SYLVIA KIWUWA MUYINGO

Adherence to Antiretroviral Therapy (ART) in the DART Trial in Uganda and Zimbabwe

Statistical analysis for Predictors and Consequences of Poor Adherence

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ACADEMIC DISSERTATION
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Dedicated to my Parents Bruno and Maria Kiwuwa and to my husband Anthony and children Malone, Maria and Vincent

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Abstract

The aim of this doctoral thesis was to explore existing statistical methods and develop new tools to analyse adherence data. In addition to the development and description of statistical methods, this research tries to find answers to several important epidemiological questions. Analysis and understanding of adherence data is a big challenge for investigators and researchers. Poor medication adherence, for example, can lead to under-reporting of both therapeutic and adverse effects and undermine the results of the otherwise well-designed studies. In some clinical trials, optimal adherence cannot often be reached, and therefore adherence has a dual role in data analysis as an outcome and an important explanatory variable.

In this work, we analyse the data from a large cohort ($n = 3316$) of previously untreated African individuals initiating ART in rural and urban centres in Uganda and Zimbabwe. Participants were randomly assigned to receive laboratory and clinical monitoring (LCM), or clinically driven monitoring (CDM). We observed excellent clinic attendance over the first year on antiretroviral therapy (ART). Our follow-up included 93% of those enrolled. Adherence measured by drug possession ratio (DPR) was high at each visit. Only 12% of patients maintained consistently high adherence over the course of the first year. Most patients had high adherence most of the time, with only one or two visits with less than 95% adherence, and less than 1% of the participants never achieved high adherence during the first year. Regardless of the measure, adherence increases over the first year.

In this work we first explore different methods of summarising adherence data collected over a time interval. We consider traditional averaging approaches and quantile based classifications or groups of patients based on these. We also consider adherence data as a realization of a Markov chain, and use the estimated transition probabilities calculated separately for each individual as summary measures. Hierarchical clustering using these summary statistics is then used to classify the patients. Different classifications are compared by their interpretations and by cross-tabulations, the associations between group memberships and the relevant background variables are described, and the group memberships are used to predict the mortality and CD4 failures.

Generalized estimating equations (GEE) were used to model for optimal adherence during the first 48 weeks (12 visits). The impact of adherence during the first 48 weeks separately on time to death and time to CD4 failure was modeled with Cox proportional hazard models. Four different adherence

classifications were used as explaining factors, and comparisons were made between the models. Finally, a dynamic logistic model was used to study the association between adherence and mortality. The model allows that the probability of dying between two clinic visits is explained by recent adherence history before the latest visit (assessed again at scheduled 4-weekly clinic visits) as well as by other (time dependent or baseline) covariates. In addition to the estimates of effects at the individual level, the approach also allows for the estimation of the population attributable fraction (PAF) a population level measure of the effect of adherence on mortality.

Based on our findings, a group of individuals (those with low CD4, reporting sexual partners 3 months prior to ART initiation, and low education) could be targeted for adherence-enhancing interventions both at ART initiation and in those not adhering well after a year on ART.

Worst adherence class based on Markov chain (MC) approach seems to predict mortality and CD4 failure independently of the worst class based on drug possession ratio (DPR). Whilst MC modeling is best suited to a research setting, DPR can be directly calculated from late return to clinic and self-reports of 4-day/weekend a simple (does not require calculation) measure are therefore most suited to a clinical setting.

The estimated population attributable fractions (PAF) based on the dynamic logistic regression model, that is, the estimated proportions of deaths that could have been avoided with optimal adherence in the LCM and CDM groups during the 5 years follow-up period were 16.0% (90% CI (-0.7,31.6)) and 33.1% (20.5,44.8), respectively. The estimated proportions of deaths on long-term ART that could be delayed at a population level (by eliminating non-optimal adherence) are similar to benefits from CD4 cell count monitoring of ART. In the absence of CD4 or viral load monitoring, individuals with optimal adherence experienced similar survival to those with customary adherence with CD4 monitoring suggesting that an alternative potential role of CD4 monitoring would be to reinforce adherence.

Contents

Abbreviations

ABC	Abacavir
AIDS	Acquired immune deficiency syndrome
ART	Antiretroviral Therapy
CDM	Clinical disease monitoring
CT	Continuous treatment
DPR	Drug possession ratio
EDM	Electronic Device monitoring
HAART	Highly active antiretroviral therapy
HIV	Human Immunodeficiency Virus
LCM	Laboratory and clinical disease monitoring
MC	Markov chain
MEMS	Medication Event Monitoring
NNRT	Non-nucleoside reverse transcriptase inhibitors
NRTI	Nucleoside reverse transcriptase inhibitors
NVP	Nevirapine
PAF	Population attributable Fraction
PC	Pill count
PI	Protease inhibitors
SAE	Serious adverse events
SSA	Sub-saharan Africa
STI	Structured treatment interruption
TDF	Tenofovir
VAS	Visual Analogue Scale
WHO	World Health Organisation

List of Original Publications

I. Muyingo SK, Walker AS, Reid A, Munderi P, Gibb DM, Ssali F, Levin J, Katabira E, Gilks C and Todd J; DART Trial Team, Patterns of Individual and Population-Level Adherence to Antiretroviral Therapy and Risk Factors for Poor Adherence in the First Year of the DART Trial in Uganda and Zimbabwe, *J Acquir Immune Defic Syndr*, Vol. 48(4)(468-75) (2008).

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1 Introduction

1.1 The concept of adherence

The concept of adherence (or compliance) has been defined as the extent to which the behaviour of the patient (in terms of taking medication, following a diet, attending clinics, or modifying habits such as smoking/drug/alcohol abuse, risky sexual behavior, or insufficient levels of physical activity) coincides with medical or health advice, or, more specifically, as the extent to which patients follow instructions for prescribed treatment necessary to achieve the full treatment benefits (Osterberg & Blaschke 2005). Poor adherence to medication then means, for example, that the patient skips entire doses, stretches prescribed time between doses, modifies doses, does not take medication for 3 or more days, unintentionally misses doses for reasons such as forgetting to take pills, not having a prescription filled or refilled, or discontinuing the medication.

Over the last half century, research in the management of many chronic and acute illnesses with medications has grown rapidly. The process of adherence to medication is a complex endeavour which involves (i) keeping the scheduled appointment, (ii) accepting a prescription for a medication, (iii) filling the prescription at pharmacy, (iv) taking the medication as prescribed (dose taking, dose timing), (v) maintaining an adequate supply by filling prescription in timely manner, and (vi) returning to the provider for ongoing monitoring (Osterberg & Blaschke 2005).The success of long-term medication use depends strongly on adherence to medication to maximize treatment benefits. In other words,

> "Drugs don't work if people don't take them". *C. Everrett Koop, 1985*

The increasing prevalence of chronic diseases with self-managed medication has led to interest and rapid developments in adherence research. Several studies have shown that patients often do not take their medications despite the evidence that non-adherence causes preventable morbidity (hospitalizations, developing complications, disease progression) and mortality, and wastes health care resources (Dimatteo 2004). In the US alone, it was estimated that approximately 125,000 yearly (McCarthy 1998) deaths occur prematurely due to poor adherence, 14-21% of patients never fill their original prescriptions, 60% cannot identify their medication, 25% of nursing home admissions are due to improper self management of their medication, 12-20%

take other people's medication, and annual hospital costs due to poor adherence are 8.5 billion US dollars.

In contrast to other chronic diseases, the rapid mutation and replication of HIV require that high levels of adherence are maintained ($\geq$ 95%) to achieve durable suppression (Sabate 2003). The introduction of antiretroviral therapy (ART) for HIV infection led to dramatic decline in HIV-related morbidity and mortality by 50% in the early 1990's. ART consists of usually two nucleoside reverse transcriptase inhibitors (NRTI's) and combined with one non-nucleoside reverse transcriptase (NNRTI), protease inhibitors (PI) or both. The potent and effective new combinations of ART regimens known as highly active antiretroviral therapy (HAART) have proven effective in reducing viral load and in improving clinical and immunological outcomes. Full virologic and clinical benefit requires high levels of adherence to ensure effective viral suppression, to avoid the emergence of cross-resistance, and to subsequently lower mortality (Moore et al. 2006, 2005, Bangsberg 2006*a*). In the presence of suboptimal drug levels due to poor adherence, these benefits may not be realised and may result in viral replication and viral rebound which in turn leads to immunological or clinical failure. Similarly adherence to ART based interventions will be critical in achieving the goal of reducing HIV transmission rates among at-risk populations using pre-exposure prophylaxis (PreP) and other biomedical interventions. However, the pill burden, complicated dosing requirements, and suboptimal tolerability due to side effects make adherence difficult (Bangsberg et al. 2000).

However resistance to drugs which results from poor adherence to ART depends on pattern of missed doses, frequency of interruptions and nature or characteristic of drug holiday (short or long) (Bangsberg et al. 2004). Studies have shown that adherence-outcome relationship varies with measurements, drug regimens and diseases. In this research work, we examine variations in patients adherence behavior to ART medication recommendations; and analyse patterns of adherence and congruence between the measures as a function of methodological and contextual factors in adherence research in Sub-saharan Africa (SSA).

1.2 Measurement of adherence

Results in studies of consequences and predictors of poor and good adherence as well as evaluations of interventions depend strongly on how adherence is quantified. In the following review we focus on medication adherence. The measures of medication adherence should be objective, practical, inexpensive, and acceptable to patients. Methods to describe adherence can be divided into direct and indirect methods.

1.2.1 Direct methods

Direct (objective) measures which include biological assays of active drug, metabolite or other markers in urine, blood or body fluids that confirm ac-

tive drug ingestion (Dimatteo 2004), have been used to assess drug adherence with varying utility. The direct methods are typically expensive and not feasible especially in resource limited settings. The methods also only give a snapshot of how much drug has been ingested during a few previous days. Direct methods are prone to overestimate adherence. In the so called "white coat" compliance, patients tend to take medication more regularly close to the visit to the clinic.

1.2.2 Indirect methods

Indirect adherence assessment methods may be based on electronic device monitoring (EDM), pill counts, self-administered questionnaires or interviews, patient diaries to assess adherence levels of the patient. A primary measure of adherence in both adults and children has been a self-report including interviews, structured questionnaires, and diaries. Indirect methods are often subject to recall bias (Bangsberg 2006*a*, Berg & Arnsten 2006). Each method naturally has its advantages and disadvantages. The following overview is based on Simoni et al (Simoni, Kurth, Pearson, Pantalone, Merrill & Frick 2006).

The Electronic Device monitoring (EDM) technology such as the medication event monotoring system (MEMS) is an objective adherence measure that uses a medicine container with an in-built microchip in the cap to detect the times and date of each opening. This method is a clear improvement to self-reports in terms of reliability.White coat adherence, for example, can be easily recognized with this method. Electronic monitoring is often considered as a proxy gold standard but its use is limited by its high cost and intrusive nature. Its intrinsic effects on adherence are still unknown. We of course expect that medication is removed and consumed upon each opening but that can not be verified. The method also assumes that medication is stored in a container, a correct number of pills is removed at each opening, the container is opened only during dosing and closed after each dose. EDM is also subject to technological malfunctions which may also cause underestimation. These devices obtain an advantage over pill count methods in providing data on dose timing.

Performing pill counts (PC) is thus another source of adherence data. The unannounced pill counts could be performed in a clinic setting or at home. Pill count measures the quantity of ART pills that the individual has taken between two ART pickups, often divided by the length of the time interval between the two pickups. This method tends to overestimate adherence due to pill dumping. In contrast unannounced pill counts also an objective method conducted at an individual's home tends to be costly. Conducting unannounced pill counts by telephone may be more viable in assessing patient medication adherence (Kalichman et al. 2007). Also rates of refilling prescriptions is another measure for overall adherence applicable in a closed pharmacy system where refills are measured at several points in time (Steiner & Prochazka 1997). Pharmacy refills or pill pickups (McMahon et al.

2011) can provide clinicians or scientists with readily available objective information on rates of prescription refill that can be used to assess overall adherence to medication. These data can also be used to support individual responses on questionnaires.

Patient self-report could be based on telephone or personal interviews, or on a written questionnaire which could be self-administered or administered by a nurse. Self-report could also be given in the form of diaries where patients record their taken pills, or by a visual analogue scale (VAS), a linear scale ranging from 0 to 100% indicating patients' best guess of how much medication they have taken. The VAS is appealing in resource limited settings (see (Bangsberg et al. 2004, Oyugi et al. 2007). More recent improvements to self-reports, such as private computer assisted interviewing devices (e.g. audio computer assisted self interviewing (A-CASI)) present a financial and technological burden in resource limited settings. The response format in interviews and questionnaires range from open ended questions (see e.g. (Golin et al. 2002)) to closed standardized questions (see (Chesney et al. 1999, 2000, Morisky et al. 1986)). Self-report can naturally vary in the lengths of the recall periods as well as in formulations of the questions. In self-reporting, low costs, convenience and easy acceptability compensate for the problems in accuracy, stability and comprehensiveness of the measurement (Gao & Nau 2000).

There is no generally accepted battery of standard questions to measure adherence, which makes the comparison of different adherence studies demanding. EDM is perhaps the most informative way of measuring adherence so far, and can be considered as a gold standard. Combining data from various sources, however, may provide invaluable insight into understanding the process of adherence. Indirect methods complement direct methods but dose taking and timing do not tell the whole story. Patient interviews, diaries, and questionnaires can provide additional information on other aspects of adherence, unknown risk factors, etc. The main drawback of EDM is its cost. Self-report is more appealing due to its low cost and convenience. The rates for poor adherence from self-reports are on the average, however, lower than those from EDM and their ability to explain (virological) outcomes is inferior to EDM. Patient self-report of the number of missed doses is often inaccurate and underestimates the true value; the self-report on missing a dose is more reliable (Pearson et al. 2007, Simoni, Kurth, Pearson, Pantalone, Merrill & Frick 2006).

1.2.3 Summary statistics

The various aspects of drug taking behavior over time are quantified with adherence indices. Several studies describing adherence over a treatment period have defined percentages or proportions of prescribed doses taken, percentages of days with prescribed number of doses taken, percentages of missed

doses, numbers or percentages of drug holidays, etc (Bangsberg et al. 2004, Simoni, Kurth, Pearson, Pantalone, Merrill & Frick 2006).

1. **Percentage doses taken** is 100 times the ratio of the number of taken doses to the number of prescribed doses. The measurements of the number of doses may be direct (EDM) or indirect (self-report).

2. **Percentage of compliant days** is the percentage of follow-up days with correct doses taken. The measurements of compliant days may be direct (EDM) or indirect (self-report).

3. **Pill counts (PC)** is defined as 100 times the ratio of the number of pills taken to the ratio of the prescribed pills over the follow-up interval. The measurements of the number of taken pills may be direct (EDM) or indirect (self-report).

4. **Medication or drug possession ratio (MPR/DPR)** is defined as the supply of drugs available (prescribed at last visit) minus the drugs returned divided by the number of days between clinic visits. The measurements are indirect (reports from clinics).

5. **Pharmacy refills or pill pick-up**. The measurements are indirect (reports from pharmacies).

6. **Missed appointments** are the appointments with doctors or nurses or the planned visits to clinics that are canceled or rescheduled, often reported as ratios or percentages.

7. **Percentage of drug holidays** refers to an interruption in dosing for three or more days (Ette & Ahmad 2007). This measure could also be obtained from questionnaires or EDM data.

1.3 Literature review of adherence

There is a growing interest in the public health implications of antiretroviral therapy (ART) as it has dramatically decreased the number of new HIV cases in many countries in the developing world. However, the preventive impact of ART will depend on several factors including adherence. Poor adherence is an important driver of virological failure, emergence of drug resistance, immunological failure and, ultimately, disease progression to death. In 2010 2.7 million people acquired new HIV infection, contributing to 34 million people living with HIV and the number of people dying from AIDS related causes was 1.9 million. In mid-2010s, about 68% of all people living with HIV resided in Sub-saharan Africa, a region with only 12% of the global population. Of the 2.7 million new infections, 1.9 million people were from Sub-saharan Africa representing 70% of all people who acquired HIV infection. Access to ART in low and middle income countries increased from 400,000 in 2003 to 6.65 million in 2010 representing 47% coverage of people eligible to treatment resulting in substantial declines in the number of people dying from AIDS related causes

in the last decade. Increased access to ART also substantially contributes to decline in number of new infections *(WHO HIV/AIDS Progress Report, 2011)*. Sub-saharan Africa accounts for the vast majority of deaths averted.

In the treatment of individuals with HIV infection or AIDS , the use of ART consists of two or more complex medications with possibly different dosing frequencies and requirements such as requirements with food. The drug regimens are designed to maintain optimal drug levels in patients body to achieve the desired therapeutic power with acceptable level of side effects. It is very important that patients take the medication as prescribed in order to suppress viral replication and avoid emergence of resistance (Nachega et al. 2007). Achieving good adherence is a challenge because patients regimen involves multiple, complex, and often expensive drugs and may have dietary requirements and side effects that result in poor tolerability. Given the consequences on nonadherence to ART, including rapid onset of viraemia and development of resistant virus that is transmittable to others (Simoni et al. 2010), an unprecedented amount of research has been undertaken to understand and promote ART adherence.

1.3.1 Poor adherence

There is no general consensus on what constitutes good adherence. Approximately half of patients with a chronic disease do not adhere to the extent that they are unable to obtain optimal clinical benefits (It is clear that full benefits of many effective medications that are available will be achieved only if patients follow prescribed treatment regimens reasonable closely). Adherence rates usually reported as percentages of good adherence typically vary across diseases, regimens, adherence measures and clinical or research setting. Adherence rate in clinical trial tend to be 40-80% on the average, due to the attention patients receive and to the selection process of the participants. In some trials, the rate can be as high as 80% and even 95% for serious chronic conditions such as HIV infection (Osterberg & Blaschke 2005). For HIV infected individuals taking antiretroviral therapy (ART) the rates tend to be 80-95% , yet ART adherence levels above 90% are recommended. However more potent ART drugs such as non-nucleoside reverse transcriptase inhibitors (NNRTI) suppress HIV at moderate adherence compared to unboosted Protease inhibitors (PI), see (Bangsberg 2006*a*). Differences in rates may also be partially caused by the differences in measurement methods, such as electronic devices, self-reports and directly observed treatments (DOTS). Key methodological concerns around the measurement and understanding patient adherence behaviour have been the recent focus for researchers and scientists (Dimatteo 2004).

Adherence behaviour varies over the course of treatment, especially for chronic disorders. In some studies, the discontinuation rates are high over the first several months in treatment. For those who remain, the treatment rates tend to decline for medication and keeping the appointments. In addition to these long term changes, adherence also has been shown to decline between clinic visits (Liu et al. 2006, Byakika-Tusiime et al. 2009). One of the

key questions then is whether the behaviour of the patient outside the ART clinic is different from the reported adherence. New strategies of measurement and analysis of adherence are needed for advances in the field of adherence research.

1.3.2 Reasons for poor adherence

The ART medication adherence is naturally strongly associated with *regimen related factors* such as pill burden, side effects, duration of treatment, duration of disease and complexity of regimen, e.g. dose frequency and timing and, in some cases, medication restrictions such as restrictions with food. Regimens that involve close supervision, lifestyle changes, and side effects lead to treatment fatigue and poor adherence. *Disease related conditions* such as symptoms, side effects from drug toxicity, have also been associated with poor adherence (Ingersoll & Cohen 2008). Disease factors include chronicity, symptom prominence and response to treatment. *Health care system and clinical factors* such as waiting time for appointments or medications, cost of drugs, drug supply, clear communication and patient-clinician relationship all affect adherence rates. *Patient related factors* such as gender, age, low education, stigma, perceived benefits, treatment companions, partnerships and other social support mechanisms play a role in poor adherence. Other patient factors include depression, health literacy, alcohol or drug abuse, wrong beliefs about medication (ART). Of recent *environmental and socio-economical factors* such as weather, poverty, migration and homelessnes have received more attention. Similarly to many psychosocial problems, social support affects adherence. Such mechanisms as treatment partners, peer counseling, ability to fit into daily routines may play a role in achieving good adherence.

As a conclusion, a large number of factors may have an impact on adherence and its variation in different populations for different regimens and adherence measures. A further complication is that, in some studies, different predictors are found for different adherence measures even in the same population.

1.3.3 Consequences of poor adherence

Poor adherence to medication results in increased use of medical resources, such as physician visits, laboratory tests, unnecessary additional treatments or more costly treatments and escalates adverse events. Poor adherence accounts to over half of unnecessary hospitalisations (Osterberg & Blaschke 2005), emergency room admissions (McDonnell & Jacobs 2002).

An association exists between poor adherence and multiple adverse clinical outcomes. Much of the recent work on adverse outcomes and patients with HIV infection has been published. For HIV infected individuals, the introduction of potent regimens led to significant reduction in mortality and morbidity, 50% in well-resourced countries. ART Adherence is the most important predictor of viral suppression, disease progression and mortality. Full virological

and clinical benefit requires high levels of adherence to ensure effective viral suppression, to avoid the emergence of cross-resistance (Bangsberg et al. 2001, Gross et al. 2006), and to subsequently lower mortality and maximize survival gains (Nachega et al. 2006, Lima et al. 2009, Chi et al. 2009). Specifically poor adherence to ART leads to virological failure, drug resistance and or immunological and (Bangsberg et al. 2003, Nachega et al. 2007, Oyugi et al. 2007, Chi et al. 2009). In some studies, adherence-viral relationships change over time (Rosenblum et al. 2009).

Improved therapies have raised the interest in the quality of life. Quality of life is considered a significant and desirable outcome for persons with chronic disease. Quality of life may reinforce adherence through increased efficacy of treatment but may also impair adherence when side effects and a complex regimen accompany the treatment. Consistent adherence is shown to be associated with better quality of life, immunological response and costs of health care (Honghong et al. 2009).

There is a growing importance of adherence as advances in medication adherence for various (chronic) diseases and cost of medications increases and as the use of medications increases with an aging population. Although consequences vary, poor adherence clearly poses a threat that must be addressed to reduce the gap between potential and actual healthcare quality. There is still a lot of debate about the risk of clinical and immunological failure following sub-optimal adherence. Sub-optimal adherence can lead to resistant virus strains and the need for expensive second and third line treatments in patients who may have initially controlled the infection. In sub-Saharan Africa these impacts could be difficult to manage, and could reverse the gains made in the past few years.

Several methods for an analysis of adherence data, including EDM data, have been proposed in the literature. Different summary statistics provide easily understandable measures of adherence. As Virjens (Vrijens & Goetghebeur 1997) pointed out, no one summary statistic however captures all the information contained in longitudinal measures. Aggregate measures over time simplify the structure of data, but often ignore information on the time order of the events. Therefore the researcher must balance the parsimony of data structure with the retention of possibly valuable information.

The method of summarizing or aggregating adherence data is influential to methodological and modeling issues. Several questions arise such as (i) what adherence variables are used, (ii) what to do with overlapping prescriptions, and (iii) how to treat missing visits or appointments in the analysis. Most studies use measures that take the average over time points, while others use models for multivariate (repeated) measures (Reynolds 2004, Simoni, Kurth, Pearson, Pantalone, Merrill & Frick 2006). For the evaluation of change in adherence behaviour over time, repeated measures or longitudinal data methods are used (Glass et al. 2009, Lazo et al. 2007, Byakika-Tusiime et al. 2009). Longitudinal methods tend to be more flexible and can accommodate complex structures with correlated measurement errors, non-normal er-

ror distributions, and missing data. For short series, marginal models and random effects models are good candidates for capturing temporal variability of adherence. They can be viable with some forms of missing data, can incorporate time-varying covariates, can handle both continuous and categorical dependent variables, and allow for error distributions other than normal (e.g., binomial and Poisson distributions). Vrijens and Goetghebeur (Vrijens & Goetghebeur 1997) also applied a generalized estimation equation (GEE) approach to analyse marginal models of the longitudinal binary adherence data based on daily EEM indices (1 indicating sufficient dose and 0 otherwise). One limitation cited for GEE approach is that it cannot handle long time series of repeated measures such as daily measures of adherence. However Smith et al modeled binary adherence data to extend the approach using a latent approach (assuming that a subject's likelihood to adhere at any given time is governed by the value of an underlying latent stationary continuous process and covariates at the time) - rarely used.

In examining the association between adherence and outcome, a number of approaches are possible. Much previous work (Vanhove et al. 1996, Bangsberg et al. 2000, Knobel et al. 2002, Gross et al. 2001, Masquelier et al. 2002) has explained aggregate outcome measures (e.g. proportion of viral load measures below detectable, viral load at a certain time point, or CD4CT-cell increase since start of therapy) with similar long-term average measures of adherence to establish the broad importance of adherence to successful therapeutic outcome.

1.3.4 Intervention strategies to enhance adherence

Based on the review by Osterberg (Osterberg & Blaschke 2005), the methods to improve adherence can be grouped into four broad categories, namely, (i) patient education, (ii) improved dosing schedule, (iii) increased opening hours of the clinic and shorter waiting time, and (iv) improved communication between physicians and patients. Patients who often miss appointments could benefit from assisted clinic schedule. The involvement of other healthcare workers such as pharmacists, nurses and behavioural specialists improve adherence (Simoni et al. 2010).

Most methods to improve adherence involve a combination of behavioural intervention approaches (and reinforcing other aspects of convenience), Or educational interventions about disease and treatment or a combination of the two methods. Adherence interventions are considered quite costly in terms of implementation and follow-up for longer term self-sustaining programs.

Interventions focusing on pharmacists-led support, educational and cognitive-behavioural aspects have demonstrated support. A meta-analysis conducted 1994-2004 (Amico et al. 2006) showed larger effects for studies that enrolled participants with adherence problems. Some studies focused on clinical trials such as Simoni et al (Simoni, Pearson, Pantalone, Marks & Crepaz 2006)

and an extended meta-analysis by Amico found support for behavioural interventions. Simoni (Simoni et al. 2010) also found support for intervention impact on adherence and two studies failed to find support for any adherence metric (followed intensive interventions in the control arm), Sampaio (Sampaio-Sa et al. 2008) and Whol et al (Wohl et al. 2006). Successful approaches incorporated pharmacy care, group based peer support, nurse delivered interview and one on one approach covering specific aspects of adherence. Recent interventions include phone text messages, personalised information, medication vials with an alarm. However the interventions demonstrate small and transient effects. Educational, behavioural and cognitive affective interventions to enhance adherence - work are needed to determine the sustaining effects of adherence promoting strategies.

In the prevention of chronic disease, the attributable fraction has been used as a practical tool in applied epidemiology and public health. Towards a public health approach, population attributable fraction (PAF) is a tool in evaluating the effectiveness of adherence interventions. PAF for population mortality, for example, is defined as the proportional reduction in population mortality that would occur if exposure to a risk factor (e.g., adherence) were changed to an ideal exposure scenario (e.g., optimal adherence).

2 Data and problem

2.1 Study design and participants

The adherence data analysed in this thesis study are provided by DART trial (Development of AntiRetroviral Therapy in Africa). DART was an open-label, multi-centre, (non-inferiority factorial) randomized trial comparing ART treatment management approaches relevant to resource-limited settings. In the DART study the main problems were whether HIV drugs can be given safely in the absence of routine laboratory tests and whether HIV drugs can be given intermittently rather than continuously to provide a similar level of benefit but with less toxicity.

The DART protocol, any modifications and with sample informed consent documents were reviewed by ethics committee from each participating site as well as ethics committees in the UK. Regulatory approval for conduct of the trial and use of antiretroviral drugs was obtained. Informed consent was obtained for the screening for the DART trial and another consent for the DART trial. The ethical clearance was renewed annually with protocol reviews. DART received ethics committee approval in Uganda, Zimbabwe and the UK (ISCRTN 13968779). DART trial was funded by the UK Medical Research Council, the UK Department for International Development (DFID), and the Rockefeller Foundation. GlaxoSmithKline, Gilead and Boehringer-Ingelheim donated first-line drugs for DART.

2.1.1 Recruitment of the participants

Participants were enrolled between Jan 15, 2003, and Oct 24, 2004, from two centres in Uganda and one centre in Zimbabwe, namely,

- The Medical Research Council DART clinic is located in Entebbe, Uganda and affiliated to Entebbe/hospital a district government hospital and the clinic was established in 2003. It serves both paediatric and adult patients from the municipality of Entebbe and surrounding communities (with total adult patient population of approximately 2000 patients).

- The JCRC and satellite Academic alliance (AA) DART clinics in Kampala, Uganda located on the outskirts of Kampala serve both paediatric and adult patients from surrounding communities. Both centres of excellence, JCRC is an HIV/AIDS care research institution established in 1990 and 2004 respectively to respond to the challenge of HIV.

- The DART clinic in Harare, Zimbabwe located at the Clinical Research Centre, University of Zimbabwe (UZ-CRC). A tertiary teaching hospital affiliated with the University of Zimbabwe and established in 2002.

Our study population mostly rural or semi-urban travelling long distances to clinics and characterised by high background burden of malaria and other pathogens. Similarly to clinics the hospitals are occupied by semi-urban and rural communities.

2.1.2 Inclusion and exclusion criteria

Eligible patients were adults (aged at least 18 years) with documented HIV infection, symptomatic HIV disease (WHO stage 2, 3 or 4) and CD4 cell counts <200 cells/μL, no prior ART. The patients that were unlikely to attend clinic (e.g., residence too far from study centre), showed poor compliance to previous medication, had current acute infections, or clinical or laboratory abnormalities were excluded.

2.1.3 Medications (Drug regimens)

All patients initiated first line triple combination of ART with (coformulated zidovudine-lamivudine (Combivir) and either tenofovir disoproxil fumarate (TDF) (3 pills a day), or abacavir (ABC) (4 pills a day), or nevirapine (NVP) (4 pills a day) and received combivir as part of their first-line regimen. The third drug for 600 patients enrolled in the NORA substudy was blinded Abacavir or Nevirapine plus placebo (see DART trial). For all patients not enrolled in the substudy TDF and NVP were available as the third first line drug for patients allocated equally between Zimbabwe and Uganda.

2.1.4 Randomisation and masking/blinding

At enrolment, all participants were randomly assigned to receive either clinically driven monitoring (CDM) or laboratory plus clinical monitoring (LCM) for toxic effects (haematology and biochemistry) and efficacy (CD4-cell counts). In the CDM arm they received clinical monitoring only, but no information on the CD4 counts. For all patients clinicians were notified about serious adverse events, and took appropriate clinical decisions for the patients best treatment. HIV viral loads were not done in real-time, in accordance with WHO guidelines and national norms. The hypothesis was that CDM would result in similar outcomes to LCM (non-inferiority). In the first substudy randomisation, 600 participants were randomly assigned to different first-line ART regimens in the nested (in the DART trial) Nevirapine or Abacavir (NORA) substudy (a placebo-controlled NORA substudy primary endpoint of toxicity at 24 weeks) (Dart Trial Team 2008*b*); all other participants received open-label first-line ART.

In the second substudy a further partial factorial (conditional) randomization was implemented within DART comparing structured treatment interruptions (STI) with continuous ART (CT)in participants with good early

response (CD4>300 cells/μL) after 48 or 72 weeks on continuous ART.(This followed a small non-randomised pilot study of one or two 12-week STIs with 4-weekly CD4 counts in 137 patients to inform the design of STI's in this setting; these pilot patients were excluded from all analyses.) The aim was to investigate the strategy of intermittent ART or structured treatment interruptions with a possibility of reducing toxicity, improving adherence and reducing cost of ART while maintaining clinical and immunological well being of patients. The 813 good early response participants were randomized (allocated in a 1:1 ratio) between continuous therapy (CT) and cycles of 12 weeks on/off ART structured treatment interruptions (STIs). The randomisations occurred between July 2004 and March 2006. However, the CT/STI randomizations were stopped early, in March 2006, due to inferiority of STIs, and all patients randomised to STIs returned to continuous treatment (DART Trial Team 2008*a*). (See Figure 2.1.)

Randomisations were stratified by centre, screening CD4 cell count and first line ART. A computer-generated sequentially numbered randomisation list was prepared by the trial statistician and incorporated in the database at each trial centre. This allowed trial managers to access the next number but not the whole list. Randomisations were undertaken by clinicians phoning local trials centre (DART Trial Team 2010).

2.1.5 Health care plan

At enrolment all participants received counseling about medication adherence and drug side effects from a nurse or a doctor and had group counseling sessions. This counseling was also reinforced at each clinic visit. At the first randomisation to CDM or LCM, eligible participants initiating ART were told their allocation on the day of randomisation.

At screening, 4 and 12 weeks then every 12 weeks, all participants in LCM and CDM groups were seen by a doctor and had a routine full blood count, tests of liver and kidney function, and measurement of lymphocyte subsets but total lymphocyte or CD4-cell counts were not returned for the latter group(See Figure 2.2). For all participants, clinicians were notified about serious adverse events, and took appropriate clinical decisions for the patients best treatment.

The second substudy randomisation commenced after the advising STI pilot study had been completed. CD4 cell counts were undertaken for all patients irrespective of allocated strategy at 48 weeks (or at 72 weeks if the patient was not randomised at week 52) and the results were returned to the clinician. For CDM patients, the results were reported as either CD4<300 or >300 cells/mm3. If CD4 was <300μL at week 48(or 72 weeks if not randomised at week 52), participants continued to be followed under their evaluation strategy see Figure 2.1. Patients were randomised to continuous ART

or to ART with STI (cycles of 12 weeks off followed by 12 weeks on ART, repeating the schedule until the end of the trial)- Following a review by the Trial Steering Committee (TSC) and Data and Safety Monitoring Committee (DSMC), STI was stopped early in March 2006.

Serious adverse events (SAE) according to the ICH Harmonised Tripartite Guidelines for Clinical Safety Data Management were documented reported to Medical Research Council(UK), clinical trials unit (MRC, CTU) as soon as they occurred. An independent Endpoints Review Committee members following pre-specified criteria reviewed all WHO staging events, deaths and serious adverse events and were masked to randomisation allocation. Whilst grade 3 and 4 adverse events were reported at next scheduled visit. For adverse events ART drugs could be substituted within the same drug class. However treatment switching to second line ART (for treatment failure) was based on clinical criteria in both LCM and CDM groups or CD4 cell counts (confirmed <100 or <50 cells before 2006) in the former group.

