
Never ☐	Once a month or less ☐	Once a week ☐	More than once a week ☐
B6. How often do you run out of money because you spent it all on drinks?			
Never ☐	Once a month or less ☐	Once a week ☐	More than once a week ☐

SECTION C: INFORMATION ABOUT HIV TREATMENT			
C1. Have you ever taken HIV tablets?	No ☐	*This completes the questionnaire, thank you*	
Yes	☐	*Please continue to the following questions*	
C2. When did you start taking HIV tablets?			
C3. Are you still taking HIV tablets?	No ☐	When did you stop?	
Yes ☐	Which tablets are you taking?		
How often do you take them?	Once a day ☐	Twice a day ☐	
C4. Since you first started HIV treatment, did you ever stop taking the HIV tablets for more than 3 days in a row for any reason?			
No, never ☐ Once ☐	2 to 3 times ☐	More than 3 times ☐	Not sure ☐
C5. If you stopped taking the tablets for more than 3 days in a row, how long did you stop for?			
A few days ☐ A few weeks ☐	A few months ☐	One year or more ☐	
Why did you stop? (give all the reasons)			

C6. People don't always manage to take all of their HIV tablets. What is your best guess about how much of the HIV treatment you took in the PAST 3 MONTHS?

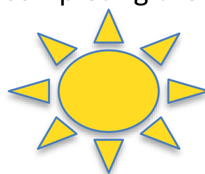
Please put an "X" on the line below at the point showing your guess:



Zero **10%** **20%** **30%** **40%** **50%** **60%** **70%** **80%** **90%** **All pills**

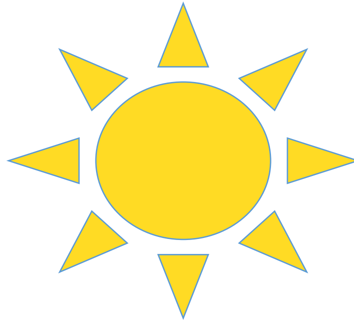
This is the end of the questionnaire. Please use this space for any comment you wish to make

Thank you for completing the questionnaire



APPENDIX 4: OPTIMISE STUDY QUESTIONNAIRE 2

OPTIMISE



ADHERENCE REVIEW FORM

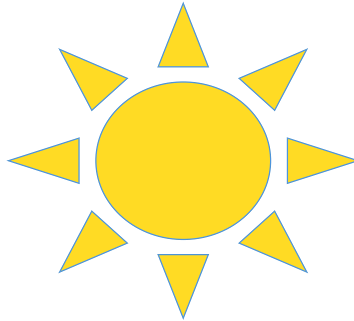
Instructions for the provider of adherence support: This form is destined to the patients that have reported incomplete adherence in the questionnaire. It is meant to explore possible reasons of suboptimal adherence. Please read the questions to the patient and encourage them to recall all possible reasons they may be missing doses. Please record ALL reasons provided by the patient

Study No.		**Initials**		**Date**	

Questions			
1. In the LAST WEEK, how many doses of the HIV treatment have you missed?			
None ☐	1 dose ☐	2 or 3 doses ☐	more than 3 doses ☐
2. In the LAST MONTH, how many doses of the HIV treatment have you missed?			
None ☐	1 dose ☐	2 or 3 doses ☐	more than 3 doses ☐
3. When you did not manage to take all of your pills, what was the reason? **Please indicate all that apply**			
The pills were making me ill		Yes ☐	
I forgot to take the pills		Yes ☐	
I was away from home and did not bring my pills		Yes ☐	
I ran out of pills		Yes ☐	
I was in a place where people could see me taking the pills		Yes ☐	
I was tired of taking pills		Yes ☐	
I was feeling ill		Yes ☐	
I was feeling sad		Yes ☐	
I took traditional medicines instead		Yes ☐	
Other (specify):			
4. Does anyone help you to remember to take the drugs?			
Yes, my partner ☐	Yes, someone in my family ☐	Yes, a friend ☐	No ☐
5. Do you have any other comment?			

APPENDIX 5: OPTIMISE STUDY QUESTIONNAIRE 3

OPTIMISE



II VISIT QUESTIONNAIRE

Instructions: This form is destined to the patients that had a detectable HIV viral load at the time of the first OPTIMISE visit in February 2018 and received an adherence support intervention. It is meant to record any change in adherence behaviour and HIV viral load after two months. Please read the questions to the patient and encourage them to recall all possible reasons they may be missing doses. Please record ALL reasons provided by the patient.

Study No.		**Initials**		**Date**	

Questions

1. In the LAST WEEK, how many doses of the HIV treatment have you missed?

None ☐ 1 dose ☐ 2 or 3 doses ☐ more than 3 doses ☐

2. In the LAST MONTH, how many doses of the HIV treatment have you missed?

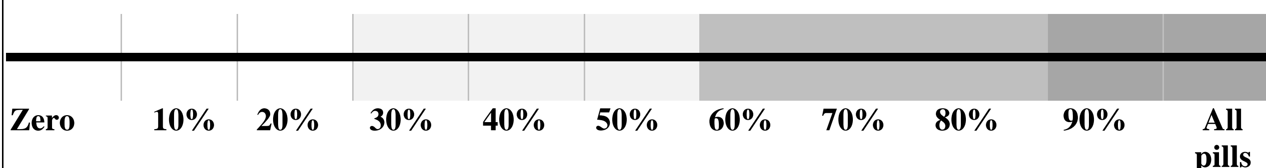
None ☐ 1 dose ☐ 2 or 3 doses ☐ more than 3 doses ☐

3. When you did not manage to take all of your pills, what was the reason?
Please indicate all that apply

The pills were making me ill	Yes ☐
I forgot to take the pills	Yes ☐
I was away from home and did not bring my pills	Yes ☐
I ran out of pills	Yes ☐
I was in a place where people could see me taking the pills	Yes ☐
I was tired of taking pills	Yes ☐
I was feeling ill	Yes ☐
I was feeling sad	Yes ☐
I took traditional medicines instead	Yes ☐

Other (specify):

4. People don't always manage to take all of their HIV tablets. What is your best guess about how much of the HIV treatment you took in the PAST 2 MONTHS?
Please put an "X" on the line below at the point showing your guess:



Zero **10%** **20%** **30%** **40%** **50%** **60%** **70%** **80%** **90%** **All pills**

5. Does anyone help you to remember to take the drugs?

Yes, my partner ☐ Yes, someone in my family ☐ Yes, a friend ☐ No ☐

6. During last visit, we discussed with you how you take your HIV treatment. How did you find it?

Very helpful ☐ Helpful ☐ Not helpful ☐ Not helpful and I did not like it ☐

7. Do you have any other comment?