Viral loads were performed retrospectively at baseline and at weeks 4, 12, 24, 36, and 48 on a subset(n=300) of subjects taking zidovudine, lamivudine, and tenofovir. (This was done shortly after the trial started; 100 consecutive subjects were selected from each of the Entebbe, Harare, and Kampala centers divided equally between those with CD4 cell counts 0–99 and 100–199 cells/mm3 at ART initiation (DART Virology Group and Trial Team. 2006).)

In addition, at each full assessment the nurse administered a symptom checklist, medical history since last visit including signs and symptoms of HIV disease and WHO staging; weight; assessment of adherence by pill count and questionnaire; pregnancy tests for women of child bearing age undergoing STI's; recording of compliance with allocated management strategy and adverse events. The severity and likely relationship of events to ART was documented by a doctor.

Switching for severe clinical or laboratory toxicity followed guidelines, based on clinical and laboratory grading of toxicities and according to treating physician. A symptom checklist included questions on nausea/vomiting, rash, headache, fever, jaundice, abdominal pain etc. Physicians were encouraged not to switch ART before 48 weeks on continuous ART, or within 12 weeks after recommencing ART after a planned STI. Clinical criteria for consideration of switching therapy include: the development of a new WHO stage 4 diagnosis; CD4 cell count <50 cells/mm3 on 2 occasions, while on ART; consideration should be given to switching if the CD4 count on 2 consecutive occasions is below 100 cells/mm3. All women of childbearing age were continually advised about avoiding pregnancy.

Participant could withdraw from the study for any reason and clinical data including weight, symptoms, full blood count, biochemistry and T-cell measurements taken at the time of withdrawal. Similarly to participants withdrawing, information on those not in follow-up for unscheduled periods was collected. Information on those who died or were lost to follow-up or left the study were obtained at each clinic visit.

2.2 Data collection procedures

2.2.1 Screening

Information on medical history, clinical exams, WHO stage 2, 3, or 4, and weight recorded, and T cell subsets (CD4, CD8, CD3 and total lymphocyte count), haematology, biochemistry and pregnancy tests were performed on all consenting participants at screening. Women of reproductive age were given information about the risks of pregnancy in the trial and encouraged to avoid pregnancy.

2.2.2 Baseline information - week 0

The time between enrolment and screening was approximately 2-weeks and maximum 4 weeks. Written informed consent was obtained for screening and enrolment for participants in the trial for both randomisations. The trial approved by ethics research committees in Uganda, Zimbabwe and the UK. A trial register was kept at each clinical site with records of all eligible patients with the name, date of birth and trial number for consenting individuals whilst reasons were recorded for those refusing consent.

2.2.3 4-weekly measurements

Clinic visits were scheduled at 2 and 4 weeks after enrollment and then every 4 weeks. Participants were asked to return to the clinic at any time if they did not feel well. At each clinic visit, participants were given a new 4-week supply of drugs (no extra pills for late attendance), unused pills from the previous period were counted and recorded, and a structured adherence questionnaire was completed. At each 4 week visit a symptom checklist to detect inter-current illness, HIV disease progression or adverse events to ART. Medical history since last visit which includes signs and symptoms and physical exam which includes weight and WHO staging for HIV were done.

2.2.4 12-weekly measurements

In addition, at each 12-weeks a full assessment included adherence assessment, medical history including WHO staging and weight, nurse symptom checklist, and participants were seen by a doctor had haematology, biochemistry and lymphocyte (see Table 2.2). The severity and likely relationship of events to ART was documented by a doctor. Also, changes in ART or opportunistic infection prophylaxis and other concomitant medications documented and compliance to allocated strategy was recorded.

2.2.5 Assessment of trial endpoints

The primary endpoints for the main randomisation were progression to a new WHO stage 4 HIV event or death (efficacy) and any serious adverse events (not HIV-related). The secondary endpoints were

Table 2.1: *Variables used in the study*

(Variables)	Item
	Any previous ART
	Sex
	Age in years or DOB
	Predominant exposure category
	Date of first HIV positive test
Clinical	
	CD4 cell count
	CD8, CD3
	Total lymphocite count
	Haematology (includes haemoglobin, platelets, neutrophils)
	WHO disease stage
	Body mass index
	Viral load
	Biochemistry (includes urea,creatinine, AST, bilirubin)
Socio-economic	
	How many(own)children have you had?
	How many children still alive?
	How many children dependent on you
	Highest education level
	Predominant occupation
	Health affected work last month
	Current employment status
	Disclosed HIV status to anyone
	Admitted to hospital in the last year
	Number of pregnancy related admissions
	Marital status
	Cohabiting
	Regular sexual partnerships
	Length of relationship with current main partner
	Condom use with regular or other partners
Adherence	
	Are you late for this visit
	During the past 4 days, on how many days have you missed taking all (or part) of your HIV drug doses?
	Do any of your anti-HIV drugs have special instructions, such as "take with food", or "on an empty stomach
	Did you miss any of your anti-HIV drugs last weekend
	Which one (if any) of your anti-HIV drugs is the easiest to take?
	Which one (if any) of your anti-HIV drugs is the most difficult to take?
	When was the last time you missed any of your anti-HIV drugs
	How many pills has the patient returned? (specify drug)
	Reasons for missing ART drugs in those reporting missed dose
	Symptom checklist (fever, vomiting, skin itch, difficult breathing, Herpes Zoster, numbness, genital ulcers,lipodystrophy etc)

- Progression to a new WHO stage 4 HIV event or death from 6 weeks after randomisation,
- Adherence as measured by questionnaire and pill counts,
- Any grade 3 or 4 adverse events
- Time to cessation of first-line regimen for failure
- CD4 count at 3 years
- HIV RNA viral load (performed retrospectively) at 3 years

2.3 Assessment of adherence/measurements

The measures of adherence were based on data collected at 4-weekly routine clinic visits, an objective nurse pill count (total number of unused pills for each ART drug prescribed since the last visit) and self-reported responses to a structured adherence questionnaire.

Secondary measures were taken from a structured administered adherence questionnaire. The structured questionnaire contained three key questions about adherence, (a) "How many times in last 4 days have you missed all (or part) of your HIV medication?", (b) " Did you miss any of your anti-HIV drugs last weekend (Saturday or Sunday)? " and (c) " When did you last miss any of your anti-HIV drugs?". The other questions included

- Are you late for this scheduled visit?
- Do any of your ART drugs have special instructions, such as 'take with food', or 'on an empty stomach'?
- Which one (if any) of your anti-HIV drugs is the easiest to take?
- Which one (if any) of your anti-HIV drugs is the most difficult to take?

In those individuals reporting missed drugs several reasons assessed included; away from home; too busy; simply forgot; had too many pills to take; avoid side effects; did not want people to notice; change in daily routine; felt drug was toxic; slept through dose time; felt sick; depressed; ran out of pills or felt good.

Adherence data of a participant at a visit is missing if (i) the participant missed his/her visit ("missing"), or if (ii) the participant had his/her visit but did not, for some unknown reason, respond to these questions in the questionnaire ("non-response"), or if (iii) the participant was at that visit an STI trial participant ("randomized to STI").

Participants were followed up under CDM or LCM strategies until Dec 31, 2008. At their next visit in January, 2009, participants received all masked results and those with low CD4 -cell counts were switched to second-line ART.

2.4 The problem

Good adherence is essential for successful antiretroviral therapy (ART) provision. In this thesis work, the aim is to describe different alternative summaries of adherence and their associations in the first year on antiretroviral therapy (ART) and the subsequent risk of mortality and CD4 failure, in order to identify patients at high risk due to early adherence behavior. Also, we assess the factors that are associated with poor adherence. The aim is also to study, in a dynamic way, the effect of recent adherence history on the risk of mortality at the individual level (as given by odds ratios from dynamic logistic regression model), and at population level (population attributable fraction (PAF) based on this model). The findings have important implications for clinical practice and developments of more focused adherence-enhancing interventions. We consider the public health implications and future perspectives of our work.

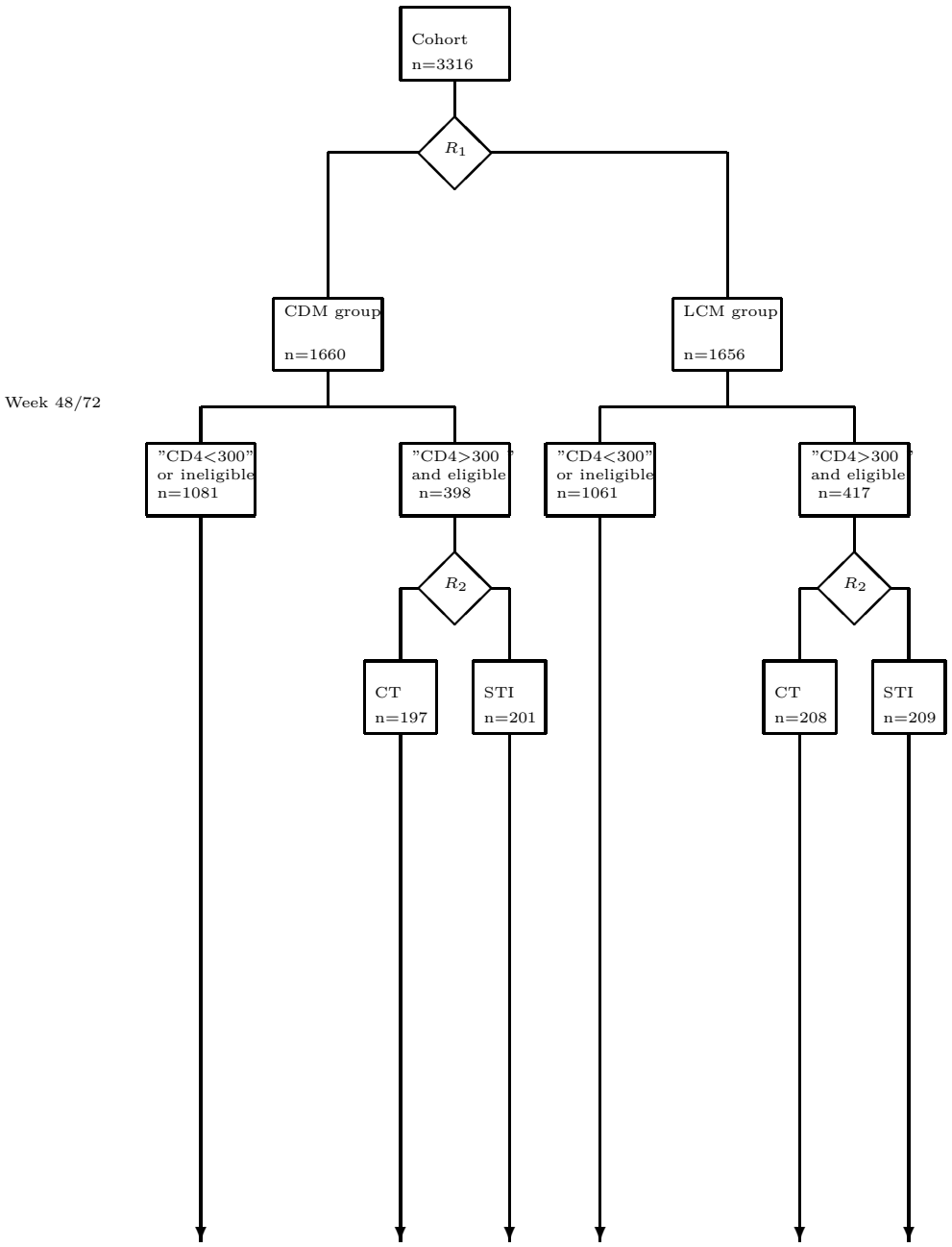

Figure 2.1: Illustration of the first and second randomization of the participants of the DART trial (356 died/lost to follow-up or excluded in first year). The latter confounds adherence measurements: 813 good early response participants were randomized between continuous therapy (CT) and cycles of 12 weeks on/off ART structured treatment interruptions (STI).

Table 2.2: *Laboratory and clinical monitoring (LCM) and clinical disease monitoring (CDM)(+/-STI co-enrolment). Patients return 4-weekly to see nurse or doctor; return containers and unused drugs(except STI). History and physical includes weight and WHO staging;Haematology includes Hb, MCV, WBC, Lymphocytes, Neutrophils, and platelets. Biochemistry includes urea, creatinine, AST or ALT, Bilirubin. CD4, CD8, CD3 percentage and absolute, total lymphocyte count*

	Events		Week in Trial												
Doctor/nurse visit*	screening	start therapy	2	4	8	12	16	20	24	28	32	36	40	44	48
Nurse visit	week-2	Week 0													
Doctor only visit*															
CD4≥300 at week 48															
Adherence assessment and 4 weeks ART supply*		X	X	X	X	X	X	X	X	X	X	X	X	X	
Consent to screening and patient information sheet	X														
Informed consent		X													
History physical[1])	X	X	X	X	X	X	X			X	X		X		X
Symptom checklist		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pregnancy test	X								X						X
Haematology	X			X		X			X			X			X
Biochemistry	X			X		X			X			X			X
Lymphocite subsets	X					X			X			X			X

Events	Week in Trial								
52	56	60	64	68	72	76	80	switch	
Start STI			Restart ART			Start STI			
STI or X	STI or X	STI or X	X	X	X	STI or X	STI or X	X	
									X
X		X			X			X	
X	X	X	X	X	X	X	X	X	X
				X					
		X			X			X	
		X			X			X	
		X			X			X	

3 Adherence behavior

3.1 Background

Measurement of adherence is an important tool to understand and improve adherence although there is no gold standard for measuring adherence. There is a significant interest in identifying who is at risk for poor adherence with the objective of designing preventive strategies. Given the absence of viral load monitoring in most African settings, measurement of adherence is even more important to manage response to treatment and provide interventions for patients having difficulties with medication.

3.2 "Raw" adherence data

First, we introduce simple summary measures of adherence to gain a quantitative understanding of variability of adherence within the patient population. Using the two primary measures of adherence, namely, PC and DPR, we define complete adherence as 100% adherence and good adherence as at least 95% adherence. Secondary measures of adherence were taken from the structured adherence questionnaire, namely, (i) missing any ART doses in the 4 days before the clinic visit, (ii) missing any ART dose in the past month, and (iii) forgetting the dose at weekends documented by the nurse in the clinic.

Figure 3.1 shows that the proportion of patients with at least 95% and 100% DPR adherence over the previous 4 weeks increased continuously over the first year on ART and declines later in time as expected. However the proportion reporting late for each visit is less 10% at each visit with a slow increase with time. From the structured questionnaire, proportion reporting missed dose in last month at each clinic visit improves over time (20% to less than 10%) while the proportions reporting missed dose 4-day/weekend seems to be stable (less than 5%) through the follow-up, see Figure 3.2.

We also summarised 5-year adherence using the 4-weekly question "missed a dose in the last month", because it was most strongly associated with viral load Muyingo et al. (2008). Due to the possibility of missing values and non-response, the adherence variable can then take, at each visit, the following four values

"poor", "good", "non-response", and "missing",

where "poor" and "good" mean "missed a dose in the last month" and "did not miss a dose in the last month", respectively. Adherence measures 1-5

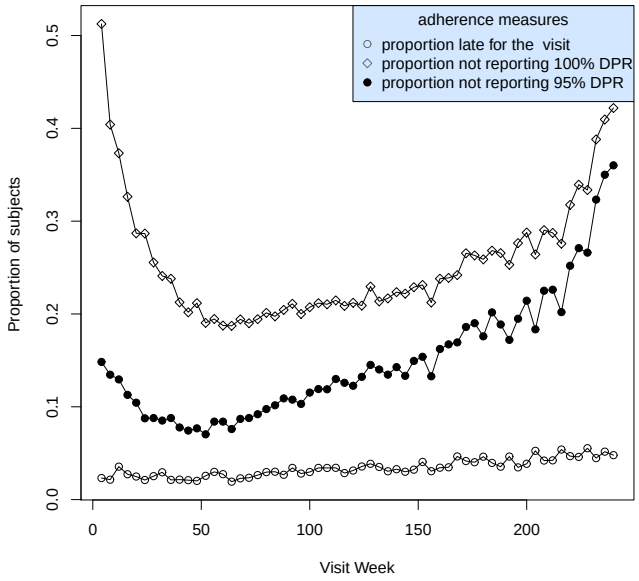

Figure 3.1: 4-weekly proportions of participants that are not late or not reporting at least 95% or 100% DPR.

years by randomisation arm are presented in Figures 3.3 and Figure 3.4. The proportion of individuals reporting good adherence at each visit remained very high and stable over most of the five year period with little difference in the adherence profiles between the LCM and CDM groups. However, the number of missing visits increased with time, at least in part because, after approximately 3 years on ART, a small number of participants moved to 12-weekly visits, with telephone nurse visits in-between (without adherence data).

3.3 Adherence seen as a Markov chain (MC)

We next assume that the 4-weekly measurements of adherence obtain finite number of possible values $1, ..., S$. These values are here called *states*. Continuous and or multivariate adherence measurements must first be categorized for the following analysis. Note also that missing data at a visit can be treated as one of the states.

The observed values of a patient at T time points $1, ..., T$, may then be seen as a sequence of random variables $X_1, ..., X_T$. Recall that the adherence measurements over time points $1, ..., T$ are usually combined by averaging over the entire period to give the *estimated probabilities* (proportions) of being in each state,

$$\hat{P}_s = \frac{1}{T}\sum_{t=1}^{T} I(X_t = s), \quad s = 1, ..., S$$

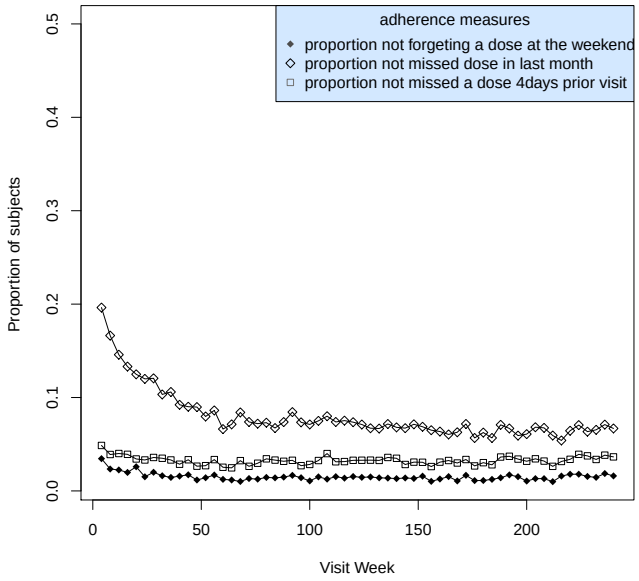

Figure 3.2: 4-weekly proportion of participant not reporting missed doses at the weekend, in last month, or 4 days prior the visit.

where $I(X_t = s) = 1$ if $X_t = s$ and zero otherwise. In this approach, the dependencies between the observations are ignored.

Another approach is to assume that the adherence process is a homogeneous Markov chain with the *transition probabilities* between states

$$p_{ij} = P\left(X_{t+1} = j | X_t = i\right), \quad i, j \in \{1, ..., S\}.$$

See Chapter 6 in Grimmett & Stirzaker (1992), for example. Natural estimates of transition probabilities p_{ij} are given by

$$\hat{p}_{ij} = \frac{\sum_{t=1}^{T-1} I(X_t = i, X_{t+1} = j)}{\sum_{t=1}^{T-1} I(X_t = i)}$$

for $\sum_{t=1}^{T-1} I(X_t = i) > 0$.

Sometimes a more realistic model to describe the adherence behavior is to use the *Markov chain of order 2* with transition probabilities

$$p_{ijl} = P\left(X_{t+2} = l | X_t = i, X_{t+1} = j\right), \quad i, j, l \in \{1, ..., S\}.$$

Also, it is sometimes possible that the Markov chain is *non-homogeneous* in the sense that the transition probabilities change at a time point t_1 so that the transition probabilities are given by two $S \times S$ matrices, say,

$$P_1 \text{ for } t = 1, ..., t_1 \quad \text{and} \quad P_2 \text{ for } t = t_1 + 1, ..., T.$$

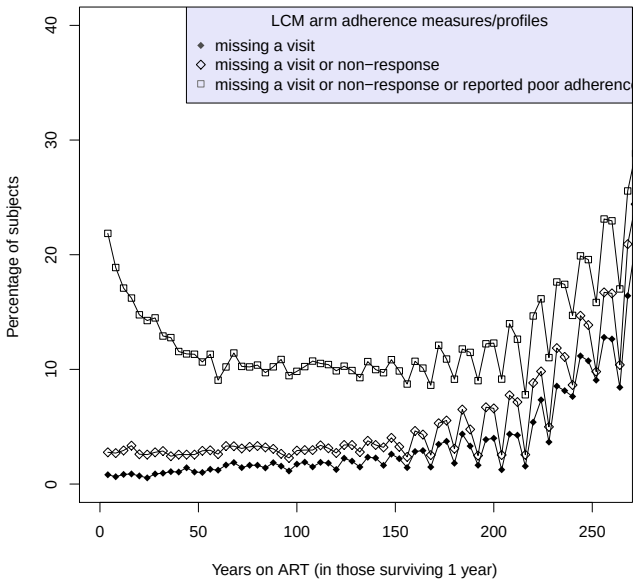

Figure 3.3: 4-weekly proportions of participants in the LCM group who (i) miss a visit, (ii) miss a visit or do not respond to the adherence question, and (iii) miss a visit, do not respond, or report poor adherence (upper curve). The periodicity in the curves after 3 years is partly due to a small group of patients who moved to 12-weekly ART refills.

3.4 Clustering based on MC approach

The aim in clustering is to divide a collection of individuals (patients) into subsets or "clusters" so that individuals within each cluster are more closely related to one another than individuals assigned to different clusters. Clustering is done on the basis of similarities or distances (dissimilarities). Clustering can be used to discover interesting groupings in the data, or to verify suitability of predefined classes. The cluster memberships can then be used as a categorical variable in further analyses. We next assume that the adherence behavior of each individual follows a Markov chain model with unknown transition probabilities. We also assume that the population of the patients can be divided into subpopulations or clusters such that within a cluster the transition probabilities are the same. The unknown cluster memberships can then be estimated from the data.

The problem then is how to identify or estimate the clusters using the patientwise measurements

$$X_1, ..., X_T.$$

We explain the procedure in the case of the homogeneous Markov chain model. First we find the matrix $Q = (q_{ij})$ with elements

$$q_{ij} = \frac{1}{T-1} \sum_{t=1}^{T-1} I(X_t = i, X_{t+1} = j), \quad i, j = 1, ..., S$$

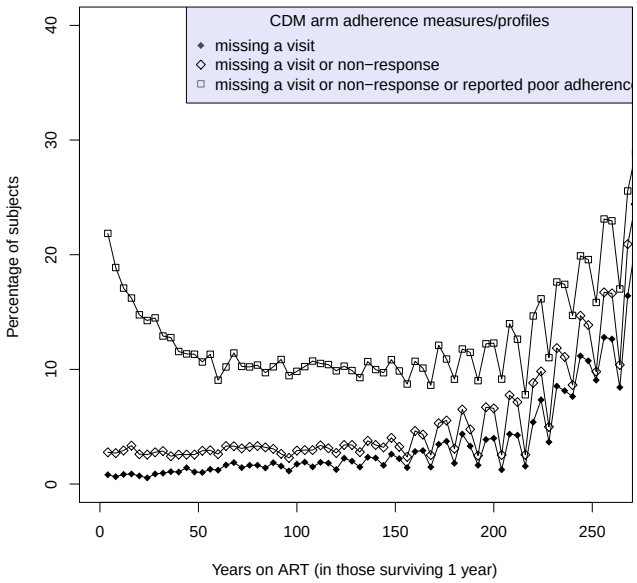

Figure 3.4: 4-weekly proportions of participants in the CDM group who (i) miss a visit, (ii) miss a visit or do not respond to the adherence question, or (iii) miss a visit, do not respond, or report poor adherence. The periodicity in the curves after 3 years is partly due to a small group of patients who moved to 12-weekly ART refills.

and then vectorize Q to get a S^2-variate observation vector

$$Z = vec(Q).$$

Note that the estimates of the transition probabilities in P can be obtained by Q just by dividing each row of Q by its row sum. The observed S^2-variate vectors Z are then used instead of the original $X_1, ..., X_T$ to cluster the data. Note that the marginal variables in Z are probabilities and therefore on the same underlying scale.

A measure of dissimilarity or distance between classes is needed for the clustering procedure. Commonly used measures of distance include Euclidean and Manhattan distances. Let index sets I and J, with $I, J \subset \{1, ..., N\}$, now refer to the indices in two clusters, and let n_I and n_J be the corresponding cluster sizes. In our study, the popular *Ward's minimum variance method* of linkage compares the between and within squared *Euclidean distances* with

$$d(I, J) = \sum_{i \in I \cup J} ||Z_i - \bar{Z}||^2 - \sum_{i \in I} ||Z_i - \bar{Z}_I||^2 - \sum_{j \in J} ||Z_j - \bar{Z}_J||^2$$

where $\bar{Z}$, $\bar{Z}_I$ and $\bar{Z}_J$ are the sample mean vectors over the subsets with indices in $I \cup J$, I and J, respectively. See Chapter 7 in Seber (1984).

We use the hierarchical clustering technique which proceeds as follows

1. Start with N clusters (individuals), that is, each individual is a cluster.

2. Find the shortest distance between two clusters, and join these two clusters.

3. Repeat step 2 until there is just a single cluster or the desired number of clusters is found.

The above defines the *agglomerative* algorithm, the other divisive method is rarely used. This gives a *tree of clusters* which can be illustrated with a *dendrogram*, see Figure 3.5 for an illustration. The clusters should not be too small and they should have natural interpretations. Natural interpretations may be found by considering conditional transition probabilities in the clusters.

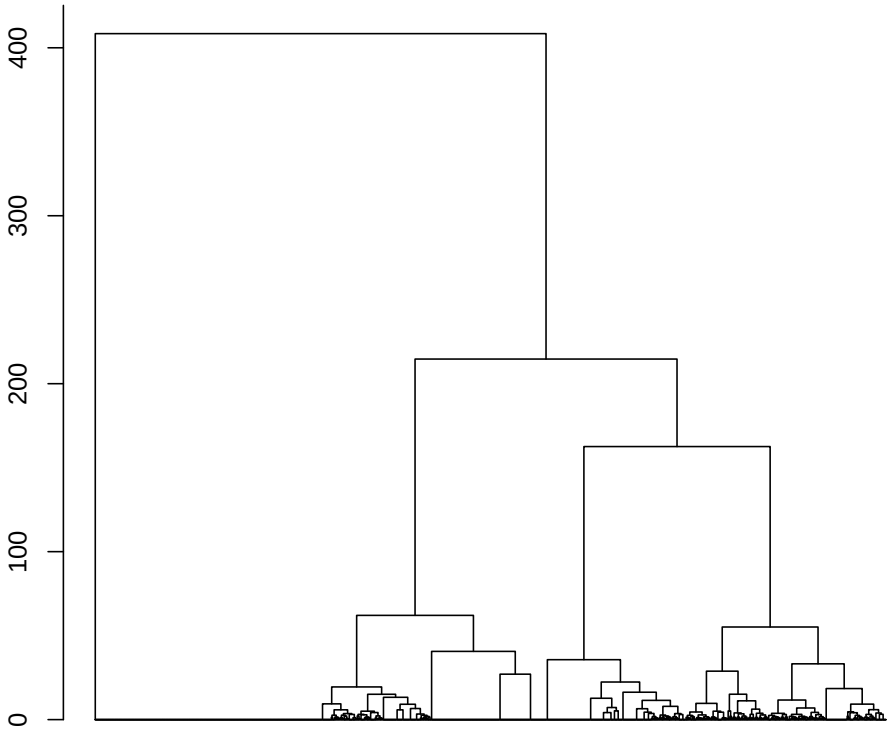

Figure 3.5: Dendrogram based on the data over twelve months (MC3) 18 variables

In our study, adherence was assessed at 4-weekly visits and we consider the adherence data collected during the first year of the follow-up period ($T = 12$ visits). The adherence patterns or clusters of patients found in this

Table 3.1: *The probabilities of being in state 0, 1, and 9 in six clusters based the i.i.d. model (MC1) ordered from best to worst.*

	State		
	0	1	9
Cluster 1 ($n = 891$)	0	1	0
Cluster 2 ($n = 618$)	.083	.917	.000
Cluster 3 ($n = 360$)	.167	.833	.000
Cluster 4 ($n = 469$)	.065	.829	.107
Cluster 5 ($n = 426$)	.280	.688	.032
Cluster 6 ($n = 196$)	.489	.426	.085

way were then used to predict the mortality during the remaining follow-up period. Adherence data at a visit were missing either because the patient (i) totally missed a visit, or (ii) attended but did not complete the adherence questionnaire. In our analysis, we only used a simple binary variable 'missed any dose in the last month' with the third possibility of missing data for this question for any reason. We then use the Markov chain with $S = 3$ states,

$$0 \textit{ (poor)}, \qquad 1 \textit{ (good)}, \qquad \text{and} \qquad 9 \textit{ (missing)}.$$

Note that the adherence values $(X_1, ..., X_T)$ may also be seen as one classificatory variable with $S^T = 3^{12} = 531,441$ classes (profiles).

We consider and compare three different models, namely,

(MC1) the i.i.d. model, that is, $X_1, ..., X_T$ identically and independently distributed,

(MC2) the homogeneous Markov chain model, and

(MC3) the non-homogeneous Markov chain model with a change point at 6 months.

To compare the clustering obtained using the above three models (MC1)-(MC3), we found six clusters in each case. In the first case (MC1), the probabilities of being in states 0,1, and 9 in each cluster are reported in Table 3.1. The probabilities are then read as follows: Cluster 4, for example, has the highest proportion for missing data but the proportion for good behavior is also high 83%. Cluster 6 is the poorest one as the proportion for good behavior is only 43 %. The first cluster 1 consists of 891 optimally behaving patients.

Secondly, for the clustering based on the 9-variate variable and the homogeneous Markov chain model (MC2), the transition probabilities between states 0, 1, and 9 are reported in Table 3.2. The transition probabilities are then read as follows. 52 % of those patients who reported good adherence in the previous month achieved good adherence also in this month, 31 % of those having missing data in previous month reported good adherence in this month, and so on. Cluster 6 is clearly the poorest one, as the proportion

Table 3.2: *Conditional transition probabilities in six clusters based on homogenous Markov chain model (MC2)*

	Cluster 1 ($n = 850$)				Cluster 2 ($n = 301$)		
	0	1	9		0	1	9
0	0	.	.	0	0	1	0
1	0	1.000	0	1	0.008	0.950	0.042
9	.	.	.	9	0	1	0

	Cluster 3 ($n = 469$)				Cluster 4 ($n = 463$)		
	0	1	9		0	1	9
Σ							
0	0	1.000	0	0	0.177	0.765	0.057
1	0.091	0.909	0	1	0.073	0.871	0.056
9	.	.	.	9	0.131	0.652	0.217

	Cluster 5 ($n = 596$)				Cluster 6 ($n = 281$)		
	0	1	9		0	1	9
0	0.253	0.723	0.024	0	0.425	0.523	0.052
1	0.207	0.770	0.023	1	0.436	0.516	0.049
9	0.222	0.684	0.094	9	0.235	0.307	0.458

maintaining good adherence from one month to the next is the lowest whilst individuals in cluster 1 behave in an optimal way.

Thirdly, we also clustered the data using 18-variate variable $Z = vec(Q_1, Q_2)$ based on the heterogeneous Markov chain model (MC3). The conditional transition probabilities were then allowed to be different over different periods (with a change point at six months). The estimated transition probabilities with six clusters are given in Table 3.3.

The clusters in MC3 can be roughly characterised in the following way.

- Cluster 1: Good adherence getting worse
- Cluster 2: Less than adequate adherence in both periods
- Cluster 3: From less than adequate to almost optimal
- Cluster 4: Moderate adherence in both periods
- Cluster 5: From good to optimal usage
- Cluster 6: Consistent optimal users

The groupings based on (MC2) and (M3C) describe the adherence behaviour in more versatile ways. One can see that the groups are genuinely different from the description of clusters, Table 3.4 gives a cross-tabulation of cluster memberships in the three clustering based on models (MC1), (MC2), and (MC3). Kendall's rank correlation were 0.80 (between MC1 and MC2) and 0.88 between MC2 and MC3, as expected as these are nested and represent dynamic behavior(Table 3.5).

Remark 1. Note that the three models are nested so that (M1) $\Rightarrow$ (M2) $\Rightarrow$ (M3). Likelihood ratio tests can be used to discriminate between the models. Note also that the estimated transition probabilities p_{ij} could be further modeled so that $g(p_{ij})$ is a linear function of a vector of explaining variables with a suitable link function g.

3.5 Classification based on other summaries

The adherence classification based on this Markov chain approach

(Method M1) the classification based on non-homogeneous Markov chain model (MC3),

is compared with three other classifications based on traditional "averaged" adherence measures, namely

(Method M2) the mean DPR for the first 12 visits (DPR approach),

(Method M3) the proportion of the first 12 visits not reporting any missed dose in last month

Table 3.3: *Average transition probabilities in six clusters based on heterogeneous Markov chain model (M3)*

Adherence class 1 ($n = 850$)

	Period 1					Period 2			
	0	1	9	Σ		0	1	9	Σ
0	**.**	.	.	.	0	.	.	.	.
1	.	**1.000**	**.**	1.000	1	.	**1.000**	**.**	1.000
9	.	.	.	.	9	.	.	.	.

Adherence class 2 ($n = 433$)

	Period 1					Period 2			
	0	1	9	Σ		0	1	9	Σ
0	**0.000**	1.000	0.000	1.000	0	**.**	.	.	.
1	0.118	**0.853**	0.029	1.000	1	.	**1.000**	**.**	1.000
9	0.000	1.000	**0.000**	1.000	9	.	.	**.**	.

Adherence class 3 ($n = 519$)

	Period 1					Period 2			
	0	1	9	Σ		0	1	9	Σ
0	**0.027**	0.960	0.133	1.000	0	**0.095**	0.866	0.039	1.000
1	0.034	**0.953**	**0.013**	1.000	1	0.115	**0.824**	0.061	1.000
9	0.117	0.860	**0.023**	1.000	9	0.051	0.800	**0.149**	1.000

Adherence class 4 ($n = 408$)

	Period 1					Period 2			
	0	1	9	Σ		0	1	9	Σ
0	**0.275**	0.684	0.041	1.000	0	**0.000**	1.000	0.000	1.000
1	0.246	**0.690**	0.063	1.000	1	0.015	**0.978**	0.007	1.000
9	0.226	0.598	**0.177**	1.000	9	0.067	0.933	**0.000**	1.000

Adherence class 5 ($n = 441$)

	Period 1					Period 2			
	0	1	9	Σ		0	1	9	Σ
0	**0.285**	0.681	0.035	1.000	0	**0.191**	0.781	0.028	1.000
1	0.163	**0.799**	0.039	1.000	1	0.273	**0.697**	0.030	1.000
9	0.202	0.556	**0.242**	1.000	9	0.261	0.620	**0.120**	1.000

Adherence class 6 ($n = 309$)

	Period 1					Period 2			
	0	1	9	Σ		0	1	9	Σ
0	**0.431**	0.526	0.043	1.000	0	**0.403**	0.549	0.048	1.000
1	0.497	**0.458**	0.045	1.000	1	0.279	**0.672**	0.049	1.000
9	0.264	0.373	**0.364**	1.000	9	0.123	0.352	**0.519**	1.000

Table 3.4: Contingency tables for cluster categories when clusters are based on (a) models (MC1) and (MC2), (b) models (MC1) and (MC3), and (c) (MC2) and (MC3). First row: (a) and (b). Second row: (c). In all case, the categories are ordered from best to worst.

	1	2	3	4	5	6
1	850	41	0	0	0	0
2	0	114	469	35	0	0
3	0	0	0	172	188	0
4	0	146	0	250	72	1
5	0	0	0	6	309	111
6	0	0	0	0	27	169

	1	2	3	4	5	6
1	850	0	41	0	0	0
2	0	370	223	19	6	0
3	0	0	63	187	109	1
4	0	63	187	116	89	14
5	0	0	5	82	212	127
6	0	0	0	4	25	167

	1	2	3	4	5	6
1	850	0	0	0	0	0
2	0	177	124	0	0	0
3	0	256	213	0	0	0
4	0	0	158	252	51	2
5	0	0	24	156	332	84
6	0	0	0	0	58	223

Table 3.5: Kendall's rank correlations between the ordered categorical adherence variables based on models MC1-MC3 as described in Table 3.4.

	MC1	MC2	MC3
MC1	.	0.80	0.80
MC2	.	.	0.88

Table 3.6: *Four adherence classifications.*

Method	Item	4-weekly response	Summary	Classification
Method M1	Questionnaire administered by a nurse (4-weekly)	Did you miss any dose in the last month? No=1, yes 0, missing 9	estimated transition probabilities based on model (MC3)	Classes based on hierachical clustering of transition probabilities in 0-6 and 6-12 months
Method M2	Pill counts by a nurse (4-weekly)	Drug possession ratio (DPR) in (0,1)	Mean M2 (DPR) over 12 visits	Quantile based classes: (0.998,1](n=461), (0.994,0.998](n=518), (0.988,0.994](n=489), (0.975,0.988](n=490), (0.912,0.975](n=489), (0,0.912](n=490)
Method M3	Questionnaire administered by a nurse (4-weekly)	Did you miss any dose in the last month? No=1, otherwise (Yes or missing) 0.	Mean M4 over 12 visits	Quantile based classes: (0.917,1](n=888), (0.833,0.917](n=767), (0.75,0.833](n=523), (0.667,0.75](n=309), (0.5,0.667](n=284), [0,0.5](n=166)
Method M4	Questionnaire administered by a nurse (4-weekly)	Did you miss any dose in last 4 days or at weekend (0 or 1)? No=1, otherwise (Yes or missing) 0.	Mean M3 over 12 visits	Quantile based classes: [1,1](n=1380), (0.833,1)(n=878), (0.75,0.833](n=399), [0,0.75](n=280)

(Method M4) the proportion of the first 12 visits not reporting any missed dose in last 4 days and not reporting any missed doses at weekend prior to clinic visit. (missed visit/non-response treated as missed dose), and (missed visit/non-response treated as missed dose).

Methods M1 and M3 are based on the same underlying data, but only method M1 takes the dynamic nature of adherence behaviour into account. For methods M1-M3, we use 6 classes. In method M4 we use only 4 classes; with 6 classes some of the class sizes would be too small for the comparison. Descriptions of the classifications are shown in Table 3.6. Similarly to MC1-MC3, as the adherence classes are ordered, Kendall's tau was used as a measure of concordance between different classifications.

Table 3.7: *Baseline characteristics according to different adherence classifications - categories are ordered from best to worst.*

	Pre-ART CD4 cell count				Highest Education level			Drug Initiated ART		
	0-49	50-99	100-149	150-199	none/ primary	secondary	university/ technical	TDF	NVP	ABC
M1		p=0.07				$p < 0.001$			p=0.001	
class 1	28.9	27.9	30.5	27.4	25.8	29.7	32.8	30.1	25.8	24.7
class 2	16.5	12.7	14.1	14.5	15.4	14.0	14.6	13.6	19.6	13.4
class 3	16.9	16.6	16.9	20.3	16.3	19.2	15.9	16.6	20.9	18.4
class 4	13.4	16.9	11.0	13.9	13.5	14.0	13.8	13.7	14.3	13.4
class 5	13.4	16.3	15.7	14.7	15.1	15.0	13.8	15.2	11.4	18.0
class 6	10.8	9.5	12.0	9.2	13.9	8.1	9.1	10.8	8.0	12.0
Σ	100	100	100	100	100	100	100	100	100	100
M2		p=0.02				p=0.03			$p < 0.001$	
class 1	17.8	18.1	15.3	14.6	16.9	16.2	17.7	18.8	11.5	9.8
class 2	15.7	17.4	18.2	15.8	18.1	16.0	15.3	16.8	13.7	20.7
class 3	15.9	19.4	15.2	16.4	19.3	14.5	16.5	16.7	18.2	13.3
class 4	16.7	14.7	17.4	17.6	15.8	17.6	16.3	16.3	18.4	16.8
class 5	19.2	16.4	16.8	18.1	16.7	18.7	16.8	16.9	20.3	19.0
class 6	14.8	14.1	17.1	17.6	13.3	17.0	17.4	14.5	18.0	20.4
Σ	100	100	100	100	100	100	100	100	100	100
M3		p=0.01				$p < 0.001$			p=0.01	
class 1	30.4	29.2	31.6	29.6	26.8	31.3	34.5	31.3	27.6	27.2
class 2	27.6	23.5	26.6	26.3	25.4	26.8	25.6	24.7	32.3	25.8
class 3	16.8	20.9	15.3	18.6	17.3	18.3	18.1	17.7	18.8	16.6
class 4	9.5	10.5	12.1	10.5	10.9	10.8	8.8	10.6	9.4	11.7
class 5	8.6	11.2	8.2	11.4	10.9	8.8	9.1	9.5	9.2	11.7
class 6	7.1	4.8	6.3	3.6	8.7	4.0	3.9	6.2	2.7	7.1
Σ	100	100	100	100	100	100	100	100	100	100
M4		p=0.78				$p = 0.003$			$p < 0.001$	
class 1	45.8	47.9	48.4	45.5	43.0	48.2	50.8	43.7	55.5	55.1
class 2	31.4	27.5	28.4	32.3	30.0	30.2	29.2	30.5	31.1	23.5
class 3	13.5	14.4	13.2	13.2	14.5	13.6	12.4	14.9	9.6	11.6
class 4	9.3	10.2	10.1	8.7	12.6	8.0	7.6	10.9	3.9	9.8
Σ	100	100	100	100	100	100	100	100	100	100

3.6 Comparison of classifications

Consider first the associations between categorical adherence variables and some baseline measurements. Statistically significant (p<.05) differences between adherence classes (M1-M4) were found for the level of education, for initial first-line drug regimen, and for pre-ART CD4 cell counts (M2 and M3 only) (Table 3.7). Optimal adherers were more likely to have had university/technical education and poor adherers more likely to have had none/primary education consistently across adherence classifications based on self-reported missing pills, although not on DPR (M2). Although differences were significant, trends with pre-ART CD4 and initial drug regimen were less consistent, again illustrating the fact that the different classifications are identifying different adherence behaviors likely with different predictors.

Figure 3.6 shows that, whilst each summary is trying to capture the same underlying concept of "good adherence", individuals may be classified very

differently. Highest values of Kendall's rank correlation were 0.88 (between M1 and M3, as expected as these are based on the same underlying question), 0.50 (between M4(4-day/weekend) and M3(28-day)), and 0.47 (between M1(MC) and M4(4-day/weekend)) (Table 3.8).

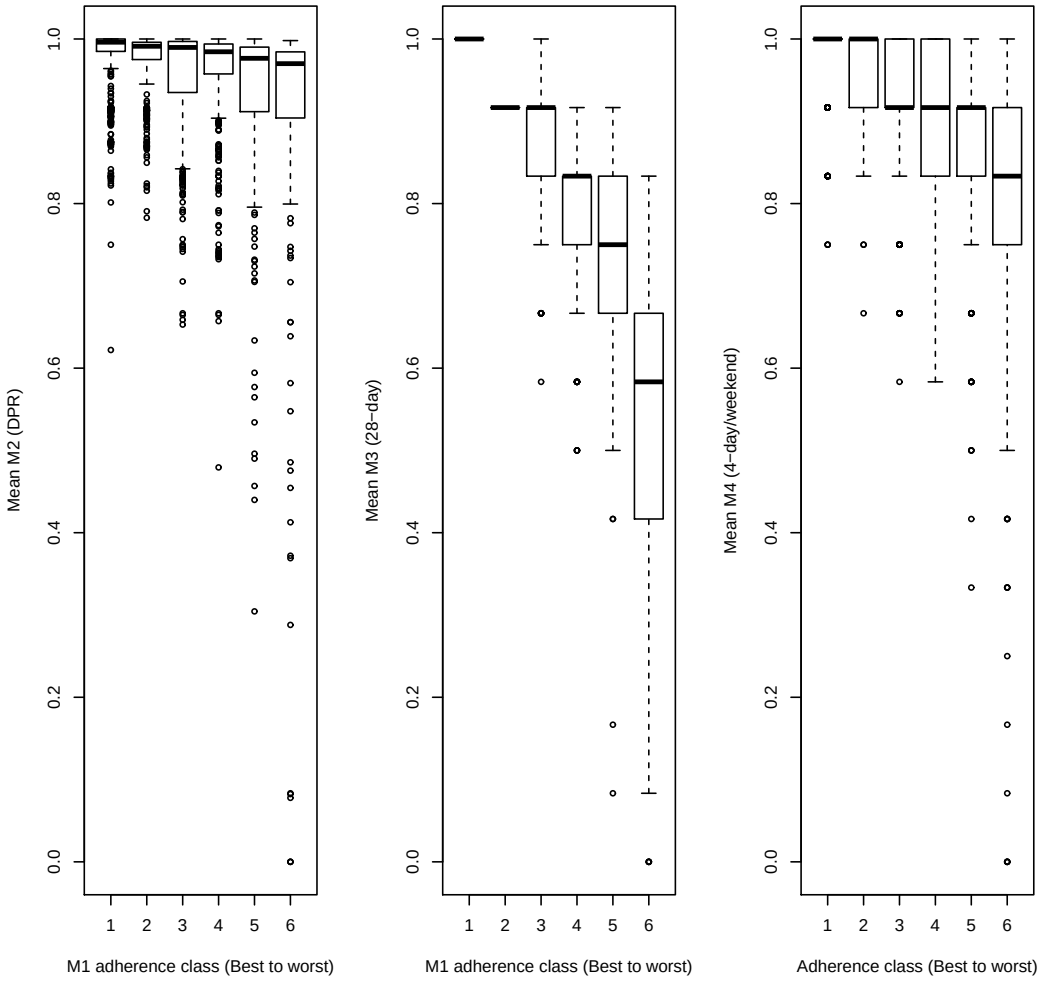

Figure 3.6: Box plots of mean M2, mean M3, and mean M4 by M1 adherence classes. For methods M1, M2, M3 and M4, see Table 3.6

We also used Kappa statistic to assess the agreement between different methods of determining adherence (PC, DPR, Missed dose 4-days, forget at weekend). We further assessed how well the other measures predicted DPR in GEE approach and define sensitivity and specificity in this context. The kappa for agreement between 100% DPR and patient reporting not missing any dose in the last month was 83% ($\kappa = 0.44$) suggesting moderate agreement, but was smaller for other adherence measures. In the model assessing the association between different measures of adherence from the structured questionnaire, the only independent predictor of 100% DPR was not missing doses in the last month, which had a specificity of 97% at identifying complete adherence

Table 3.8: Kendall's rank correlations between the ordered categorical adherence variables M1-M4 as described in Table 3.6.

	M1	M2	M3	M4
M1	.	0.32	0.88	0.47
M2	.	.	0.35	0.35
M3	.	.	.	0.5

(100% DPR) and a sensitivity of 40% at identifying poor adherence (less than 100% DPR adherence). Defining non-adherence by reporting missed doses in last month, OR at weekends did not change specificity/sensitivity (97%, 41%, respectively).

3.7 Predictors of complete adherence

To find the predictors for complete (100%) DPR adherence during the first year of the follow-up, generalized estimating equations (GEE) with exchangeable correlation structure were used adjusted for relevant baseline social, demographic, and clinical characteristics across study visits. See paper I. If Y_{it} is the indicator for poor adherence for individual i at visit t , then we assumed that

$$logit(P(Y_{it} = 1)) = \alpha + \beta^T x_i + \gamma_1 t + \gamma_2 t I(t > 12)$$

where x_i is the vector of explaining baseline variables for individual i. Correlation between Y_{it} and $Y_{it'}$ was assumed to be a constant ρ. The effect of time on ART was estimated using 2 linear slopes with a change-point at 12 weeks.

Multivariate models were selected based on backward elimination (P = 0.2). Odds ratios (ORs), adjusted odds ratios (aORs), and 95% confidence intervals (95% CIs) are presented in paper I. Complete DPR adherence increased over time, with a 9% increase every 4 weeks over the first 12 weeks [aOR = 1.09, 95% CI (1.08, 1.10)] and a 2% increase from weeks 12 to 52 [aOR = 1.02, (1.02, 1.03)] independently of adjustment for other factors. Complete DPR adherence was also significantly more frequent in those with higher CD4 counts at enrollment [aOR = 1.08/100 cells higher, (1.01, 1.16)], higher in patients initiating ART later 2004 versus 2003, and there were differences between clinical centres. The only social predictor of adherence was reporting other sexual partners in the 3 months before starting ART [aOR = 0.72, 95% CI (0.60, 0.87)]. None of the other factors were independently associated with

complete DPR%, although univariably being female, higher education status and not having been admitted to hospital in the last year showed strong associations but these did not remain after adjustment for factors center, CD4 count, and time since ART.

4 Adherence as an explanatory factor

4.1 The use of first year adherence

4.1.1 Methods

The categorical adherence variables introduced in Chapter 3 can be used to describe variation in adherence, to find predictors for adherence, and to predict future disease progression or other outcomes. We assessed the adherence classification based on the Markov chain approach (Method M1) and three other classifications based on traditional "averaged" adherence measures, namely methods M2-M4. The variables were all based on 12 first visits only. For definitions, see Table 3.6. We considered the impact of adherence (M1-M4) separately on time to death and time to CD4 failure using Kaplan-Meier survival curves with log-rank tests, and Cox proportional hazard models stratified by randomized arm, centre, and initial first-line ART, and adjusted for most important confounding factors at ART initiation.

CD4 failure was defined as the earliest time with either CD4 count $\leq$50 cells/μL, or two successive CD4 counts $\leq$100 cells/μL (the immunological criteria for switch to second-line in LCM). Participants with CD4 <50 at time zero (week 48) were included as an event at time 0, (92/575 CD4 failures). Potential confounding factors were sex (male/female) and age (18-35,35-50,50+), WHO stage (2,3,4), CD4 counts (0-49,50-99, 100-149,150-199), body mass index (-20,20+), and socio-demographic factors at ART initiation. We did not adjust for measurements obtained at the 12 visits during the first year, as these can be seen as intermediate factors between our main exposure variable and the outcome.

4.1.2 Results

Observed survival probabilities were 0.99, 0.97, 0.96, 0.94, 0.92 at 1-5 years (after 48 weeks on ART) and were clearly lowest in the worst adherence class for all approaches (Figure 4.1). Differences between other adherence classes were smaller for all approaches so that the global log-rank test was of marginal statistical significance for categories M1, M3, and M4 (p=0.08, 0.08, 0.09) with the exception of M2 (p=0.01). The adjusted and unadjusted estimates of class effects were similar suggesting the effects were not modified by pre-ART characteristics (Table 4.1). The worst adherence class for methods M1-M4 having significantly greater mortality in adjusted analysis with hazard ratios

(95% confidence intervals) 2.01 (1.21,3.32), 1.73 (1.09, 2.76), 1.77 (1.11,2.83), and 2.46 (1.37,4.42), respectively.

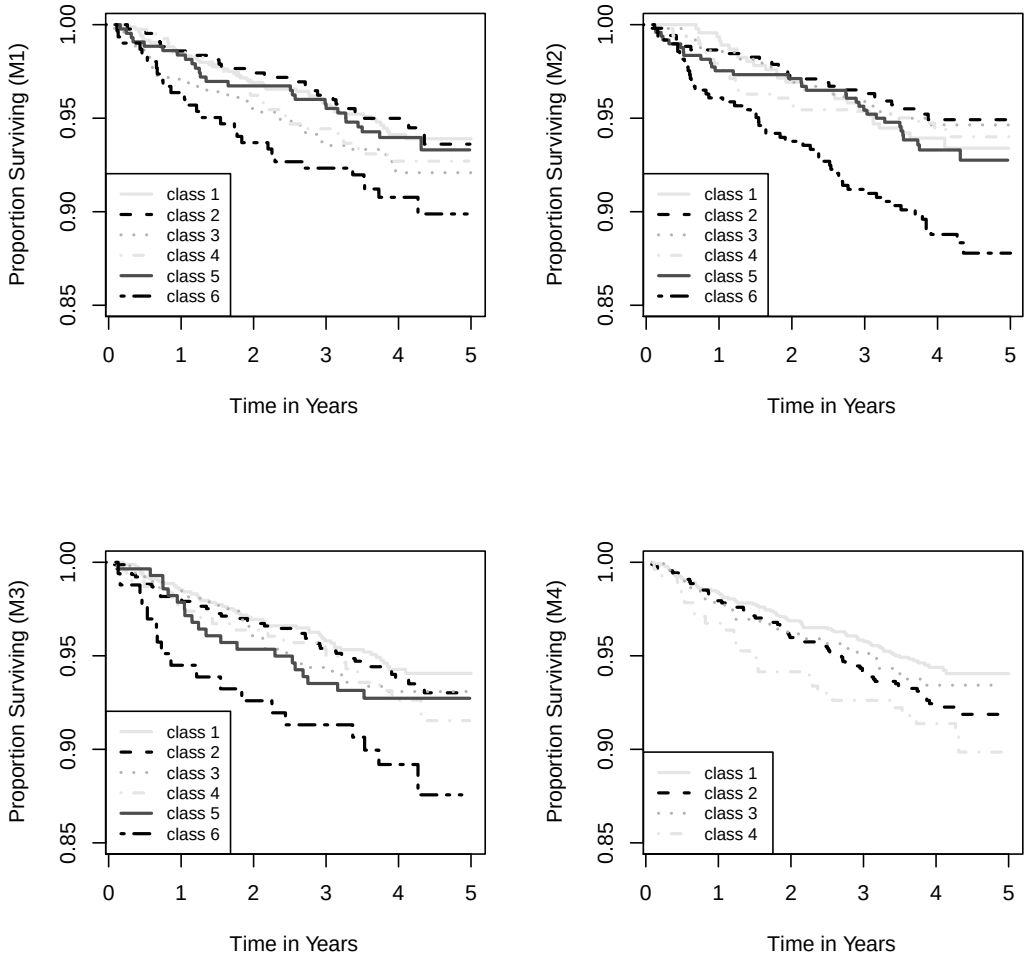

Figure 4.1: Estimated survival curves for mortality by different adherence classes based on methods M1-M4.

In a further analysis, we also fitted a combined model using simple indicators for being in the worst adherence class for each of M1-M4. The MC3 (M1) associated with mortality independently of DPR (M2) with HR=1.57 (1.02, 2.42) (p=0.04) and 1.82 (1.32, 2.51) (p=0.01) respectively, suggesting that the 2 ways of defining adherence add independent information. Survival curves showed clear evidence of non-proportional hazards which may be due to the fact that the effect of first year adherence changes with time. Therefore fitting a separate model with a censoring at 2 years showed stronger effects in the worst adherence class for all four approaches (Table 4.1), but overall tests of association were similar to the pooled analysis. And when modeling for years 2-5, the effect of the worst adherence class then weakened as expected.

Of 2960 participants included, 575 (19%) had CD4 failure (single count

Table 4.1: *Estimated hazard ratios (with 95 % confidence intervals) for the effects of four categorical adherence variables (methods M1-M4) on mortality. The estimates are obtained from a fitted Cox proportional hazard model. The model is stratified by randomized arms, center, and initial first-line ART, and the estimates are adjusted for pre-ART characteristics (CD4 cell count, BMI, WHO disease stage, age and sex).*

Adherence class	(0-5] years (Unadjusted) HR	p p	(0-5] years Adjusted HR(95% CI)	p	(0-2] years Adjusted HR(95% CI)	p	(2-5]years Adjusted HR(95% CI)	p
M1								
1	1(ref)	0.08	1(ref)	0.09	1(ref)	0.04	1(ref)	0.9
2	0.98(0.60, 1.62)		0.95(0.58, 1.57)		0.86(0.43, 1.75)		1.02(0.50,2.08)	
3	1.42(0.93, 2.19)		1.41(0.92, 2.17)		1.68(0.96, 2.94)		1.10(0.56, 2.17)	
4	1.38(0.86, 2.22)		1.36(0.84, 2.18)		1.35(0.71, 2.57)		1.33(0.66, 0.68)	
5	1.17(0.72, 1.91)		1.17(0.72, 1.90)		1.20(0.62, 2.31)		1.09(0.53, 2.25)	
6	2.05(1.24, 3.37)		2.01(1.21, 3.32)		2.48(1.31, 5.22)		1.43(0.63, 3.29)	
M2								
1	1(ref)	0.004	1(ref)	0.01	1(ref)	0.08	1(ref)	0.02
2	0.78(0.45, 1.34)		0.76(0.44, 1.30)		0.95(0.45, 1.98)		0.56(0.25, 1.29)	
3	0.88(0.51, 1.51)		0.86(0.50, 1.50)		1.07(0.51, 2.24)		0.66(0.29, 1.51)	
4	0.96(0.56, 1.65)		0.93(0.54, 1.60)		1.47(0.74, 2.94)		0.40(0.15, 1.05)	
5	1.11(0.66, 1.86)		1.06(0.63, 1.79)		0.98(0.46, 2.08)		1.09(0.53, 2.24)	
6	1.84(1.16, 2.93)		1.73(1.09, 2.76)		1.99(1.04,3.79)		1.44(0.73, 2.84)	
M3								
1	1(ref)	0.08	1(ref)	0.09	1(ref)	0.09	1(ref)	0.7
2	1.17(0.79, 1.76)		1.16(0.77, 1.73)		1.14(0.66, 1.97)		1.14(0.63, 2.07)	
3	1.33(0.86, 2.06)		1.30(0.84, 2.02)		1.45(0.81, 2.58)		1.09(0.55, 2.15)	
4	1.47(0.88, 2.45)		1.46(0.88, 2.44)		1.33(0.66, 2.71)		1.59(0.76, 3.33)	
5	1.42(0.74, 2.70)		1.57(0.91, 2.70)		1.91(0.96, 3.81)		1.17(0.49, 2.84)	
6	2.29(1.37, 3.82)		2.46(1.37, 4.42)		2.88(1.38,6.04)		1.92(0.73, 5.06)	
M4								
1	1(ref)	0.09	1(ref)	0.08	1(ref)	0.2	1(ref)	0.4
2	1.37(0.98, 1.91)		1.36(0.98, 1.91)		1.27(0.81, 2.00)		1.47(0.90. 2.44)	
3	1.22(0.77, 1.93)		1.18(0.75, 1.86)		1.21(0.67, 2.19)		1.13(0.55, 2.32)	
4	1.75(1.10, 2.79)		1.77(1.11, 2.83)		1.85(1.02,3.36)		1.65(0.77, 3.54)	

<50 or confirmed <100 cells/μL) during follow-up at or after 48 weeks. The estimated probabilities of remaining CD4 failure-free were 0.93, 0.87, 0.82, 0.81, 0.79 with 210, 163, 112, 49 and 32 events in the 1-5 years. Similarly to survival, risk of CD4 failure was greatest in the poorest adherence class and there were no substantial differences between other adherence classes for methods M1-M4 (Figure 4.2, Table 4.2). Interestingly, missing doses in the 4 days before clinic or at weekends (M3) appeared to have the greatest discrimination in terms of risks of CD4 failure, consistent with short interruptions (missing doses regularly at weekends) having higher risks of virological and hence CD4 failure. As for mortality, results from unadjusted and adjusted analyses were similar (Table 4.2), with only the difference between the last (worst) class and the first (best) class being statistically significant with adjusted HR-estimates 1.59 (1.17,2.15), 1.54 (1.15,2.05), 1.69 (1.29,2.22), and 1.83 (1.29,2.61), respectively).

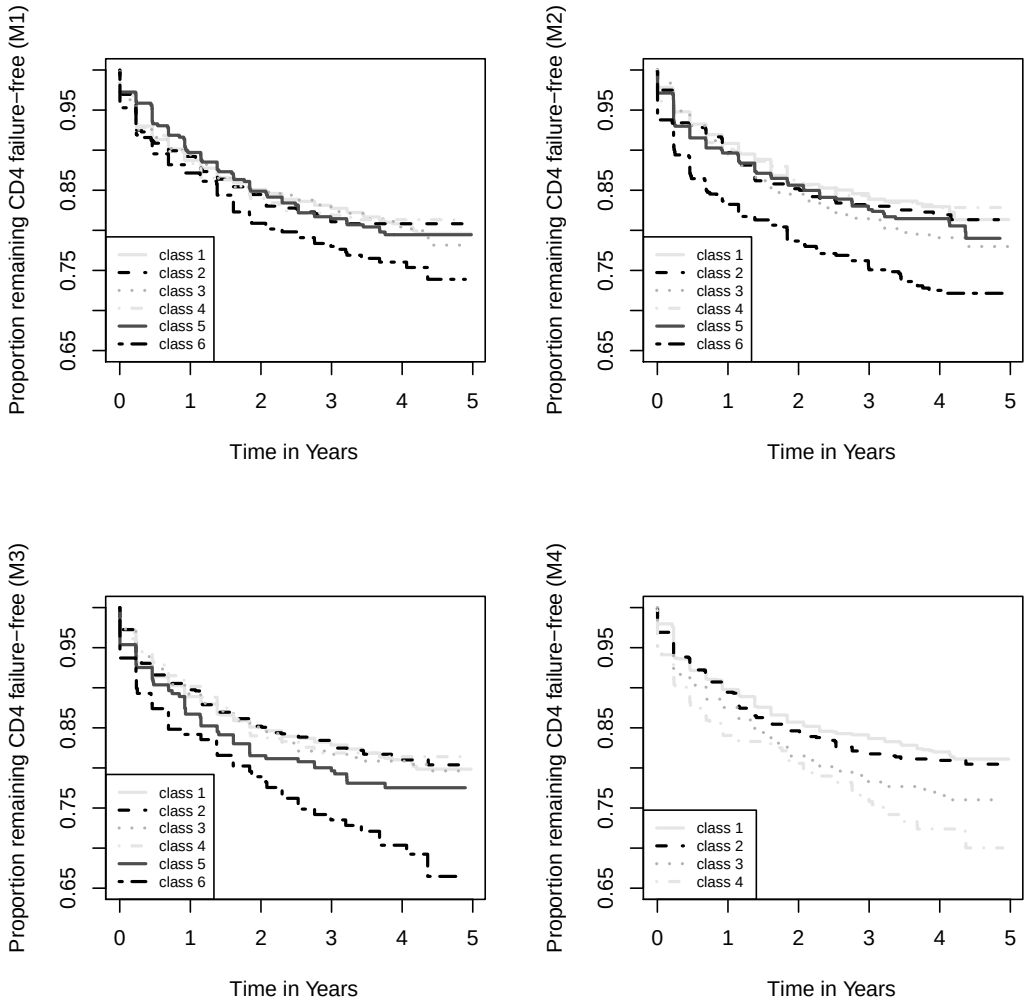

Figure 4.2: Estimated survival curves for CD4 cell count failures by adherence classes based on methods M1-M4.

Table 4.2: *Estimated hazard ratios (with 95 % confidence intervals) for the effects of four categorical adherence variables (methods M1-M4) on CD4 failure. The estimates are obtained from a fitted Cox proportional hazard model. The model is stratified by randomized arms, center, and initial first-line ART, and the estimates are adjusted for pre-ART characteristics (CD4 cell count, BMI, WHO disease stage, age and sex).*

Adherence class	(0-5] years Unadjusted HR	p	(0-5] years Adjusted HR(95% CI)	p	(0-2] years Adjusted HR(95% CI)	p	(2-5]years Adjusted HR(95% CI)	p
M1								
1	1(ref)	0.15	1(ref)	0.11	1(ref)	0.26	1(ref)	0.37
2	1.10(0.84, 1.43)		1.04(0.80, 1.36)		1.07(0.79,1.45)		0.94(0.51, 1.73)	
3	1.08(0.84, 1.39)		1.12(0.87, 1.44)		1.11(0.83, 1.48)		1.08(0.63, 1.85)	
4	1.07(0.81, 1.42)		1.05(0.80, 1.39)		1.17(0.86, 1.59)		0.64(0.32, 1.32)	
5	1.11(0.85, 1.45)		1.11(0.85, 1.45)		1.09(0.80, 1.48)		1.18(0.68, 2.05)	
6	1.55(1.15, 2.09)		1.59(1.17, 2.15)		1.55(1.10, 2.19)		1.62(0.84,3.14)	
M2								
1	1(ref)	0.01	1(ref)	0.02	1(ref)	0.09	1(ref)	0.11
2	1.09(0.80, 1.48)		1.03(0.76, 1.40)		1.01(0.72, 1.41)		1.20(0.57, 2.53)	
3	1.28(0.95, 1.73)		1.21(0.89, 1.64)		1.03(0.73, 1.44)		2.10(1.06,4.17)	
4	1.02(0.74, 1.40)		1.02(0.74, 1.40)		0.96(00.68, 1.36)		1.28(0.60, 2.75)	
5	1.15(0.85, 1.57)		1.10(0.81, 1.50)		0.94(0.66, 1.34)		1.86(0.92,3.76)	
6	1.61(1.21, 2.14)		1.54(1.15, 2.05)		1.40(1.02, 1.93)		2.12(1.08,4.17)	
M3								
1	1(ref)	0.003	1(ref)	0.01	1(ref)	0.05	1(ref)	0.01
2	1.04(0.83, 1.31)		1.02(0.81, 1.28)		1.02(0.79, 1.32)		1.01(0.62, 1.66)	
3	1.10(0.86, 1.42)		1.10(0.85, 1.41)		1.07(0.80, 1.42)		1.15(0.67, 1.96)	
4	1.05(0.77, 1.43)		1.09(0.80, 1.48)		1.21(0.87,1.70)		0.65(0.28, 1.46)	
5	1.41(1.04, 1.91)		1.55(1.14, 2.11)		1.63(1.16,2.30)		1.10(0.53, 2.29)	
6	2.06(1.45, 2.91)		1.83(1.29, 2.61)		1.56(1.03,2.36)		3.10(1.56, 6.17)	
M4								
1	1(ref)	0.01	1(ref)	<0.001	1(ref)	0.04	1(ref)	0.01
2	1.07(0.87, 1.30)		1.08(0.88, 1.32)		1.08(0.86, 1.35)		1.05(0.67, 1.66)	
3	1.31(1.03, 1.68)		1.36(1.06, 1.74)		1.35(1.02, 1.77)		1.41(0.81, 2.44)	
4	1.57(1.20, 2.05)		1.69(1.29, 2.22)		1.46(1.06, 2.01)		2.46(1.45, 4.17)	

4.2 Dynamic logistic regression model

4.2.1 Methods

A dynamic logistic model was used to study the association between adherence and mortality for HIV-infected adults. As before, adherence was assessed at scheduled 4-weekly clinic visits. The model allows that the probability of dying between two clinic visits is explained by recent adherence history before the latest visit as well as by other (time dependent or time-invariant) covariates (See paper IV). In addition to the estimates of effects at the individual level, the approach also allows for the estimation of the population attributable fraction (PAF) a population level measure of the effect of adherence on mortality. Our primary outcome is mortality in those surviving the first year on ART, censored by their last follow-up visit or 31 December 2008. We also assess the time delay in the effect of adherence on the risk of mortality. As the main trial results demonstrated a small but statistically significant difference in mortality between the LCM and CDM groups, see (DART Trial Team 2010), analyses were done separately for both groups, and estimates adjusted for relevant pre-ART confounding factors.

To explain the model, consider a patient i, $i = 1, ..., n$ and let T_i be the total planned number of clinic visits during the follow-up. For patient i, the response y_{it} is a binary outcome which indicates whether patient i has died before the tth visit. Let t_i be the observed follow-up time for the ith patient: If patient i does not die during the full follow-up period then $t_i = T_i$, otherwise $t_i = \min\{t : y_{it} = 1\}$. Let x_{it} be the time-dependent vector of explanatory variables for y_{it}, $t = 1, ..., t_i$. In a dynamic logistic model we assume that the probability of $y_{it} = 1$ conditional on $y_{i,t-1} = 0$ is $r_{it}(\beta) = \left(1 + \exp(-\beta^T x_{ij})\right)^{-1}$ and the full likelihood function is

$$L(\beta) = \prod_{i=1}^{n} \prod_{t=1}^{t_i} r_{it}^{y_{it}} (1 - r_{it})^{1-y_{it}}.$$

The model can be fitted using standard logistic regression algorithms to give the maximum likelihood (ML) estimate $\hat{\beta}$ with its estimated covariance matrix. Standard arguments can be used to prove the limiting multivariate normality of $\hat{\beta}$. The dynamic logistic regression model was first proposed by Bonney et al.(Bonney 1987) and used in Alho et al.(Alho et al. 1996). Here we use dynamic logistic regression model to investigate the relationship between adherence to ART and mortality. The estimated odds ratios are for the risk of dying in the current 4-weekly interval and the results are similar to those from time-dependent Cox proportional hazard models.

If the full covariate history $x_i = (x_{i1}, ..., x_{iT_i})^T$ of individual i were known, then the individual survival function is

$$S_{it}(\beta) = \prod_{j=1}^{t} (1 - r_{ij}(\beta)).$$

The population survival function with n covariate histories given in $X = \{x_1, ..., x_n\}$ is

$$S_t(\beta, X) = \frac{1}{n} \sum_{i=1}^{n} S_{it}(\beta),$$

and $S_t(\beta, X)$ thus gives the expected proportion of individuals alive at visit t, $t = 1, 2, ...$ If the covariate histories in $X = \{x_1, ..., x_n\}$ are changed to take the values in $X^* = \{x_1^*, ..., x_n^*\}$, then the population survival curve $S_t(\beta, X)$ will transform to $S_t(\beta, X^*)$, and the ratio

$$PAF_t = \frac{S_t(\beta, X^*) - S_t(\beta, X)}{1 - S_t(\beta, X)},$$

the population attributable fraction (PAF), gives the (theoretical) proportion of deaths which could have been avoided with the manipulation by time $t = 1, 2, ...$ Naturally, PAF_t can be estimated by

$$\widehat{PAF}_t = \frac{S_t(\hat{\beta}, X^*) - S_t(\hat{\beta}, X)}{1 - S_t(\hat{\beta}, X)},$$

and the delta method can be used to construct limiting confidence intervals, $t = 1, 2, ...$ (Leung & Kupper 1981, Oja et al. 1996).

Here, we are interested in the impact of adherence behavior on the risk of death. The covariate vector x_i can then be decomposed as $x_i = (x_{i1}^T, x_{i2}^T)^T$ where x_{i1} is a subvector of the adherence variables and x_{i2} contains other covariates (confounders). (Figure 4.3). For the population attributable fraction, the manipulation, is then

$$x_i = \begin{pmatrix} x_{i1} \\ x_{i2} \end{pmatrix} \quad \rightarrow \quad x_i^* = \begin{pmatrix} x_{i1}^* \\ x_{i2} \end{pmatrix}$$

In order to estimate the population attributable fraction due to non-optimal adherence, x_{i1} will be set to x_{i1}^* (optimal adherence) while x_{i2} will be left unchanged. Unfortunately, the full adherence history of patient i who dies before the end of the follow-up is not known so that $S_t(\hat{\beta}, X)$ is partially unknown. We therefore use the estimated (Kaplan-Maier) population survival curve from the original data (excluding STI patients after STI randomisation and upweighting equivalent patients randomised to CT), and the curve $S_t(\hat{\beta}, X^*)$ to find an estimate $\widehat{PAF}_t$, $t = 1, 2, ...$ Bootstrap samples can be taken to find a confidence interval for PAF_t, $t = 1, 2, ...$

At each visit $t = 1, 2, ...,$ we consider the adherence variable that takes the following four values

"poor", "good", "non-response", and "missing",

where "poor" and "good" mean "missed a dose in the last month" and "did not miss a dose in the last month", respectively. The history of adherence

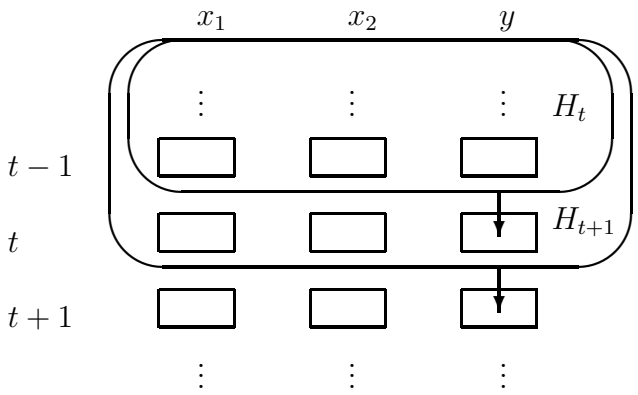

Figure 4.3: Illustration of the dynamic model: The probability $P(y_t = 1|y_1 = ..., y_{t-1} = 0)$ is modeled using history H_t, that is, the values $x_{1,1}, ..., x_{1,t-1}$ and $x_{2,1}, ..., x_{2,t-1}$.

behavior up to the visit t is then summarized as follows. First, we consider the visits $t-8, t-7, ..., t-3$ (6 visits, that is, 6 months) to calculate the following three independent indicator variables

"poor at least once", "non-response at least once", "missing at least once",

for visits t. These three adherence variables are not mutually exclusive, that is, any combination of zeros and ones is possible. Whilst this is only one way to combine 4-weekly adherence measurements, it is a simple summary which retains much of the historical information. (The adherence measurements at visits $t-2$ and $t-1$ are seen rather as intermediate factors between adherence history and death. A preliminary analysis showed that patients dying between visits $t-1$ and t often missed visits $t-2$ and $t-1$ for reasons that were clearly more related to their mortality risk than their adherence behavior.) The adherence measurement are further confounded by the CT/STI randomization. We therefore considered a fourth indicator in our model

"randomized to STI"

which is 1 at visit t if the patient has been randomized to STI before visit t. If it gets value 1, then the three adherence indicators above lose their interpretation and are all set to be 0. Note that, if all four indicators get the value zero, then the patient is not randomized to STI and adherence behavior is "optimal"; this is thus the reference class in the modeling.

We fitted an adjusted model including the following fixed pre-ART characteristics: age, sex, WHO disease stage, body mass index (BMI), and CD4 cell count, categorizing continuous variables to allow for non-linearity. Time-dependent CD4 cell count can be seen as an intermediate variable, and therefore only pre-ART CD4 count was included in the model (similarly for WHO

disease stage and BMI). To adjust for the effect of time on ART, we allowed yearly changes in the risks to die. For the estimation of population attributable fraction (PAF), we calculated first a weighted Kaplan-Maier survival curve estimate for the original population assuming all patients had been intended to take ART continuously (ie if the CT/STI randomization had not occurred), by using weights 2 and 0 for those randomized to CT and STI respectively (after randomization), and 1 for non-randomized patients (and before STI/CT randomisation). Second, model-based hypothetical estimated survival curves for the same population but with optimal adherence history (all four indicators constantly zero, confounded variables as in the population) were calculated. The PAF estimates are then based on the values of these curves at 5 years. All the analyses were done separately in the LCM and CDM groups.

4.2.2 Results

Median (IQR) follow-up after 1 year on ART in the 2960 patients surviving 12 months was a further 3.9 (3.5-4.3) years on ART. The proportion of individuals reporting good adherence at each visit remained very high and stable over most of the five year period (Figure 3.3 and Figure 3.4), with little difference in the adherence profiles between the LCM and CDM groups. However, the number of missing visits did increase with time, at least in part because, after approximately 3 years on ART, a small number of participants moved to 12-weekly visits, with telephone nurse visits in-between (without adherence data). This may result in a small bias in the estimates of the effect of missing visits on mortality.

The estimated odds ratios (OR) with 95% CI for different risk factors for mortality from the dynamic logistic model in LCM and CDM groups are given in Tables 4.3 and 4.4, respectively, and demonstrate a clear association between poorer adherence and 4-weekly probability of dying. The largest differences between LCM and CDM groups can be seen in the odds ratios for poor vs. optimal (1.30 and 2.18 for LCM and CDM groups, correspondingly) and for randomized to STI vs optimal (0.86 and 2.07) but none of the differences were statistically significant when fitted using interaction tests ($p>0.10$).

For those monitored following LCM, mortality risks seemed somewhat lower at visits either 2-3 or 4-5 years on ART ($p=0.06$), none of the other pre-ART factors were significantly associated with mortality risk after 1 year on ART ($p>0.3$). In contrast, in the CDM group the post-1-year mortality risks were higher for patients with pre-ART <150 cells/μL ($p=0.01$ for categorical pre-ART CD4), and for patients aged >50 years at ART initiation ($p=0.07$ for categorical variable age).

To consider the bias caused by the participants who moved to 12-weekly visits (with telephone nurse visits in-between), we also fitted interactions between time indicator (4,6] and adherence variables. There was no statistically significant interactions in the LCM group. The only statistically significant interaction term ($p=0.004$) in the CDM group was for the interaction between

Table 4.3: *Estimated unadjusted and adjusted odds ratios (OR) with 95 percent confident intervals for the risk of death obtained from the dynamic logistic regression model and based on 1478 individuals in the LCM group alive after the first year of follow-up. Adherence history is given by 4 time-dependent indicators with "optimal" adherence as a reference class.*

	Unadjusted OR	95% CI	Adjusted OR	95% CI	P-value
Adherence history:					
poor at least once	1.34	(0.81, 2.17)	1.30	(0.78, 2.10)	0.30
non-response at least once	1.99	(1.01, 3.63)	1.98	(1.00, 3.62)	0.03
missing at least once	3.26	(1.65, 5.96)	3.60	(1.80, 6.65)	<0.001
randomized to STI	0.73	(0.25, 1.69)	0.86	(0.29, 2.05)	0.8
Pre-ART					
WHO disease stage					
stage 2	1(ref)		1(ref)		0.8
stage 3	1.08	(0.61, 2.02)	1.02	(0.57, 1.91)	
stage 4	1.48	(0.76, 2.92)	1.19	(0.60, 2.41)	
Pre-ART					
CD4 cell count					
0-49	1.69	(0.88, 3.51)	1.43	(0.72, 3.07)	
50-99	1.27	(0.61, 2.77)	1.18	(0.55, 2.61)	
100-149	1.07	(0.49, 2.41)	1.01	(0.45, 2.29)	
150-199	1(ref)		1(ref)		0.6
Pre-ART					
Body mass index					
<20	1.35	(0.82, 2.18)	1.31	(0.79, 2.14)	
20-27	1(ref)		1(ref)		0.5
>27	1.37	(0.59, 2.79)	1.33	(0.57 ,2.77)	
Age at ART					
initiation groups					
18-35	1(ref)		1(ref)		0.9
35-50	0.84	(0.53, 1.35)	0.92	(0.57, 1.50)	
50+	0.89	(0.31, 2.09)	1.03	(0.35, 2.48)	
Sex					
Female vs Male	1.23	(0.76, 2.04)	1.31	(0.79, 2.22)	0.3
Time since ART					
initiation in years					
≤2	1(ref)		1(ref)		0.06
2-3	0.37	(0.17, 0.73)	0.40	(0.18, 0.79)	
3-4	0.57	(0.30, 1.04)	0.60	(0.31, 1.10)	
4-5	0.49	(0.24, 0.93)	0.47	(0.23, 0.91)	
>5	0.76	(0.34, 1.54)	0.65	(0.29, 1.34)	

Table 4.4: *Estimated unadjusted and adjusted odds ratios (OR) with 95 percent confident intervals for the risk of death obtained from the dynamic logistic regression model and based on 1482 individuals in the CDM group alive after the first year of the follow-up. Adherence history is given by 4 time-dependent indicators with "optimal" adherence as a reference class.*

	Unadjusted OR	95% CI	Adjusted OR	95% CI	P-value
Adherence history:					
poor at least once	2.14	(1.45, 3.14)	2.18	(1.47, 3.22)	<0.001
non-response at least once	2.16	(1.29, 3.52)	2.09	(1.22, 3.40)	<0.001
missing at least once	3.46	(2.07, 5.54)	3.65	(2.15, 5.92)	0.005
randomized to STI	1.60	(0.81, 2.92)	2.07	(1.03, 3.85)	0.03
Pre-ART					
WHO disease stage					
stage 2	1(ref)		1(ref)		0.51
stage 3	1.67	(0.99, 3.03)	1.39	(0.82, 2.55)	
stage 4	1.61	(0.88, 3.08)	1.28	(0.68, 2.48)	
Pre ART					
CD4 cell count					
0-49	3.61	(1.88, 7.85)	3.43	(1.74, 7.62)	
50-99	2.75	(1.36, 6.14)	2.62	(1.28, 5.90)	
100-149	2.49	(1.20, 5.63)	2.45	(1.18, 5.57)	
150-199	1(ref)		1(ref)		0.01
Pre-ART					
Body mass index					
<20	1.63	(1.12, 2.38)	1.33	(0.90, 1.96)	
20-27	1(ref)		1(ref)		0.35
>27	0.98	(0.43, 1.93)	1.07	(0.46 ,2.15)	
Age at ART					
initiation groups					
18-35	1(ref)		1(ref)		0.07
35-50	0.72	(0.49, 1.05)	0.83	(0.56, 1.24)	
50+	1.45	(0.74, 2.63)	1.74	(0.87, 3.21)	
Sex					
Female vs Male	0.62	(0.43, 0.90)	0.72	(0.49, 1.06)	0.10
Time since ART					
initiation in years					
≤2	1(ref)		1(ref)		0.13
2-3	1.65	(0.98, 2.84)	1.66	(0.98, 2.86)	
3-4	1.10	(0.61, 1.99)	1.10	(0.61, 1.98)	
4-5	1.64	(0.96, 2.86)	1.56	(0.90, 2.74)	
>5	0.91	(0.38, 1.96)	0.80	(0.33, 1.73)	

non-response and time. The adjusted odds ratio for non-response vs. optimal then changed from 3.72(95% CI (2.07,6.35)) to 0.10(95% CI (0.01,0.38)). This change may be due to the fact that the subjects with 12-weekly visits are classified as non-responders but in fact they are good adherers and therefore eligible for 12-weekly refills. The adherence history in the dynamic logistic regression model may naturally be quantified in several ways. We found adherence between 3 and 9 months prior to the death was the most useful choice for our model. Different alternative measures of adherence at each visit may be summarized using different time delays. In each study, one should consider carefully the best way to do this as the results may be sensitive to the choices.

The estimates of PAF, that is, the estimated proportions of deaths that could have avoided by optimal adherence in the LCM and CDM groups were 16% and 33%, respectively. In the absence of laboratory tests, patients in the CDM with optimal adherence had similar survival to those in LCM arm who had customary adherence.

5 Discussion and conclusions

In contrast to other studies in sub-Saharan Africa, all adherence measures used in this study showed improving trend over the first year of ART. The most significant improvement was seen in the first 12 weeks. These data show that there are several socioeconomic (education) and medical (depression, side effects) factors influencing adherence to ART, and more frequently reported reasons were due to personal circumstances (forgot, etc). For different classifications, the poorest adherence class experienced the highest risk of death and CD4 failure regardless of definition: The results were consistent with other studies of non-virological and immunological outcomes (Chi et al. 2009, Abaasa et al. 2008, Hogg et al. 2002, Nachega et al. 2006). The poorest adherence class had lowest level of education for all classifications based on self-report, highlighting that this group could be targeted for adherence-enhancing interventions both at ART initiation and in those not adhering well after a year on ART. Classification based on Markov chain approach is predictive of mortality and CD4 cell count failure independently of DPR. The classifications could be useful in understanding adherence, targeting focused interventions, and improving long-term adherence to therapy.

The Markov chain model is perhaps the simplest model for categorical repeated measurements. Transition probabilities describe the dynamics of adherence to ART. Whilst simple summary measures can relate adherence to the factors that are relatively unchanging in a patient's life, they are unable to take into account the full history of past medical conditions. The other key benefit of a Markov chain model is its ability to handle missing or unobserved data. Even without missing data, it would be complicated to think up a battery of "past history" variables which captured poor adherence completely, including intermit poor adherence, repeated poor adherence, and how often poor adherence occurred. Transition probabilities may enable a better understanding of adherence patterns compared to the traditional methods of just averaging the raw adherence data. We showed that although adherence looked very high overall in the first year in DART, this masked an inconsistent adherence behavior at the individual level. Our motivation for this approach was based on comparison of different adherence profiles. Consider, for example, three individuals with very different profiles ((9, 9, 9, 0, 0, 0, 0, 0, 1, 1, 1, 1), (9, 0, 1, 1, 1, 9, 1, 0, 0, 0, 0, 9), and (9, 1, 0, 0, 0, 9, 0, 0, 1, 1, 1, 9)) but similar overall adherence (measured as proportions). The differences between the individuals cannot be explained with model (MC1) and not even with model (MC2). Only model (MC3) observes the differences between these three individuals. As with any refined and appropriate statistical methodology, one could obtain evidence of effects when more standard

and perhaps simplistic approaches would fail to do so.

The advantage of the approach based on Markov chain is its versatility and simplicity. Several variables can then be used together to construct the states needed for Markov chain model. Our approach can naturally be applied to different data sources (patients diaries, electronic event monitoring, drug possession ratio), and to the adherence to several drugs simultaneously. One could transform data for several drugs taken simultaneously to a single binary indicator or categorical variable, if we can define what patterns are acceptable or what are not. Using different adherence variables (if not highly correlated) would produce different clusters with different predictive powers for mortality. The choice of adherence variables is therefore a crucial step, and depends on the data and application. As an estimation problem, the underlying assumption is that the data set used in the analysis is a random sample from a population with subpopulations having different adherence behavior. Then the cluster memberships naturally estimate the unknown subpopulation memberships, and the odds ratios using cluster memberships estimate the unknown odds ratios for the difference in mortality in the subpopulations. Of course, without this assumption, one can still consider the predictive power of the whole procedure (area under the ROC curve). Naturally, various criteria may be used for the ranking of clusters from best to worst. The cross classification of clusters between models should tend to have largest numbers along the diagonal. However many cases can also be found in the off-diagonals indicating that the ranking is not entirely unambiguous. Although the ranking criteria is somewhat subjective, in other studies one should consider different choices of ranking and the sensitivity of the results to small changes in the criteria and this is of interest in our future work.

Dynamic logistic regression can be used to examine the delayed effect of predictors on death, which may be seen several months after the reported poor adherence, similarly to a time-dependent Cox model. The model also easily allows estimation of the achievable effects of risk factor manipulation at the population level, i.e. the proportion of deaths that could have been avoided with optimal adherence. The major advantage of the dynamic logistic regression model is that although the survival under optimal adherence is unknown in this context, it can be estimated with an estimated model. Only modest absolute differences in mortality risk were observed (1% in LCM and 3% in CDM at 6 years from ART initiation), but the estimated proportions of deaths that could have been delayed (by eliminating non-optimal adherence) within 1-6 years after initiating ART were remarkably high in both groups - 16% in the LCM group and 33% in the CDM group. However, the differences between LCM and CDM under optimal adherence were also narrower than observed in the trial itself, suggesting that, as well as its role in detecting ART failure earlier, one major role of CD4 monitoring could be to re-inforce good adherence behavior - or identify those with adherence issues.

The adherence history in the dynamic logistic regression model may naturally be quantified in several ways. We found adherence between 3 and 9

months prior to the death was the most useful choice for our model. Different alternative measures of adherence at each visit may be summarized using different time delays. In each study, one should consider carefully the best way to do this as the results may be sensitive to the choices. One can naturally also try other time intervals $t-u, ..., t-v$ with different choices of u and v but then the incidence and interpretation of "poor at least once", for example depends strongly on the length $u-v+1$. The comparison of different fitted models is therefore difficult, and we tried to keep the model as simple as possible. There is also an issue with our adherence assessment of how to account for those who come late for scheduled visits. However our measure is conservative, which implies that the actual impact on mortality could be larger.

These data show that overall adherence to ART in this setting is good and improved over the first year of treatment. However, the number of missing visits and poor adherers (not reporting at least 95% or 100% DPR) did increase with time, at least in part because, after approximately 3 years on ART, a small number of participants moved to 12-weekly visits, with telephone nurse visits in-between (without adherence data). In the first year, all patients were on first-line drugs but adherence may be more complicated with second-line treatment regimens. Despite high adherence at the population level, many patients have periods when they do not achieve good adherence. Patients with these problems are associated with lower baseline CD4 counts, side effects, or lack of support mechanisms. DPR and simple questions on missed doses could be useful tools in identifying when patients have problems with adherence and are feasible for large numbers of patients attending routine clinics. Accurate, valid measures of adherence are needed from regular ART clinics to reinforce the ongoing monitoring of adherence and to evaluate interventions to enhance successful use of ART.

Our findings suggest that different approaches may be potentially useful in practical data analysis, and that overall (mean) adherence may not be enough when dealing with ART adherence. In paper III, we compare the predictive powers of the procedures based on models M1, M2, and M3 for mortality with ROC curves. We could not find any big differences between the procedures, but this may just be due to the short period for the measurements of adherence. We expect bigger improvements in the longer term. There are several extensions and possibilities to develop and deepen the analysis of DART trial data: Another extension to this approach would be to use two or more measures of reported adherence for the states in the Markov chain model. Continuous measurements such as the drug possession ratio could be categorized and used in the Markov chain model. One could look at trends over time and/or over a longer period of 3 years. We only considered three states, good, poor and missing. One could consider more states such as lost to follow-up or missed visit and look at different transition probabilities between those states. For example in clinical trials, non-response or drop-out are important outcomes in their own right and should be distinguished from

incomplete forms or poor documentation. However, even with three states we have learned a lot about adherence, and Markov chain models provide a new insight. Another extension of the model used here would be the use of the Markov chain model of order 2. Statistical tools are needed for the model selection. Statistical tests and estimates are needed for the change point in a non-homogeneous Markov chain model and for the order of the model. One can easily build likelihood ratio tests for our nested parametric families of distributions.

Our findings demonstrate clearly that a group of participants with particularly poor adherence, regardless of whether assessed with DPR or self-report over the first year on ART, could be targeted for continued frequent follow-up and enhanced adherence intervention, whereas other patients would probably have done as well without the intensive follow-up provided in this trial. However what works in a trial setting may not work in clinical setting with challenges such as limited staff, inadequate resources, diverse patient population and disease burden. The 4-day/weekend or 28-day self-report approaches are easy to implement in a clinical setting, and even DPR can be approximated by late return to clinic. The identification of different risk classes could be useful in understanding and evaluating adherence, targeting focused interventions in clinical and research settings and improving long term adherence to therapy. The estimated proportions of deaths on long-term ART that could be delayed at population level (by eliminating non-optimal adherence) are similar to benefits from CD4 cell count monitoring of ART and thus high enough to warrant considerable efforts on interventions to support and improve adherence at a programme level, particularly for patients not being monitored using CD4 counts or viral loads. In the absence of laboratory tests, one mechanism through which laboratory monitoring may improve outcomes appears to be to mitigate some of the negative consequences of poor adherence by identifying poor adherers earlier and enabling interventions.

There are several ways to measure adherence to ART medication, however there is no gold standard. Although self-report is subject to recall bias and/or social desirability bias with a tendency to overestimate optimal adherence, it was nevertheless strongly associated with mortality, indicating that the effect of true adherence would likely be even greater if this could have been measured using other more accurate adherence measures. In common with other studies, we validated these measures against viral load outcomes, which were associated with 100% DPR adherence but not 95% adherence. Some have argued that lack of virologic suppression may be an inadequate indicator of nonadherence, as other factors such as mutations, regimen potency, and pharmacokinetics can affect virological suppression. Thus, adherence measures associated with viral load suppression may not provide very accurate determinants of adherence behavior. We only investigated viral load in patients on Combivir plus tenofovir DF, but the association could be different for other drugs. It has also been argued that virologic failure is an inadequate indicator of non-adherence, as several other factors (e.g., viral mutations, HIV

viraemia at initiation of therapy, potency of the therapy prescribed, individual differences in absorption, and interactions) mediate virologic outcomes, see Nieuwkerk et al. (Nieuwkerk & Oort 2005). Assessments of content validity (i.e., how well the measurement items represent the entire universe of items or domain being assessed) and construct validity (i.e., how well a measure reflects some underlying construct or latent variable) are rare, Bangsberg et al. (Bangsberg 2006*b*); and have only been evaluated in few and small studies in sub-Saharan African study (Oyugi et al. 2004).

Summaries of Original Publications

I. In Africa there have been only few studies that have attempted to describe adherence to ART. Adherence is an essential part of successful ART, and yet ways of measuring adherence were not assessed or validated in African patients. Collecting adherence data is important as it can be used in managing patients response to treatment, and in proactively identifying interventions for particular patients having difficulties with their medication. In the first article *Patterns of Individual and Population-Level Adherence to Antiretroviral Therapy and Risk Factors for Poor Adherence in the First Year of the DART Trial in Uganda and Zimbabwe* (Muyingo SK, Walker AS, Reid A, Munderi P, Gibb DM, Ssali F, Levin J, Katabira E, Gilks C and Todd J; DART Trial Team) we explore the adherence profile in the first year and assess the association of different measures of adherence on viral load suppression using the generalised estimating equations (GEE) approach. We consider viral load as a continuous variable using a more sensitive normal interval regression approach. Using a GEE approach, we identify subgroups of individuals who would benefit from enhanced adherence interventions.

II. In an alternative approach patient adherence measurements at visits to clinics are seen as a stochastic process as described by Girard at al, Wong et al, and Sun et al [16], [17] and [18]. Here we develop new statistical tools to characterize and understand the adherence behavior of the individuals, and illustrate the approach with real data. In this second article *Clustering Based on Adherence Data , Epidemiologic Perspectives Innovations* (Sylvia Kiwuwa-Muyingo, Hannu Oja, Sarah A Walker, Pauliina Ilmonen, Jonathan Levin and Jim Todd) we assume that the adherence behavior of each individual is a Markov chain with unknown transition probabilities. This is natural if the population of the patients can be divided into subpopulations or clusters such that within a cluster the transition probabilities are the same. The estimated cluster memberships can then be used as a categorical variable in further analysis. We compare three different Markov chain models for the first year measurements to classify the patients into subclasses (clusters) with similar adherence behavior. The ability of the resulting three classificatory adherence variables to predict mortality in the 2-3 years on ART with their relative merits were discussed.

III. In the third article *The impact of first year adherence to ART on long*

term clinical and immunological outcomes in the DART Trial in Uganda and Zimbabwe, (Kiwuwa-Muyingo S., Walker A. S., Oja H, Levin J, Miiro G., Katabira E., Kityo C., Jim Todd) we assess whether the classification of adherence based on Markov chain model introduced in paper II can be used to predict, using Cox proportional hazard models, long-term clinical and immunological outcomes, mortality and CD4 failure in the second to fifth years after ART initiation. We compare these results with those from average summaries of traditional adherence measures (such as drug possession ratio (DPR) and self-reported adherence). Different measures for poor adherence should be used depending on whether a simple measure for busy ART clinics or a good predictor for research purposes is needed.

IV. We propose the use of the dynamic logistic model for studying the association between adherence and mortality in an antiretroviral therapy (ART) study for HIV-infected adults. In our study, the adherence was assessed at scheduled 4-weekly clinic visits. In the last article *Dynamic logistic regression model and population attributable fraction to investigate the association between adherence and mortality: An antiretroviral therapy study for HIV-infected adults* (Kiwuwa-Muyingo S, Oja H, Walker AS, Ilmonen P, Levin J, Mambule I, Reid A, Mugyenyi P, Todd J, and the DART Trial team) we use a dynamic logistic model allowing that the probability of dying between two visits is explained by recent adherence history before the latest visit as well as by other (time dependent) covariates, similarly to a time-dependent Cox model. Apart from the estimates of effects at the individual level, the odds ratios, the approach also allows the estimation of the population attributable fraction (PAF) which is a population level measure of the effect of adherence on mortality. Different alternative measures of adherence at each visit may be summarized using different time delays.

References

Abaasa, A. M., Todd, J., Ekoru, K., Kalyango, J. N., Levin, J., Odeke, E. & Karamagi, C. A. (2008), 'Good adherence to haart and improved survival in a community hiv/aids treatment and care programme: the experience of the aids support organization (taso), kampala, uganda.', *BMC Health Serv Res* **8**, 241.

Alho, O. P., Läärä, E. & Oja, H. (1996), 'Public health impact of various risk factors for acute otitis media in northern finland.', *Am. J. Epidemiol.* **143**(11), 1149–1156.

Amico, K. R., Harman, J. J. & Johnson, B. T. (2006), 'Efficacy of antiretroviral therapy adherence interventions: a research synthesis of trials, 1996 to 2004.', *J Acquir Immune Defic Syndr.* **41**(3), 285–297.

Bangsberg, D. R. (2006*a*), 'Less than 95% adherence to nonnucleoside reverse-transcriptase inhibitor therapy can lead to viral suppression.', *Clin Infect Dis* **43**(7), 939–941.

Bangsberg, D. R. (2006*b*), 'Monitoring adherence to hiv antiretroviral therapy in routine clinical practice: The past, the present, and the future.', *AIDS Behav.* **10**(3), 249–251.

Bangsberg, D. R., Charlebois, E. D., Grant, R. M., Holodniy, M., Deeks, S. G., Perry, S. & et al. (2003), 'High levels of adherence do not prevent accumulation of hiv drug resistance mutations.', *AIDS* **17**, 1925–1932.

Bangsberg, D. R., Hecht, F., Charlebois, E. D., Zolopa, A. R., Holodniy, M., Sheiner, L., Bamberger, J. D., Chesney, M. A. & ., M. A. (2000), 'Adherence to protease inhibitors, hiv-1 viral load, and development of drug resistance in an indigent population.', *AIDS* **14**(4), 357–366.

Bangsberg, D. R., Moss, A. R. & Deeks, S. G. (2004), 'Paradoxes of adherence and drug resistance to hiv antiretroviral therapy.', *Journal of Antimicrobial Chemotherapy* **53**(5), 696–699.

Bangsberg, D. R., Perry, S., Charlebois, E. D., Clark, R. A., Roberston, M., Zolopa, A. R. & A., M. (2001), 'Adherence to protease inhibitors, hiv-1 viral load, and development of drug resistance in an indigent population.', *AIDS* **15**(9), 1181–1183.

Berg, K. M. & Arnsten, J. H. (2006), 'Practical and conceptual challenges in measuring antiretroviral adherence.', *J Acquir Immune Defic Syndr* **Suppl 1**, 79–87.

Bonney, G. E. (1987), 'Logistic regression for dependent binary observations.', *Biometrics* **43**(4), 951–973.

Byakika-Tusiime, J., Crane, J., Oyugi, J. H., Ragland, K., Kawuma, A., Musoke, P. & Bangsberg, D. R. (2009), 'Longitudinal antiretroviral adherence in hiv+ ugandan parents and their children initiating haart in the mtct-plus family treatment model: role of depression in declining adherence over time.', *Ann Intern Med.* .

Chesney, M. A., Ickovics, J., Hecht, F. M., Sikipa, G. & Rabkin, J. (1999), 'Adherence: a necessity for successful hiv combination therapy.', *AIDS* **13**(Suppl A), S271–8.

Chesney, M. A., Ickovics, J. R., Chambers, D. B., Gifford, A. L., Neidig, J., Zwickl, B. & Wu, A. W. (2000), 'Self-reported adherence to antiretroviral medications among participants in hiv clinical trials: the aactg adherence instruments. patient care committee adherence working group of the outcomes committee of the adult aids clinical trials group (aactg).', *AIDS Care* **12**(3), 255–266.

Chi, B. H., Cantrell, R. A., Zulu, I., Mulenga, L. B., Levy, J. W., Tambatamba, B. C., Reid, S., Mwango, A., Bulterys, M., Saag, M. S. & Stringer, J. S. (2009), 'Adherence to first-line antiretroviral therapy affects non-virologic outcomes among patients on treatment for more than 12 months in lusaka, zambia.', *Int J Epidemiol* **38**, 746–756.

DART Trial Team (2008*a*), 'Fixed duration structured treatment interruptions is inferior to continuous treatment in african hiv-infected adults initiating therapy with cd4<200 cells/mm3.', *AIDS* **22**(2), 237–247.

Dart Trial Team (2008*b*), 'Twenty-four-week safety and tolerability of nevirapine vs. abacavir in combination with zidovudine/lamivudine as first-line antiretroviral therapy: a randomized double-blind trial (nora)*.', *Tropical Medicine International Health* **13**(1), 1365–3156.

DART Trial Team (2010), 'Routine versus clinically driven laboratory monitoring of hiv antiretroviral therapy in africa (dart): a randomised non-inferiority trial.', *Lancet* **375**, 123–131.

DART Virology Group and Trial Team. (2006), 'Virological response to a triple nucleoside/nucleotide analogue regimen over 48 weeks in hiv-1-infected adults in africa.', *AIDS* **20**(10), 1391–1399.

Dimatteo, M. R. (2004), 'Variations in patients' adherence to medical recommendations: a quantitative review of 50 years of research.', *Med Care* **42**(3), 200–209.

Ette, E. I. & Ahmad, A. (2007), Estimating the dynamics of drug regimen compliance, in pharmacometrics: The science of quantitative pharmacology, *in* E. I. Ette & P. J. Williams, eds, 'Origins of Plastids', Wiley, Hoboken, NJ, USA, pp. 165–181.

Gao, X. & Nau, D. P. (2000), 'Congruence of three self-report measures of medication adherence among hiv patients.', *Ann Pharmacother.* **34**(10), 1117–1122.

Glass, T. R., Battegay, M., Cavassini, M., De Geest, S., Furrer, H., Vernazza, P. L. & et al. (2009), 'Longitudinal analysis of patterns and predictors of changes in self-reported adherence to antiretroviral therapy: Swiss hiv cohort study.', *J Acquir Immune Defic Syndr* **9**.

Golin, C. E., Liu, H., Hays, R. D., Miller, L. G., Beck, C. K., Ickovics, J., Kaplan, A. H. & Wenger, N. S. (2002), 'A prospective study of predictors of adherence to combination antiretroviral medication.', *J Gen Intern Med.* **17**(10), 756–765.

Grimmett, G. R. & Stirzaker, D. (1992), *Probability and Random Processes*, Oxford University Press, New York.

Gross, R., Bilker, W. B., Friedman, H. M. & Strom, B. L. (2001), 'Effect of adherence to newly initiated antiretroviral therapy on plasma viral load.', *AIDS* **15**(16), 2109–2117.

Gross, R., Yip, B., Lo Re, V. r., Wood, E., Alexander, C. S., Harrigan, P. R., Bangsberg, D. R., Montaner, J. S. & Hogg, R. S. (2006), 'A simple, dynamic measure of antiretroviral therapy adherence predicts failure to maintain hiv-1 suppression.', *J Infect Dis* **198**(8), 1108–14.

Hogg, R. S., Heath, K., Bangsberg, D., Yip, B., Press, N., O'Shaughnessy, M. V. & Montaner, J. S. (2002), 'Intermittent use of triple-combination therapy is predictive of mortality at baseline and after 1 year of follow-up.', *AIDS* **16**, 1051–1058.

Honghong, W., Jun, Z., Gouping, H., Yang, L., Xianhong, L., Aiyun, Y., Kristopher, F., & Ann, B. W. (2009), 'Consistent art adherence is associated with improved quality of life, cd4 counts, and reduced hospital costs in central china.', *AIDS Research and Human Retroviruses.* **25**(8), 757–763.

Ingersoll, K. S. & Cohen, J. (2008), 'The impact of medication regimen factors on adherence to chronic treatment: a review of literature.', *J Behav Med.* **31**(3), 213–224.

Kalichman, S. C., Amaral, C. M., Stearns, H., White, D., Flanagan, J., Pope, H., Cherry, C., Cain, D., Eaton, L. & Kalichman, M. O. (2007), 'Disease progression in patients with virological suppression in response to haart is associated with the degree of immunological response.', *J Gen Intern Med.* **22**, 1003–1006.

Knobel, H., Alonso, J., Casado, J. L., Collazos, J., González, J., Ruiz, I., Kindelan, J. M., Carmona, A., Juega, J., Ocampo, A. & Group, G. S. (2002), 'Validation of a simplified medication adherence questionnaire in a large cohort of hiv-infected patients: the geema study.', *AIDS* **16**(4), 605–613.

Lazo, M., Gange, S. J., Wilson, T. E., Anastos, K., Ostrow, D. G., Witt, M. D. & Jacobson, L. P. (2007), 'Patterns and predictors of changes in adherence to highly active antiretroviral therapy: longitudinal study of men and women.', *Clin Infect Dis* **45**(10), 1377–1385.

Leung, H. M. & Kupper, L. L. (1981), 'Comparisons of confidence intervals for attributable risk.', *Biometrics* **37**(2), 293–302.

Lima, V. D., Harrigan, R., Bangsberg, D. R., Hogg, R. S., Gross, R., Yip, B. & Montaner, J. S. (2009), 'The combined effect of modern highly active antiretroviral therapy regimens and adherence on mortality over time.', *J Acquir Immune Defic Syndr.* **50**(5), 529–536.

Liu, H., Miller, L. G., Hays, R. D., Golin, C. E., Wu, T., Wenger, N. S. & Kaplan, A. H. (2006), 'Repeated measures longitudinal analyses of hiv virologic response as a function of percent adherence, dose timing, genotypic sensitivity, and other factors.', *J Acquir Immune Defic Syndr.* **41**(3), 315–322.

Masquelier, B., Peytavin, G., Leport, C., Droz, C., Duran, S., Verdon, R., Besnier, J. M., Chêne, G., Raffi, F. & Brun-Vézinet, F. A. S. G. (2002), 'Mechanisms of early virologic failure in antiretroviral-naive patients starting protease inhibitor-containing regimens: the aprovir study.', *J Infect Dis.* **186**(10), 1503–1507.

McCarthy, R. (1998), 'The price you pay for the drug not taken.', *Bus Health.* **16**(10), 27–28.

McDonnell, P. J. & Jacobs, M. R. (2002), 'Hospital admissions resulting from preventable adverse drug reactions.', *Ann Pharmacother.* **36**(9), 1331–1336.

McMahon, J. H., Jordan, M. R., Kelley, K., Bertagnolio, S., Hong, S. Y., Wanke, C. A., Lewin, S. R. & Elliott, J. H. (2011), 'Pharmacy adherence measures to assess adherence to antiretroviral therapy: review of the literature and implications for treatment monitoring.', *Clin Infect Dis.* **52**(4), 493–506.

Moore, D. M., Hogg, R. S., Chan, K., Tyndall, M., Yip, B. & Montaner, J. S. (2006), 'Disease progression in patients with virological suppression in response to haart is associated with the degree of immunological response.', *AIDS* **20**(3), 371–377.

Moore, D. M., Hogg, R. S., Yip, B., Wood, E., Tyndall, M., Braitstein, P. & Montaner, J. S. (2005), 'Discordant immunologic and virologic responses to highly active antiretroviral therapy are associated with increased mortality and poor adherence to therapy.', *J Acquir Immune Defic Syndr.* **40**(3), 288–293.

Morisky, D. E., Green, L. W. & Levine, D. M. (1986), 'Concurrent and predictive validity of a self-reported measure of medication adherence.', *Med Care* **24**(1), 67–74.

Muyingo, S. K., Walker, A. S., Reid, A., Munderi, P., Gibb, D. M., Ssali, F. & et al. (2008), 'Patterns of individual and population-level adherence to antiretroviral therapy and risk factors for poor adherence in the first year of the dart trial in uganda and zimbabwe.', *J Acquir Immune Defic Syndr* **48**, 468–475.

Nachega, J. B., Hislop, M., Dowdy, D. W., Chaisson, R. E., Regensberg, L. & G., M. (2007), 'Adherence to nonnucleoside reverse transcriptase inhibitor-based hiv therapy and virologic outcomes.', *Ann Intern Med* **146**, 564–573.

Nachega, J. B., Hislop, M., Dowdy, D. W., Lo, M., Omer, S. B., Regensberg, L. & et al. (2006), 'Adherence to highly active antiretroviral therapy assessed by pharmacy claims predicts survival in hiv-infected south african adults.', *J Acquir Immune Defic Syndr* **43**, 78–84.

Nieuwkerk, P. T. & Oort, F. J. (2005), 'Self-reported adherence to antiretroviral therapy for hiv-1 infection and virologic treatment response: a meta-analysis.', *J Acquir Immune Defic Syndr.* **38**(4), 445–448.

Oja, H., Alho, O. P. & Läärä, E. (1996), 'Model-based estimation of the excess fraction (attributable fraction): day care and middle ear infection.', *Statistics in Medicine* **15**(2), 1519–1534.

Osterberg, L. & Blaschke, T. (2005), 'Adherence to medication.', *N Engl J Med* **353**(5), 487–497.

Oyugi, J. H., Byakika-Tusiime, J., Charlebois, E. D., Kityo, C., Mugerwa, R., Mugyenyi, P. & Bangsberg, D. R. (2004), '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.* **36**(5), 1100–1102.

Oyugi, J. H., Byakika-Tusiime, J., Ragland, K., Laeyendecker, O., Mugerwa, R., Kityo, C. & et al. (2007), 'Treatment interruptions predict resistance in hiv-positive individuals purchasing fixed-dose combination antiretroviral therapy in kampala uganda.', *AIDS* **21**, 965–971.

Pearson, C. R., Micek, M. A., Simoni, J. M., Hoff, P. D., Matediana, E., Martin, D. P. & Gloyd, S. S. (2007), 'Randomized control trial of peer-delivered, modified directly observed therapy for haart in mozambique.', *JAIDS* **46**(2), 238 – 244.

Reynolds, N. R. (2004), 'Adherence to antiretroviral therapies: state of the science.', *Curr HIV Res.* **2**(3), 207–214.

Rosenblum, M., Deeks, S. G., van der Laan, M. & Bangsberg, D. R. (2009), 'The risk of virologic failure decreases with duration of hiv suppression, at greater than 50% adherence to antiretroviral therapy.', *PLoS One.* **4**(9), e7196.

Sabate, E: World Health organisation Adherence to long term therapies:Evidence for action. Geneva, S. (2003).

Sampaio-Sa, M., Page-Shafer, K., Bangsberg, D. R., Evans, J., Dourado, M. L., Teixeira, C., Netto, E. & Brites, C. (2008), '100% adherence study: educational workshops vs. video sessions to improve adherence among art-naïve patients in salvador, brazil.', *AIDS Behav.* **4 Suppl**, S54–62.

Seber, G. (1984), *Multivariate observations*, Wiley, New York.

Simoni, J., Amico, K., Smith, L. & Nelson, K. (2010), 'Antiretroviral adherence interventions: Translating research findings to the real world clinic', *Current HIV/AIDS Reports* **7**, 44–51.

Simoni, J. M., Kurth, A. E., Pearson, C. R., Pantalone, D. W., Merrill, J. O. & Frick, P. A. (2006), 'Self-report measures of antiretroviral therapy adherence: A review with recommendations for hiv research and clinical management.', *AIDS Behav* **10**(3), 227–245.

Simoni, J. M., Pearson, C. R., Pantalone, D. W., Marks, G. & Crepaz, N. (2006), 'Efficacy of interventions in improving highly active antiretroviral therapy adherence and hiv-1 rna viral load. a meta-analytic review of randomized controlled trials.', *J Acquir Immune Defic Syndr.* .

Steiner, J. F. & Prochazka, A. V. (1997), 'The assessment of refill compliance using pharmacy records: methods, validity, and applications.', *J Clin Epidemiol.* **50**(1), 105–116.

Vanhove, G. F., Schapiro, J. M., Winters, M. A., Merigan, T. C. & Blaschke, T. F. (1996), 'Patient compliance and drug failure in protease inhibitor monotherapy.', *JAMA.* **276**(24), 1955–1956.

Vrijens, B. & Goetghebeur, E. (1997), 'Comparing compliance patterns between randomized treatments.', *Controlled Clinical Trials* **18**(3), 187 – 203.

Wohl, A. R., Garland, W. H., Valencia, R., Squires, K., Witt, M. D., Kovacs, A., Larsen, R., Hader, S., Anthony, M. N. & Weidle, P. J. (2006), 'A randomized trial of directly administered antiretroviral therapy and adherence case management intervention.', *Clin Infect Dis.* **42**(11), 1619 – 1627.

Patterns of Individual and Population-Level Adherence to Antiretroviral Therapy and Risk Factors for Poor Adherence in the First Year of the DART Trial in Uganda and Zimbabwe

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Background: Good adherence is essential for successful antiretroviral therapy (ART) provision, but simple measures have rarely been validated in Africa.

Methods: This was an observational analysis of an open multicenter randomized HIV/AIDS management trial in Uganda and Zimbabwe. At 4-weekly clinic visits, ART drugs were provided and adherence measured through pill usage and questionnaire. Viral load response was assessed in a subset of patients. Drug possession ratio (percentage of drugs taken between visits) defined complete (100%) and good (≥95%) adherence.

Results: In 2957 patients, 90% had pill counts at every visit. Good adherence increased from 87%, 4 weeks after ART initiation, to 94% at 48 weeks, but only 1454 (49%) patients achieved good adherence at every visit in the first year. Complete adherence was associated with 0.32 greater reduction in $\log_{10}$ viral load (95% confidence interval 0.05, 0.60 $P = 0.02$) and was independently associated with higher baseline CD4 count, starting ART later in the trial, reporting a single regular sexual partner, clinical center, and time on ART.

Conclusions: Population level adherence improved over time suggesting an association with clinical experience. Most patients had at least one visit in the year on which they reported not having good adherence, showing the need for continued adherence interventions.

Key Words: HIV, Africa, adherence, ART

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INTRODUCTION

Since 1995, the introduction of antiretroviral therapy (ART) has led to substantial reductions in HIV–associated mortality and morbidity in industrialized countries.[1–3] There is a strong relationship between adherence, effective viral suppression, and survival.[4–6] Although some patients maintain viral suppression with moderate levels of adherence to newer boosted protease inhibitor and nonnucleoside reverse transcriptase inhibitors,[7,8] the potential for the emergence of resistance supports maximal adherence for optimal long-term outcomes.

Recent expansion of ART in resource-limited settings has led to similar improvements in mortality. Early reports suggest high levels of adherence in Cote d'Ivoire, Senegal, Uganda, and South Africa.[9–12] However, most reports have considered adherence at a population level, that is, what proportion of the population on treatment is adherent at various times after initiation of therapy, and have shown this population level adherence declining or unchanging as time on ART increases.[13,14] Analysis at an individual level is of more interest, whether there is a small subset of patients consistently adhering poorly or more patients have poor adherence for a limited period of time. The challenge is maintaining good adherence over the longer term at the individual level. This may be difficult to sustain without cultural and social support in resource-limited settings, support which may not be scalable to the estimated 4.6 million people needing ART in Africa alone.[15] In addition, good adherence is more than compliance to medication but includes following instructions for prescriptions and attendance at scheduled appointments.[16]

There is no gold standard for measuring adherence. In developed countries, self-report and pill count (PC) seem to overestimate adherence but have been significantly associated with viral load suppression.[13] Given the absence of viral load monitoring in most African settings, measurement of adherence is even more important to manage response to treatment and provide interventions for patients having difficulties with medication. There have been few reports of validation of adherence measurements in Africa. Nonadherence has been associated with the drug regimen, personal factors, stigma, side effects, and travel away from home.[10,17,18]

This paper describes adherence to ART in HIV-infected subjects in Uganda and Zimbabwe enrolled in the Development of Antiretroviral Therapy in Africa (DART) trial over the

first year on ART,[19] comparing different measures of adherence, their relationship with viral load suppression in a subset of participants, and assessing risk factors for poor adherence.

METHODS

Study Design

DART is an open, multicenter, randomized trial, comparing management approaches relevant to resource-limited settings: clinical monitoring only versus laboratory plus clinical monitoring.[19,20] Eligible subjects were ART-naive adults (18 years and older), with documented advanced HIV infection [World Health Organization (WHO) clinical stage 2, 3, or 4] and CD4 count <200 cells/μL in 2 centers in Uganda (1 with an additional satellite site) and 1 center in Zimbabwe. Subjects were ineligible if they had current acute infections, were receiving chemotherapy for malignancies, or were pregnant or breast-feeding. At enrollment, all participants received counseling about medication adherence and drug side effects from a nurse or doctor and had group counseling sessions. This counseling was also reinforced at each clinic visit. All participants initiated first-line triple drug therapy with coformulated zidovudine/lamivudine (Combivir) and tenofovir disoproxil fumarate (DF) (3 pills/d), nevirapine (4 pills/d), or abacavir (4 pills/d) as recommended in World Health Organization guidelines at the time the trial started.[21]

Clinic visits were scheduled at 2 and 4 weeks after enrollment and then every 4 weeks. Participants were asked to return to the clinic if they felt unwell at any time. At each clinic visit, participants were given a new 4-week supply of drugs (no extra pills for late attendance), unused pills from the previous period were counted and recorded, and a structured adherence questionnaire was completed. Every 12 weeks, all participants were seen by a doctor and had a routine full blood count, tests of liver and kidney function, and measurement of lymphocyte subsets.

Viral loads were performed at baseline and at weeks 4, 12, 24, 36, and 48 on a subset of subjects taking zidovudine, lamivudine, and tenofovir. Shortly after the trial started, 100 consecutive subjects were selected from each of the Entebbe, Harare, and Kampala centers divided equally between those with CD4 cell counts 0–99 and 100–199 cells/mm^3 at ART initiation.[22]

Adherence Definitions

The 2 primary measures of adherence were based on the total number of unused pills (for all ART drugs prescribed, including substitutions for toxicity) at each 4-week visit documented by the nurse in the clinic. The PC adherence was defined as 1 minus the proportion of pills dispensed that were returned. Drug possession ratio (DPR) was defined as the days' supply of drugs delivered minus the days' supply of drugs returned divided by the number of days between clinic visits, assuming that ART was used continuously throughout the period between the clinic visits.[6,23] DPR takes into account the date of the clinic visits and adjusts for late return to clinic (meaning pills had been missed) and early return to clinic (meaning the patient should have pills to return). For both measures, we defined complete adherence as 100% adherence and good adherence as at least 95% adherence.[24]

Secondary measures of adherence were taken from the structured adherence questionnaire, namely, reporting late for the scheduled clinic visit, missing any ART doses in the 4 days before the clinic visit, missing an ART dose in the past month, or forgetting the dose at weekends.

Selection of Participants

This analysis considers the first 52 weeks after ART initiation, excluding those lost to follow-up or dying in the first year. For each participant, the number and proportion of clinic visits that achieved complete (100%) adherence or good (≥95%) adherence were calculated per quarter and over the whole year for each measure of adherence, treating missing observations as not achieving complete/good adherence.

Statistical Analyses

Data were analyzed in STATA 9.0. The effect of each adherence measure summarized over the preceding 12 weeks on viral load suppression (<400 or <50 copies/mL) at 12, 24, 36, and 48 weeks was estimated using generalized estimating equations adjusting for time on ART. Similar models using normal interval regression[25] were used to investigate the effect of different measures of adherence on absolute $\log_{10}$ HIV-1 RNA viral load adjusted for baseline viral load and CD4 count.

Generalized estimating equations (GEE) with exchangeable correlation structure were used to estimate associations between complete (100%) DPR adherence and baseline social, demographic, and clinical characteristics across study visits. The effect of time on ART was estimated using 2 linear slopes with a change-point at 12 weeks. Multivariable models were selected based on backward elimination ($P = 0.2$). Odds ratios (ORs), adjusted odds ratios (aORs), and 95% confidence intervals (95% CIs) are presented.

RESULTS

From January 5, 2003 to October 28, 2004, the DART trial enrolled 3316 ART-naive adults, 2156 (65%) women, and 1160 (35%) men.[19] For this analysis focusing on cumulative adherence to ART during the first year after initiation, we excluded 137 (4%) participants who entered a pilot structured treatment interruption study at 28 weeks, 3 participants who subsequently reported ART use before enrollment, 171 (5%) who died in the first year (85 died in the first 3 months), and 48 (1%) who were permanently lost to follow-up before the end of the first year. There were no significant differences in age, sex, center of recruitment, or WHO clinical stage between those excluded and included in the subsequent analysis. However, 53% of those who died or were lost to follow-up had a CD4 count <50 cells/mL at enrollment.

Of the 2957 participants still under follow-up at 52 weeks, 252 individuals (8.5%) missed a total of 393 of the 38,441 scheduled 4-weekly clinic visits during the course of the year (range 0.9%–1.7% over visit weeks). PCs were not performed at 455 (1.2%) of the 38,048 visits that occurred (range 0.6–2.1%), leaving PCs observed at 37,593 clinic visits: missing PCs and/or visits was treated as nonadherent (see Methods). Structured

adherence questionnaires were not completed at 282 visits (0.7%), leaving 37,766 responses for analysis. Nine hundred seventy-nine (2.5%) visits occurred one or more days late (range 1.9%–3.7%). The 2957 patients initiated ART with zidovudine/lamivudine (as Combivir) plus tenofovir DF (2161, 73%), abacavir (285, 10%), or nevirapine (511, 17%).

Adherence Measures

Across all 38,048 PCs, 28,652 (75%) showed 100% adherence, 35,377 (93%) ≥95% adherence, and only 1475 (4%) with <80% adherence. Figure 1 demonstrates that the proportion of patients with ≥95% and 100% DPR adherence over the previous 4 weeks increased progressively over the first year on ART. At week 4, 2581 (87%) participants had ≥95% adherence, increasing to 2745 (93%) at 28 weeks and 2785 (94%) at week 52. Similar increases were seen for other measures of adherence.

Table 1 summarizes the adherence data for participants across 13 clinic visits in the first year. Although good (≥95%) DPR adherence was seen at 93% of all visits in the first year, only 1454 (49%) participants achieved good adherence at every clinic visit (13/13), 76% at at least 12 of the 13 visits, and 87% at at least 11 visits. From the structured questionnaire, during the first year, 1852 (62%) of patients never reported being late for a clinic visit, 1418 (48%) never reported missing a dose in the preceding 4 days, 1938 (66%) never reported forgetting to take ART at weekends, and 816 (28%) never reported missing any dose in the previous month. Two thousand one hundred thirty-seven participants reported 14,595 reasons for missing doses, the most common being: forgot 1072 (N, 50% of participants reporting reasons), away from home 953 (N, 45%), ran out of drugs 678 (N, 32%), too busy 425 (N, 20%), sick 380 (N, 18%), avoiding side effects 282 (N, 13%), change in routine 248 (N, 12%), slept through dose 235 (N, 11%), and too many pills 187 (N, 9%).

Association Between Adherence and Viral Load

Viral load data were available for 278 (9.4%) of the 2957 subjects at baseline. In each subsequent 12-week period, complete adherers were defined as those achieving 100% DPR at all 3 clinic visits preceding the viral load measurement, with 73/267 (27%), 115/274 (42%), 136/270 (50%), and 151/273 (55%) achieving this at 12, 24, 36, and 48 weeks, respectively (denominators changing because of missed visits/no samples). Differences in viral load suppression <50 and <400 copies/mL (the primary outcomes of the virology substudy) between those with 100% DPR and those with lower adherence in the last 12 weeks widened with increasing time on ART, reaching formal statistical significance at week 48 for <50 copies/mL ($P = 0.01$) but not for <400 copies/mL ($P = 0.11$) (Fig. 2). Pooling data across all 4 quarters, the unadjusted GEE analysis showed greater viral suppression in complete adherers for <50 copies/mL [OR = 1.34, 95% CI (1.05, 1.71), $P = 0.02$; adjusted for baseline HIV-1 RNA and CD4 aOR = 1.29 (0.97, 1.70) $P = 0.08$] but not for <400 copies/mL [OR = 1.09 (0.83, 1.44), $P = 0.54$; aOR = 1.13 (0.88, 1.45), $P = 0.33$]. There was no evidence that the effect of complete adherence on viral load varied over time (heterogeneity $P = 0.97$ and 0.29, respectively). None of the other adherence measures were significantly associated with viral load suppression to either <400 or <50 copies/mL.

In an exploratory analysis with greater power, more sensitive normal interval regression models for change in HIV-1 RNA viral load from baseline, we found independent associations for complete DPR and 2 other measures of adherence over the preceding 3 clinic visits, not missing pills at the weekend and not missing pills in the last month (Table 2). The magnitude of the association between log viral load and 100% DPR was similar between the first 6 visits and the later 7.

Association Between Different Measures of Adherence

Across individual visits, the kappa agreement between 100% DPR and patient reporting not missing any dose in the last month was 83% ($\kappa = 0.44$) suggesting moderate agreement, but kappa <0.15 for other adherence measures.

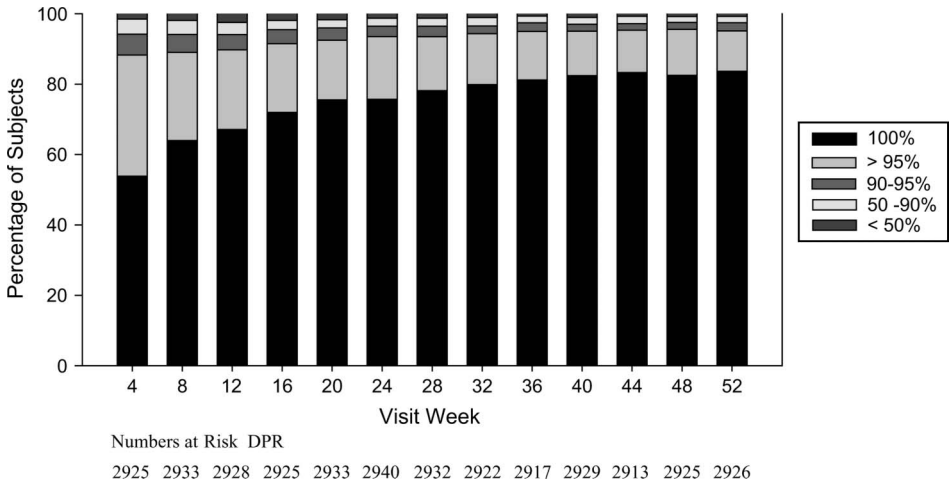

FIGURE 1. Adherence to ART by the DPR at 13 regular clinic visits over the first year in 2957 participants enrolled in the DART trial. Denominators taken from all participants attending clinic in that 4-week period.

TABLE 1. Cumulative Adherence Over the First 13 Clinic Visits (52 Weeks) in DART

No. Visits Achieving This Level of Adherence During the First Year on ART	Adherence Based on PCs				Adherence Based on Structured Questionnaire			
	100% PC	100% DPR	PC ≥ 95%	DPR ≥ 95%	Not Late for Visit	Not Missed dose in Last 4 days	Not Forgot to Take ART at Weekend	Not Missed any ART in Last Month
	Patients (%)	Patients (%)	Patients (%)	Patients (%)	Patients (%)	Patients (%)	Patients (%)	Patients (%)
13/13 (100%)	257 (9)	362 (12)	956 (32)	1454 (49)	1852 (62)	1418 (48)	1938 (66)	816 (28)
12/13 (92%)	481 (16)	491 (17)	985 (33)	806 (27)	725 (25)	921 (31)	700 (24)	787 (27)
11/13 (85%)	555 (19)	505 (17)	553 (19)	323 (11)	226 (8)	376 (13)	210 (7)	522 (18)
10/13 (77%)	496 (17)	426 (14)	270 (9)	178 (6)	82 (3)	136 (5)	63 (2)	321 (11)
9/13 (69%)	398 (13)	328 (11)	105 (4)	81 (3)	35 (1)	51 (2)	20 (0.7)	202 (7)
8/13 (62%)	300 (10)	283 (10)	44 (1.5)	47 (2)	18 (0.6)	26 (1)	10 (0.3)	118 (4)
7/13 (54%)	189 (6)	203 (7)	15 (0.5)	25 (1)	8 (0.3)	15 (0.5)	5 (0.2)	70 (2)
6/13 (46%)	121 (4)	124 (4)	14 (0.5)	9 (0.3)	3 (0.1)	6 (0.2)	3 (0.1)	50 (2)
1–5/13 (<46%)	158 (5)	221 (7)	13 (0.5)	31 (1)	6 (0.2)	6 (0.2)	8 (0.3)	69 (2)
None	2 (0.1)	14 (0.5)	2 (0.1)	3 (0.1)	2 (0.1)	2 (0.1)	0	2 (0.1)
Total patients	2957 (100)	2957 (100)	2957 (100)	2957 (100)	2957 (100)	2957 (100)	2957 (100)	2957 (100)
Total visits with this level of adherence	28846 (75)	28652 (75)	34565 (91)	35377 (93)	36638 (97)	35812 (95)	36872 (98)	32833 (87)

Table shows the number and proportion of participants achieving adherence goals at various numbers of visits during the first year.

From the 4 questions in the structured questionnaire, the only independent predictor of 100% DPR was not missing doses in the last month, which had a specificity of 97% at identifying complete adherence (100% DPR) and a sensitivity of 40% at identifying poor adherence (<100% DPR adherence). Defining nonadherence by reporting missed doses in last month, OR at weekends did not change specificity/sensitivity (97%, 41%, respectively).

Effect of Baseline Characteristics on Adherence Using DPR

Independently, complete (100%) DPR adherence was significantly higher in Center C (OR = 1.89) and in Center D (OR = 1.68) compared with Center A ($P < 0.001$, Table 3). Patients initiating ART in 2004 were significantly more likely to achieve complete DPR adherence than those initiating ART in 2003, and the size of this effect increased after adjustment

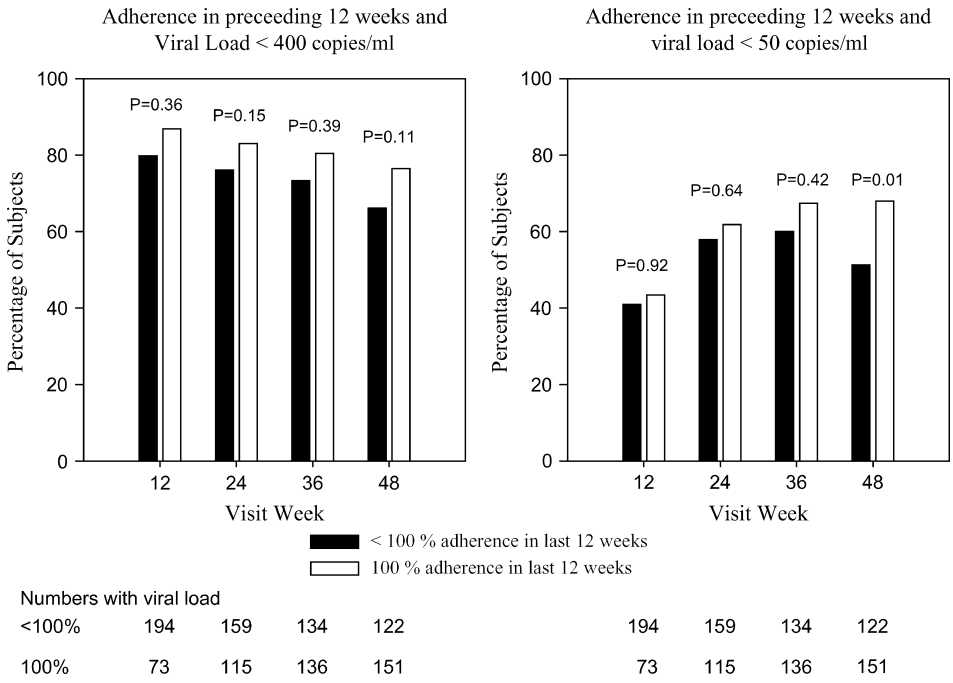

FIGURE 2. HIV-1 RNA suppression by DPR in the preceding 12 weeks in a subset of 278 participants. *P* values from χ^2 test. Global (GEE) *P* value for difference between adherence categories across all 4 time points is 0.08 (<400 copies/ml) and 0.33 (<50 copies/ml), respectively, adjusting for baseline HIV-1 RNA and CD4 (cells/mm^3). GEEs, generalized estimating equations.

TABLE 2. Change in Log_{10} HIV-1 RNA Viral Load from Baseline at 12, 24, 36, and 48 Weeks and Association With Different Adherence Measures in the Preceding 12 Weeks*

Factor	Univariable Model Log (95% CI)	P†	Multivariable Model Log (95% CI)	P‡
100% DPR	−0.31 (−0.58, −0.03)	0.03	−0.32 (−0.60, −0.05)	0.020
95% DPR	−0.23 (−0.59, 0.12)	0.20	−0.28 (−0.62, 0.06)	0.11
100% PC	0.05 (−0.00, 0.10)	0.07	0.04 (−0.01, 0.09)	0.10
95% PC	−0.01 (−0.33, 0.32)	0.96	0.01 (−0.31, 0.33)	0.96
Not reported late for scheduled visit	−0.10 (−0.67, 0.48)	0.74	−0.17 (−0.80, 0.45)	0.58
Not reported missed treatment dose in last 4 days	−0.20 (−0.57, 0.18)	0.31	−0.27 (−0.65, 0.10)	0.16
Not reported missed any ART within last month	−0.23 (−0.53, 0.07)	0.13	−0.30 (−0.59, −0.003)	0.048
Not reported forgetting to take pills at the weekend	−0.51 (−1.02, 0.01)	0.05	−0.58 (−1.09, −0.07)	0.025

*Analysis using interval regression with generalized estimating equations for viral load at 12, 24, 36, and 48 weeks.
†Adjusting for baseline viral load only.
‡Adjusting for baseline viral load, baseline CD4 counts, and time on ART.

for center, CD4 count, and time since ART. Although lower adherence was observed in Center A, exclusion of this center did not change the overall results.

Complete DPR adherence increased over time, with a 9% increase every 4 weeks over the first 12 weeks [aOR = 1.09, 95% CI (1.08, 1.10), $P < 0.001$] and a 2% increase from weeks 12 to 52 [aOR = 1.02, (1.02, 1.03), $P < 0.001$] independently of adjustment for other factors. Complete DPR adherence was also significantly more frequent in those with higher CD4 counts at enrollment [adjusted aOR = 1.08/100 cells higher, (1.01, 1.16), $P = 0.025$].

Complete DPR adherence was not significantly associated with education, the number of child dependents, or the length of the relationship with the current partner. Although univariably there were significant associations between greater adherence and higher previous educational attainment, female gender not disclosing HIV status and not having been admitted to hospital in the last year ($P < 0.05$), these did not persist after adjustment for center, baseline CD4 count, and year of randomization ($P > 0.2$). The only independent social predictor of complete DPR adherence was reporting other sexual partners in the 3 months before starting ART, associated with lower rates of 100% DPR adherence [aOR = 0.72, 95% CI (0.60, 0.87), $P = 0.001$].

DISCUSSION

This paper shows excellent clinic attendance and high adherence to ART over the first year of treatment in a large cohort of previously untreated African individuals similar to other studies in sub-Saharan Africa.[17,26] In contrast to other studies, population level adherence increased during the course of the first year. Although 90% of clinic visits showed good adherence, only 49% of participants achieved this level of adherence at every clinic visit over the first year, highlighting the fact that individuals may find it difficult to maintain good adherence for long periods of time, as demonstrated from chronic disease studies.[27] Our results demonstrate that high levels of adherence reported at a population level may mask considerably more variable adherence at the individual level and emphasize the need for better assessment and promotion of adherence by health care workers in Africa.

Accurate measurement of adherence is difficult: electronic-based monitoring of pill bottle opening is often considered the proxy gold standard but is expensive and intrusive, and its intrinsic effect on underlying adherence is also unknown.[28] Clinic-based PCs,[24,29] drug refills, and self-reported measures of adherence have therefore generally been used to identify poor adherence patterns, and meta-analysis has demonstrated that self-reported measures can distinguish between virologically meaningful patterns of medication use.[30] There are several ways to measure adherence to ART medication; as our aim was to consider convenient measures of adherence to use in routine ART clinics, we used DPR, the proportion of drugs possessed by the patient between clinic visits, and responses to structured questions. In common with other studies, we validated these measures against viral load outcomes, which were associated with 100% DPR adherence but not 95% adherence. Some have argued that lack of virologic suppression may be an inadequate indicator of nonadherence, as other factors such as mutations, regimen potency, and pharmacokinetics can affect virological suppression.[30] Thus, adherence measures associated with viral load suppression may not provide very accurate determinants of adherence behavior. We only investigated viral load in patients on Combivir + tenofovir DF, but the association could be different for other drugs.

We found that reductions in HIV RNA viral load were significantly greater in patients with 100% DPR. Although only 9% of subjects had HIV viral load measurements, these patients were selected according to baseline CD4 and clinical center[22] and thus should not bias the assessment of any differences in adherence level. The numbers provide more than 90% power to detect a difference of 20% in those with undetectable viral load, between different adherence levels. Responses to simple questions about when the last dose was missed and missing doses at weekends independently predicted viral load changes, and reporting missing a dose in the last month also predicted 100% DPR. We recommend that these 2 questions, as the best self-reported measures, are used by African health care workers on a regular (monthly) basis when the DPR cannot be calculated[6,31] and viral load monitoring is not available.

TABLE 3. ORs for Baseline Predictors of Complete, 100% DPR Over the Previous 4 Weeks on ART

Baseline Characteristics		n (%)	Univariable Model OR (95% CI) *P**	Multivariable Model† OR (95% CI) *P**
Center	A	893 (30)	1.00	1.00
	B	872 (29)	1.27 (1.15, 1.40) <0.001	1.32 (1.20, 1.47)
	C	928 (31)	1.89 (1.72, 2.07) <0.001	1.89 (1.71, 2.10)
	D	264 (9)	1.68 (1.41, 2.00) <0.001	1.70 (1.42, 2.03) <0.001
Sex	Male	1038 (35)	1.00	—
	Female	1919 (65)	1.08 (0.99, 1.17) 0.069	—
Age (yrs)	18–30	478 (16)	1.00	—
	30–35	703 (24)	0.99 (0.87, 1.13) 0.93	—
	35–40	754 (26)	1.03 (0.91, 1.17) 0.65	—
	40–45	546 (18)	0.99 (0.87, 1.13) 0.92	—
	45–50	287 (10)	0.85 (0.73, 0.99) 0.04	—
	50+	189 (6)	0.93 (0.78, 1.10) 0.39	—
CD4 (cells/mm^3)‡	Median (IQR)	85 (32, 139)	1.06 (1.01, 1.13) 0.056	1.08 (1.01, 1.16) 0.025
Time (for each 4 wks later)§	Before 12 wks		1.09 (1.08, 1.10) <0.001	1.09 (1.08, 1.10) <0.001
	After 12 wks		1.02 (1.02, 1.03) <0.001	1.02 (1.02, 1.03) <0.001
Initiated ART with Combivir +	Tenofovir	2161 (73)	1.00	—
	Nevirapine	511 (17)	1.20 (1.09, 1.33) <0.001	—
	Abacavir	285 (10)	0.90 (0.78, 1.02) 0.108	—
Year of ART initiation	2003	1622 (55)	1.00	1.00
	2004	1335 (45)	1.18 (1.09, 1.28) <0.001	1.27 (1.17, 1.38) <0.001
Education	None	91 (3)	1.00	—
	Primary	993 (34)	1.04 (0.83, 1.31) 0.721	—
	Secondary	1312 (44)	1.32 (1.05, 1.66) 0.018	—
	Further	540 (18)	1.23 (0.97, 1.57) 0.088	—
Child dependents	None	435 (15)	1.00	—
	1–2	1163 (40)	1.10 (0.97, 1.23) 0.128	—
	3–5	993 (35)	0.99 (0.88, 1.12) 0.883	—
	6+	286 (10)	0.83 (0.71, 0.98) 0.025	—
HIV disclosure	No	127 (4)	1.00	—
	Yes	2830 (96)	0.75 (0.62, 0.91) 0.003	—
Admission to hospital in year before ART	No	2186 (74)	1.00	—
	Yes	694 (23)	0.90 (0.82, 0.99) 0.035	—
Length of relationship with current main partner	No partner	1595 (54)	1.00	—
	≤1 yr	78 (3)	0.79 (0.62, 1.01) 0.063	—
	1–2 yrs	102 (3)	0.86 (0.69, 1.06) 0.159	—
	3–5 yrs	290 (10)	1.03 (0.89, 1.17) 0.700	—
	>6 yrs	864 (29)	1.07 (0.98, 1.17) 0.116	—
Other sexual partners in 3 months before ART‖	None	2612 (88)	1.00	1.00
	1 or more	124 (4)	0.70 (0.59, 0.84) <0.001	0.72 (0.60, 0.87) 0.001
No. times used condoms in last 3 mos with main partner	No partner	1555 (53)	1.00	—
	Sometimes	124 (4)	0.88 (0.78, 0.99) 0.034	—
	Always	549 (19)	0.86 (0.74, 1.00) 0.051	—
	Never	433 (15)	1.10 (0.99, 1.22) 0.071	—

IQR, interquartile range.
**P* values from Wald tests.
†Adjusted for center, year of ART initiation, CD4 count at baseline, and time since ART initiation. Model selection based on backward selection with *P*= 0.2 on social and clinical factors.
‡ORs of 1.06 and 1.08 refer to an increase of 100 in CD4 cell count.
§Time since ART initiation analyzed as 2 linear trends, allowing the effect of increasing time on ART to change at 12 weeks.
‖Sexual partners other than regular or main partner.

Our finding that self-report of missing pills at the weekend predicted viral load is intriguing in the light of recent observations that 90% of missed doses are treatment interruptions of at least 48 hours. These interruptions are more likely associated with drug resistance, at least when using fixed-dose combinations.[32] Nevertheless, the majority of patients, even without good adherence, achieved viral load suppression in agreement with previous reports of excellent

virological suppression with adherence to NNRTIs as low as 54%.[33]

In contrast to other studies in sub-Saharan Africa,[34] all measures used in this study showed adherence improving over the first year of ART, with the most significant improvement seen in the first 12 weeks. Possible explanations include increased social support and health improvements, making adherence and clinic attendance easier with returning strength, and increased ability to manage drug side effects. This accords with the observation that adherence was significantly better in patients with higher CD4 counts at ART initiation, highlighting the need for prompt diagnosis of HIV-positive individuals in resource-limited countries to enable them to access ART as soon as they reach thresholds recommended in national guidelines.[35]

We observed a strong learning effect of calendar time, likely explained by improved experience of counseling and support for patients within the DART trial. This highlights the importance of prioritizing adherence counseling when setting up new ART clinics in resource-limited settings. The best adherence was seen in an urban center and lowest in a rural center, which may indicate that urban, easily accessible clinics are an important component to maintaining adherence. Support from the regular partner and family is also likely to be important, and any lack of trust may impinge negatively on adherence, perhaps explaining why adherence was significantly lower in those who had another sexual partner other than their main/regular partner 3 months before initiating ART.

The reasons for missing pills reported by patients reflected 2 main problems. The first of these was drug related, when patients felt sick, depressed, or side effects from ART. Other reasons were more frequent and came from their personal circumstances when they forgot to take the drugs or were unable to take the drugs correctly because of absence from home or being asleep. These reasons are similar to previous studies in both resource-rich and -limited settings.[36,37] Some patients said they did not adhere because they had run out of pills, and this could reflect poor attendance at the clinic or pill sharing.

In this study, clinical care, ART drugs, and transport were provided without cost to study patients. Other studies have shown that cost of drugs and clinical care are important predictors of adherence in sub-Saharan Africa,[17,18] and therefore, adherence may be higher in our study than in other settings. Our findings are based on clinic-based PCs and self-reported responses to questionnaires: We did not verify whether or not the patients actually took their drugs or if they had problems with dosing interval or dietary requirements. There are also inevitable errors in our estimates of adherence—for example, extra pills were occasionally given to patients who knew beforehand that they would be late for the next clinic visit, but this was not recorded systematically. Other studies have reported that some patients gave or sold drugs to others or threw away their drugs if they experienced difficulties or side effects[38]: Such activities would lead us to overestimate adherence. Patient retention in care is also higher (90%) in DART than reported in other ART programs in sub-Saharan Africa,[39] perhaps due to the greater resources available in this study.

These data show that overall adherence to ART in this setting is good and improved over the first year of treatment. Further analysis of the longer term adherence of patients in the DART trial is ongoing to explore whether good adherence continues up to 4 years of follow-up. In the first year, all patients were on first-line drugs but adherence may be more complicated with second-line treatment regimens. Despite high adherence at the population level, many patients have periods when they do not achieve good adherence. Patients with these problems are associated with lower baseline CD4 counts, side effects, or lack of support mechanisms. DPR and simple questions on missed doses could be useful tools in identifying when patients have problems with adherence and are feasible for large numbers of patients attending routine clinics. Accurate, valid measures of adherence are needed from regular ART clinics to reinforce the ongoing monitoring of adherence and to evaluate interventions to enhance successful use of ART.

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REFERENCES

1. Palella FJ Jr, Delaney KM, Moorman AC, et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. *N Engl J Med*. 1998;338: 853–860.
2. Hogg RS, Heath KV, Yip B, et al. Improved survival among HIV-infected individuals following initiation of antiretroviral therapy. *JAMA*. 1998;279: 450–454.
3. Lima VD, Hogg RS, Harrigan PR, et al. Continued improvement in survival among HIV-infected individuals with newer forms of highly active antiretroviral therapy. *AIDS*. 2007;21:685–692.
4. Bangsberg DR, Perry S, Charlebois ED, et al. Non-adherence to highly active antiretroviral therapy predicts progression to AIDS. *AIDS*. 2001;15: 1181–1183.
5. Nachega JB, Hislop M, Dowdy DW, et al. Adherence to highly active antiretroviral therapy assessed by pharmacy claims predicts survival in HIV-infected South African adults. *J Acquir Immune Defic Syndr*. 2006; 43:78–84.
6. Gross R, Yip B, Re VL III, et al. A simple, dynamic measure of antiretroviral therapy adherence predicts failure to maintain HIV-1 suppression. *J Infect Dis*. 2006;194:1108–1114.
7. Shuter J, Sarlo JA, Kanmaz TJ, et al. HIV-infected patients receiving lopinavir/ritonavir-based antiretroviral therapy achieve high rates of virologic suppression despite adherence rates less than 95%. *J Acquir Immune Defic Syndr*. 2007;45:4–8.
8. Nachega JB, Hislop M, Dowdy DW, et al. Adherence to nonnucleoside reverse transcriptase inhibitor-based HIV therapy and virologic outcomes. *Ann Intern Med*. 2007;146:564–573.
9. Katzenstein D, Laga M, Moatti JP. The evaluation of the HIV/AIDS drug access initiatives in Cote d'Ivoire, Senegal and Uganda: how access to antiretroviral treatment can become feasible in Africa. *AIDS*. 2003;17 (Suppl 3):S1–S4.
10. Laniece I, Ciss M, Desclaux A, et al. Adherence to HAART and its principal determinants in a cohort of Senegalese adults. *AIDS*. 2003;17 (Suppl 3):S103–S108.
11. Gill CJ, Hamer DH, Simon JL, et al. No room for complacency about adherence to antiretroviral therapy in sub-Saharan Africa. *AIDS*. 2005;19: 1243–1249.
12. Orrell C, Bangsberg DR, Badri M, et al. Adherence is not a barrier to successful antiretroviral therapy in South Africa. *AIDS*. 2003;17:1369–1375.
13. Mannheimer S, Friedland G, Matts J, et al. The consistency of adherence to antiretroviral therapy predicts biologic outcomes for human immunodeficiency virus-infected persons in clinical trials. *Clin Infect Dis*. 2002; 34:1115–1121.
14. Howard AA, Arnsten JH, Lo Y, et al. A prospective study of adherence and viral load in a large multi-center cohort of HIV-infected women. *AIDS*. 2002;16:2175–2182.
15. World Health Organisation Report. *Towards Universal Access: Scaling Up Priority HIV/AIDS Interventions in the Health Sector*. Geneva, Switzerland: The World Health Organisation; 2006.
16. Simoni JM, Frick PA, Pantalone DW, et al. Antiretroviral adherence interventions: a review of current literature and ongoing studies. *Top HIV Med*. 2003;11:185–198.
17. Weiser S, Wolfe W, Bangsberg D, et al. Barriers to antiretroviral adherence for patients living with HIV infection and AIDS in Botswana. *J Acquir Immune Defic Syndr*. 2003;34:281–288.
18. Byakika-Tusiime J, Oyugi JH, Tumwikirize WA, et al. Adherence to HIV antiretroviral therapy in HIV+ Ugandan patients purchasing therapy. *Int J STD AIDS*. 2005;16:38–41.
19. Reid A, Munderi P, Kityo C, et al. A randomised trial of monitoring practice and structured treatment interruptions in the management of antiretroviral therapy in adults with HIV infection in Africa. Paper presented at: 15th World AIDS Conference; July 11–16, 2004; Bangkok, Thailand. Abstract TuPeB4495.
20. Ssali F, Stohr W, Munderi P, et al. Prevalence, incidence and predictors of severe anaemia with zidovudine-containing regimens in African adults with HIV infection within the DART trial. *Antivir Ther*. 2006;11: 741–749.
21. WHO. *World Health Organization Scaling Up Antiviral Therapy in Resource Limited Settings: Guidelines for a Public Health Approach*. Geneva, Switzerland; 2002.
22. DART Virology Group and Trial Team. Virological response to a triple nucleoside/nucleotide analogue regimen over 48 weeks in HIV-1-infected adults in Africa. *AIDS*. 2006;20:1391–1399.
23. Sikka R, Xia F, Aubert RE. Estimating medication persistency using administrative claims data. *Am J Manag Care*. 2005;11:449–457.
24. Mills EJ, Nachega JB, Buchan I, et al. Adherence to antiretroviral therapy in sub-Saharan Africa and North America: a meta-analysis. *JAMA*. 2006; 296:679–690.
25. Marschner IC, Betensky RA, DeGruttola V, et al. Clinical trials using HIV-1 RNA-based primary endpoints: statistical analysis and potential biases. *J Acquir Immune Defic Syndr Hum Retrovirol*. 1999;20:220–227.
26. 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;36:1100–1102.
27. Reid SE, Reid CA, Vermund SH. Antiretroviral therapy in sub-Saharan Africa: adherence lessons from tuberculosis and leprosy. *Int J STD AIDS*. 2004;15:713–716.
28. Chesney MA. The elusive gold standard. Future perspectives for HIV adherence assessment and intervention. *J Acquir Immune Defic Syndr*. 2006;43 (Suppl 1):S149–S155.
29. Bangsberg DR, Hecht FM, Clague H, et al. Provider assessment of adherence to HIV antiretroviral therapy. *J Acquir Immune Defic Syndr*. 2001;26:435–442.
30. Nieuwkerk PT, Oort FJ. Self-reported adherence to antiretroviral therapy for HIV-1 infection and virologic treatment response: a meta-analysis. *J Acquir Immune Defic Syndr*. 2005;38:445–448.
31. Glass TR, De Geest S, Weber R, et al. Correlates of self-reported nonadherence to antiretroviral therapy in HIV-infected patients: the Swiss HIV Cohort Study. *J Acquir Immune Defic Syndr*. 2006;41:385–392.
32. Oyugi JH, Byakika-Tusiime J, Ragland K, et al. Treatment interruptions predict resistance in HIV-positive individuals purchasing fixed-dose combination antiretroviral therapy in Kampala, Uganda. *AIDS*. 2007;21: 965–971.
33. Bangsberg DR. Less than 95% adherence to nonnucleoside reverse-transcriptase inhibitor therapy can lead to viral suppression. *Clin Infect Dis*. 2006;43:939–941.
34. Laurent C, Diakhate N, Gueye NF, et al. The Senegalese government's highly active antiretroviral therapy initiative: an 18-month follow-up study. *AIDS*. 2002;16:1363–1370.
35. Wood E, Hogg RS, Yip B, et al. The impact of adherence on CD4 cell count responses among HIV-infected patients. *J Acquir Immune Defic Syndr*. 2004;35:261–268.
36. Ammassari A, Trotta MP, Murri R, et al. Correlates and predictors of adherence to highly active antiretroviral therapy: overview of published literature. *J Acquir Immune Defic Syndr*. 2002;31 (Suppl 3):S123–S127.
37. Bartlett JA. Addressing the challenges of adherence. *J Acquir Immune Defic Syndr*. 2002;29 (Suppl 1):S2–S10.
38. Weidle PJ, Wamai N, Solberg P, et al. Adherence to antiretroviral therapy in a home-based AIDS care programme in rural Uganda. *Lancet*. 2006; 368:1587–1594.
39. Rosen S, Fox MP, Gill CJ. Patient retention in antiretroviral therapy programs in sub-Saharan Africa: a systematic review. *PLoS Med*. 2007;4:e298.

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METHODOLOGY Open Access

Clustering based on adherence data

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Abstract

Adherence to a medical treatment means the extent to which a patient follows the instructions or recommendations by health professionals. There are direct and indirect ways to measure adherence which have been used for clinical management and research. Typically adherence measures are monitored over a long follow-up or treatment period, and some measurements may be missing due to death or other reasons. A natural question then is how to describe adherence behavior over the whole period in a simple way. In the literature, measurements over a period are usually combined just by using averages like percentages of compliant days or percentages of doses taken. In the paper we adapt an approach where patient adherence measures are seen as a stochastic process. Repeated measures are then analyzed as a Markov chain with finite number of states rather than as independent and identically distributed observations, and the transition probabilities between the states are assumed to fully describe the behavior of a patient. The patients can then be clustered or classified using their estimated transition probabilities. These natural clusters can be used to describe the adherence of the patients, to find predictors for adherence, and to predict the future events. The new approach is illustrated and shown to be useful with a simple analysis of a data set from the DART (Development of AntiRetroviral Therapy in Africa) trial in Uganda and Zimbabwe.

Introduction

Adherence is defined as the extent to which patients follow instructions for prescribed treatment necessary to achieve the full treatment benefits [1]. Treatment adherence is known to affect the outcome, but adherence behavior differs not only between patients, but also over time [2]. Also there is no standard measure of adherence, and different adherence measures (variables) are used in different settings and for different treatments [3]. For chronic diseases requiring continuous adherence to treatment a single measure is rarely useful, and one should use combined measures of adherence and consider both mean adherence and the variability in adherence over time [4].

To be clinically relevant, adherence measures should naturally be prominent predictors for future outcomes. With anti-retroviral therapy (ART) for HIV infection, some patients maintain viral suppression and achieve good outcomes with moderate levels of adherence to the newer drugs (boosted protease inhibitor and nonnucleoside reverse transcriptase inhibitors) [5]. However, good adherence is needed to minimise the potential for the emergence of resistance strains of the virus, and to support maximal survival benefit in the long-term [6].

In the analysis of adherence data we often use adherence as a predictor of future outcomes. In assessing the effect of an intervention, measures of adherence are also valuable in describing how well the intervention is received (which may change over time). One therefore wishes to understand and describe in a natural way the relationships

explanatory variables → adherence → responsevariables

For example, in HIV infection several predictors of poor adherence to anti-HIV drugs can be found, including low socio-economic status of the patient [7], low education [8], regimen complexity [9], dosing frequency, cost of drugs and transport [10]. Non-adherence has also been associated with the drug regimen, personal factors, stigma, side effects, and travel away from home [11]. The impact of different patterns of adherence differs by drug class [5,12,13]. Various statistical methods have been used to predict adherence. Linear regression, logistic regression, or multinomial models have been used when adherence is expressed as a percentage, as a dichotomized variable (good vs poor adherence), or as a categorical or categorized

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ordinal variable (good, moderate, poor; 1,2,3+; etc.). Marginal models have been used for an analysis of repeated measures adherence data [14].

In the HIV studies, for example, adherence to ART is an important predictor of mortality, disease progression and virological failure [3,5,15]. Poor adherence to ART raises public health concerns of increased prevalence of disease, more potential for transmission of drug resistant virus to uninfected partners and minimizes the cost benefit of ART. However one difficulty with the analysis of adherence data is how to model the dynamic changes in adherence over time, and how to relate changes in adherence to patient characteristics, and to patient outcomes.

However since adherence is a dynamic and complex human behavior, the key is not so much the individual, observed values themselves, as whether we can characterize the underlying behavior of the patient outside the ART clinic from the observed pattern of reported adherence. In an alternative approach patient adherence measures are seen as a stochastic process, as described by Girard at al, Wong et al, and Sun et al [16], [17] and [18]. Stochastic models have the advantage of taking into account variability in adherence over time, being able to incorporate and distinguish missing data, and flexibility over the type of adherence measure used at each time point.

In our approach to develop new statistical tools to characterize and understand the adherence behavior of the HIV patients treated with ART, and to illustrate the use of these tool with real data. To do this the adherence measures at each time point are first categorized to a variable with finite number of values or "states". Repeated measures over time are then analyzed as a Markov chain of order 1, and the transition probabilities between the states are thought to describe the behavior of a patient. The patients are then clustered using their estimated transition probabilities between the various adherence states. These natural clusters can be used to describe variation in adherence, to find predictors for adherence, and to predict future disease progression or other outcomes. The new approach is illustrated with a simple analysis of a data set from the Development of Antiretroviral Therapy in Africa (DART) trial in Uganda and Zimbabwe (see http://www.ctu.mrc.ac.uk/dart). We compare the predictive powers of different models for mortality with adherence as a continuous and categorical explanatory variable under different Markov chain model assumptions. The comparisons are made using the ROC curves and areas under the ROC curves.

The paper is organized as follows. In Section 2 we describe the repeated adherence measurements on each individual as a Markov chain, and assume that the population consists of a finite number of clusters of patients with the same transition probabilities. In Section 3 the hierarchical clustering procedure based on the transition probabilities is described. Section 4 provides an example of DART trial data. We use three different models (repeated measurements are (i) independent and identically distributed, or distributed according to a (ii) homogeneous or (iii) non-homogeneous Markov chain model) to analyse the data, we describe and interpret the clusters and compare their ability to predict mortality. A discussion of the relative merits of this new approach is given in section 5.

Adherence seen as a Markov chain

We assume that the adherence is measured by a discrete variable with finite number of possible values 1, ..., S. The values are here called *states*. For each individual the *states* are recorded at T time points 1, ..., T. The observed states are then denoted by X_1, ..., X_T , and the whole process can be seen as one classificatory variable with S^T classes or *profiles*. Note that if the adherence measurements are continuous or multivariate, they must first be categorized for the analysis. Note also that *missing data* at some time point can be treated as one of the states.

The adherence measurements over time points 1, ..., T are usually combined by averaging over the entire period to give the *estimated probabilities* (proportions) of being in each state,

$$\hat{P}_s = \frac{1}{T}\sum_{t=1}^{T} I(X_t = s), \quad s = 1, ..., S$$

where $I(X_t = s) = 1$ if $X_t = s$ and zero otherwise. Note that, if X_1, ..., X_T are independent and identically distributed categorical random variables, this would be a sufficient way to describe the adherence behavior over the follow-up period. In this paper, we rather see the adherence as a process, as a (homogeneous) Markov chain with the *transition probabilities* between states

$$p_{ij} = P(X_{t+1} = j | X_t = i), \quad i, j \in \{1, ..., S\}$$

See Chapter 6 in [19], for example. Matrix

$$P = (p_{ij})$$

is then called the *transition matrix*. A natural estimate of p_{ij}, if $\sum_{t=1}^{T-1} I(X_t = i) > 0$, is

$$\hat{p}_{ij} = \frac{\sum_{t=1}^{T-1} I(X_t = i, X_{t+1} = j)}{\sum_{t=1}^{T-1} I(X_t = i)}$$

A more complicated, and sometimes more realistic model to describe the adherence behavior is to use the *Markov chain of order 2* with transition probabilities

$$p_{ijl} = P(X_{t+2} = l | X_t = i, X_{t+1} = j), \quad i, j, l \in \{1, ..., S\}.$$

Also, it is sometimes possible that the the Markov chain is *non-homogeneous* in the sense that the transition probabilities change at a time point t_1 so that the transition probabilities are given by two $S \times S$ matrices, say,

$$P_1 \text{ for } t = 1, ..., t_1 \quad \text{and} \quad P_2 \text{ for } t = t_1 + 1, ..., T.$$

Clustering based on Markov chain approach

In the paper we assume that the adherence behavior of each individual is a Markov chain with unknown transition probabilities. We also assume that the population of the patients can be divided into subpopulations or clusters such that within a cluster the transition probabilities are the same. The cluster memberships can then be used as a categorical variable in further analysis. The unknown cluster memberships must naturally be estimated from the data.

The problem then is how to identify or estimate the clusters using the measurements

$$X_1, ..., X_T.$$

We explain the procedure in the case of the homogeneous Markov chain model. First we find the matrix $Q = (q_{ij})$ with elements

$$q_{ij} = \frac{1}{T-1} \sum_{t=1}^{T-1} I(X_t = i, X_{t+1} = j), \quad i, j = 1, ..., S$$

and then vectorize Q to get a S^2-variate observation vector

$$Z = vec(Q).$$

The vector Z is thus obtained by stacking the columns of Q on top of each other. Note that the estimates of the transition probabilities in P can be obtained by Q just by dividing each row of Q by its row sum. To avoid the possible divisions by zero we use Q instead of P in our analysis. The observed vectors Z are then used instead of the original $X_1, ..., X_T$ to cluster the data.

In the following we use the S^2-variate observations $Z_1, ..., Z_N$ to cluster the N individuals in the data. (The vectors Z_i, $i = 1, ..., N$, are in fact lying in the $(S^2 - 1)$-variate space as the sum of the components is one. We however prefer to use the whole vector Z_i in our analysis instead of any choice of a subvector.) The marginal variables are often standardized for the cluster analysis but that is not natural here; the marginal variables are here probabilities and therefore already on the same underlying scale. We use the hierarchical clustering technique which starts with N clusters (individuals) and then iteratively joins the two most similar clusters until there is just a single cluster. This gives a *tree of clusters* with can be illustrated with a *dendrogram*; one can then cut the tree to have a suitable number of clusters. The clusters should not be too small and they should have relevant interpretations. Natural interpretations may be obtained via joint conditional transition probabilities in the cluster. A measure of dissimilarity or distance between classes is needed for the clustering procedure. Let two distinct index sets I and J, with $I, J \subset \{1, ..., N\}$, give the indices corresponding to two clusters, and let n_I and n_J be the corresponding cluster sizes. The popular *Ward's minimum variance method* of linkage compares the between and within squared *Euclidean distances* with

$$d(I,J) = \sum_{i \in I \cup J} \left\| Z_i - \bar{Z} \right\|^2 - \sum_{i \in I} \left\| Z_i - \bar{Z}_I \right\|^2 - \sum_{j \in J} \left\| Z_j - \bar{Z}_J \right\|^2$$

where $\bar{Z}$, $\bar{Z}_I$ and $\bar{Z}_J$ are the sample mean vectors over the subsets with indices in $I \cup J$, I and J, respectively. See Chapter 7 in [20]. R software was used in the practical analysis of data.

If $X_1, ..., X_T$ are identically and independently distributed then the clustering should be based on a S-vector $Z = (P_1, ..., P_S)$ only where $P_s = [1/T] \sum_{t=1}^{T} I(X_t = s)$, $s = 1, ..., S$. In case of the non-homogeneous Markov chain, one may consider two matrices of estimated probabilities, Q_1 and Q_2, which correspond to measurements at time points 1, ..., t_1 and $t_1 + 1$, ..., T, respectively. The clustering algorithm is then based on the $2S^2$-vector $Z = vec(Q_1, Q_2)$. The interpretations for the clusters can then made using two matrices of transition probabilities, P_1 and P_2. In our application, the change point t_1 is assumed to be fixed and known.

An example: The DART trial in Uganda and Zimbabwe

The data and the problem

We illustrate the clustering procedure and its use with a cohort data set of 2960 participants in the DART trial in Uganda and Zimbabwe. The trial started in January 2003, and the patients were followed until the end of December 2008. Participants' adherence to the treatment was assessed by pill counts and a structured questionnaire administered at each scheduled 4-weekly clinic visit. Participants were asked questions on whether they had missed any dose in the last month, were late for the visit, had forgotten to take any dose at the weekend or missed any ART in the four days prior to the clinic visit. Drug possession ratio (DPR) previously defined as the days'supply of drugs delivered minus the days'supply of drugs returned divided by the number of days between clinic visits, assuming that ART was used continuously throughout the period between the clinic visits was obtained from clinic based pill counts [21]. Also

missed clinic visits, death and loss to follow-up or withdrawals from the study were recorded.

The objective of this analysis was thus to find groups of DART patients with a similar adherence behavior (same proportions of being in each state, or same transition probabilities in homogeneous or non-homogeneous Markov chains) during the first year of the follow-up. We then considered whether the cluster membership could be explained by age or gender (chi-square tests for independence) and whether the risk of death during the second and third year of the follow-up was different in different clusters (chi-square goodness-of-fit tests, logistic analysis).

Adherence variables in this study

In this study we consider the adherence data collected during the first year of the follow-up period (T = 12 visits). Analysis was restricted to the N = 2960 patients who were alive at the end of the first year of the trial, as described in Muyingo et al [21]. We have previously shown that of all self reported measures, 'missed any dose in the last month' most strongly associated with Viral load [21]. The adherence patterns of patients alive at 12 months were later used to predict the mortality during the second and third year. Adherence data were missing at a visit either because the patient (i) totally missed a visit, or (ii) attended but did not complete the adherence questionnaire. For the illustration of our approach here we only use a simple binary variable 'missed any dose in the last month' with the third possibility of missing data for this question for any reason. We then use the Markov chain with S = 3 states,

$$0\ (poor),\quad 1\ (good),\quad and\quad 9\ (missing)$$

Note that the adherence values $(X_1, ..., X_T)$ may be seen as one classificatory variable with $S^T = 3^{12} = 531,441$ classes (profiles). Clustering is a way to reduce the number of classes in a rational way.

The rational and efficient use of the observed values $X_1, ..., X_T$ in the clustering naturally depends on the true statistical model. In the following we consider and compare three different models, namely,

(M1) the i.i.d. model, that is, $X_1, ..., X_T$ identically and independently distributed,

(M2) the homogeneous Markov chain model, and

(M3) the non-homogeneous Markov chain model.

In the model (M3) we assume that the change point is at six months time. Note that the models are nested so that (M1) ⇒ (M2) ⇒ (M3). Likelihood ratio tests can be used to discriminate between the models.

To illustrate the differences between the approaches, consider three individuals, i_1, i_2, and i_3, with the following observed values of $X_1, ..., X_{12}$.

$$i_1 : 9, 9, 9, 0, 0, 0, 0, 0, 1, 1, 1, 1$$
$$i_2 : 9, 0, 1, 1, 1, 9, 1, 0, 0, 0, 0, 9$$
$$i_3 : 9, 1, 0, 0, 0, 9, 0, 0, 1, 1, 1, 9$$

The profiles of the individuals look very different but, if we assume that the model (M1) is true and therefore use estimated probabilities of being in each state (sufficient statistic), the we get

$$P_{i_1} = P_{i_2} = P_{i_3} = (0.417, 0.333, 0.250)'$$

and the three individuals are treated identically in the analysis ($z_{i_1} = Z_{i_2} = Z_{i_3}$).

If one assumes that the second model (M2) is true then one should use the matrix $Q = (q_{ij})$ with elements

$$q_{ij} = \frac{1}{T-1}\sum_{t=1}^{T-1} I(X_t = i, X_{t+1} = j), \quad i, j = 1, ..., S,$$

(a sufficient statistic) to condense the data. For the three individuals we then get

$$Q_{i_1} = \begin{pmatrix} 0.364 & 0.091 & 0.000 \\ 0.000 & 0.273 & 0.000 \\ 0.091 & 0.000 & 0.182 \end{pmatrix} \quad \text{and}$$

$$Q_{i_2} = Q_{i_3} = \begin{pmatrix} 0.273 & 0.091 & 0.091 \\ 0.091 & 0.182 & 0.091 \\ 0.091 & 0.091 & 0.000 \end{pmatrix},$$

and individuals i_2 and i_3 again get the same value $Z = vec(Q)$. Finally, in the third model (M3), the three values of $Z = vec(Q_1, Q_2)$ are different, namely,

$$Z_{i_1} = (0.4, 0.0, 0.2, 0.0, 0.0, 0.0, 0.0, 0.0, 0.4, 0.2, 0.0, 0.2, 0.3, 0.0, 0.0, 0.0, 0.0)',$$
$$Z_{i_2} = (0.0, 0.0, 0.2, 0.2, 0.4, 0.0, 0.0, 0.2, 0.0, 0.6, 0.2, 0.0, 0.0, 0.0, 0.0, 0.2, 0.0, 0.0)', \text{ and}$$
$$Z_{i_3} = (0.4, 0.2, 0.0, 0.0, 0.0, 0.2, 0.2, 0.0, 0.0, 0.2, 0.0, 0.0, 0.2, 0.4, 0.0, 0.0, 0.2, 0.0)',$$

Depending on the chosen model one then uses the adherence variable

$$Z = P\,(\text{model (M1)}),\ Z = vec\,(Q)\ \ (\text{model (M2)}),\ \text{or}$$
$$Z = vec(Q_1, Q_2)\ \ (\text{model (M3)}),$$

in the analysis. The variable is then 3-, 9- or 18-variate, respectively.

Clustering based on the models (M1), (M2), and (M3)

We use hierarchical clustering as explained in Section. The clustering is first based on the 3-variate variable $Z = P$, and the number of clusters was chosen to be six. The probabilities of being in states 0,1, and 9 in each cluster are reported in Table 1. Cluster 1, for example, has the highest proportion for missing data but the proportion for good behavior is also high 83%. Cluster 5 is the poorest one as the proportion for good behavior is only 43%. The last cluster 6 consists of 891 optimally behaving patients.

Table 1 The probabilities of being in state 0, 1, and 9 in six clusters based the i.i.d. model (M1)

	State			
	0	1	9	Σ
Cluster 1 (n = 469)	.065	.829	.107	1.000
Cluster 2 (n = 426)	.280	.688	.032	1.000
Cluster 3 (n = 360)	.167	.833	.000	1.000
Cluster 4 (n = 618)	.083	.917	.000	1.000
Cluster 5 (n = 196)	.489	.426	.085	1.000
Cluster 6 (n = 891)	0	1	0	1.000

The clustering is next based on the 9-variate variable $Z = vec(Q)$ and the homogeneous Markov chain model. For the comparison we use again six clusters here. The transition probabilities between states 0, 1, and 9 in six clusters are reported in Table 2. The probabilities in the table for cluster 2, for example, are read as follows. 52% of those patients who reported good adherence in the previous month achieved good adherence also in this month, 31% of those having missing data in previous month reported good adherence in this month, and so on.

Cluster 2 is clearly the poorest one, as the proportion maintaining good adherence from one month to the next is the lowest. Patients in cluster 6 behave in an optimal way.

Third, we also clustered the data using 18-variate variable $Z = vec(Q_1, Q_2)$ based on the heterogeneous Markov chain model. The conditional transition probabilities were then allowed to be different over different periods (with a change point at six months). The estimated transition probabilities with six clusters are given in Table 3. The clusters can be roughly characterized in the following way.

- Cluster 1: Good adherence - getting worse
- Cluster 2: Poor adherence with missing data - getting slightly better
- Cluster 3: First poor with some missing data - then very good

Table 2 Conditional transition probabilities in six clusters based on homogenous Markov chain model (M2)

	Cluster 1 (*n* = 301)					Cluster 2 (*n* = 281)			
	0	1	9	Σ		0	1	9	Σ
0	0	1	0	1.000	0	0.425	0.523	0.052	1.000
1	0.008	0.950	0.042	1.000	1	0.436	0.516	0.049	1.000
9	0	1	0	1.000	9	0.235	0.307	0.458	1.000
	Cluster 3 (*n* = 463)					**Cluster 4 (*n* = 596)**			
	0	1	9	Σ		0	1	9	Σ
0	0.177	0.765	0.057	1.000	0	0.253	0.723	0.024	1.000
1	0.073	0.871	0.056	1.000	1	0.207	0.770	0.023	1.000
9	0.131	0.652	0.217	1.000	9	0.222	0.684	0.094	1.000
	Cluster 5 (*n* = 469)					**Cluster 6 (*n* = 850)**			
	0	1	9	Σ		0	1	9	Σ
0	0	1.000	0	1.000	0	.	.	.	.
1	0.091	0.909	0	1.000	1	0	1.000	0	1.000
9	.	.	.	.	9	.	.	.	.

Table 3 Conditional transition probabilities in six clusters based on heterogeneous Markov chain model (M3)

	Cluster 1 (*n* = 519)							
	Period 1				Period 2			
	0	1	9	Σ	0	1	9	Σ
0	0.027	0.960	0.133	1.000	0.095	0.866	0.039	1.000
1	0.034	0.953	0.013	1.000	0.115	0.824	0.061	1.000
9	0.117	0.860	0.023	1.000	0.051	0.800	0.149	1.000
	Cluster 2 (*n* = 309)							
	Period 1				Period 2			
	0	1	9	Σ	0	1	9	Σ
0	0.431	0.526	0.043	1.000	0.403	0.549	0.048	1.000
1	0.497	0.458	0.045	1.000	0.279	0.672	0.049	1.000
9	0.264	0.373	0.364	1.000	0.123	0.352	0.519	1.000
	Cluster 3 (*n* = 408)							
	Period 1				Period 2			
	0	1	9	Σ	0	1	9	Σ
0	0.275	0.684	0.041	1.000	0.000	1.000	0.000	1.000
1	0.246	0.690	0.063	1.000	0.015	0.978	0.007	1.000
9	0.226	0.598	0.177	1.000	0.067	0.933	0.000	1.000
	Cluster 4 (*n* = 441)							
	Period 1				Period 2			
	0	1	9	Σ	0	1	9	Σ
0	0.285	0.681	0.035	1.000	0.191	0.781	0.028	1.000
1	0.163	0.799	0.039	1.000	0.273	0.697	0.030	1.000
9	0.202	0.556	0.242	1.000	0.261	0.620	0.120	1.000
	Cluster 5 (*n* = 433)							
	Period 1				Period 2			
	0	1	9	Σ	0	1	9	Σ
0	0.000	1.000	0.000	1.000	.	.	.	.
1	0.118	0.853	0.029	1.000	.	1.000	.	1.000
9	0.000	1.000	0.000	1.000	.	.	.	.
	Cluster 6 (*n* = 850)							
	Period 1				Period 2			
	0	1	9	Σ	0	1	9	Σ
0	.	.	.	.	.	.	.	.
1	.	1.000	.	1.000	.	1.000	.	1.000
9	.	.	.	.	.	.	.	.

Table 4 Contingency tables for cluster categories when clusters are based on (a) models (M1) and (M2), (b) models (M1) and (M3), and (c) (M2) and (M3)

		(a) (M2)					
		1	**2**	**3**	**4**	**5**	**6**
(M1)	1	146	1	250	72	0	0
	2	0	11	6	309	0	0
	3	0	0	172	188	0	0
	4	114	0	35	0	469	0
	5	0	169	0	27	0	0
	6	41	0	0	0	0	850
		(b) (M3)					
		1	**2**	**3**	**4**	**5**	**6**
	1	187	14	116	89	63	0
	2	5	127	82	212	0	0
	3	63	1	187	109	0	0
	4	223	0	19	6	370	0
	5	0	167	4	25	0	0
	6	41	0	0	0	0	850
		(c) (M3)					
		1	**2**	**3**	**4**	**5**	**6**
(M2)	1	124	0	0	0	177	0
	2	0	223	0	58	0	0
	3	158	2	252	51	0	0
	4	24	84	156	332	0	0
	5	213	0	0	0	256	0
	6	0	0	0	0	0	850

• Cluster 4: Poor with some missing data - no big changes

• Cluster 5: First good - then optimal

• Cluster 6: Optimal in both periods

Table 4 gives a cross-tabulation of cluster memberships in the three clustering based on models (M1), (M2), and (M3). One can see that the groups are genuinely different and, as seen from the description of clusters above, the groupings based on (M2) and (M3) describe the adherence behavior in more versatile ways.

Adherence clusters, predictors and explanatory variables

As an illustration of the use of the clusters in a further analysis, we considered the relationship between age and sex and adherence which was categorized using clusters based on non-homogeneous Markov chain model (M3). We also considered how the risks of death in the second and third year of follow-up were associated with adherence behavior during the first year. We thus follow the scheme

$$\boxed{\text{gender, age}} \rightarrow \boxed{\text{adherence}} \rightarrow \boxed{\text{death}}$$

The results in the analyses for clusters coming from heterogeneous Markov chain model (Z = vec(Q_1, Q_2)) are given in Tables 5 and 6. No difference was found between the proportions of women or between the age distributions. There were 100 deaths in the second and third year, individuals in cluster 2 were 2.72 (95% CI:1.42 to 5.18) times more likely to die and in cluster 4 were 2.08 (95% CI:1.42 to 5.18) times more likely to die as compared to Cluster 6 with optimal adherence. Individuals in Clusters 1 and 3 were 1.41 (95% CI:0.72 to 2.71) and 1.47 (95% CI:0.72 to 2.92) times more likely to die whilst in cluster 5, were 0.88 (95% CI:0.72 to 2.71) times likely to die compared to the optimal cluster 6. Adjusting for age and sex did not change the OR estimates. (Age and sex are not confounding factors in this analysis.) Again, R software was used in these analyses.

Table 5 Clusterwise mortality in the second and third year on ART, proportion of women and proportion of patients in three age groups

				Age at ART initiation		
	n	**deaths**	**women**	**18-35**	**35-45**	**45+**
Cluster 1	(*n* = 519)	.033	.65	.41	.42	.17
Cluster 2	(*n* = 309)	.061	.62	.39	.42	.19
Cluster 3	(*n* = 408)	.034	.65	.41	.42	.16
Cluster 4	(*n* = 441)	.048	.65	.41	.43	.16
Cluster 5	(*n* = 433)	.020	.65	.37	.45	.15
Cluster 6	(*n* = 850)	.024	.65	.40	.46	.15

Six clusters are based on the heterogeneous Markov chain model.

Finally, we also compared the categorical cluster variables based on the three models (M1), (M2), and (M3) as predictors of mortality during the second and third year of ART. If linear predictor $\beta' Z_i$ with the rule $\beta' Z_i > c$ is used as a predictor for the death of individual i, then the receiver operating characteristic (ROC) curve is a graphical tool for illustrating the trade off between the false negative (sensitivity) and false positive rates (specificity) for all possible cut off points c, and the area under the ROC curve is a numerical measure of that. There are no big differences between the predictive powers of the three categorical cluster variables based on models (M1), (M2), and (M3) as seen in Figure 1. For the comparison, also the ROC curves from the

Table 6 Estimated odds ratios (OR) with 95 percent confident intervals to compare the risk of deaths in different clusters, also adjusted for age and sex

	OR	**95% CI**	**Adjusted OR**	**95% CI**
Cluster 1	1.4	(0.72, 2.71)	1.4	(0.71, 2.68)
Cluster 2	2.7	(1.42, 5.18)	2.7	(1.41, 5.17)
Cluster 3	1.5	(0.72, 2.93)	1.5	(0.71, 2.91)
Cluster 4	2.1	(1.11, 3.89)	2.1	(1.10, 3.87)
Cluster 5	0.9	(0.38, 1.90)	0.88	(0.38, 1.90)
Cluster 6	1		1	

Cluster 6 serves as a reference class. The clusters are based on the heterogeneous Markov chain model.

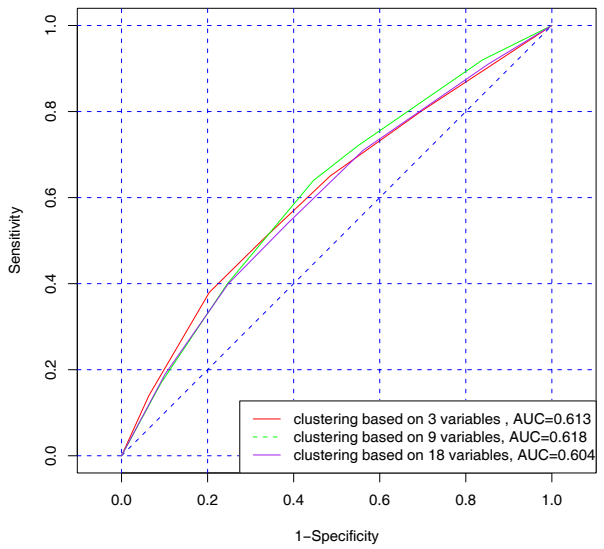

Figure 1 ROC curves for predicting mortality using categorigal cluster variable based on model (M1) (3 variables), model (M2) (9 variables) and model (M3) (18 variables).

conventional logistic regression with explaining variables $Z = P$, $Z = vec(Q)$, and $Z = vec(Q_1, Q_2)$ used as linear predictors in the conventional logistic regression model are given in Figure 2. (Of course the linearity assumption may not be realistic.) The predictive powers in these cases were again very similar. This may be due to

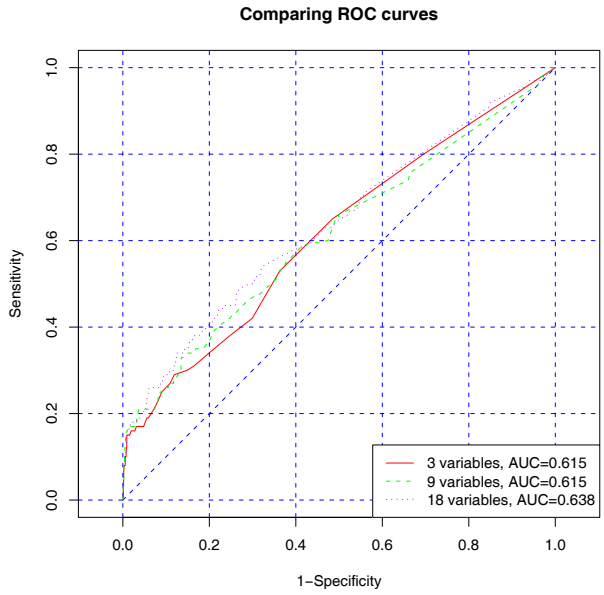

Figure 2 ROC curves for predicting mortality using continuous *Z*-variable based on model (M1) (3-variate), model (M2) (9-variate) and model (M3) (18-variate).

the very short follow-up time of 12 months to assess adherence.

Discussion

The main motivation of this paper was to develop and illustrate new statistical tools to characterize and understand the adherence behavior of the HIV patients treated with ART, and to illustrate these tools with data from the DART study. The Markov chain model is perhaps the simplest model for dependent categorical repeated measurements. In this work, estimated proportions and transition probabilities in a three-state Markov chain model were used to cluster the individuals into groups with different adherence patterns. Transition probabilities represent the dynamics of adherence to ART, and the non-homogeneous Markov chain model allows the patients to change their adherence behaviour over time. Relating patient characteristics to transition probabilities may enable a better understanding of adherence patterns compared to the traditional methods of just averaging the raw adherence data.

We illustrated the approach using one variable - "missed any dose of ART in the last month" - but the approach could be extended to a any number of adherence variables. Several variables can then be used together to construct the states needed for Markov chain model. Our approach can naturally be applied to different data sources (patients diaries, electronic event monitoring, drug possession ratio %), and to the adherence to several drugs simultaneously. Using different adherence variables (if not highly correlated) would produce different clusters with different predictive powers for mortality. The choice of adherence variables is therefore a crucial step, and depends on the data and application.

In our approach, the adherence observations X_1, ..., X_T at time points 1, ..., T , are seen as a realization of a random process. Data analysts often implicitly assume that the observed values X_1, ..., X_T are independent and identically distributed (iid). We think that such assumptions should be explicitly stated, and that it is unrealistic to assume that there is no dependence and no changes in distributions of X_t. With our Markov chain model we have explicitly stated and assumed a certain simple dependency between the observations. In the paper, we compare three different models, namely the iid model (M1), the homogenous Markov chain model (M2), and the non-homogenous Markov chain model (M3). Note that both (M1) and (M2) assume that there is no change in the adherence behavior over the follow-up period. In model (M3) this is allowed. If we assume constant transition probabilities over time we may lose information and important aspects of the phenomenon. It also

important to note that the three models are nested within each other, so that the regular likelihood ratio tests can be used to distinguish between the models; this will be studied in our future work. In the paper we are interested in modeling adherence behavior in general rather than in modelling the changes in adherence behavior as in Lazo et al [22]. However, our model (M3) is flexible enough for modeling the changes as well. In model (M3), the transition probabilities can naturally be made to depend on explaining (modifiable) factors; this is however beyond the scope of this paper.

In our earlier paper [21] we showed that although adherence looked very high overall in the first year in DART, this masked an inconsistent adherence behavior at the individual level. For the illustration and comparison of different adherence profiles we gave an example of three individuals with very different profiles ((9, 9, 9, 0, 0, 0, 0, 0, 1, 1, 1, 1), (9, 0, 1, 1, 1, 9, 1, 0, 0, 0, 0, 9), and (9, 1, 0, 0, 0, 9, 0, 0, 1, 1, 1, 9)) but similar overall adherence (measured as proportions). The differences between the individuals cannot be explained with model (M1) and not even with model (M2). Only model (M3) can analyse the differences between these three individuals. For the DART data set, we found six clusters based on models (M1), (M2), and (M3). The clusters were genuinely different with different interpretations. Also clusters with changing behavior could be found (which supports the use of model (M3)). Our findings suggest that different approaches may be potentially useful in practical data analysis, and that overall (mean) adherence may not be enough when dealing with ART adherence. We compare the predictive powers of the procedures based on models (M1), (M2), and (M3) for mortality with ROC curves. We could not find any big differences between the procedures, but this may just be due to the short period for the measurements of adherence. Whilst viral load is of major importance to participants, this was not done in real-time, so not available for all participants. Death was also relatively infrequent in the second and third year (proportion of deaths = 3%) and so the power to distinguish different effects of M1 from M2 or M3, M2 from M3 was low. However the fact that M2 and M3 classified people differently illustrates potential of our approach.

The DART trial has collected data on virological failures and immunological failures as well. Those outcome variables will be considered in future analyses looking at the predictive value of these adherence clusters. We did not find any significant association between the adherence groups and age or gender. It is possible, however, that other socio-demographic variables are associated with the adherence groups. This approach could then be used in the future to identify individuals at risk of poor adherence. It is of course important to validate this new approach against outcomes that are associated with adherence to ART. In this work, the clustering variable based on the non-homogeneous Markov model was seen to be associated with the risk of death in the second and third year of ART. Those reporting poor or less than adequate adherence had a significantly higher mortality in the second and third year than those that achieved optimal adherence, with the good and adequate users somewhere in-between. The worst cluster has the highest mortality risk.

Our analysis was restricted to those who survived the first year of the trial [21]. The majority of deaths in the first year occurred in the first 3 months (50%). The patients with early deaths did not have the opportunity to fully demonstrate their adherence behavior. We also reasoned that poor patient outcome associated with adherence would likely manifest later in the course of treatment. We considered mortality during the second or third year as the outcome that adherence might predict. The causal pathway is that poor adherence leads to viral replication in the presence of low levels of drug which leads to drug resistance which leads to viral rebound which leads to CD4 decline, and finally leads to morbidity/mortality. It is precisely this process which takes several months, and motivates our prediction model where adherence in the first year predicts mortality in the second or third year.

Because we have used a relatively short time period for the assessment of adherence, the vector Z (with dimensions 3, 9, and 18 in different models) that we use for clustering seems to have a higher dimension than the vector of original observations (with dimension 12). However, the original adherence measurements yield in fact 3^{12} different profiles, and the idea here is to classify these profiles in a rational way. The transformed variables Z provide sufficient statistics in different models; if Z is known then X_1, ..., X_T does not carry any additional information on the model. Our clustering procedures were based on Euclidean distances and Ward's minimum variance method as they seemed to work well in our case. Alternative linkage methods and distance measures should be used to generate the clusters for the comparison. In determining the distances between the individuals we could for example assign different weights to different transition probabilities. This will be a part of our future work on the use of stochastic adherence models and their use to predict future events.

It is of course not always clear what population quantities we are estimating when we report the odds ratio estimates for mortality for the six clusters we have obtained. The underlying assumption is that the data set used in the analysis is a random sample from a population with six subpopulations having different adherence behavior, then the cluster memberships (with six

clusters) estimate the unknown subpopulation memberships, and the odds ratios using cluster memberships estimate the unknown odds ratios for the difference in mortality in the subpopulations. Under the above strict assumptions one may hope that the estimates are consistent to population values. However, if one does not believe in this assumption, one can still consider the predictive power of the whole procedure (area under the ROC curve) and use that for meta-analysis.

There are several extensions and possibilities to develop and deepen the analysis of DART trial data: Another extension to this approach would be to use two or more measures of reported adherence for the states in the Markov chain model. Continuous measurements such as the drug possession ratio could be categorized and used in the Markov chain model. One could look at trends over time and/or over a longer period of 3 years. One could use more states such as lost in the follow-up. In our analysis the problem of drop-outs did not arise as all patients who died or were lost to follow-up in the first year of the trial had been excluded from the dataset [21]. Only 968(2.7%) clinic visits had missing data, 653 were due to forms not being completed by the adherence nurse and 315 were due to missed visits. These numbers were small and were not divided further in this application but could easily be divided if the analysis required it.

For example in clinical trials, non-response or drop-out are important outcomes in their own right and should be distinguished from incomplete forms or poor documentation. Another extension of the model used here would be the use of the Markov chain model of order 2. Statistical tools are needed for the model selection: Statistical tests and estimates for the change point in a non-homogeneous Markov chain model, and tests and estimates for the order of the model. We could easily build likelihood ratio tests for our nested parametric families of distributions. To show whether our Markov chain fits better than an independence model and more specifically test for homogeneity; if our non-homogeneous Markov chain of order 2 fits better than the homogeneous one.

Our aim in this paper was to develop and illustrate some new ideas on how to classify patients based on adherence data using a stochastic model and to illustrate how this analysis could be carried out on real data. Further detailed analyses will be undertaken using the full DART dataset, in which we will explore the extensions to the basic model, develop ways of testing different models and evaluate factors that influence the transition probabilities. We believe this may develop a new way of looking at adherence and in better analysing adherence data.

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Authors' contributions

All authors read and approved the manuscript prior to submission, and approved the final manuscript. All authors have agreed with revisions to the manuscript during the submission process. SK-M wrote the first draft of the manuscript. SK-M and PI did the statistical analyses of the data. SK-M and HO conceived the use of Markov chain models in the analysis of adherence data. SK-M, JL, JT and SW conceived the analysis of adherence data from the DART trial. SK-M and SW prepared the data from the DART trial for analysis.

Competing interests

The authors declare that they have no competing interests.

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References

1. Osterberg L, Blaschke T: **Adherence to medication.** *N Engl J Med* 2005, **353(5)**:487-497.
2. Dimatteo MR: **Variations in patients' adherence to medical recommendations: a quantitative review of 50 years of research.** *Med Care* 2004, **42(3)**:200-209.
3. Simoni JM, Kurth AE, Pearson CR, Pantalone DW, Merrill JO, Frick PA: **Self-report measures of antiretroviral therapy adherence: A review with recommendations for HIV research and clinical management.** *AIDS Behav* 2006, **10(3)**:227-245.
4. Berg KM, Arnsten JH: **Practical and conceptual challenges in measuring antiretroviral adherence.** *J Acquir Immune Defic Syndr* 2006, , **Suppl 1**: 79-87.
5. Bangsberg DR: **Less than 95% adherence to nonnucleoside reverse-transcriptase inhibitor therapy can lead to viral suppression.** *Clin Infect Dis* 2006, **43(7)**:939-941.
6. Bangsberg DR: **Preventing HIV antiretroviral resistance through better monitoring of treatment adherence.** *J Infect Dis* 2008, **197(Suppl 3)**:272-278.
7. Wood E, Montaner JS, Chan K, Tyndall MW, Schechter MT, Bangsberg D, O'shaughnessy MV, Hogg RS: **Socioeconomic status, access to triple therapy, and survival from HIV-disease since 1996.** *AIDS* 2002, **16(15)**:2065-2072.
8. Glass TR, DE Geest S, Weber R, Vernazza PL, Rickenbach M, Furrer H, Bernasconi E, Cavassini M, Hirschel B, Battegay M, Bucher HC: **Correlates of self-reported nonadherence to antiretroviral therapy in HIV-infected patients: the Swiss HIV Cohort Study.** *J Acquir Immune Defic Syndr* 2006, **41(3)**:385-392.
9. Stone VE, Jordan J, Tolson J, Miller R, Pilon T: **Perspectives on adherence and simplicity for HIV-infected patients on antiretroviral therapy: self-report of the relative importance of multiple attributes of highly active antiretroviral therapy (HAART) regimens in predicting adherence.** *J Acquir Immune Defic Syndr* 2004, **36(3)**:808-816.
10. Byakika-Tusiime J, Oyugi JH, Tumwikirize WA, Katabira ET, Mugyenyi PN, Bangsberg DR: **Adherence to HIV antiretroviral therapy in HIV+ Ugandan patients purchasing therapy.** *Int J STD AIDS* 2005, **16**:38-41.
11. Weiser S, Wolfe W, Bangsberg D, Thior I, Gilbert P, MAKHEMA J, Kebaabetswe P, Dickenson D, Mompati K, Essex M, Marlink R: **Barriers to antiretroviral adherence for patients living with HIV infection and AIDS in Botswana.** *J Acquir Immune Defic Syndr* 2003, **34(3)**:281-288.
12. Maggiolo F, Ravasio L, Ripamonti D, Gregis G, Quinzan G, Arici C, Airoldi M, SUTER F: **Similar adherence rates favor different virologic outcomes for patients treated with nonnucleoside analogues or protease inhibitors.** *Clin Infect Dis* 2005, **40**:158-163.
13. Harrigan PR, Hogg RS, Dong WW, Yip B, Wynhoven B, Woodward J, Brumme CJ, Brumme ZL, Mo T, Alexander CS, Montaner JS: **Predictors of HIV drug-resistance mutations in a large antiretroviral-naive cohort initiating triple antiretroviral therapy.** *J Infect Dis* 2005, **191(3)**:339-347.
14. Mellins CA, Chu C, Malee K, Allison S, Smith R, Harris L, Higgins A, Zorrilla C, Landesman S, Serchuck L, LARUSSA P: **Adherence to antiretroviral treatment among pregnant and postpartum HIV-infected women.** *AIDS Care* 2008, **20(8)**:958-968.
15. Nachega JB, Hislop M, Dowdy DW, Lo M, Omer SB, Regensberg L, Chaisson RE, Maartens G: **Adherence to highly active antiretroviral therapy assessed by pharmacy claims predicts survival in HIV-infected South African adults.** *J Acquir Immune Defic Syndr* 2006, **43**:78-84.
16. Girard P, Blaschke TF, Kastrissios H, Sheiner LB: **A Markov mixed effect regression model for drug compliance.** *Stat Med* 1998, **17(20)**:2313-2333.
17. Wong D, Modi R, Ramanathan M: **Assessment of Markov-Dependent Stochastic Models for Drug Administration Compliance.** *Clin Pharmacokinet* 2003, **42**:193-204.
18. Sun J, Nagaraj HN, Reynolds NR: **Discrete Stochastic Models for Compliance Analysis Based on an AIDS Clinical Trial Group (ACTG) Study.** *Biomedical Journal* 2003, **49**:731-741.
19. Grimmett GR, Stirzaker DR: *Probability and Random Processes* New York: Oxford University Press; 1992.
20. Seber GAF: *Multivariate observations* New York: Wiley; 1984.
21. Muyingo SK, Walker AS, Reid A, Munderi P, Gibb DM, Ssali F, Levin J, Katabira E, Gilks C, Todd J: **Patterns of individual and population-level adherence to antiretroviral therapy and risk factors for poor adherence in the first year of the DART trial in Uganda and Zimbabwe.** *J Acquir Immune Defic Syndr* 2008, **48(4)**:468-475.
22. Lazo M, Gange SJ, Wilson TE, Anastos K, Ostrow DG, Witt MD, Jacobson LP: **Patterns and predictors of changes in adherence to highly active antiretroviral therapy: longitudinal study of men and women.** *Clin Infect Dis* 2007, **45(10)**:1377-1385.

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The impact of first year adherence to ART on long term clinical and immunological outcomes in the DART Trial in Uganda and Zimbabwe

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Summary.
Objectives: To describe associations between different summaries of adherence in the first year on antiretroviral therapy (ART) and the subsequent risk of mortality, in order to identify patients at high risk due to early adherence behavior.
Methods: We previously described an approach where adherence behavior at successive clinic visits during the first year on ART was seen as a Markov chain (MC), and the individually estimated transition probabilities between "good", "poor" and "non-response" adherence states were used to classify HIV-infected adults in the DART trial into subgroups with similar behavior. The impact of this classification and classifications based on traditional "averaged" measures (mean drug possession ratio (DPR) and self-reported adherence) were compared in terms of their impact on longer-term mortality over the 2-5 years on ART using Cox proportional hazards models.
Results: Of 2960 participants in follow-up after 1 year on ART, 849(29%) never having missed pills in the last month and 300(11%) had 100% DPR throughout the first year. The poorest adherers by self-reported measures were more likely to have only none/primary education ($p < 0.01$). Being in the poorest adherence subgroup by MC and DPR were independently associated with increased mortality (HR=1.57(95% CI 1.02, 2.42); 1.82(1.32, 2.51) respectively)
Conclusions: Classification based on dynamic adherence behavior is associated with mortality independently of DPR. The classifications could be useful in understanding adherence, targeting focused interventions, and improving longer- term adherence to therapy.

1. Introduction

By December 2009, approximately 5.25 million people were receiving antiretroviral therapy (ART) in low/middle-income countries, representing 36% of those in need of ART in these settings (WHO 2010). Several studies have shown that adherence to combination ART is a major predictor of viral suppression (Nachega et al. 2007, Bajunirwe et al. 2009, Arnsten et al. 2001), drug resistance (Bangsberg et al. 2003, Oyugi et al. 2007), CD4 cell count recovery (Chi et al. 2009, Nash et al. 2008), and survival (Lima et al. 2007, Chi et al. 2009, Abaasa et al. 2008, Nachega et al. 2006).

There is no gold standard measure of adherence. In low/middle-income countries, sophisticated laboratory tests are not available and simple self-report measures are commonly used instead. They do not provide an accurate measure of drug ingestion but may be useful in identifying patients at a high risk of failure. In particular, the drug possession ratio (DPR), a simple measure

based on drugs prescribed, days between visits and returned pill counts, is associated with viral suppression (Gross et al. 2006, Muyingo et al. 2008).

Here we use adherence data from HIV-infected adults in Uganda/Zimbabwe initiating combination ART in the DART trial where adherence was assessed every 4 weeks by nurse count of unused pill counts at each clinic visit (patients were asked to return all unused pills) and a structured questionnaire (DART Trial team. 2010). We previously developed an alternative approach to describe adherence behavior (Kiwuwa-Muyingo et al. 2011) to the more commonly used summary means which simply average adherence behavior. The repeated adherence measurements such as "good adherence", "poor adherence", or "no response" at successive clinic visits were seen as states in a Markov chain. Each individual's estimated transition probabilities over the first year (12 clinic visits) were then used to group patients with similar adherence behavior, using hierarchical clustering techniques. Here we assess the impact of this adherence classification on longer-term mortality in the second-fifth years on ART, and compare with traditional "averaged" methods for describing adherence.

2. Methods

2.1. Study Population

DART was an open-label, multi-center, randomized trial which primarily compared clinically driven monitoring (CDM) versus laboratory and clinical monitoring(LCM) of ART (DART Trial team. 2010). The trial enrolled previously untreated HIV-infected symptomatic (WHO stage 2, 3, or 4) adults (18 years) with CD4 cell count <200 cells/μL in two centers in Uganda (plus a satellite centre) and one in Zimbabwe between January 2003-October 2004. LCM participants received routine 12-weekly laboratory (CD4 count, haematology, biochemistry) and clinical monitoring. In CDM these tests were done, but CD4 counts were never returned and haematology/biochemistry results could be requested only if clinically indicated, that is, monitoring was clinically driven. Viral load was not tested in real-time in either arm. DART received ethics committee approval in Uganda, Zimbabwe and the UK (ISCRTN 13968779).

All participants initiated first-line therapy with co-formulated zidovudine/lamivudine (Combivir) and either tenofovir (3 pills/day), nevirapine (4 pills/day), or abacavir (4 pills/day). As our objective is to investigate the association between first year adherence and long-term ART outcomes, here we exclude patients who died or were lost to follow-up in the first year, and who participated in a pilot structured treatment interruption study during the first year.

At enrolment pre-ART characteristics were recorded including clinical and socio-demographic factors CD4 cell count, WHO disease stage, age, sex, partnerships, and education level. Clinic visits were scheduled 2 and 4 weeks after enrolment, and every 4 weeks thereafter. Every 12 weeks, all participants were seen by a doctor and had a routine full blood count, tests of liver and kidney functioning, and measurement of lymphocyte subsets. Participants were followed up under their randomised strategies until 31 December 2008.

2.2. Adherence measures

Adherence data were collected using two methods at 4-weekly routine clinic visits, an objective nurse pill count (total number of unused pills for each ART drug prescribed since the last visit) and self-reported responses to a structured adherence questionnaire. Based on prescribed and returned

pill counts, drug possession ratio (DPR) was defined as the days supply of drugs delivered minus the days supply of drugs returned, divided by the number of days between clinic visits, i.e the percentage of time the patient could have taken the (correct) medication between clinic visits (Muyingo et al. 2008).

The structured questionnaire contained three key questions about adherence, (a) "How many times in last 4 days have you missed all (or part) of your HIV medication?", (b) " Did you miss any of your anti-HIV drugs last weekend (Saturday or Sunday)? " and (c) " When did you last miss any of your anti-HIV drugs?".

Here, we consider the adherence during the first year on ART, i.e. the first 12 visits. Data at a visit were missing either because the patient totally missed a visit, or attended but did not complete the questionnaire. Based on its association with viral load (Muyingo et al. 2008), we used a simple indicator variable of whether or not the patient had missed any dose in the last month from (c) above. Then, for each visit a patient may be in one of three possible states

$$0 \textit{ (poor adherence)}, \quad 1 \textit{ (good adherence)}, \quad \text{and} \quad 9 \textit{ (missing data)}.$$

In our approach, the 12 first observations on each individual (excluding the week 2 visit) are seen as a non-homogeneous Markov chain (MC) allowing a possible change point after six visits, and their dynamic adherence behavior is fully characterized by their transition probabilities estimated from the observed data. These estimated transition probabilities are then used to cluster the individuals into six classes with similar adherence patterns (Table 1). For example, in class 3, 95% (82%) of those patients reporting good adherence at a visit will also report good adherence at next their visit in 0-6 (6-12)months on ART. For details, see (Kiwuwa-Muyingo et al. 2011).
.

The adherence classification based on this Markov chain approach **(Method M1, MC)** was compared to two other classifications based on traditional "averaged" adherence measures, namely

(Method M2, DPR) the mean DPR for the first 12 visits,

(Method M3, PROP) the proportion of the first 12 visits not reporting any missed dose in last month.

For M3 missed visit/non-response was treated as missed dose. Methods M1 and M3 are based on the same underlying data, but only M1 takes the dynamic nature of adherence behavior into account. For methods M1-M3, we use 6 classes (Table 2).

2.3. Exposures, outcomes and statistical analysis

In all analyses, the main exposure variable was six adherence classes from the first year on ART, that is, our 3 different classification methods (M1, M2, and M3). As the adherence classes are ordered, Kendall's tau was used as a measure of concordance between different classifications. Pre-ART socio-demographic and clinical characteristics were compared between adherence classes using chi-squared (categorical) and Kruskal-Wallis (continuous) tests. Follow-up for the longer-term outcomes started 48 weeks after ART initiation, i.e., just after the 12th clinic visit.

We considered the impact of adherence during the first 48 weeks (12 visits) on time to death using Kaplan-Meier survival curves with log-rank tests, and Cox proportional hazard models stratified by randomized arm, centre, and initial first-line ART, and adjusted for most important confounding factors at ART initiation. In further analyses, we compared the worst adherence class to all others for each of the three classifications M1, M2 and M3. Follow-up was censored at the earliest of lost to follow-up or December 31, 2008.

Table 1. *Mean transition probabilities in six adherence classes based on heterogeneous Markov chain model over the first 12 visits. Period 1 (2) covers the first (last) 6 visits.*

Adherence class 1 ($n = 850$)
consistent optimal users

	0-24 weeks on ART					24-48 weeks on ART			
	next visit:					next visit:			
this visit	poor	good	missing	*Sum*		poor	good	missing	*Sum*
poor	**.**	.	.	.	0	.	.	.	.
good	.	**1.000**	**.**	1.000	1	.	**1.000**	**.**	1.000
missing	.	.	.	.	9	.	.	.	.

Adherence class 2 ($n = 433$)
from good to optimal usage

	0-24 weeks on ART					24-48 weeks on ART			
	next visit:					next visit:			
this visit	poor	good	missing	*Sum*		poor	good	missing	*Sum*
poor	**0.000**	1.000	0.000	1.000	0	**.**	.	.	.
good	0.118	**0.853**	0.029	1.000	1	.	**1.000**	**.**	1.000
missing	0.000	1.000	**0.000**	1.000	9	.	.	**.**	.

Adherence class 3 ($n = 519$)
good adherence getting worse

	0-24 weeks on ART					24-48 weeks on ART			
	next visit:					next visit:			
this visit	poor	good	missing	*Sum*		poor	good	missing	*Sum*
poor	**0.027**	0.960	0.133	1.000	0	**0.095**	0.866	0.039	1.000
good	0.034	**0.953**	**0.013**	1.000	1	0.115	**0.824**	0.061	1.000
missing	0.117	0.860	**0.023**	1.000	9	0.051	0.800	**0.149**	1.000

Adherence class 4 ($n = 408$)
from less than adequate to almost optimal

	0-24 weeks on ART					24-48 weeks on ART			
	next visit:					next visit:			
this visit	poor	good	missing	*Sum*		poor	good	missing	*Sum*
poor	**0.275**	0.684	0.041	1.000	0	**0.000**	1.000	0.000	1.000
good	0.246	**0.690**	0.063	1.000	1	0.015	**0.978**	0.007	1.000
missing	0.226	0.598	**0.177**	1.000	9	0.067	0.933	**0.000**	1.000

Adherence class 5 ($n = 441$)
moderate adherence in both periods

	0-24 weeks on ART					24-48 weeks on ART			
	next visit:					next visit:			
this visit	poor	good	missing	*Sum*		poor	good	missing	*Sum*
poor	**0.285**	0.681	0.035	1.000	0	**0.191**	0.781	0.028	1.000
good	0.163	**0.799**	0.039	1.000	1	0.273	**0.697**	0.030	1.000
missing	0.202	0.556	**0.242**	1.000	9	0.261	0.620	**0.120**	1.000

Adherence class 6 ($n = 309$)
less than adequate adherence in both periods

	0-24 weeks on ART					24-48 weeks on ART			
	next visit:					next visit:			
this visit	poor	good	missing	*Sum*		poor	good	missing	*Sum*
poor	**0.431**	0.526	0.043	1.000	0	**0.403**	0.549	0.048	1.000
good	0.497	**0.458**	0.045	1.000	1	0.279	**0.672**	0.049	1.000
missing	0.264	0.373	**0.364**	1.000	9	0.123	0.352	**0.519**	1.000

Table 2. *Three adherence classifications.*

Method	Item	4-weekly response	Summary	Classification
Method M1	Questionnaire administered by a nurse (4-weekly)	Did you miss any dose in the last month? No=1, yes 0, missing 9	estimated transition probabilities	Classes based on hierachical clustering of transition probabilities in 0-6 and 6-12 months
Method M2	Pill counts by a nurse (4-weekly)	Drug possession ratio (DPR) in (0,1)	Mean DPR over 12 visits	Quantile based classes: (0.998,1](n=461), (0.994,0.998](n=518), (0.988,0.994](n=489), (0.975,0.988](n=490), (0.912,0.975](n=489), (0,0.912](n=490)
Method M3	Questionnaire administered by a nurse (4-weekly)	Did you miss any dose in the last month? No=1, otherwise (Yes or missing) 0.	Mean proportion over 12 visits	Quantile based classes: (0.917,1](n=888), (0.833,0.917](n=767), (0.75,0.833](n=523), (0.667,0.75](n=309), (0.5,0.667](n=284), [0,0.5](n=166)

Potential confounding factors were sex (male/female) and age (18-35,35-50,50+), WHO stage (2,3,4), CD4 counts (0-49, 50-99, 100-149, 150-199), body mass index (-20,20+), and socio-demographic factors at ART initiation. We did not adjust for measurements obtained at the 12 visits during the first year, as these can be seen as intermediate factors between our main exposure variable and the outcome. Statistical analyses were carried out using Stata 10.1 and R 2.12.2.

3. Results

3.1. Participants included

2960 of 3316 participants enrolled in DART were alive and still in follow-up after the first year where they had been intended to be on continuous ART. Excluded patients had died (n=171), been lost to follow-up (n=48), or entered a pilot structured treatment interruption study (n=137) in the first year. Overall 65% of participants were women and the median age at ART initiation was 36 years. There were no significant differences in age, sex or center between excluded and included patients ($p>0.3$), but 53% of those excluded had pre-ART CD4 <50 cells/μL at enrolment versus 33% of those included ($p<0.001$), as expected given the strong association between pre-ART CD4 and early mortality.

3.2. Adherence classes and other characteristics of the patients

In previous development of the Markov chain approach to analyse the adherence measure "missed any dose in last month" described above, we obtained six adherence classes in Table 1 which

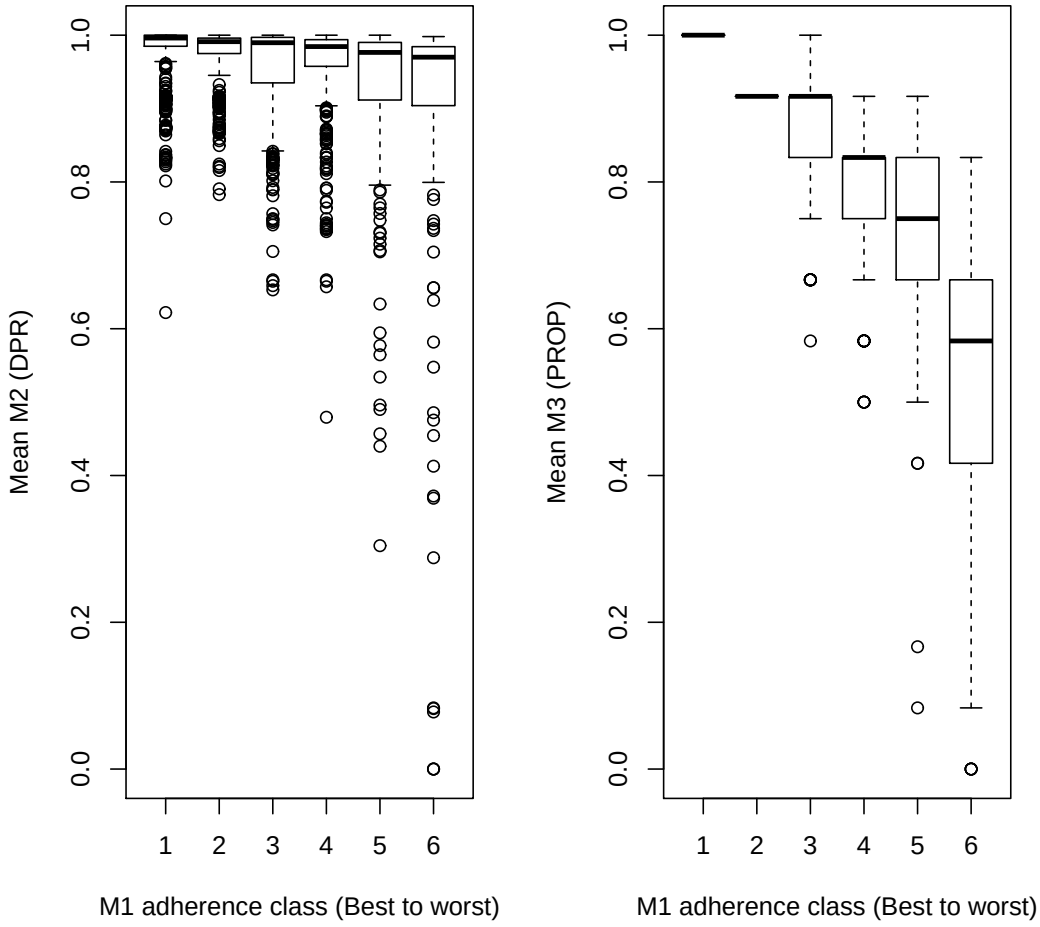

Fig. 1. Box plots of mean M2 and mean M3 by M1 adherence classes. For methods M1, M2 and M3, see Table 2

described different patterns of adherence in the first year on ART: 1) consistent optimal adherence (n=849, 29%), 2) good to optimal adherence (n=433, 15%), 3) good adherers getting worse (n=517, 17%), 4) from less than adequate to almost optimal (n=408, 14%), 5) moderate adherence throughout (n=441, 15%), 6)less than adequate adherence throughout (n=309, 10%). The quantile based adherence classes obtained from M2 and M3, are summarized in Table 2. In particular, in our classification, perfect adherence was less common for M1/M3 (29%) and M2 (10%).

Figure 1 shows that, whilst each summary is trying to capture the same underlying concept of "good adherence", individuals may be classified very differently. Highest values of Kendall's rank correlation were 0.88 (between M1 and M3, as expected as these are based on the same underlying question), 0.35 (between M2 and M3), and 0.32 (between M1 and M2).

The worst adherence classes are in practice most interesting. In the worst adherence class by M1 (MC: n=309, 10%), good adherence at one visit was followed by good adherence at the next visit 46% and 67% of the time in 0-6 and 6-12 months on ART respectively (Table 1). In the worst class

Table 3. *Baseline characteristics according to different adherence classifications - categories are ordered from best to worst.*

Characteristics	Pre-ART CD4 cell count				Highest Education level			Drug Initiated ART		
	0-49	50-99	100-149	150-199	none/ primary	secondary	university/ technical	Tenofovir	Nevirapine	Abacavir
Method M1 (MC)		p=0.07				$p < 0.001$			p=0.001	
class 1	28.9	27.9	30.5	27.4	25.8	29.7	32.8	30.1	25.8	24.7
class 2	16.5	12.7	14.1	14.5	15.4	14.0	14.6	13.6	19.6	13.4
class 3	16.9	16.6	16.9	20.3	16.3	19.2	15.9	16.6	20.9	18.4
class 4	13.4	16.9	11.0	13.9	13.5	14.0	13.8	13.7	14.3	13.4
class 5	13.4	16.3	15.7	14.7	15.1	15.0	13.8	15.2	11.4	18.0
class 6	10.8	9.5	12.0	9.2	13.9	8.1	9.1	10.8	8.0	12.0
Σ	100	100	100	100	100	100	100	100	100	100
Method M2 (DPR)		p=0.02				p=0.03			$p < 0.001$	
class 1	17.8	18.1	15.3	14.6	16.9	16.2	17.7	18.8	11.5	9.8
class 2	15.7	17.4	18.2	15.8	18.1	16.0	15.3	16.8	13.7	20.7
class 3	15.9	19.4	15.2	16.4	19.3	14.5	16.5	16.7	18.2	13.3
class 4	16.7	14.7	17.4	17.6	15.8	17.6	16.3	16.3	18.4	16.8
class 5	19.2	16.4	16.8	18.1	16.7	18.7	16.8	16.9	20.3	19.0
class 6	14.8	14.1	17.1	17.6	13.3	17.0	17.4	14.5	18.0	20.4
Σ	100	100	100	100	100	100	100	100	100	100
Method M3 (PROP)		p=0.01				$p < 0.001$			p=0.01	
class 1	30.4	29.2	31.6	29.6	26.8	31.3	34.5	31.3	27.6	27.2
class 2	27.6	23.5	26.6	26.3	25.4	26.8	25.6	24.7	32.3	25.8
class 3	16.8	20.9	15.3	18.6	17.3	18.3	18.1	17.7	18.8	16.6
class 4	9.5	10.5	12.1	10.5	10.9	10.8	8.8	10.6	9.4	11.7
class 5	8.6	11.2	8.2	11.4	10.9	8.8	9.1	9.5	9.2	11.7
class 6	7.1	4.8	6.3	3.6	8.7	4.0	3.9	6.2	2.7	7.1
Σ	100	100	100	100	100	100	100	100	100	100

for M2 (DPR: n=490, 17%), median of DPR over the first year was only 87%. For M3 (PROP: n=166, 6%), worst adherers reported not missing any dose in last 28 days in 6% of the visits. In particular, these worst adherence classes consisted of genuinely different patients: only 50 (1.7%) were in the worst adherence class in all classifications.

Statistically significant (p<.05) differences between adherence classes were found for the level of education, for initial first-line drug regimen, and for pre-ART CD4 cell counts (M2 and M3 only) (Table 3). For education level, optimal adherers were more likely to have had university/technical education and poor adherers more likely to have had none/primary education consistently across adherence classifications based on self-reported missing pills, although not on DPR (M2). Although differences were significant, trends with pre-ART CD4 and initial drug regimen were less consistent, again illustrating the fact that the different classifications are identifying groups of individuals with different background variables. There were no major or significant differences between the distributions of age, sex, pre-ART WHO disease stage, or randomised group (CDM/LCM) in different adherence categories (p>0.1).

3.3. Adherence and mortality

Of the 2960 participants included, 191 patients (6.5%) subsequently died after 48 weeks. The observed survival probabilities were 0.99, 0.97, 0.96, 0.94, 0.92 at 1-5 years (after 48 weeks on ART). Survival probabilities were clearly lowest in the worst adherence class for all approaches (Figure 2). Differences between other adherence classes were smaller for all approaches so that

the global log-rank test was of marginal statistical significance for categories M1 and M3 (p=0.08, 0.08); M2 was an exception (p=0.01). The adjusted and unadjusted estimates of class effects were similar suggesting the effects were not modified by pre-ART characteristics (Table 4), with the worst adherence class having significantly greater mortality in adjusted analysis with hazard ratios (95% confidence intervals) 2.01(1.21, 3.32), 1.73(1.09, 2.76), and 2.46(1.37, 4.42) for methods M1, M2 and M3, respectively. For DPR (M2) there was little difference between adherence classes other than the worst, although MC class 3 and 4 did similarly (but non-significantly) worse in adjusted as well as unadjusted models.

In a further analysis, we replaced the categorical M1, M2, and M3 variables by three indicator (binary) variables for their worst adherence classes. When a model with M1 and M2 indicators was fitted, both HR ratio estimates, 1.57 (1.02, 2.42) and 1.82 (1.32, 2.51), respectively, were statistically significant so that M1 and M2 indicators seem to have independent effects on mortality. Naturally, this did not happen when a model with M1 and M3 indicators was fitted as they are based on the same underlying adherence measure. In the model with binary M2 and M3, the HR-estimates HR=1.80 (1.30, 2.49) and 1.69 (1.00, 2.87) are again both statistically significant.

However, survival curves showed clear evidence of non-proportional hazards consistent with the effect of adherence in the first year on ART having an effect which changes with time. Fitting a separate model censoring at 2 years showed stronger effects of being in the worst adherence class for all four approaches (Table 4), but overall tests of association were similar to the pooled analysis. In 2-5 years, the effect of being in the worst adherence class in the first year on ART weakened as expected.

4. Discussion

Our main objective was to examine how adherence classes in the first year (developed in an earlier study (Muyingo et al. 2011)) impact on the subsequent risk of mortality and compared this classification to other traditional "averaged" methods. The large numbers accessing ART in resource-limited settings places substantial resource demands on struggling healthcare sectors: identifying patients who have received 1 year of ART who would either likely do well with lower intensity clinical input, or might need enhanced support in order to maximise ART benefits could target limited resources to greatest effect. Viewing adherence as a dynamic process led to identification of 6 adherence classes: which had relatively low concordance with adherence classes defined by mean DPR adherence, highlighting the fact that different summary measures capture different aspects of adherence behaviour which is not homogenous.

Our MC approach (M1), differs from most previous studies on adherence to ART particularly as it demonstrates differences in adherence behavior over time, with 3 of the classes defined by differing adherence between 0-24 and 24-48 weeks on ART. Traditional "averaged" approaches can mask important differences in the underlying behavior - for example MC classes 2 and 3 have similar overall proportions of year 1 visits reporting not missing doses in the last month (93% vs 89%), but in the former adherence is getting better, whereas in the latter it is getting worse over the year. The MC method may therefore unmask important individual differences and provide insight into approaches of improving long-term adherence.

The poorest adherence class experienced the highest risk of death regardless of definition, consistent with other studies of non-virological and immunological outcomes ((Chi et al. 2009, Abaasa et al. 2008, Hogg et al. 2002, Nachega et al. 2006). Importantly 49% of this adherence class

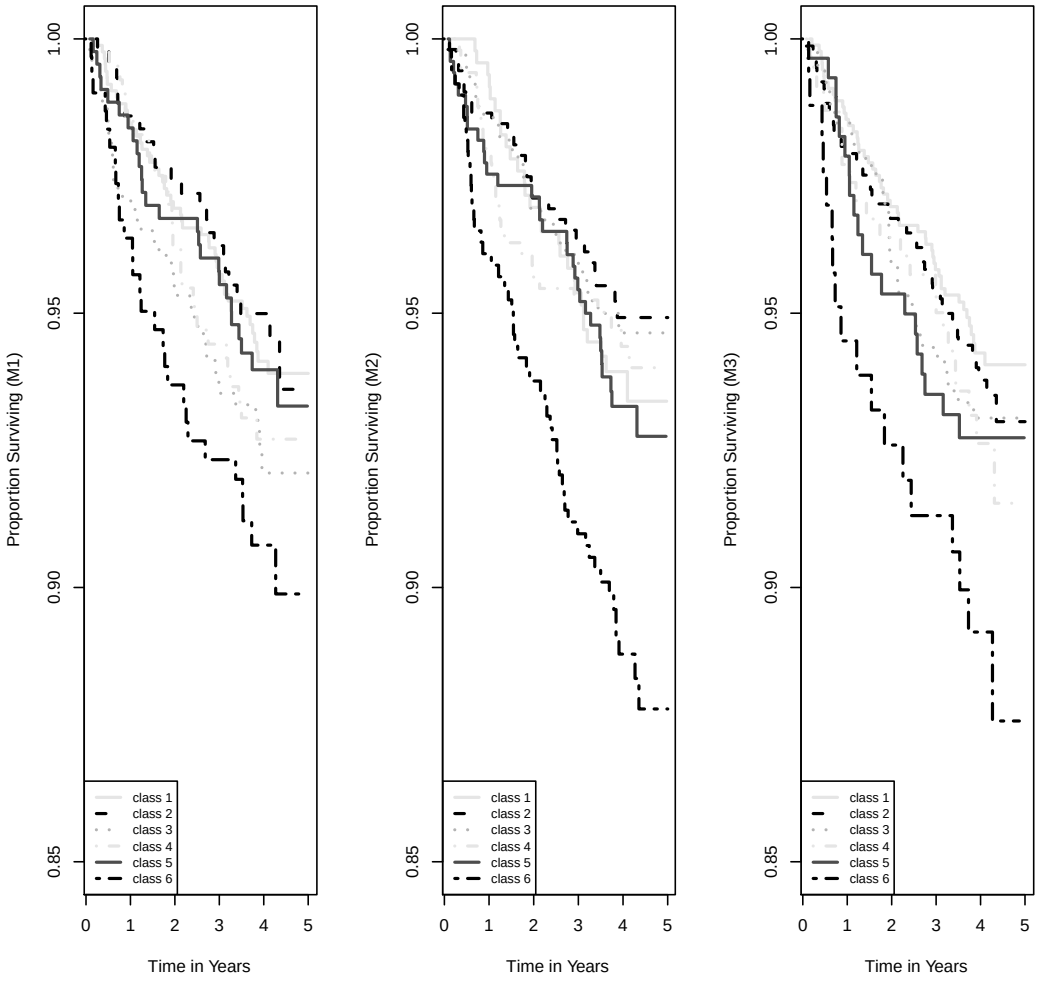

Fig. 2. Estimated Kaplan Meier survival curves for mortality by different adherence classes in methods M1-M3.

had no/only primary education, compared to 33% of the optimal adherence class, and in fact the poorest adherence class had lowest level of education for all definitions based on self-report, highlighting that this group could be targeted for adherence-enhancing interventions both at ART initiation and in those not adhering well after a year on ART. Although MC adherence class was associated with DPR, DPR measures whether patients had drugs available to take (also linked to stock outs and weak drug supply in other studies but not this trial), while MC adherence class is based on dynamic behavior of reporting 'missed dose in last month'. We found that the poorest adherers by MC (M1), DPR (M2) and PROP (M3) respectively had significant independent effects on mortality, suggesting targeting those doing worst on DPR (M2) or MC (M1) could be beneficial.

Associations between MC (M1) adherence class and pre-ART CD4 count were less clear, but those with lowest pre-ART CD4 appeared to be over-represented in class 2 (good to optimal first year adherence) and those with highest pre-ART CD4 appeared to be over-represented in

Table 4. *Estimated hazard ratios (with 95 % confidence intervals) for the effects of three categorical adherence variables (methods M1, M2 and M3) on mortality. The estimates are obtained from a fitted Cox proportional hazard model. The model is stratified by randomized arms, center, and initial first-line ART, and the estimates are adjusted for pre-ART characteristics (CD4 cell count, BMI, WHO disease stage, age and sex). The p-values are for categorical variables with 6 categories.*

Adherence class	(0-5] years (Unadjusted)	p	(0-5] years Adjusted HR(95% CI)	p	(0-2] years Adjusted HR(95% CI)	p	(2-5]years Adjusted HR(95% CI)	p
Method M1								
1	1(ref)	0.08	1(ref)	0.09	1(ref)	0.04	1(ref)	0.9
2	0.98(0.60, 1.62)		0.95(0.58, 1.57)		0.86(0.43, 1.75)		1.02(0.50,2.08)	
3	1.42(0.93, 2.19)		1.41(0.92, 2.17)		1.68(0.96, 2.94)		1.10(0.56, 2.17)	
4	1.38(0.86, 2.22)		1.36(0.84, 2.18)		1.35(0.71, 2.57)		1.33(0.66, 0.68)	
5	1.17(0.72, 1.91)		1.17(0.72, 1.90)		1.20(0.62, 2.31)		1.09(0.53, 2.25)	
6	2.05(1.24, 3.37)		2.01(1.21, 3.32)		2.48(1.31, 5.22)		1.43(0.63, 3.29)	
Method M2								
1	1(ref)	0.004	1(ref)	0.01	1(ref)	0.08	1(ref)	0.02
2	0.78(0.45, 1.34)		0.76(0.44, 1.30)		0.95(0.45, 1.98)		0.56(0.25, 1.29)	
3	0.88(0.51, 1.51)		0.86(0.50, 1.50)		1.07(0.51, 2.24)		0.66(0.29, 1.51)	
4	0.96(0.56, 1.65)		0.93(0.54, 1.60)		1.47(0.74, 2.94)		0.40(0.15, 1.05)	
5	1.11(0.66, 1.86)		1.06(0.63, 1.79)		0.98(0.46, 2.08)		1.09(0.53, 2.24)	
6	1.84(1.16, 2.93)		1.73(1.09, 2.76)		1.99(1.04,3.79)		1.44(0.73, 2.84)	
Method M3								
1	1(ref)	0.08	1(ref)	0.09	1(ref)	0.09	1(ref)	0.7
2	1.17(0.79, 1.76)		1.16(0.77, 1.73)		1.14(0.66, 1.97)		1.14(0.63, 2.07)	
3	1.33(0.86, 2.06)		1.30(0.84, 2.02)		1.45(0.81, 2.58)		1.09(0.55, 2.15)	
4	1.47(0.88, 2.45)		1.46(0.88, 2.44)		1.33(0.66, 2.71)		1.59(0.76, 3.33)	
5	1.42(0.74, 2.70)		1.57(0.91, 2.70)		1.91(0.96, 3.81)		1.17(0.49, 2.84)	
6	2.29(1.37, 3.82)		2.46(1.37, 4.42)		2.88(1.38,6.04)		1.92(0.73, 5.06)	

class 3 (good adherence getting worse) and also less likely to be optimal adherers, with similar trends for DPR. Patients with very low pre-ART CD4 counts may initially struggle to adhere (eg due to food insecurity coupled with increased hunger on ART) (Weiser et al. 2010), but long-term their motivation to adhere may be extremely high having typically experienced severe HIV-related morbidity: in contrast, those initiating ART with higher CD4 counts may have less motivation over the longer-term. However long-term mortality risk in class 2 was not much different from the optimal adherence class 1, demonstrating that providing they survive the first year on ART, with excellent adherence their long-term survival is very good. Our findings with regard to adherence class 5 are more difficult to explain. It is not clear why this class - with only moderate adherence and tendency towards missed visits should have done well with mortality similar to adherence classes 1 and 2. One possible explanation is that these patients were identified in the clinics and responded to more intensive adherence interventions, in contrast to those with the poorest adherence who might have had more pervasive structural barriers to improving adherence.

We have analysed the impact of adherence during the first 48 weeks on time to CD4 failure (defined as the earliest time with either CD4 count $\leq$50 cells/μL, or two successive CD4 counts $\leq$100 cells/μL using Cox proportional hazard models to adjust for potential confounders (data not shown.) The results showed a similar pattern to the results on mortality risk, with the poorest adherence class experiencing highest risk of CD4 failure. We did not analyse viral load as an outcome as these were not available. Virological failure can occur a long time before immunological failure or clinical failure, and conversely, immunological or clinical failure can occur in patients with suppressed viral load (Walker & Gibb 2011). It is therefore possible that there are greater associations between adherence classes and longer-term viral load suppression. We focussed only on initial adherence, as our goal was to identify patients for targeted interventions, but assessing whether the adherence patterns observed in the first year remain stable or change over longer-term

ART would be useful.

Our analysis was restricted to those surviving the first year on ART (Muyingo et al. 2008). The majority of deaths (50%) in the first year occurred in the first three months. The individuals who died in the first year did not have the opportunity to fully demonstrate their adherence profile. The causal pathway - starting with poor adherence leads to viral replication and viral rebound in the presence of sub-optimal drug levels which leads to CD4 failure and death may take several months. We focused on adherence history in the first year, as our goal was to identify patients for targeted interventions, by assessing whether the adherence patterns observed in the first year can explain subsequent adverse outcomes.

In the MC approach, several possibilities of cut-points could be explored. Our choice of 6 month cut-point in the MC approach was a priori the more natural appropriate choice for dividing the first 12 months on ART into 2 periods. The estimated transition probabilities with choices of cut-points less than 6 months, would have greater variation and therefore are less stable.

As adherence class is based on simple self-reported measures/DPR, our adherence measures do not capture dose frequency/timing and do not show actual drug ingestion. However the association of self-reported measures and DPR with long-term clinical outcomes supports its validity in resource-limited settings (Simoni et al. 2006, Chi et al. 2009, Weidle et al. 2006).

Our findings demonstrate clearly that a group of participants with particularly poor adherence, regardless of whether assessed with DPR or self-report over the first year on ART, could be targeted for continued frequent follow-up and enhanced adherence intervention, whereas other patients would probably have done as well without the intensive follow-up provided in this trial. Low education level is the strongest predictor of being in this poorest adherence group. Our study also shows that looking at dynamic adherence behavior unmasks important individual differences even in the first year which predict subsequent mortality and CD4 failure over the longer-term independently of DPR. The 28-day self-report approach which is the only simple (that does not require calculation) measure assessed from self-report is easy to implement in a clinical setting, and even DPR can be approximated by late return to clinic. Whilst formal construction of the MC classes requires statistical modeling and so is probably best suited to a research setting, if regular self-reported data are available. Therefore it is still reasonably simple to identify substantial variation in reporting and frequent reports of missing doses in the last month which together would suggest poorer adherence class. The identification of different risk classes could be useful in understanding and evaluating adherence, targeting focused interventions in clinical and research settings and improving long term adherence to therapy.

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References

Abaasa, A. M., Todd, J., Ekoru, K., Kalyango, J. N., Levin, J., Odeke, E. & Karamagi, C. A. (2008), 'Good adherence to haart and improved survival in a community hiv/aids treatment and care programme: the experience of the aids support organization (taso), kampala, uganda.', *BMC Health Serv Res* **8**, 241.

Arnsten, J. H., Demas, P. A., Farzadegan, H., Grant, R. W., Gourevitch, M. N., Chang, C. J., Buono, D., Eckholdt, H., Howard, A. A. & Schoenbaum, E. E. (2001), 'Antiretroviral therapy adherence and viral suppression in hiv-infected drug users: comparison of self-report and electronic monitoring.', *Clin Infect Dis.* **33**(8), 1417–1423.

Bajunirwe, F., Arts, E. J., Tisch, D. J., King, C. H., Debanne, S. M. & Sethi, A. K. (2009), 'Adherence and treatment response among hiv-1-infected adults receiving antiretroviral therapy in a rural government hospital in southwestern uganda.', *J Int Assoc Physicians AIDS Care (Chic Ill)* **8**, 139–147.

Bangsberg, D. R., Charlebois, E. D., Grant, R. M., Holodniy, M., Deeks, S. G., Perry, S. & et al. (2003), 'High levels of adherence do not prevent accumulation of hiv drug resistance mutations.', *AIDS* **17**, 1925–1932.

Chi, B. H., Cantrell, R. A., Zulu, I., Mulenga, L. B., Levy, J. W., Tambatamba, B. C. & et al. (2009), 'Adherence to first-line antiretroviral therapy affects non-virologic outcomes among patients on treatment for more than 12 months in lusaka, zambia.', *Int J Epidemiol* **38**, 746–756.

DART Trial team. (2010), 'Routine versus clinically driven laboratory monitoring of hiv antiretroviral therapy in africa (dart): a randomised non-inferiority trial.', *Lancet* **375**, 123–131.

Gross, R., Yip, B., Lo Re, V. r., Wood, E., Alexander, C. S., Harrigan, P. R., Bangsberg, D. R., Montaner, J. S. & Hogg, R. S. (2006), 'A simple, dynamic measure of antiretroviral therapy adherence predicts failure to maintain hiv-1 suppression.', *J Infect Dis* **198**(8), 1108–14.

Hogg, R. S., Heath, K., Bangsberg, D., Yip, B., Press, N., O'Shaughnessy, M. V. & Montaner, J. S. (2002), 'Intermittent use of triple-combination therapy is predictive of mortality at baseline and after 1 year of follow-up.', *AIDS* **16**, 1051–1058.

Kiwuwa-Muyingo, S., Oja, H., Walker, A. S., Ilmonen, P., Levin, J. & Todd, J. (2011), 'Clustering based on adherence data.', *Epidemiological Perspectives and Innovations* **8**(3).

Lima, V. D., Hogg, R. S., Harrigan, P. R., Moore, D., Yip, B., Wood, E. & Montaner, J. S. (2007), 'Continued improvement in survival among hiv-infected individuals with newer forms of highly active antiretroviral therapy.', *AIDS* **21**, 685–692.

Muyingo, S. K., Walker, A. S., Reid, A., Munderi, P., Gibb, D. M., Ssali, F. & et al. (2008), 'Patterns of individual and population-level adherence to antiretroviral therapy and risk factors for poor adherence in the first year of the dart trial in uganda and zimbabwe.', *J Acquir Immune Defic Syndr* **48**, 468–475.

Nachega, J. B., Hislop, M., Dowdy, D. W., Chaisson, R. E., Regensberg, L. & G., M. (2007), 'Adherence to nonnucleoside reverse transcriptase inhibitor-based hiv therapy and virologic outcomes.', *Ann Intern Med* **146**, 564–573.

Nachega, J. B., Hislop, M., Dowdy, D. W., Lo, M., Omer, S. B., Regensberg, L. & et al. (2006), 'Adherence to highly active antiretroviral therapy assessed by pharmacy claims predicts survival in hiv-infected south african adults.', *J Acquir Immune Defic Syndr* **43**, 78–84.

Nash, D., Katyal, M., Brinkhof, M. W., Keiser, O., May, M., Hughes, R. & et al. (2008), 'Long-term immunologic response to antiretroviral therapy in low-income countries: a collaborative analysis of prospective studies.', *AIDS* **22**, 2291–2302.

Oyugi, J. H., Byakika-Tusiime, J., Ragland, K., Laeyendecker, O., Mugerwa, R., Kityo, C. & et al. (2007), 'Treatment interruptions predict resistance in hiv-positive individuals purchasing fixed-dose combination antiretroviral therapy in kampala uganda.', *AIDS* **21**, 965–971.

Simoni, J. M., Kurth, A. E., Pearson, C. R., Pantalone, D. W., Merrill, J. O. & Frick, P. A. (2006), 'Self-report measures of antiretroviral therapy adherence: A review with recommendations for hiv research and clinical management.', *AIDS Behav* **10**(3), 227–245.

Walker, A. S. & Gibb, D. M. (2011), 'Food insecurity as a barrier to sustained antiretroviral therapy adherence in uganda.', *Curr Opin Infect Dis.* **24**(1), 27–33.

Weidle, P. J., Wamai, N., Solberg, P., Liechty, C., Sendagala, S., Were, W. & et al. (2006), 'Adherence to antiretroviral therapy in a home-based aids care programme in rural uganda.', *Lancet* **368**, 1587–1594.

Weiser, S. D., Tuller, D. M., Frongillo, E. A., Senkungu, J., Mukiibi, N. & Bangsberg, D. R. (2010), 'Food insecurity as a barrier to sustained antiretroviral therapy adherence in uganda.', *PLoS One* **5**(4), e10340.

WHO (2010), *World Health Organisation Towards universal access: Scaling up priority HIV/AIDS interventions in the health sector, Progress report 2010 (n.d.).*